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Endometriosis: an improper name for two different disorders

Andy Petroianu[1*]

The suffix osis (from ancient Greek, ωσις) in medical terms denotes a state of morphological and functional disorder, in general with degenerative consequences such as osteoporosis, arthrosis, diverticulosis, cirrhosis, myocardosis, apoptosis, and necrosis[1]. Even when this word is used to define an increasing process, for example, leukocytosis, fibromatosis, tuberculosis, and mycosis, deteriorating consequences occur in cells, tissues, and organs as well[1]. Endometriosis may be used as endometrial hypoplasia or atrophy, which occurs with the age inside uterus, starting in the climacteric period. However, this term has been used as the presence of endometrial tissue not only in abnormal sites, mostly in the pelvis, but also in other parts of the body. Endometrial tissue has been described in peritoneum, omentum, liver, kidney, lung, heart, spine, eyes, neck, and even central nervous system. This tissue is frequently found in pelvic tissues, including ovary, sigmoid, rectum, and abdominal wall after surgical procedures on the uterus[2].

The presence of endometrial tissue outside the uterus is not a deteriorating process or an increased disorder of the endometrium. The correct term for the presence of any tissue far from its origin is "teleplasia" (τῆλε, at a distance, far away, or far from; and πλάσις, molding, formation), but this word was not included in the medical terminology. Since the first studies of pathology in the 19th century, metaplasia (μετά, after, beyond, changed, or altered) has been adopted. The pathogenesis of any metaplasia, including metastasis, is not known, but it is not due to a modification of local mature tissue to another type of cell. Only the stem cells are able to create other cells and probably the metaplasia of any tissue[3]. None of theories that explained this disorder has been proven. Thus, the correct term for endometrial tissue far from the uterus is endometrial metaplasia no matter its origin since the embryonic stage or from stem cells[4,5].

On the other hand, the iatrogenic implant of endometrial tissue in pelvic organs (peritoneum, ovary, urinary bladder, sigmoid, rectum) and in the surgical wound during a procedure on the uterus, mainly cesarean, biopsies, and intrauterine fetal surgeries, cannot be named endometrial metaplasia. The pathogenesis of this disorder has been established as a surgical event, and it should be named endometrial implant. These implants are self-limited, occur near the uterus, and do not spread to distal sites[6,7].

In conclusion, spontaneous presence of endometrial tissue outside the uterus should be named endometrial metaplasia, and when the ectopic endometrial tissue is due to a surgical procedure, it is an endometrial implant, but not endometriosis.

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Severe atopic dermatitis and dupilumab

Wanderley Bernardo[1,2*], Luca Silveira Bernardo[3], Juliana Hegedus Baroni[4], Ricardo dos Santos Simões[2]

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct, and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.

QUESTION

Is there an indication for the drug dupilumab in the treatment of severe atopic dermatitis? Is its constant use effective and safe?

METHODS

Eligibility criteria

The eligibility criteria used to select the evidence and data were as follows: patients with severe atopic dermatitis (refractory to conventional treatment); intervention: dupilumab; comparison: placebo; outcomes: reduction of the effects of the disease and adverse events; study design: randomized clinical trials and no period and language restriction.

Base consulted and strategy used

Searches were performed in the Medline database via PubMed, using the following search strategy: (Dermatitis, Atopic OR Atopic Dermatitides OR Atopic Dermatitis OR Atopic Neurodermatitides OR Atopic Neurodermatitis OR Disseminated Neurodermatitides OR Disseminated Neurodermatitis OR Atopic Eczema) AND (dupilumab) AND Random*.

Extracted data

The studies selected according to the eligibility criteria had their full texts accessed, from which the following variables were extracted: author's name, year of publication, study design, description of the population, intervention, comparison, outcomes, and follow-up time.

Risk of bias

The biases evaluated were: randomization and allocation methods, double and rater blinding, losses, appropriate outcomes, prognostic characteristics of the compared groups, ITT analysis, presence of sample calculation, early interruption, selection bias, and "confounding bias." This risk was estimated as very high, high, or low.

Quality of evidence analysis by outcome

The quality of evidence analysis was expressed as very low, low, moderate, and high. The items considered (using GRADEpro software) were classified as very high, high, and low, using the items: risk of bias, inconsistency, precision, indirect evidence, and publication bias.

Expression of results (with meta-analysis)

Event risk difference (difference between absolute risk of intervention and comparison for each outcome) and 95% confidence interval (CI) for each risk difference are presented. Heterogeneity in I^2 ranges from 0 to 100%, with values above 50% considered high (inconsistency). Random (I^2>50%) and fixed (I^2≤50%) models were used for analysis. Sensitivity analysis was used to treat I^2 >50% in the presence of publication bias (Egger's test).

RESULTS

Works retrieved and selected

A total of 1,344 works were retrieved, of which 57 publications were selected by title and/or abstract. Nine randomized

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clinical trials were included to support the analyses and conclusions of this review[1-9].

A total of 2,606 patients submitted to dupilumab and 1,441 submitted to placebo were analyzed. The populations are children, adolescents, and adults. The dupilumab regimen used was a 300 mg subcutaneous injection once every 1, 2, or 4 weeks. The follow-up time ranged from 12 to 28 weeks. The outcomes analyzed were: Eczema Area and Severity Index (EASI) 75 and 50 (improvement ≥50% and 75%); Investigator's Global Assessment (IGA) (0–1 and/or improvement ≥2 points); numerical rating scale (NRS) pruritus (reduction ≥3 points); and treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs). Subgroup analysis by age was not performed due to the homogeneity obtained, as well as the fact that 80% of the studies were in adults.

FLUX DIAGRAM

Selection of retrieved works in the virtual databases of scientific information is detailed in Figure 1.

RESULTS BY OUTCOMES

EASI 50 (improvement ≥50%)

In this analysis (Figure 2), 1,773 patients submitted to dupilumab and 1,684 to placebo were studied. Treatment with dupilumab increases the improvement by ≥50% (EASI 50) by 40% (95%CI 37–43%) (NNT: 2) when compared to placebo. The quality of evidence is high.

EASI 75 (improvement ≥75%)

In this analysis (Figure 3), 2,606 patients submitted to dupilumab and 2,615 to placebo were studied. Treatment with dupilumab increases the improvement by ≥75% (EASI 75) by 37% (95%CI 34–39%) (NNT: 3) when compared to placebo. The quality of evidence is high.

IGA (0–1 and improvement ≥2 points)

In this analysis (Figure 4), 2,606 patients submitted to dupilumab and 2,615 to placebo were studied. Treatment with

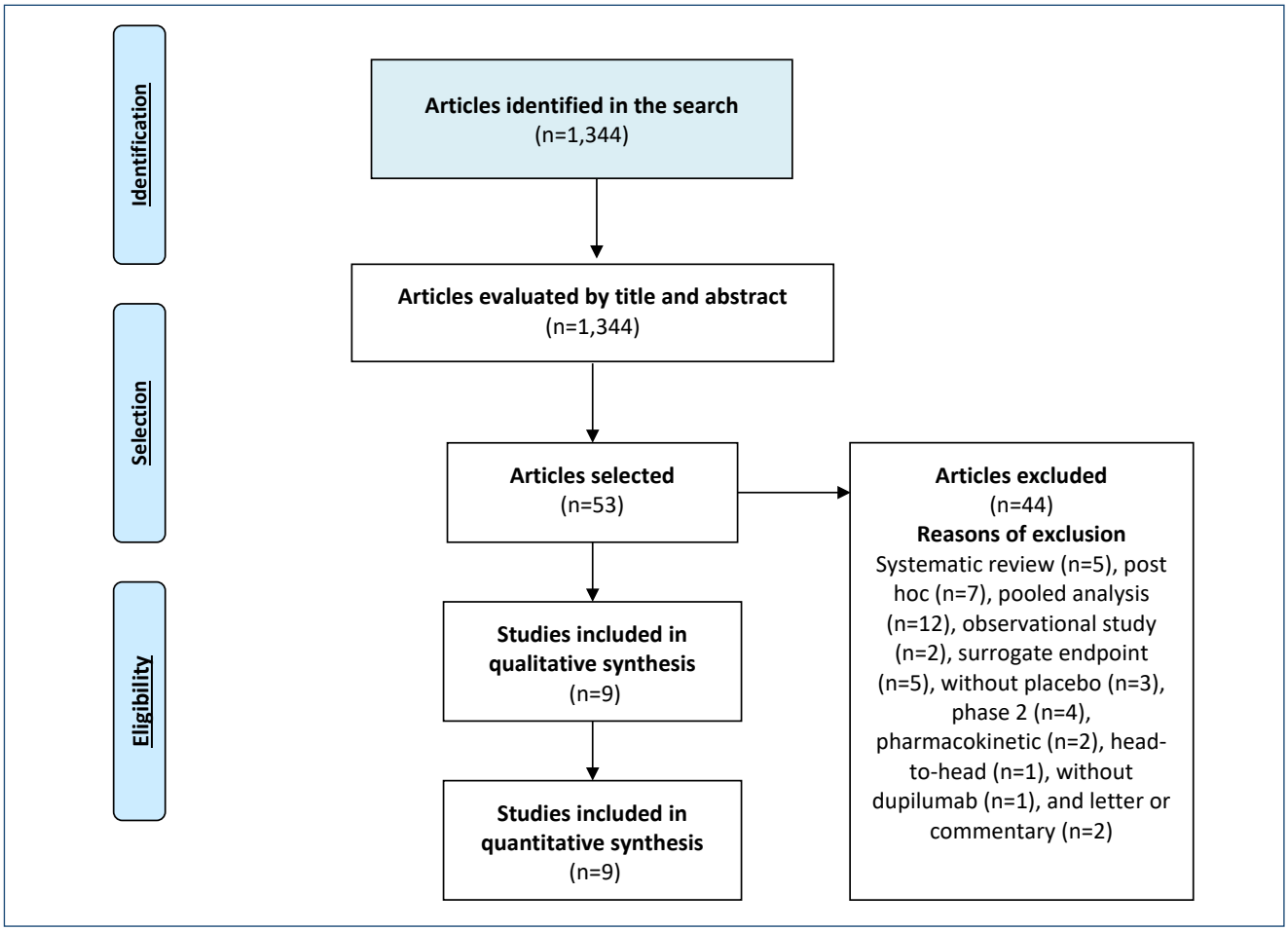

Figure 1. Flow diagram of evidence retrieved, selected, and included, with exclusion reasons.

dupilumab increases the improvement measured by IGA by 25% (95%CI 23–27%) (NNT: 4) when compared to placebo. The quality of evidence is high.

NRS pruritus (reduction ≥3 points)

In this analysis (Figure 5), 2,126 patients submitted to dupilumab and 1,931 to placebo were studied. Treatment with dupilumab reduces pruritus measured by the NRS by 21% (95%CI 5–36%) (NNT: 5) when compared to placebo. The quality of evidence is low.

Treatment-emergent adverse events

In this analysis (Figure 6), 748 patients submitted to dupilumab and 664 to placebo were studied. There is no difference in the risk of TEAEs with dupilumab treatment compared to placebo. The quality of evidence is high.

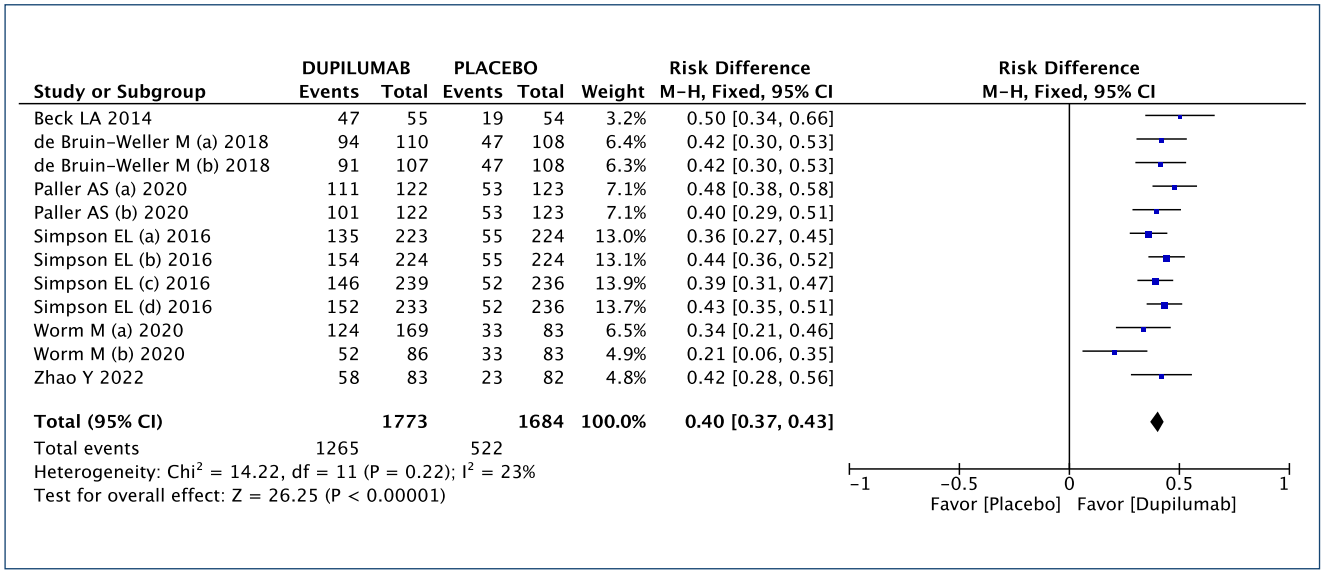

Figure 2. Analysis of the Eczema Area and Severity Index 50 outcome in patients with severe atopic dermatitis treated with dupilumab.

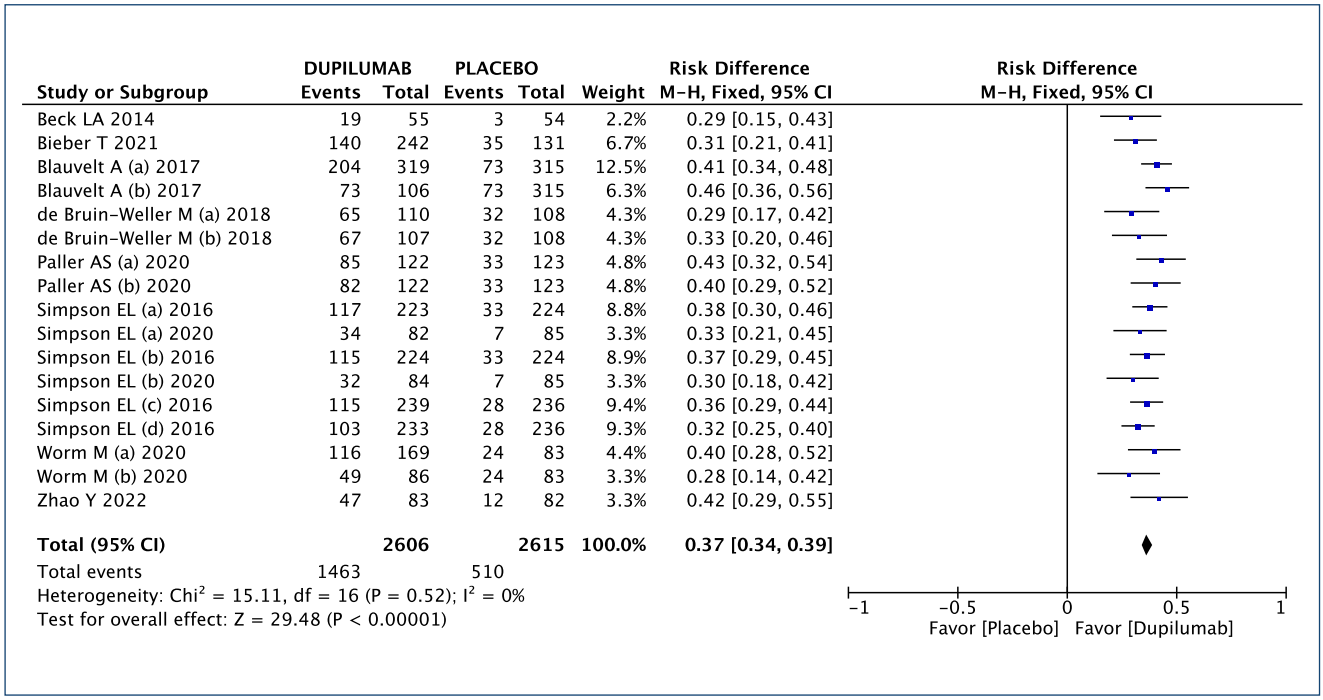

Figure 3. Analysis of the Eczema Area and Severity Index 75 outcome in patients with severe atopic dermatitis treated with dupilumab.

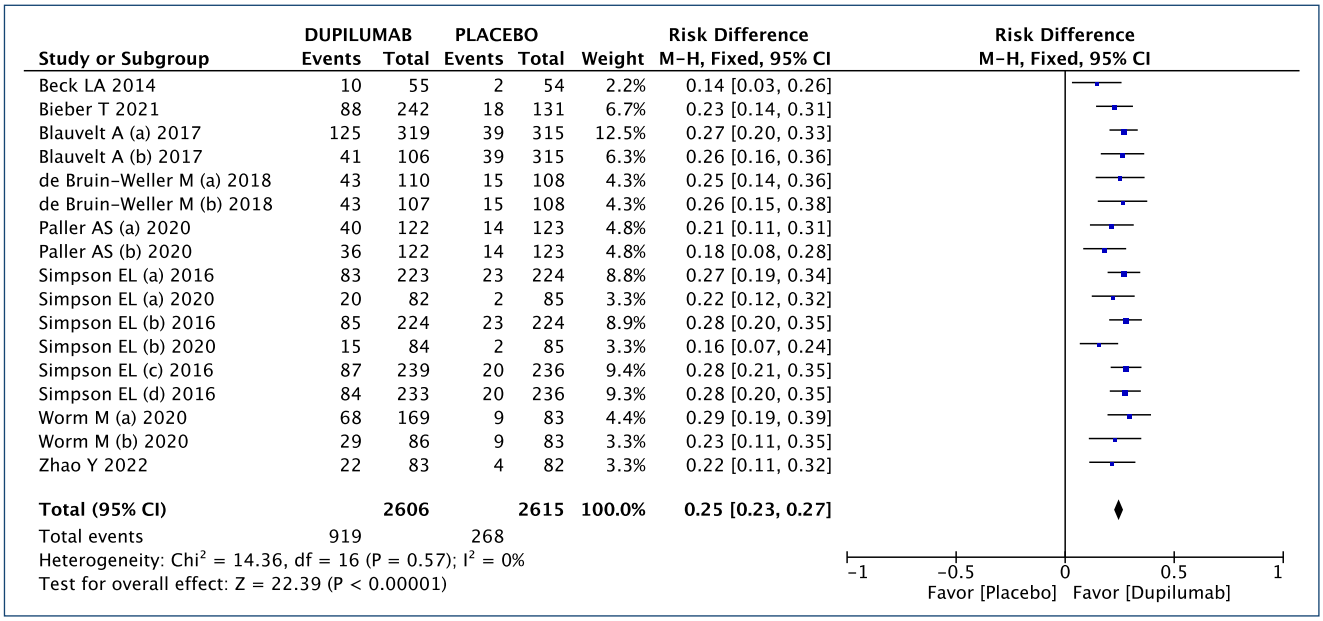

Figure 4. Analysis of the Investigator's Global Assessment outcome in patients with severe atopic dermatitis treated with dupilumab.

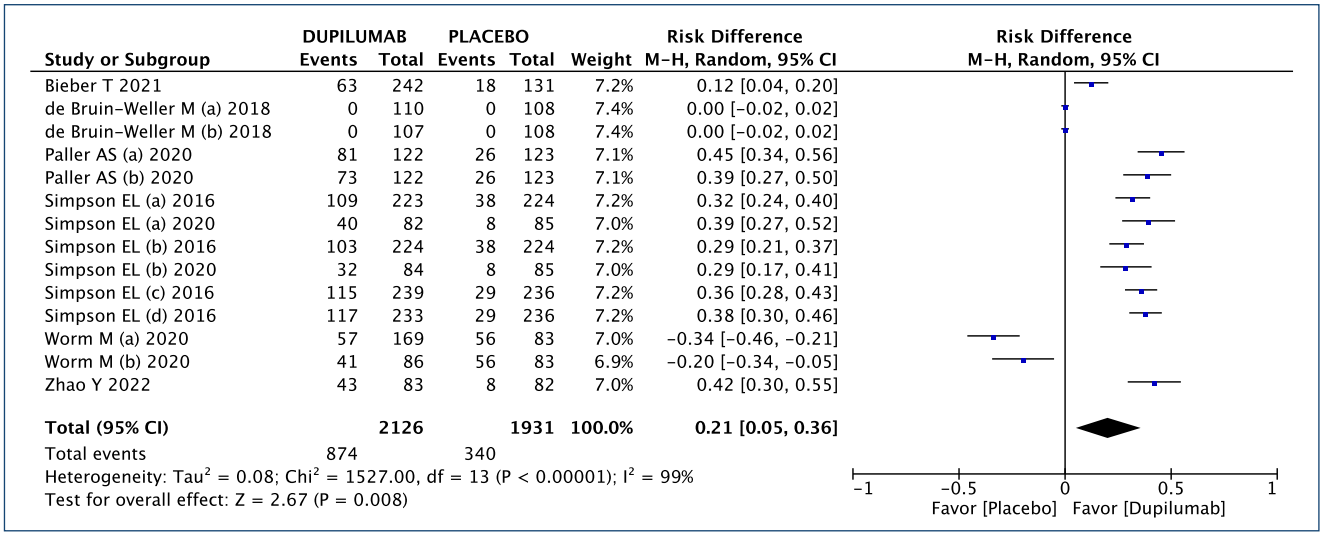

Figure 5. Analysis of the pruritus numerical rating scale outcome in patients with severe atopic dermatitis treated with dupilumab.

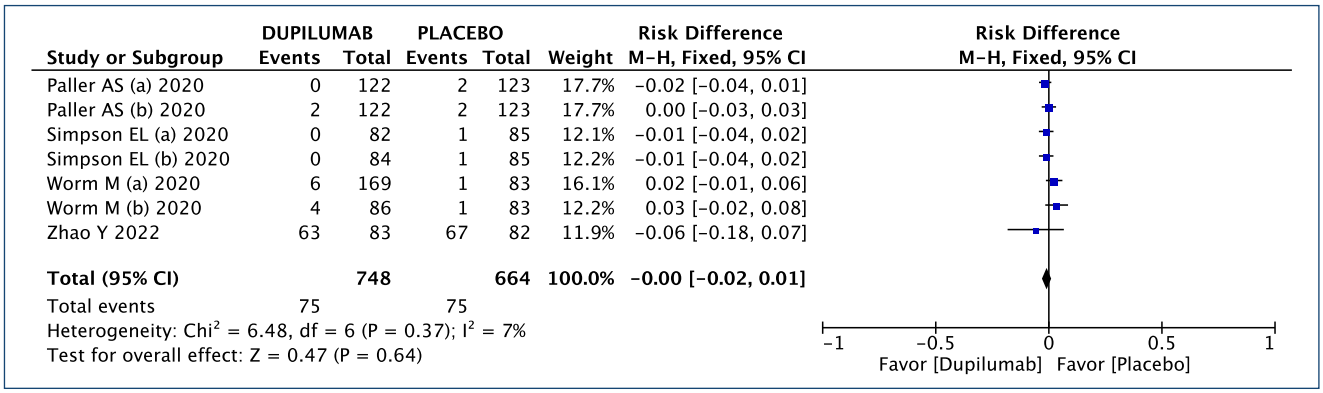

Figure 6. Analysis of treatment-emergent adverse events in patients with severe atopic dermatitis treated with dupilumab.

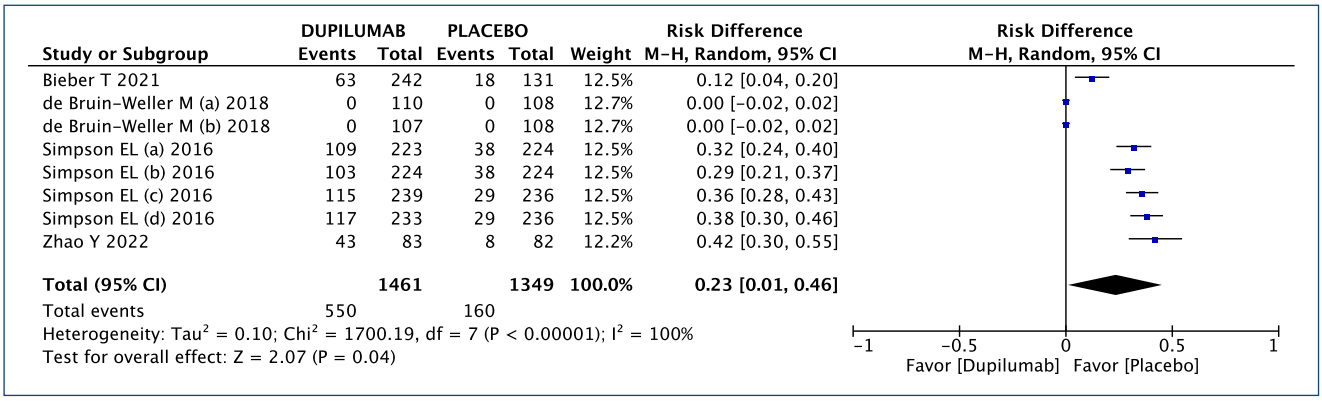

Figure 7. Analysis of serious adverse events in patients with severe atopic dermatitis treated with dupilumab.

Serious adverse events

In this analysis (Figure 7), 1,461 patients submitted to dupilumab and 1,349 to placebo were studied. Treatment with dupilumab increases the risk of serious adverse events by 23% (95%CI 1–46%) (NNH: 4) when compared to placebo. The quality of evidence is low.

SUMMARY OF EVIDENCE

The treatment of patients with severe atopic dermatitis using dupilumab at the usual doses, and with an average follow-up of 6 months, produces benefits (EASI 50/75, IGA, NRS outcomes), with an increased risk of SAEs.

AUTHORS' CONTRIBUTIONS

WB: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **LSB:** Supervision, Validation, Visualization, Writing – review & editing. **RSS:** Supervision, Validation, Visualization, Writing – review & editing. **JHB:** Supervision, Validation, Visualization, Writing – review & editing.

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Comment on "Relationship between the number of comorbidities, quality of life, and cardiac autonomic modulation in patients with coronary disease: a cross-sectional study"

Yi-Feng Wang[1], Yangfen Zhang[2], Yi Lin[2], Yi Luo[1*]

Editor,

We read with great interest the original article entitled "Relationship between the number of comorbidities, quality of life, and cardiac autonomic modulation in patients with coronary disease: a cross-sectional study," by Valente et al.[1] in *Revista da Associacao Medica Brasileira*. In this article, the authors used the Medical Outcome Study 36-Item—Short Form Health Survey (SF-36) to assess the quality of life of the subjects. Their results found that the number of comorbidities is inversely related to the pain domain of the SF-36, suggesting a stronger association between higher pain levels and the number of comorbidities in patients with coronary artery disease. Although discussed in detail, our team still believes that there are some issues that need further in-depth study.

First, the authors used the RR interval as an important indicator of cardiac function to measure heart rate. However, the heart beat frequency is affected by many factors, and hormones in the body are one of the factors that need to be considered. For example, thyroid disease[2], pituitary disease[3], and kidney or adrenal disease[3,4] will directly or indirectly affect the basal metabolic rate of the human body, which in turn affects the beating frequency of the heart and hence reflects the difference in value, that is, RR.

In addition, we noted that during the subjects' baseline data collection, the researchers collected only general demographic characteristics such as age, gender, and BMI , which were incomplete. Items such as occupation, culture, income, and permanent residence were not collected. Occupation is the determinant of a person's living habits, and poor living habits may lead to the decline of the pain domain of the SF-36. Therefore, occupational factors may be another potential factor that causes difference in the pain domain of the SF-36[5,6].

In general, we believe that the baseline data of the patients are not complete enough, and the comparability of the groups is weak. We suggest that the authors improve the baseline data of the subjects and conduct random grouping so that the observation group (experimental group) and the control group are balanced.

AUTHORS' CONTRIBUTIONS

Y-FW: Conceptualization, Data curation. **YZ:** Writing – original draft. **YL:** Writing – review & editing.

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Comment on "Serum vascular endothelial growth factor as a marker for tubal pregnancy"

Zhijiao Li[1], Jie Tang[1*]

Dear Editor,

We are very happy to read the article entitled "Serum vascular endothelial growth factor as a marker for tubal pregnancy" by Cabar et al.[1] In this study, the authors investigated the diagnostic value of serum vascular endothelial growth factor (VEGF) in tubal pregnancy. The authors classified all participants into three groups: abnormal intrauterine pregnancy, tubal pregnancy, and normal intrauterine pregnancy. Their results found that patients with tubal pregnancy had significantly higher serum VEGF concentrations than the other two groups. In addition, when the serum VEGF concentration was >188.7 ng/mL, it had a higher diagnostic value for tubal pregnancy, with a sensitivity and specificity of 96.7 and 95.0%, respectively. We really appreciate for their great contribution, as the findings of this study provide an important basis for the early diagnosis of tubal pregnancy. However, according to our opinion, there are some concerns that deserve further elucidation.

First, the information on the female participants included in this study[1] is not comprehensive. Did the participants included in this study have a history of recurrent pregnancy loss (RPL)? Results from a previous study[2] indicated that women with a history of RPL had significantly higher serum VEGF concentrations than the control group (210.33±108.23 pg/mL versus 123.91±18.8 pg/mL, $p<0.05$). This finding suggests that elevated maternal serum VEGF concentrations are associated with RPL. However, it is unclear whether the participants in this study had a history of RPL. In the absence of RPL information, one possible hypothesis is that participants with tubal pregnancy had more RPL, resulting in significantly higher serum VEGF concentrations than other groups. Therefore, it is necessary to clearly describe the differences in RPL between groups.

Second, from the statement included in this study "*There was no difference in maternal age between the three subgroups (p=0.633), but gestational age was significantly different between the subgroups (p=0.003),*" it is noted that there was a significant difference in gestational age among the three groups. It should also be noted that gestational age also has a significant effect on serum VEGF concentrations. Evans et al.[3] demonstrated that serum VEGF concentration was positively correlated with gestational age, and this correlation continued up to 10 weeks of pregnancy. In addition, the gestational age in this study was between 42 and 56 days, which indicates the range of 10 weeks of pregnancy. In that case, gestational age rather than tubal pregnancy may be the underlying factor leading to the significant increase in serum VEGF concentration. Therefore, it is necessary to balance the differences in gestational age between groups.

AUTHORS' CONTRIBUTIONS

ZL: Conceptualization, Investigation Methodology, Writing – original draft. **JT:** Conceptualization, Investigation, Writing – review & editing.

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Feedback in medical education: beyond the traditional evaluation

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FEEDBACK CONCEPT

Feedback is an information provided by an agent (teacher, colleague, relative, etc.), regarding aspects related to the performance and/or understanding of an individual[1]. The concept of feedback has been used for many years in psychology, administration, and economics. In a more recent review of 2008, feedback was conceptualized as specific information about the observed performance of the learner, comparing it to a model ("standard"), provided to improve the student's performance[2]. Learning through feedback is a complex process influenced by individual and cultural factors[3].

To deepen the theme, this article will conceptualize important aspects of feedback integrated into teaching, in order to make this tool increasingly used and improved by educators, aiming for a teaching method focused on student development.

APPLICATION OF FEEDBACK IN MEDICAL EDUCATION

In the medical education environment, a student is often insufficiently provided with feedback[4,5]. Medical educators use to believe that they provide feedback to their students, although learners report that they are rarely provided with them[2]. When feedback is given, the information contained in it is generally too vague, even neutral, limited to be considered useful[6,7]. Isolated or singular feedbacks are not enough. This practice must be developed continuously throughout the whole training in an effective way[8]. Therefore, to be powerful, there must be a learning context in which the feedback is provided[1].

Education is not only the information we incorporate into our knowledge, but also the ability to keep learning through the process of revisiting our skills[9]. The evaluation is intrinsic to the act of teaching and the possibility to expand and improve the knowledge acquired by the student[4]. Besides the traditional method of evaluation, feedback is an essential part of the education process throughout the entire course of training, in a continuous rather than punctual way, to provide information and not judgment[10]. Therefore, the development of skills and the improvement of the student's performance through interactions with their educator (instead of judge) should be the real stimulus for effective feedback[7]. This attitude represents a concern with the progress and development of the student as a person, instead of a preoccupation only with grades or scores[10].

In medical learning involving practical tasks, many of them are passive to feedback, like clinical history, discussion of clinical cases, physical examination, teamwork, and critical thinking[2]. Observations made in clinical practice do not necessarily need to be scheduled; less formal observations are frequently more valid to obtain material for providing future feedback. The many opportunities for observation and feedback that are available as part of routine clinical activities should not go unnoticed[10].

The goal of clinical training in the medical field is to accomplish expertise and ability in patient care. Without feedback, students may not become aware of some specific subject in which they should invest more time or, still, they may not know what they can already perform well, so they can repeat the positive behavior or ability later[5]. In other words, if no feedback is provided, errors happen without correction, performance does not improve, and clinical competence is achieved obscurely or even not achieved at all[10].

FEEDBACK CONTENT

The main purpose of feedback is to reduce differences between the student's current understanding/performance and the final goal. The model ("standard") to which the student is compared must be clearly exposed[8]. This model can be based on protocols where performance is described, the previous performance of the learner himself, or the opinions of teachers about standard performance[2]. Effective feedback should answer the following three questions[1]:

Where am I going? (What the objectives are.)

How am I doing? (What progress is being made towards the goal.)

What are the next steps? (What needs to be done to achieve more progress.)

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Ideally, feedback should be initiated by asking about the student's goals and what he/she expects to receive through this interaction[11]. It is also important to initially reinforce the good practices of the student because this promotes self-confidence. Another way to involve the student more in the dialog is to question their perception of their performance; from this starting point, the feedback may contain the positive aspects of the student's performance, followed by what must be improved[5,11].

As the ultimate goal is to establish real improvements, feedback that only points out the flaws is not enough. Therefore, practical examples and suggestions should be provided. It also helps to listen to the student's plan for reworking the task in the future, that is, what he would do differently in the same activity[12]. An objective action plan can be elaborated, with necessary points to improve, scheduling a follow-up to check the progress achieved[5]. The more the student is involved in planning their learning goals, the greater the chance of improvement[13].

A teaching environment where the feedback culture is not well established leads students to draw their own conclusions and, sometimes, to give disproportionate importance to certain reactions of their teachers. As it was well exemplified in a pioneer publication about the theme by Jack Ende[10], an arched eyebrow can promote the thought "I am not performing as good as the required standard." Or, with a sudden response from a resident, one can infer that "I am really displaced in this environment"[10]. Nonverbal or verbal communication not intentionally directed to provide feedback about the student's performance is occasionally harmful; this way, the student cannot draw constructive conclusions about their evolution throughout the training.

Table 1 shows a summary of feedback considered effective or not. Feedback content is best targeted when it involves an objective problem, task, or process, but never a personal trait of the person receiving the information[1,11]. That is, feedback should be focused on behaviors that can be changed, not on the individual's personality[5].

Table 1. Feedback modalities*.

Effective	Little (or less) effective
About observable skills and abilities	About skills that are not observable
Experienced observer and/or feedback provider	Non-experienced observer and/or feedback provider
Information provided is specific	Information provided is too vague
Explicit standard models	Unclear standard models
Establishes a goal for performance improvement	No goal is established for performance improvement
Plan to observe again	No intention of observing again

*Adapted from van de Ridder et al., What is feedback in clinical education?, 2008[2].

The language used must encourage constructive criticism. Instead of using expressions like "perfect," "good," "bad," or "can improve," it may be more effective to use phrases that develop the idea, and do not just deliver unconditional praise or negative judgments. For example, "The positive aspects of your clinical exam were… and the negative ones were…" Or, "Your presentation gave us a very detailed and useful view of the problem… maybe you could also add…" Beginning the conversation by asking "What would you change or do differently" or "How do you think it went" can also open the way to reflection and have a better impact on developing ideas and action plans to achieve the determined goal[8,10]. Descriptive and nonevaluative language can bring the students closer and arouse their interest. As also well exemplified by Jack Ende in his article, statements such as "Your differential diagnosis did not include the possibility of appendicitis" may sound better than "Your elaboration of differential diagnosis is inadequate"[10]. Especially when providing negative feedback, there must be emotional distancing so that the information is better accepted[10,11].

Furthermore, when it comes to subjective aspects, which are also passive to feedback, one should be very careful and the content should be clearly expressed as subjective. In the training of difficult conversations, for example, talking "Watching this situation, I felt that you were not comfortable approaching the patient's cancer diagnosis." Putting it in another way, "You were not comfortable addressing cancer diagnosis with the patient" may suggest that this is a general perception (not only of the teacher) or even put an unsafe label on the apprentice[10].

For all types of feedback, especially the most subjective, the educator should always make sure that the message has been delivered. Encouraging the learner to paraphrase what has been said can be a useful strategy that stimulates discussion[10]. Therefore, effective feedback should be a dialog of mutual engagement, not a one-way system in delivering information[7,11]. If misunderstood, it can hurt the teacher-student relationship, making the student interpret it as a judgment of their personal value or potential, when in fact it should represent information[5].

The educator who provides feedback needs to abstain from any vanity and cannot want to be seen as a "nice" person. In other words, the educator's willingness to be well-known cannot prevent him/her from giving feedback, which is often hard. Importantly, as Adam Grant wrote in his book[9],

psychological safety for providing feedback is not a matter of relaxing standards, or giving unconditional praise; it is establishing a climate of respect, trust, and openness in which people can show themselves without fear of reprisal. It is the foundation of a learning culture[9]. The teacher's work in this situation can truly be seen as a mission, where the student is the focus, not the progress of the educator within his/her career.

Table 2 shows the desired characteristics for feedback givers and takers. The educator needs to acquire the student's trust so that they can accept and want to receive progressively more feedback[7]. The credibility of the information provided also increases when it is the educator himself who observes the tasks performed[10]. It is important that he/she is observing his/her students directly and frequently, finding a balance between supervision and student autonomy[13]. Feedback given to each person individually it is more credible than group feedback, which is usually perceived as generic and less relevant[11].

With all the guidance and content on the topic, it seems that providing feedback is like "walking on eggs"[10]. On the contrary, using precise and objective language is not as difficult as it sounds; it is only necessary training, practice, and, especially, the willingness of educators to do so.

WHERE WE CAN IMPROVE

In the traditional relationship between students and teachers, there is a tendency to consider providing feedback something that occurs "naturally" in the process, regardless of the educator's training on the topic. However, when the educator perceives himself as a learner in the evaluation process, it can promote the search for continuing education[4].

Workshops could be offered by universities for educator training and recycling. Teacher training can normalize the practice of constructive feedback by making them more acceptable to students[13]. Institutions must establish a growth-friendly learning environment and culture, increasing the frequency of feedback at all levels. Strategies that can help include creating an atmosphere that normalizes learning as a continuous process, where everyone (teachers and students) has strengths and weaknesses; stimulating the search for feedback from teachers and students; establishing long-term, trusting relationships between students and teachers; and stimulating direct observation of the student's performance[13]. There is no single recipe and no easy way to create a favorable teaching atmosphere, each institution has its particularities. But managers and educators should spend their efforts and think beyond the proposal of formal evaluation, seeking a constructive teaching mentality focused on the learning and self-development of their students.

Table 2. Qualities for effective feedback*.

Related to the student	Confident humility, resiliency, patience, knows how to listen, not in search only for appraisals, open to receive criticism, and reflects on your actions
Related to the educator	Knows how to transmit the message, knows how to listen, respectful, does not want to humiliate or intimidate, example of professionalism, empathic, and open to questioning

*Adapted from Maia et al., Adapted feedback strategy aimed at undergraduate outpatient clinics, 2018[5].

FINAL CONSIDERATIONS

I could say that this article was written under the influence of my area of expertise (endocrinology), which is full of hormonal feedback, the nature in its perfect regulatory process. But, in reality, the theme choice was motivated by a personal reflection of my learning process and its feedback models, which were much more complicated and noisier, because they depended on interpersonal relationships. With a certain maturity level now, I can see how many teaching opportunities were lost during my medical school (especially) and also during residency. Competitive environments and hidden feedback possibilities did not allow a spontaneous search for personal development, possibly involving fear of receiving a negative evaluation. I guess a good part of the students still have this behavior, because they lack the maturity to know what is necessary for their personal and professional growth, with rare exceptions. It is up to the educator to promote an appropriate environment and stimulate opportunities for the delivery of feedback, in addition to traditional evaluation. In an effort to remember the most remarkable feedback situations in my training, unfortunately, there are only few memories. There were some compliments (genuine?), almost no effectively constructive feedback, and even some catastrophic feedback attempts, which luckily did not interfere much with my professional path.

At present, in a position as a postgraduate student and with experience in preceptorship within a residency service, I can see that it is really very difficult to create favorable conditions to provide feedback. But I believe that the construction of knowledge about the theme and a movement, mainly by educators, to improve the task of performing evaluations/feedback, is the way to bring improvements in this field of the medical education process.

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"Genomic Homeopathy" proposal: use of auto-isotherapic of DNA as a modulator of gene expression in chronic diseases

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INTRODUCTION

Homeopathic treatment is based on four scientific assumptions (principle of therapeutic similitude, homeopathic pathogenetic trials, prescription of individualized medicines in dynamized doses) validated in different lines of research[1,2]. Employing the principle of therapeutic similitude[3,4] as the central nucleus, homeopathy administers individualized medicines (according to the totality of symptoms) in dynamized doses (ultra-diluted and potentized), which cause similar disorders to those intended to be treated (homeopathic pathogenetic trials)[5,6], with the aim of stimulating a vital (homeostatic) reaction of the body against its own illnesses.

In addition to these scientific assumptions, the homeopathic epistemological model uses vitalist and miasmatic philosophical concepts to broaden understanding of the human illness process (health disease process) and infer a nonmaterial and dynamic substrate that justifies the proven action of ultra-diluted medicines (Figure 1).

According to the homeopathic vitalist conception, the primary cause of diseases is in the imbalance of nonmaterial organic vital force, while the return to the state of health occurs through reestablishing the integrity of this vital principle. In contrast, the homeopathic miasmatic conception considers the dynamic action of chronic miasms as a fundamental cause for manifesting chronic diseases and the main obstacle to their natural resolution.

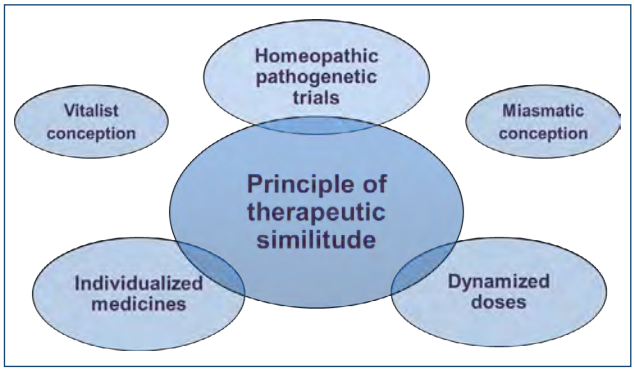

Figure 1. Scientific and philosophical assumptions of the homeopathic epistemological model.

In view of the biomedical model, the vital functions of the body are controlled by biochemical information contained in the genome (exome *plus* epigenome), and the primary cause of diseases is in disease-coding genes. In turn, these disease-promoting gene expressions are modulated by the epigenome, the non-protein-coding portion of DNA that regulates the coding portion (exome), a fundamental cause for the manifestation of chronic diseases.

Correlating these conceptions of homeopathic and biomedical models based on conceptual, functional, and experimental aspects described in detail in previous studies[7,8], we infer the hypothesis that the genome (exome *plus* epigenome) is the representation or biological substrate of the vital force or principle[7], while disease-promoting epigenetic alterations are the representation or biological substrate of chronic miasms[8]. Similarly, we infer that telomeres (terminal portion of chromosomes) are markers of the state of vital force or principle[9,10] as they are considered biomarkers of cellular vitality, aging, and the health disease process.

Assuming the premise that the biological substrate of the vital force and chronic miasms is located in the DNA or genome (exome *plus* epigenome), we have suggested using auto-isotherapic (auto-sarcode) of DNA (medicine prepared with the patient's own DNA, according to homeopathic pharmacotechnics) as a homeopathic treatment that stimulates the body's vital (homeostatic) reaction and the gene expression modulation in chronic diseases[7,8]. In contrast, we have suggested using telomere length as a marker of the effectiveness of homeopathic treatment[9,10].

Entitled "Genomic Homeopathy[11]," this innovative proposal of homeopathic treatment should be tested in basic and clinical research projects so that its safety and efficacy are verified, its methodology is improved, and it can be used as a new therapeutic approach in the future. The purpose of this preliminary communication is to disseminate this proposal to physicians

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and researchers, in general, inviting them to participate in elaborating research projects in the area, thus contributing to its validation, improvement, or refutation.

PRIMARY CAUSE OF DISEASES: CORRELATION BETWEEN VITAL FORCE AND GENOME

In the homeopathic vitalist conception[12,13], the vital force or principle is substantially united to the physical body (organic vital force) and is responsible for preserving the state of health and maintaining life. An imbalance in the vital force causes the body to become ill, and its return to health occurs through its rebalance. In view of its nonmaterial and dynamic nature, the vital principle is influenced by other related forces, as well as by emotional and psychic manifestations. *Due to their similar nonmaterial and dynamic nature, dynamized homeopathic medicines are capable of restoring vital balance*, provided they are used according to the principle of therapeutic similitude (vital curative reaction). Samuel Hahnemann did not delve into understanding the nature or essence of the vital force, although he considers it to be the *prima causa morbi* of diseases. The homeopathic vitalist model presents a set of aspects that are similar to other medical and philosophical vitalist conceptions[13,14].

According to the biomedical model, the vital functions of the body are essentially controlled by biochemical information contained in DNA (set of nucleotide sequences) or cellular genome, which transmits these characteristics to future generations and undergoes changes in the face of various stimuli and environmental factors. This genetic material is contained in highly organized nuclear structures called chromosomes, formed by extremely long DNA molecules, containing genes and other nucleotide sequences with specific functions. The genome is composed of the exome (portion of DNA encoding proteins necessary for maintaining and controlling physiological functions) and the epigenome (portion of noncoding DNA that regulates the expression of coding genes). While the exome makes up 2% of the genome, the epigenome is formed by the remaining 98% of it, showing the complexity of the cell differentiation process and the consequent homeostatic regulation. In turn, physiological imbalances and most chronic diseases are manifested as a result of changes in the patterns of this gene expression.

In correlating the vital force characteristics and functions with those of the genome, we can highlight some analogies: the vital force and the genome are the fundamental substrates for the emergence and maintenance of life (vitality of living beings); the vital principle is responsible for maintaining the balance of sensations and body functions, just as the genome stores the biochemical information that will produce the proteins responsible for maintaining vital processes and developing organisms; diseases generally occur due to vital principle dystonia, as well as disease-promoting genomic alterations, with both phenomena being affected by the same etiopathogenic factors or stimuli; among others.

In view of these correspondences, we infer the hypothesis that the genome (exome *plus* epigenome) is the representation or the biological substrate of the vital force or principle[7].

FUNDAMENTAL CAUSE OF CHRONIC DISEASES: CORRELATION BETWEEN MIASMS AND EPIGENOME

In the homeopathic miasmatic conception[15], the dynamic action of chronic miasms is the fundamental cause for manifesting chronic diseases, as well as the main obstacle to their natural resolution. Hahnemann describes a series of etiopathogenic factors or stimuli that weaken the vital force, transforming a "latent" miasm into one that is "manifested" and predisposes the emergence of chronic diseases, such as lifestyle, diet, climate change, lack of physical activity or excess of mental activity, sexual excesses, trauma, acute infectious diseases, use of drugs and alcohol, inadequate medication and treatments, emotional and psychological disorders, among others. These miasms are transmitted hereditarily.

According to the biomedical model, the epigenome is composed of a series of epigenetic alterations (DNA methylation, acetylation of histones, and micro-RNAs, among others), comprising a set of chemical processes mediated by enzymes that represent an additional mechanism for regulating individual gene expression at the transcriptional level; they modulate the genome activity and the phenotypic profile through the "activation" or "silencing" of genes, without altering the nucleotide sequence of the genetic code. The individual epigenome is heritably transmitted, influencing the health disease process of offspring and acting through interconnected regulatory networks that provide the genome with instructions on gene modulation. In addition to being reversible, these epigenetic changes can be expressed in the genome of individuals at any age as long as they come into contact with etiopathogenic factors or stimuli (inadequate habits and lifestyle, pollution and irradiation, use of drugs and alcohol, medications and hormones, inflammation, stress, and emotions, among others), promoting the "activation" or "silencing" of genes responsible for the manifestation of chronic diseases.

In correlating miasms characteristics and functions with those of epigenetic alterations, we can highlight that most chronic diseases have a miasmatic or epigenetic cause that predisposes their appearance and prevents their natural resolution;

"latency" or "manifestation" of miasms, as well as "silencing" or "activation" of disease-promoting genes are modulated by similar etiopathogenic factors or stimuli; both miasms and disease-promoting epigenetic changes are heritably transmitted.

In view of these correspondences, we infer the hypothesis that disease-promoting epigenetic alterations are the representation or biological substrate of chronic miasms[8].

HOMEOPATHIC MEDICINES ACT ON THE GENOME BY MODULATING GENE EXPRESSION (GENE REGULATORY HYPOTHESIS)

Based on experimental studies that showed the effect of homeopathic medicines in repairing chromosomal damage caused by toxic or radioactive stimuli, since 1997 Khuda-Bukhsh[16,17] defends the hypothesis that the mechanism of action of homeopathic medicines occurs through the regulation of gene expression.

Evidencing the experimental studies that demonstrate the action of homeopathic medicines in molecular biology, Dei and Bernardini[18] reaffirm the hypothesis of Khuda-Bukhsh, suggesting that the action of homeopathic medicines "is not quenched by ultrahigh dilution and proceeds through modulation of gene expressions." Analogously describing experiments that evidence the action of homeopathic medicines on gene expression, Bellavite et al.[19] suggest that "these findings support the hypothesis that homeopathic remedies could turn some important genes on or off, initiating a cascade of gene actions to correct the gene expression that has gone wrong and produced the disorder or disease."

Considering that *homeopathic medicines act in the regulation of the vital force*, these experimental studies reiterate the hypothesis that the genome (exome *plus* epigenome) is the representation or the biological substrate of the vital principle[7,8].

USE OF AUTO-ISOTHERAPIC OF DNA AS A MODULATOR OF GENE EXPRESSION IN CHRONIC DISEASES: "GENOMIC HOMEOPATHY" PROPOSAL

Although homeopathy locates the primary cause of disease in the imbalance of nonmaterial organic vital force, Hahnemann did not believe it possible to know "how the vital force causes the organism to display morbid phenomena, that is, how it produces disease" or recognize in the organic constitution "a manifest cause that excites or sustains the disease *(causa occasionalis)*" (*Organon of medicine,* paragraphs 6–20)[20].

Therefore, the homeopathic treatment was structured in the *clinically perceptible representation (symptomatic totality) of these vital and dynamic dystonias*, prescribing medicines that present similar dystonias (symptoms) according to the principle of therapeutic similitude.

However, if a biological substrate for these vital and dynamic dystonias can be identified, it will be possible to direct homeopathic treatment that stimulates an intrinsic reaction of the body against this *inner essence of diseases (causa occasionalis),* acting on the individual and profound pathophysiology.

Based on the conceptual, functional, and experimental correlations described above[7,8], we infer that the genome (exome *plus* epigenome) is the biological substrate of the vital force or principle (primary cause of diseases), while the disease-promoting epigenetic alterations are the biological substrate of chronic miasms (fundamental cause of chronic diseases). In homeopathic terms, the genome (exome *plus* epigenome) would be the *simillimum* of the vital force and the epigenetic alterations that promote chronic diseases would be the *simillimum* of miasms.

Grounded in these hypotheses and using the reactional treatment method called "isopathy" ("a method of curing a given disease by the same contagious principle that produces it") described by Hahnemann (*Organon of medicine,* note on paragraph 56)[20], we are suggesting administering the homeopathic medicine prepared with the patient's own DNA (auto-isotherapic of DNA) with the aim of awakening a dynamic, complex, and self-organizing therapeutic reaction of the vital principle or genome, thereby respectively promoting the vital balance or modulating gene expression in chronic diseases.

According to the Brazilian Homeopathic Pharmacopoeia[21], in the chapter "Biotherapics and Isotherapics," "isotherapics" are described as "medicinal preparations obtained from inputs related to the patient's pathology that are prepared following the homeopathic, pharmaco-technical method and are classified as auto-isotherapics and hetero-isotherapics." In turn, "auto-isotherapics" are "isotherapics whose active inputs are obtained from the very patient (fragments of organs and tissues, blood, secretions, excretions, calculus, feces, urine and microbial cultures, among others) and are destined to this specific patient". Following the "Minimum Requirements for the Preparation of Biotherapics and Isotherapics," complying with the biosafety norms of the Brazilian health surveillance[22], "auto-isotherapics can only be stored in alcohol at 70% (v/v) and dispensed from 12cH[21]," meaning in concentrations less than 10^{23} mol^{-1} according to homeopathic pharmacotechnics (Table 1), which are below the Avogadro limit (6.02×10^{23} mol^{-1}).

The extraction and purification of genetic material (DNA extracted from whole blood) must be performed by molecular

Table 1. Homeopathic pharmacotechnics for the preparation of medicines (dynamization or potentization) according to the Hahnemannian Centesimal Method (cH)[21].

1 part of matrix substance (of any nature or origin)+99 parts of water (or alcohol) ⇨ 100 succussions ⇨ **1cH** dynamization (10^{2} mol^{-1} of the matrix substance);
1 part of 1cH+99 parts of water ⇨ 100 succussions ⇨ **2cH** dynamization (10^{4} mol^{-1});
1 part of 2cH+99 parts of water ⇨ 100 succussions ⇨ **3cH** dynamization (10^{6} mol^{-1});
1 part of 3cH+99 parts of water ⇨ 100 succussions ⇨ **4cH** dynamization (10^{8} mol^{-1});
1 part of 4cH+99 parts of water ⇨ 100 succussions ⇨ **5cH** dynamization (10^{10} mol^{-1});
1 part of 5cH+99 parts of water ⇨ 100 succussions ⇨ **6cH** dynamization (10^{12} mol^{-1});
And so on ...
12cH dynamization ⇨ 10^{24} mol^{-1} of matrix substance (below the Avogadro limit: 6.02×10^{23} mol^{-1}) ⇨ "absence of molecule-gram" ⇨ biosafety and absence of significant adverse events.

Succussions: vigorous agitations.

biology laboratories following specific techniques and protocols[23], which will be sent to homeopathic pharmacies or laboratories to prepare the auto-isotherapic of DNA (Table 2).

It is worth mentioning that the isopathic (isotherapic) treatment method is similar to immunotherapy or vaccines, reactional treatment methods in which minimal doses of pathogens (allergens, poisons, and microorganisms, among others) are administered, usually subcutaneously and repeatedly, with the aim of stimulating modulation of the immune response against diseases caused by these agents. However, it is worth mentioning that isopathic treatment is administered orally and in doses that are tens of thousands of times more diluted than the aforementioned immunizing agents (below the Avogadro limit), making the isotherapeutic method safe and free from significant adverse events.

Table 2. Preparation of auto-isotherapic of DNA according to the homeopathic pharmacotechnics (Hahnemannian Centesimal Method or cH)[21].

1. Receive the material from the molecular biology lab (DNA soluble in buffer solution).	6. Apply 100 strong succussions to the dynamization vial, obtaining the 1cH potency.
2. Add absolute alcohol (95° to 100° GL) to precipitate the filament of DNA.	7. Transfer 1 part of 1cH potency to another vial containing 99 parts of alcohol 30° GL and apply 100 strong succussions to obtain 2cH potency.
3. Wash the filament of DNA with alcohol 70° GL several times to perform the antisepsis and remove residue of adsorbed buffer.	8. Repeat the previous procedure to obtain 3cH to 12cH potencies.
4. Dissolve the isolated DNA filament in 0.5mL of alcohol 30° GL and shake the vial. The DNA will become soluble again.	9. If 12cH potency is dispensed (start treatment), store 11cH potency in alcohol 70° GL to dispense higher potencies in the future.
5. Transfer 1 part of DNA soluble to vial of dynamization (potentization) adding 99 parts of alcohol 30° GL.	10. Repeat the process until the desired potency is obtained.

CONCLUSION

If the auto-isotherapic of DNA is able to stimulate a constitutional modulation of vital force or gene expression, it will exert systemic action in preventing and treating organic disorders and chronic diseases in general.

It is important to reiterate that this is a theoretical hypothesis and without scientific evidence so far, and its use in humans can only be disseminated after studies attest to its safety and efficacy. Therefore, the preliminary disclosure of this innovative proposal is intended to unite researchers around it, encouraging research that confirms or refutes its validity.

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ORIGINAL ARTICLE

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Relationship between platelet indices and red cell distribution width and short-term mortality in traumatic brain injury with 30-day mortality

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SUMMARY

OBJECTIVE: This study aimed at investigating whether there is a relationship between 7- or 30-day mortality and mean platelet volume, platelet distribution width, platelet count-to-total lymphocyte count ratio, or red cell distribution width in patients with traumatic brain injury.

METHODS: We retrospectively analyzed intensive care unit patients with traumatic brain injury. We recorded patients' ages; genders; diagnoses; Glasgow Coma Scale scores; length of intensive care unit stay (in days); mean platelet volume, platelet distribution width, platelet count-to-total lymphocyte count ratio, and red cell distribution width values upon hospital admission; and health on the 7th and 30th days of their stays.

RESULTS: We analyzed data from 110 patients. Of these, 84 (76.4%) were male and 26 (23.6%) were female. On the 7- and 30-day mortality evaluations, compared to the living patients, the deceased patients had a significantly higher median age and a significantly lower median Glasgow Coma Scale. Thus, increased age and lower Glasgow Coma Scale scores were associated with increased 7- and 30-day mortality rates. mean platelet volume and platelet distribution width values were similar in living and deceased patients. platelet count-to-total lymphocyte count ratio values were lower in deceased patients, but this difference was not statistically significant. Within 30 days after traumatic brain injury, deceased patients' red cell distribution width values were significantly elevated in deceased patients compared to those of living patients.

CONCLUSION: Mean platelet volume, platelet distribution width, and platelet count-to-total lymphocyte count ratio values were not associated with 7- and 30-day mortality, whereas only elevated red cell distribution width was associated with 30-day mortality.

KEYWORDS: Mean platelet volume. Mortality. Red cell distribution width. Brain injuries, traumatic.

INTRODUCTION

Coagulopathy has an essential role as a prognostic factor in traumatic brain injury (TBI). When coagulopathy develops, the risk of a poor outcome increases. Platelet dysfunction can lead to or result from coagulopathy after TBI[1,2]. Mean platelet volume (MPV), platelet distribution width (PDW), and computable platelet count-to-total lymphocyte count ratio (computable PLR) are essential, but they are straightforward parameters in monitoring platelet activation. Platelet indices play an essential role in determining specific diagnostic or therapeutic methods and in following the treatment process.

To the best of our knowledge, there have been no findings on the relationship between short-term mortality and MPV, PDW, PLR, or red cell distribution width (RDW) values in patients with TBI. The aim of this study was to investigate the relationships between 7- and 30-day mortality and these values in patients with TBI.

METHODS

Subjects

This retrospective study was approved by the local Ethics Committee (2018-E.1561). We scanned the charts of all patients who were admitted to the intensive care unit (ICU) between January 2015 and December 2017. Our 20-bed ICU is a department in which patients with a poor general health condition, hemodynamic instability, and multisystem trauma

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are hospitalized and receive third-degree treatment. In this study, we included all patients admitted to our ICU during the study period with head trauma who may have had concomitant multisystem trauma. Only children under the age of 18 years were excluded from the study. We obtained patients' ages, genders, diagnoses, numbers of ICU hospitalization days, and their health on the 7th and 30th days of their stays from the patients' ICU follow-up charts and the hospital information management system. Additionally, we obtained MPV, PDW, RDW, and computable PLR values by examining the initial hemograms taken on patients' admission to our hospital emergency service.

Statistical analysis

Descriptive analyses were performed to provide information on the study population's general characteristics. The Kolmogorov-Smirnov test assessed the normality of the numerical variables' distribution. Accordingly, the Mann-Whitney U test was used to compare the numeric variables between the two groups. The numeric variables were presented as the median [interquartile range]. A multiple logistic regression model was implemented to determine the risk factors independently associated with 7- and 30-day mortality. We considered a p-value of <0.05 statistically significant. We performed analysis using the SPSS Statistics software (IBM SPSS Statistics, version 23.0., IBM Corp., Armonk, NY, USA).

RESULTS

During the study period, 137 patients with TBI were hospitalized in the ICU. We excluded 27 patients from the study because they were younger than 18 years of age, and we performed the data analysis on 110 patients.

The participants' mean age was 47.81 (±21.5) years. In total, 84 (76.4%) patients were male, and 26 (23.6%) were female. The mean ICU stay length was 29.88±42.3 days. The mean Glasgow Coma Scale (GCS) score was 6.16±3.5. Severe TBI had occurred among 75.5% (n=83) of the patients. Of the patients, 45.5% (n=50) had subarachnoid hemorrhage (SAH) alone, 20.9% (n=23) had SAH and epidural hemorrhage (EDH), 14.5% (n=16) had subdural hemorrhage (SDH), 7.3% (n=8) had EDH, 7.3% (n=8) had intracerebral hemorrhage, and 4.5% (n=5) had SAH and SDH. The patients' 7- and 30-day mortality rates were 20.9 and 44.5%, respectively. The descriptive statistics for all variables for patients are shown in Table 1.

There was a significant difference between the patients who died by the 7th day and those who survived until the 7th day in terms of age and GCS. The median age was significantly higher in the deceased patients than that of the living patients (p=0.003). The median GCS was significantly lower in deceased patients (p<0.001). There was no significant difference between the patients who died by the 7th day and those who survived until the 7th day in terms of the other variables. Although deceased patients had higher RDW and PDW and lower MPV and PLR than living patients, these differences in values were not statistically significant (p>0.05; Table 2).

There was a significant difference between the patients who died by the 30th day and those who survived until the 30th day in terms of age, GCS, and RDW. The median age and RDW level were significantly higher in deceased patients than those in living patients (p<0.001 and p=0.007, respectively). GCS in deceased patients was significantly lower than in living patients (p<0.001). We found no significant difference between the patients who died by the 30th day and those who survived until

Table 1. Descriptive statistics for all variables.

Variables		Mean±Std	Min–Max
Age (years)		47.81±21.5	18–91
GCS		6.16±3.5	3–15
RDW (%)		15.14±1.5	12.1–20.7
MPV (fL)		7.69±1.4	5.6–12.8
PDW (%)		18.07±1.4	16.1–23.8
PLR		101.44±86.7	21.3–511.0
ICU stay (days)		29.88±42.3	1–180
Gender	Male	84 (76.4%)	
	Female	26 (23.6%)	
Diagnosis	SAH	50 (45.5%)	
	SAH+SDH	5 (4.5%)	
	ICH	8 (7.3%)	
	SDH	16 (14.5%)	
	EDH	8 (7.3%)	
	SAH+EDH	23 (20.9%)	
Degree of TBI	Mild/moderate	27 (24.5%)	
	Severe	83 (75.5%)	
Day 7	Alive	87 (79.1%)	
	Deceased	23 (20.9%)	
Day 30	Alive	61 (55.5%)	
	Deceased	49 (44.5%)	

EDH: epidural hemorrhage; GCS: Glasgow Coma Scale; ICH: intracerebral hemorrhage; ICU: intensive care unit; MPV: mean platelet volume; PDW: platelet distribution width; PLR: platelet-to-lymphocyte ratio; RDW: red cell distribution width; TBI: traumatic brain injury; SAH: subarachnoid hemorrhage; SDH: subdural hemorrhage; Std: standard deviation; Min: minimum; Max: maximum. Data were shown as number, count, and percentage.

the 30th day in terms of MPV, PDW, or PLR values (p>0.05). PLR values were lower in deceased patients, but this difference was not statistically significant (p=0.147; Table 2).

According to the logistic regression models for 7- and 30-day mortality, with a decrease of 1 point in GCS, the probability of death increased by 1.731 times and 1.615 times, respectively (Table 3). On the 7th day, the risk of mortality was 7.253 times higher in men than in women, and mortality in non-EDH diagnoses was 5.435 times higher than that in EDH diagnoses (Table 3).

DISCUSSION

This study found no relationship between MPV or PDW and 7-day or 30-day mortality. However, RDW was significantly higher in deceased patients within 30 days after TBI. The PLR was lower in deceased patients at both 7 and 30 days, but this difference was not statistically significant. Increased age and lower GCS scores were associated with both 7- and 30-day mortality.

Traumatic brain injury is an important, life-threatening public health problem and a significant cause of morbidity and mortality in ICU patients. In one study of patients with severe TBI, the 7-day mortality rate was 10%, and the 28-day mortality was 29%[3]. In a study of patients with TBI aged 65 years and older, the 14-day mortality rate was 11.2%[4]. Studies on adults without any age limit have shown that the 30-day mortality rates ranged from 15.3 to 31.5%[5,6]. In young adults between the ages of 18 and 30 years, the 30-day mortality rate has been reported to be 12%[7]. In this study, 7- and 30-day mortality rates were 20.9 and 44.5%, respectively, which are higher than the rates demonstrated in other studies. Both the mean PDW and the mean RDW values of all our living and deceased patients were higher than the standard values, reported as

Table 2. Comparison results of the characteristics and other features between two groups for 7- and 30-day mortality.

	Day 7 - alive (n=87)	Day 7 - deceased (n=23)	p	Day 30 - alive (n=61)	Day 30 - deceased (n=49)	p
Age (years)	40 [30]	64 [37]	**0.003**	38 [27.5]	63 [37]	**<0.001**
GCS	6 [7]	3 [1]	**<0.001**	8 [6]	3 [1.5]	**<0.001**
MPV (fL)	7.5 [1.9]	7.1 [1.6]	0.402	7.5 [1.8]	7.5 [1.6]	0.445
PDW (%)	17.7 [1.7]	18.1 [2.1]	0.298	17.7 [2.05]	17.8 [1.75]	0.500
PLR	83.6 [70.2]	66.2 [25.5]	0.164	85.6 [75.85]	66.8 [52.35]	0.147
RDW (%)	14.8 [1.7]	15.2 [1.9]	0.191	14.6 [1.45]	15.3 [1.8]	**0.007**
Gender (M/F)	63/24	21/2	0.095	46/15	38/11	0.971

GCS: Glasgow Coma Scale; MPV: mean platelet volume; PDW: platelet distribution width; PLR: platelet-to-lymphocyte ratio; RDW: red cell distribution width; M: male; F: female. Data were presented as median [interquartile range] for continuous variables and as number (percentage) for categorical variables. Bold indicates statistically significant p-value.

Table 3. Logistic regression model for 7- and 30-day mortality.

		β	SE (β)	p	OR	95%CI for OR
Day 7	Age	0.038	0.014	**0.008**	1.038	(1.01–1.067)
	Gender (male)	1.981	0.877	**0.024**	7.253	(1.305–41.066)
	GCS	-0.548	0.186	**0.003**	1.731	(1.202–2.492)
	EDH	-1.692	0.847	**0.046**	5.435	(1.032–28.571)
	Constant	-2.011	1.350	0.137	0.134	
Day 30	Age	0.042	0.013	**0.001**	1.043	(1.017–1.069)
	GCS	-0.479	0.110	**<0.001**	1.615	(1.302–2.003)
	SDH	0.678	0.628	0.280	1.971	(0.576–6.745)
	Constant	0.271	0.774	0.727	1.311	

GCS: Glasgow Coma Scale; EDH: epidural hemorrhage; SDH: subdural hemorrhage; β: regression coefficient; SE: standard error; OR: odds ratio; CI: confidence interval. Bold indicates statistically significant p-value.

increased mortality risk[4,8,9], and thus supporting our high mortality rate. We thought that the higher mortality rates in our study compared to those in the literature were due to the hospitalization planning of our hospital's TBI patients, our patient population's general health status, the distribution of intracranial hemorrhage diagnoses, and the presence of concurrent multisystem trauma. First, the patients with head trauma admitted to our hospital who had hemodynamic stability and high GCS scores were hospitalized in the surgical ICU, whereas the patients with poor general health, an unstable hemodynamic status, and low GCS scores were hospitalized in our ICU. Concurrent types of intracranial hemorrhage and the comorbidities are associated with high mortality[10]. Moreover, mortality is higher than normal in patients with SAH and SDH[11,12]. In our study, SAH and SDH constituted the majority (~65%) of diagnoses. The mortality increased by approximately 5.4 times in patients diagnosed with intracranial hemorrhages other than EDH. It should also be noted that our study included not only patients with isolated head trauma but also patients with head trauma and concomitant thoracic or abdominal trauma or bone fractures.

Most studies have shown that mortality increases with advancing age[13-15]. In this study, the ages of the patients who died within 7 and 30 days after TBI were significantly higher than those of the patients who survived. Furthermore, according to the logistic regression analysis, age was an independent risk factor for both 7- and 30-day mortality.

Head trauma is mostly seen in men[3,6,13]. Although gender has not been related to mortality[4,14], several studies have found that females have a higher mortality rate[15,16]. In this study, the number of males was higher than that of female patients. Although the mortality rate was similar in both men and women, the probability of death in men was approximately 6.7 times higher than that in women within the first 7 days after TBI.

Low GCS scores have been associated with an increased risk of death within 7 and 30 days following TBI[13-15,17]. In this study, the mortality rate was higher in patients with a GCS scores of 8 or lower than in patients with higher GCS scores, and a low GCS score was an independent risk factor for both 7- and 30-day mortality.

Platelets are known to be important blood cells in the coagulation system. Coagulopathy development following trauma causes changes in platelet indices[1,8]. Decreased platelet count and platelet dysfunction occur in the pathophysiological process of coagulopathy after TBI and have been associated with increased mortality[1,2,18]. Platelet indices are a group of platelet biomarkers that serve as easily measurable parameters. MPV, which is an indicator of the average platelet size, increases during platelet activation[19]. Changes in MPV values have been related to a variety of prothrombotic and pro-inflammatory diseases as prognostic factors[19]. An MPV value of greater than 11.3 fL has been reported to be an independent risk factor for mortality in ICU patients[8,9]. MPV values are significantly lower in patients with TBI than in healthy individuals[20,21]. In this study, the MPV values were statistically similar for both deceased and living patients.

Another platelet marker related to platelet activation is PDW. A PDW value between 9 and 14 fL is considered normal, and increased PDW indicates platelet anisocytosis[22]. A PDW value above 17% was an independent risk factor for mortality in ICU patients[8,9]. Zhang et al.[17] reported that PDW levels were higher in deceased patients with TBI than in survivors; nevertheless, Bobeff et al.[4] found that PDW levels were similar in both groups. In this study, the mean PDW value of all patients was above 18%, which was higher than the normal range. The deceased patients had higher PDW values than the living patients, but this difference was not statistically significant.

The PLR was higher among deceased patients than among survivors[23,24]. A higher PLR on ICU admission was associated with a poor neurological outcome at discharge in patients with non-traumatic intracerebral hemorrhage[25]. However, in TBI, a decreased platelet count is commonly seen and is associated with an increased risk of mortality[4,8]. Therefore, it is expected that the PLR will decrease after TBI. Among the ICU patients with TBI included in our study, PLR was lower in deceased patients than in living patients, but this difference was not statistically significant.

Red cell distribution width is an indicator of red blood cells' volume and size changes, and elevated RDW levels are associated with increased mortality[9] and poor neurological outcomes after aneurysmal SAH[26]. In one study, patients with traumatic SAH who died had higher RDW than those who survived[27], and mortality rates and unfavorable neurological outcomes after SAH have been found to be significantly more common in patients with high RDW on admission[28]. A study that included patients with TBI over 65 years old reported significantly increased 30-day mortality in patients with initial RDW values of 14.5 and above[4]. In this study, the mean RDW value of all patients was above 15%, and, consistent with the literature, RDW was higher in deceased patients than in living patients. Additionally, an increased RDW was associated with increased 30-day mortality.

This study has some limitations. The most important limitation is that it is a retrospective study. The limited number

of included patients due to the study being conducted within a single center represents another limitation.

CONCLUSION

Intensive care unit patients with TBI have high mortality rates. Age and low GCS scores are independent risk factors associated with increased 7- and 30-day mortality rates. Elevated RDW is associated with 30-day mortality. Contrary to what is reported in other diseases, our patients with TBI who later died demonstrated lower PLR than those who survived. However, this result of the study was not statistically significant. The results of this study can guide the literature and broaden our horizons for future research.

AUTHORS' CONTRIBUTIONS

OP: Conceptualization, Data curation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **YT**: Conceptualization, Methodology, Supervision, Writing – review & editing. **MA**: Data curation, Resources. **UE**: Formal Analysis, Validation. **ATT**: Data curation, Resources. **KOS**: Data curation, Resources. **DC**: Data curation, Resources.

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ORIGINAL ARTICLE

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Association between antibiotic prophylaxis and adverse perinatal outcomes in premature rupture of membranes

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the association between antibiotic prophylaxis and adverse perinatal outcomes in premature rupture of membranes.

METHODS: This retrospective cohort included pregnant women with premature rupture of membranes (between 24 and 33+6 weeks) who used or did not use prophylactic antibiotics. Pearson's chi-square (χ^2) test, Student's t-test, and binary logistic regression were used for statistical analysis.

RESULTS: A significant effect was observed in patients with premature rupture of membranes using prophylactic antibiotics regarding amniotic fluid index (p=0.007), deepest vertical pocket (p=0.049), duration of antibiotic therapy (p≤0.001), C-reactive protein level upon admission (p≤0.001), leukocyte count upon admission (p=0.007), and length of stay in neonatal intensive care (p=0.047). A significant association was observed between the abovementioned patients and surfactant use during the neonatal period (p=0.04). A higher prevalence of surfactant use was noted in these patients (20.0 vs. 8.7%; p=0.04).

CONCLUSION: No association was found between antibiotic prophylaxis and the presence of adverse perinatal outcomes in pregnant women with premature rupture of membranes between 24 and 33+6 weeks of gestation.

KEYWORDS: Pregnancy. Premature rupture of membrane (pregnancy). Antibiotic prophylaxis. Morbidity.

INTRODUCTION

Premature rupture of membranes (PROM) is defined as spontaneous rupture of membranes before the onset of labor. The incidence rate of PROM is approximately 10%, with 7% in full-term and 3% in preterm pregnancies. Approximately 60–95% of PROM cases progress to labor in the next 24–48 hours, which is associated with one-third of preterm deliveries[1,2].

PROM is associated with adverse perinatal outcomes. Sim et al.[3] highlighted chorioamnionitis, cesarean section rates, and maternal sepsis as the primary adverse maternal outcomes and respiratory distress syndrome (RDS), bronchopulmonary dysplasia, and neonatal sepsis as the primary neonatal morbidities.

Previous studies have shown that antibiotic prophylaxis in PROM is associated with pregnancy prolongation as well as a reduction in the number of maternal and neonatal infections and morbidities[4,5]. Thus, the use of antibiotics increases the latency period, improving perinatal conditions and problems associated with prematurity, such as RDS and neonatal sepsis.

However, some researchers have raised concerns regarding the benefits of the routine use of antibiotics, from the diagnosis of PROM to birth, as unnecessary administration has been linked with an increased rate of necrotizing enterocolitis and predisposition to antibiotic resistance. Therefore, they suggest that antibiotics should be administered only to pregnant women with clinical or laboratory signs of infection[6-8].

This study aimed to evaluate the relationship between antibiotic prophylaxis and adverse perinatal outcomes in pregnant women with PROM between 24 and 33+6 weeks of gestation.

METHODS

This was a retrospective cohort study conducted at the Clinic Hospital of the Federal University of Triângulo Mineiro (UFTM) and Mário Palmério University Hospital of the University of Uberaba (UNIUBE) between January 2014 and April 2019. The study was approved by the ethics committee (CAAE:10374919700005154) of both institutions.

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The patients included were divided into two groups: PROM with the use of antibiotics at the time of diagnosis and PROM without the use of antibiotics. All patients with single or multiple pregnancies with spontaneous PROM, as confirmed by clinical examination and/or additional tests, and gestational age between 24 and 33+6 weeks, as dated by the ultrasound of the first trimester, were included. All pregnancies with fetal malformations, as evidenced by obstetric ultrasound, and chromosomal anomalies, as confirmed by fetal karyotyping, were excluded.

The patients using antibiotics at the time of diagnosis were selected at UNIUBE, whereas those not using antibiotics were selected at UFTM. The reason for this selection was based on the institutional protocols in force at each hospital.

According to UNIUBE's protocol, the patients diagnosed with PROM were hospitalized and followed expectantly. After hospitalization, betamethasone was administered (12 mg every 24 hours for two days) to accelerate fetal lung maturity. Maternal monitoring was performed by clinical (daily) and laboratory (every three days) evaluations. For maternal infection screening, the following tests were requested: blood count, C-reactive protein level (PCR), urinalysis, urine culture, beta-hemolytic streptococci culture (rectal and vaginal swabs), and vaginal wet mount. Fetal monitoring was performed by cardiotocography (daily) and obstetric Doppler ultrasound (weekly). Prophylactic antibiotics were always administered upon admission, shortly after the PROM diagnosis. In the absence of maternal hypersensitivity, penicillin G was prescribed as a 5,000,000 IU loading dose, followed by 2,500,000 IU every four hours for seven days. Pregnancies were terminated upon obstetric indication at 34 weeks or immediately in case of clinical or laboratory signs of maternal and/or fetal infection. Magnesium sulfate was used for neuroprotection in all births with gestational age <32 weeks.

According to the UFTM protocol, the patients with PROM were also followed expectantly. For maternal infection screening, the following laboratory tests were performed every three days: complete blood count, PCR, complements C3 and C4, urinalysis, and urine culture. Fetal monitoring was performed by cardiotocography (every three days) and Doppler obstetric ultrasound (weekly). Corticotherapy was administered with betamethasone (4 mg every eight hours for 48 hours), and magnesium sulfate if gestational age <32 weeks, for fetal neuroprotection. Prophylactic antibiotics were not administered at the time of PROM diagnosis. Penicillin G at a dose of 5,000,000 IU was used every four hours, until birth, only in the presence of uterine contractions and possibility of delivery in the next few hours. Pregnancy termination was performed according to obstetric indication at 34 weeks or whenever there were clinical or laboratory signs of maternal and/or fetal infection.

In both hospitals, PROM was diagnosed in the presence of typical history of vaginal fluid leakage with characteristic odor, and clinical presence of moistened vulva associated with the visualization of clear fluid in the posterior vaginal fornix during speculum examination or a positive fern test. In some cases, diagnosis could also be performed through diagnostic amnioinfusion (observing the output of contrast, vitamin B12, through the vagina, approximately 30–60 minutes after its injection). Ultrasound was not used for the diagnosis of PROM in either hospital; however, in the presence of oligohydramnios associated with suggestive and/or doubtful clinical signs, patients were followed as if diagnosed with PROM.

The following variables were evaluated: 1- and 5-minutes Apgar score, birth weight, length of stay in neonatal intensive care unit (ICU), presence of neonatal infection (neonatal sepsis), need for oxygen therapy, use of surfactant, presence of maternal chorioamnionitis and sepsis, maternal PCR levels, duration of latency period, and type of delivery.

Data were entered and analyzed using spreadsheets in the software programs SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and MedCalc (Ostend, Belgium). Quantitative variables were subjected to the Kolmogorov–Smirnov test. The variables showing a normal distribution were presented as mean and standard deviation. To study their differences, the unpaired t-test was used. The variables that presented nonnormal distribution were demonstrated as median, and minimum and maximum values. The Mann–Whitney U test was used to study their differences. Categorical variables were described based on absolute and percentage frequencies and are presented in tables and graphs. Pearson's chi-square test (χ^2) was used to study the difference between categorical variables and their proportions. Binary logistic regression was performed to determine the best predictors of perinatal adverse outcomes and the composite perinatal outcomes between groups. Through logistic regression, the odds ratio (OR) of the development of adverse maternal and perinatal outcomes was estimated for the variables that presented statistical differences. The receiver operating characteristics (ROC) curve was used to determine the best cutoff value for the predictor variables of composite perinatal outcomes. Significance was set at $p<0.05$ for all tests.

RESULTS

In total, 1,136 pregnant women (>24 weeks) were diagnosed with PROM in both hospitals. Of them, 14.9% (169) were between 24 and 33+6 weeks of gestation. Of these 169 women, five were excluded due to fetal malformations. For the final

statistical analysis, 164 women were included and divided into two groups based on antibiotic prophylaxis (group I, n=58, and group II, n=106).

A significant effect was noted in group I regarding the number of pregnancies (p=0.018), parity (p=0.038), amniotic fluid index (AFI) (p=0.007), measurement of the largest vertical pocket (LVP) (p=0.049), duration of antibiotic therapy (p≤0.001), PCR level upon admission (p=0.001), last PCR level (p≤0.001), leukocyte count upon admission (p=0.007), and length of stay in neonatal ICU (p=0.047). However, no significant difference was observed regarding the duration of the latency period (p=0.659; Table 1).

A significant association was observed between group I and surfactant use during the neonatal period (p=0.04). A higher prevalence of surfactant use was noted in these patients (20.0 vs. 8.7%; p=0.04). No significant association was observed among antibiotic prophylaxis=0.057), oxygen use (p=0.072), and composite adverse perinatal outcomes (p=0.058; Table 2).

The following were significant predictors of adverse perinatal outcomes: gestational age at delivery [$\chi^2(1)=12.5$; p=0.002; R^2Nagelkerke=0.110; OR 1.35; 95%CI 1.170–1.165], AFI [$\chi^2(1)=5.4$; p=0.022; R^2Nagelkerke=0.081; OR 1.12; 95%CI 1.018–1.253], LVP [$\chi^2(1)=7.03$; p=0.015; R^2Nagelkerke=0.192; OR 1.65; 95%CI 1.104–2.481], and birth weight [$\chi^2(1)=23.3$; p<0.0001; R^2Nagelkerke=0.20; OR 1.0; 95%CI 1.001–1.003]. Contrastingly, maternal age (p=0.285), antibiotic prophylaxis (p=0.191), PCR level upon admission (p=0.747), last PCR level (p=0.393), leukocyte count upon admission (p=0.304),

Table 1. Clinical characteristics of the studied population.

	Group I (n=58) median (min–max)	Group II (n=106) median (min–max)	χ^2	p-value
Maternal age (years)	26 (15–41)	25 (14–43)	0.26	0.608†
GA at delivery (weeks)	32 (24.8–35.5)	32.4 (24.3–34.3)	0.03	0.859†
Number of pregnancies	1 (1–6)	2 (1–9)	5.60	0.018†
Parity	0 (0–5)	1 (0–7)	4.13	0.038†
AFI (cm)	5 (0–18.7)	2.5 (0–18)	7.18	0.007†
LVP (cm)	3.6 (0–7.6)	1.95 (0–6)	3.88	0.049†
Antibiotic prophylaxis time (hours)	72 (1–240)	6.5 (1–72)	34.6	<0.001†
PCR level on admission (mg/dL)	1.95 (0.5–23.2)	0.8 (0–36.7)	24.6	<0.001†
PCR level last (mg/dL)	2.2 (1.1–20.9)	0.4 (0–7.1)	21.2	<0.001†
Leukocyte count on admission (cells/mm^3)	10,050 (4910–21,940)	11,800 (5,980–28,700)	7.20	0.007†
Leukocyte count last (cells/mm^3)	12,055 (5,250–21,660)	13920 (3257–23,140)	0.78	0.376†
Birth weight (grams)	1,890 (940–3,570)	1,775 (630–2,955)	2.60	0.107†
Apgar score at 1st min	8 (1–10)	8 (0–9)	0.40	0.527†
Apgar score at 5th min	9 (3–10)	9 (1–10)	0.74	0.389†
Length of stay in the neonatal ICU (hours)	504 (10–2,208)	768 (3–5,520)	3.95	0.047†
Latency period (hours)	48 (4–1,560)	48 (3–620)	0.19	0.659†
Ethnicity			0.308	0.857§
White	53.6 (30/56)	49.21 (52/106)		
Black	12.5 (7/56)	13.2 (14/106)		
Mixed	33.9 (19/56)	33.7 (40/106)		
Smoking	13 (7/54)	18.1 (17/94)	0.662	0.416§
Alcoholism	5.6 (3/54)	6.4 (6/94)	0.041	0.839§
Type of delivery			2.45	0.180§
Cesarean	44.6 (25/56)	57.5 (61/106)		
Vaginal	55.4 (31/56)	42.5 (45/106)		

GA: gestational age; AFI: amniotic fluid index; LVP: largest vertical pocket; PCR: C-reactive protein; ICU: intensive care unit. †Mann-Whitney: median (minimum–maximum); χ^2: chi-square; §: percentage (absolute number/total number of cases per group); p-value: p<0.05.

Table 2. Association between use or not of prophylactic antibiotics on the premature rupture of membranes and adverse perinatal outcomes.

	Group I (n=58)			Group II (n=106)			χ^2	p-value
	n	N	%	n	N	%		
Apgar score at 1st minute <7	8	57	14	27	102	26.5	3.3	0.070
Admission at neonatal ICU	41	57	71.9	59	104	56.7	3.61	0.057
Surfactant use	11	55	20	9	104	8.7	4.21	0.040
Oxygen use	39	57	68.4	67	104	64.4	0.26	0.609
Fetal death	0	55	0	5	106	4.7	2.68	0.102
Neonatal death	5	55	9.1	12	106	11.3	0.19	0.662
Maternal death	0	0	0	0	0	0		
Neonatal sepsis	17	55	30.9	21	105	20	2.37	0.124
Maternal sepsis	0	0	0	0	0	0		
Chorioamnionitis	13	56	23.2	39	105	37.1	3.24	0.072
Composite perinatal outcomes	38	56	67.9	86	106	81.1	3.60	0.058

n: absolute number; ICU: intensive care unit; N: total number of cases per group; %: percentage; χ^2: chi-square; p-value: p<0.05.

last leukocyte count (p=0.914), and latency period (p=953) were not statically significant (Table 1).

Using ROC curves, the best cutoff value was determined for the predictor variables of composite perinatal outcomes. Table 2 presents the cutoff values for sensitivity, specificity, positive likelihood ratio (LR+), and negative LR (LR–) to better predict composite perinatal outcomes. LVP (≤3.6 cm; AUC 0.728; 95%CI 0.581–0.847; p=0.0006) and estimated fetal weight (≤1,735 grams; AUC 0.739; 95%CI 0.665–0.805; p≤0.0001) performed moderately in the prediction of composite adverse perinatal outcomes. Gestational age at delivery (≤31.9 weeks; AUC 0.652; 95%CI 0.573–0.725; p=0.0006) performed poorly, whereas AFI showed no significant performance (p=0.073) in this prediction (Figure 1).

DISCUSSION

Preterm PROM is associated with several obstetric complications, such as placental abruption, prematurity, intrapartum fetal distress, and umbilical cord prolapse[2]. Regardless of gestational age at the time of diagnosis of PROM, the risk of intrauterine infection is the most relevant complication; the earlier and longer the rupture time of membranes, the more frequent it is[4]. Antibiotic prophylaxis prolongs pregnancy and reduces maternal and neonatal morbidity. In their systematic review, Kenyon et al.[4] reported the highest frequency of antibiotic prophylaxis, showing an association with increased latency period, reduced chorioamnionitis and postpartum endometritis, and decreased neonatal morbidity. Thus, antibiotic prophylaxis has been recommended in preterm PROM cases[9]. However, in this study, no significant difference was observed in the latency period between the groups. In addition, no significant difference was noted in the reduction of maternal and neonatal morbidity.

According to Sim et al.[3], the primary predictors of neonatal survival after gestational age at PROM diagnosis and at birth were prolonged latency period, AFI, leukocyte count, and PCR levels <1 mg/dL in the first 24 hours of hospitalization. Serum PCR levels ≥1 mg/dL upon admission correlated positively with clinical signs of chorioamnionitis[7]. In a study carried out by our group in patients with PROM between 34 and 36.9weeks, patients with expectant management had a higher PCR level than those with an active conduct (5.2 vs. 1.5 mg/dL)[10]. However, similar to the results of a study by Çetin et al.[1], our study results did not find a significant association between antibiotic prophylaxis and PCR levels in predicting neonatal survival in PROM.

Gasparović et al.[11] compared two groups of pregnant women with PROM who used (n=190) and did not use (n=134) prophylactic antibiotics. They found significant differences in gestational age, birth weight, Apgar scores, maternal PCR levels, and latency period between the groups. Histological chorioamnionitis was more frequent in the group receiving prophylactic antibiotics. Dannapaneni et al.[12] observed that women with PROM <33 weeks who used prophylactic antibiotics had perinatal outcomes similar to those without PROM.

Gestational age at the time of PROM diagnosis, AFI and LVP measurements, and fetal weight at birth were the predictors for adverse perinatal outcomes. According to Esteves et al.[13], one of the primary predictors of survival was birth weight,

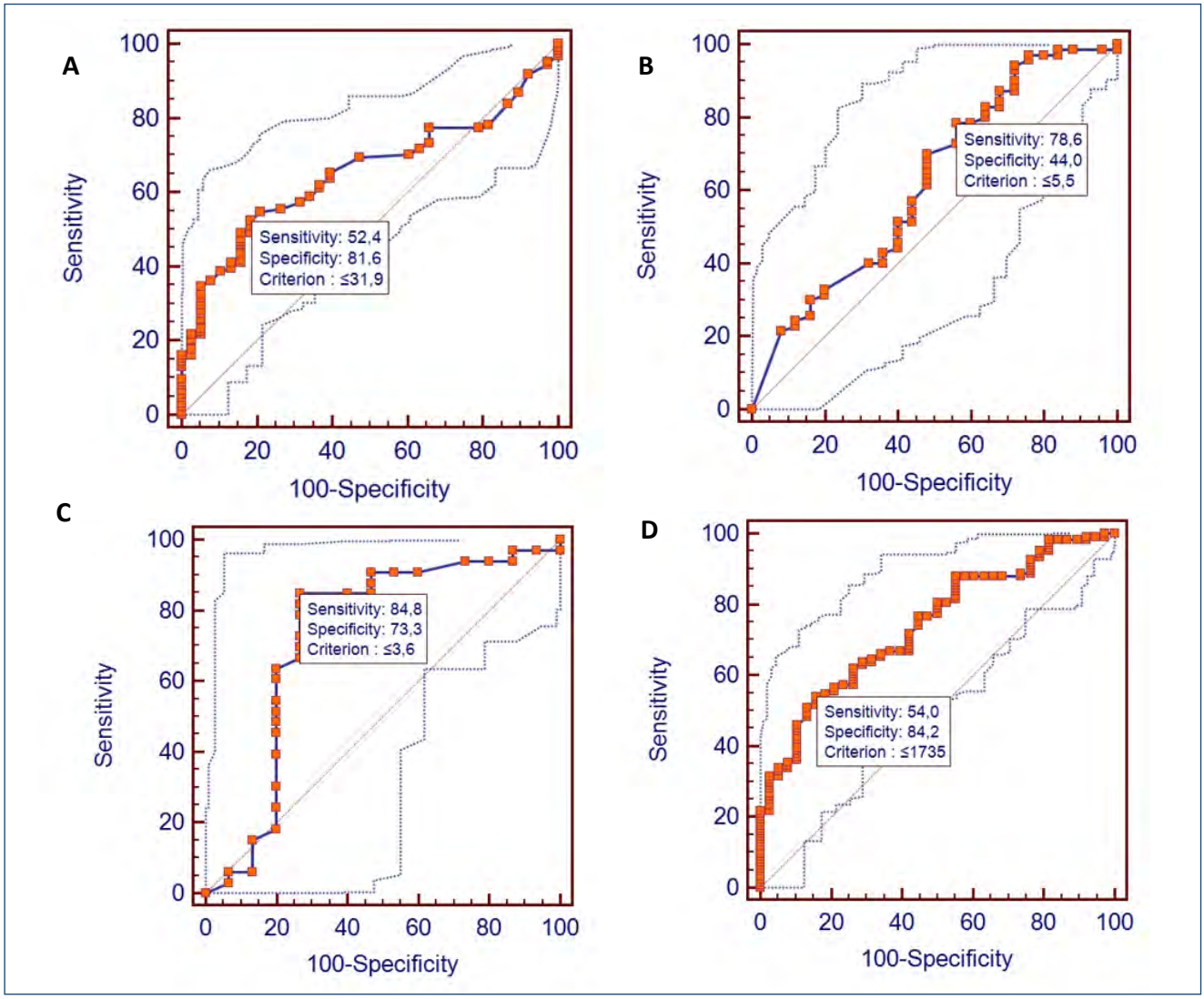

Figure 1. Receiver operating characteristics curve to establish the cutoffs for gestational age at delivery **(A)**, amniotic fluid index measurement **(B)**, largest vertical pocket measurement **(C)**, and estimated fetal weight **(D)** to predict adverse perinatal outcomes in pregnant women with premature rupture of membranes between 24 and 33+6 weeks of gestation.

and they recommended efforts to increase latency, aiming for older gestational age at delivery and birth weight >960 grams. Sayed Ahmed et al.[14] evaluated the maternal serum level of interleukin-6 (IL-6) in pregnant women with PROM between 24 and 34 weeks. Considering the IL-6 level cutoff point of 8.5 pg/mL, histological chorioamnionitis and admission to neonatal ICU were significantly higher, whereas birth weight and 1- and 5-minutes Apgar scores were significantly lower.

CONCLUSION

In conclusion, no association was found between antibiotic prophylaxis and the presence of adverse perinatal outcomes in pregnant women with PROM between 24 and 33+6 weeks of gestation.

AUTHORS' CONTRIBUTIONS

TSL: Data curation, Writing – review & editing, Visualization. **FMP:** Data curation, Writing – review & editing, Visualization. **CBB:** Methodology, Writing – review & editing, Visualization. **CGP:** Investigation, Writing – review & editing, Visualization. **MCP:** Project administration, Supervision, Writing – review & editing, Visualization. **EAJ:** Writing – original draft, Writing – review & editing, Visualization. **ABP:** Conceptualization, Formal Analysis, Visualization.

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ORIGINAL ARTICLE

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Effect of coolant spray on rib fracture pain of geriatric blunt thoracic trauma patients: a randomized controlled trial

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SUMMARY

OBJECTIVE: This study aimed to evaluate the effectiveness of cryotherapy in elderly patients with rib fractures due to blunt thoracic trauma.

METHODS: In this prospective randomized controlled study, geriatric patients were assigned to groups to receive either coolant spray (n=51) or placebo spray (n=50). The visual analog scale scores of all patients were recorded before starting spray application (V0), as well as at 10th (V1), 20th (V2), 30th (V3), 60th (V4), 120th (V5), and 360th (V6) minute. The mean decreases in the visual analog scale scores were calculated.

RESULTS: The differences between V0 and V1, V0 and V2, V0 and V3, and V0 and V4 mean visual analog scale scores measured in the coolant spray group were found to be significantly higher ($p<0.001$). In V1, V2, V3, and V4 measurements, the incidence of "clinical effectiveness" in the coolant spray group was significantly higher than in the placebo group ($p=0.001$).

CONCLUSIONS: Coolant spray therapy can be used as a component of multimodal therapy to provide adequate analgesia due to rib fractures in geriatric patients.

KEYWORDS: Acute pain, coolant spray, cryotherapy, geriatric, rib fracture, trauma

INTRODUCTION

Rapid growth in the geriatric population and their efforts to lead an independent and active life has led to a significant increase in the number of geriatric cases admitted to trauma units of emergency departments (ED)[1]. For these vulnerable patients, "pain" due to trauma is a complicated situation that affects the quality of life and behavior, impairs cognitive function, worsens the course of comorbid diseases, and can lead to death.

In the geriatric population, thoracic injuries are the second most common injury after head injuries[2]. In older patients, thoracic injuries may occur even with low-energy mechanisms due to lower bone density and reduced chest wall elasticity[3]. The most common injury due to blunt thoracic trauma in this population is the fracture of the rib(s)[4]. The pain caused by rib fractures is a serious symptom, and it is challenging to manage[5].

In daily practice, both pharmacological and invasive methods (e.g., epidural catheters, intercostal, paravertebral, and interpleural blocks) are used to ensure adequate analgesia in rib fractures[6]. Cryotherapy application is frequently used to treat acute pain due to musculoskeletal trauma. The primary mechanism in cryotherapy is to reduce the perception of both local and systemic pain by reducing nociceptive input. Although there are many different studies on the analgesic efficacy of coolant sprays, there is no study in the literature about their use in blunt thoracic trauma. This study aimed to evaluate the effectiveness of cryotherapy in the early pain treatment of elderly patients with rib fractures due to blunt thoracic trauma. The cooling spray treatment was preferred for cryotherapy because of its nonpharmacological nature and ease of use by health care workers.

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METHODS

This is a prospective, randomized, controlled, double-blind, multicenter clinical trial. Patients admitted to EDs of three hospitals between January 10, 2019, and January 10, 2020, were evaluated for eligibility. The study was approved by the Ethics Committee at Atatürk University Faculty of Medicine.

Patient selection

Patients aged 65 years and over who were referred to the EDs due to falls from the patient's own height and whose treatment and discharge was completed in ED were examined. Among these patients, those diagnosed with rib fractures due to isolated blunt thoracic trauma after radiologic imaging (e.g., chest x-ray or computed tomography) were evaluated for the study.

Inclusion criteria

1. Trauma history in 24 h.
2. Patients with fractures in less than three ribs and limited to a single hemithorax.
3. Having a visual analog scale (VAS) score of ≥5.

Exclusion criteria

1. Inability to provide informed consent.
2. Chest injury scores >11 (insufficiency of this treatment)[7].
3. Having additional trauma-related injuries, skin lesions, and/or trauma in multiple regions of the thorax.
4. Patients with fractures in ribs 1 and 2 (risk of serious injury).
5. History of regular analgesic usage, antiaggregant and anticoagulant drug usage, and/or allergy to nonsteroidal anti-inflammatory drugs (NSAIDs) and narcotic analgesics.
6. Having coagulation disorders, hematologic disease, gastrointestinal bleeding, uncontrolled heart failure, renal failure, liver failure, and chronic lung disease.
7. Patients in whom trauma-related complications developed during the ED follow-up, and patients who needed to be hospitalized.

Informed consent was obtained from patients who met the eligibility criteria. The study coordinator randomized the patients into two treatment groups using a formal randomization protocol (www.random.org/integers).

Intervention

Application of the sprays and measurement of the VAS scores were performed by ED physicians who were blinded for the study. For the placebo group, a standard saline solution refrigerated at 4°C was prepared. In the coolant spray group, Cryos® Spray (Phyto Performance, Italy) was applied at a distance of 20 cm from the injured area for 5–10 s. The first spray application was performed after the initial assessment, and the second spray application was performed at the end of the 30th minute. All patients were given intravenous (IV) dexketoprofen (50 mg in 50 mL standard saline solution) in 5 min simultaneously with the first spray application. The rescue analgesic treatment was IV fentanyl at a dose of 1 μg/kg.

Outcomes

Patients' demographics, vital signs, pain levels, chest injury scale scores, and local side effects associated with spray treatment were recorded. The VAS was used to measure the pain levels of the patients. Patients were asked to rate the pain level with a value from 0 to 10[8]. The VAS scores were recorded at admission (V0), as well as at 10th (V1), 20th (V2), 30th (V3), 60th (V4), 120th (V5), and 360th (V6) minute. The mean VAS decreases and the mean percentage VAS reductions for each measurement time were calculated. The primary outcome variable of this study was determined as a ≥50% reduction (clinical effectiveness) according to the V0. The secondary outcome variable was the frequency of patients who needed at least one dose of rescue treatment.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Efficacy analyses were performed in the per-protocol (PP) population. Safety analysis was performed on the intention-to-treat (ITT) population. Comparisons between the treatment groups were made with the unpaired t-test and the Mann-Whitney U test. The incidences of adverse events were compared using the chi-square test. The confidence interval (CI) was 95%, and a p-value of <0.05 was accepted as statistically significant.

RESULTS

Patients

There were 108 patients in the ITT population, of whom 53 were given placebo spray, and 55 were given coolant spray (Figure 1). A total of seven patients (three in the placebo spray group and four in the coolant spray group) were excluded from the study during the 6-h follow-up period of the study. Notably, 50 and 51 patients remained in the placebo and coolant spray groups for the PP population, respectively.

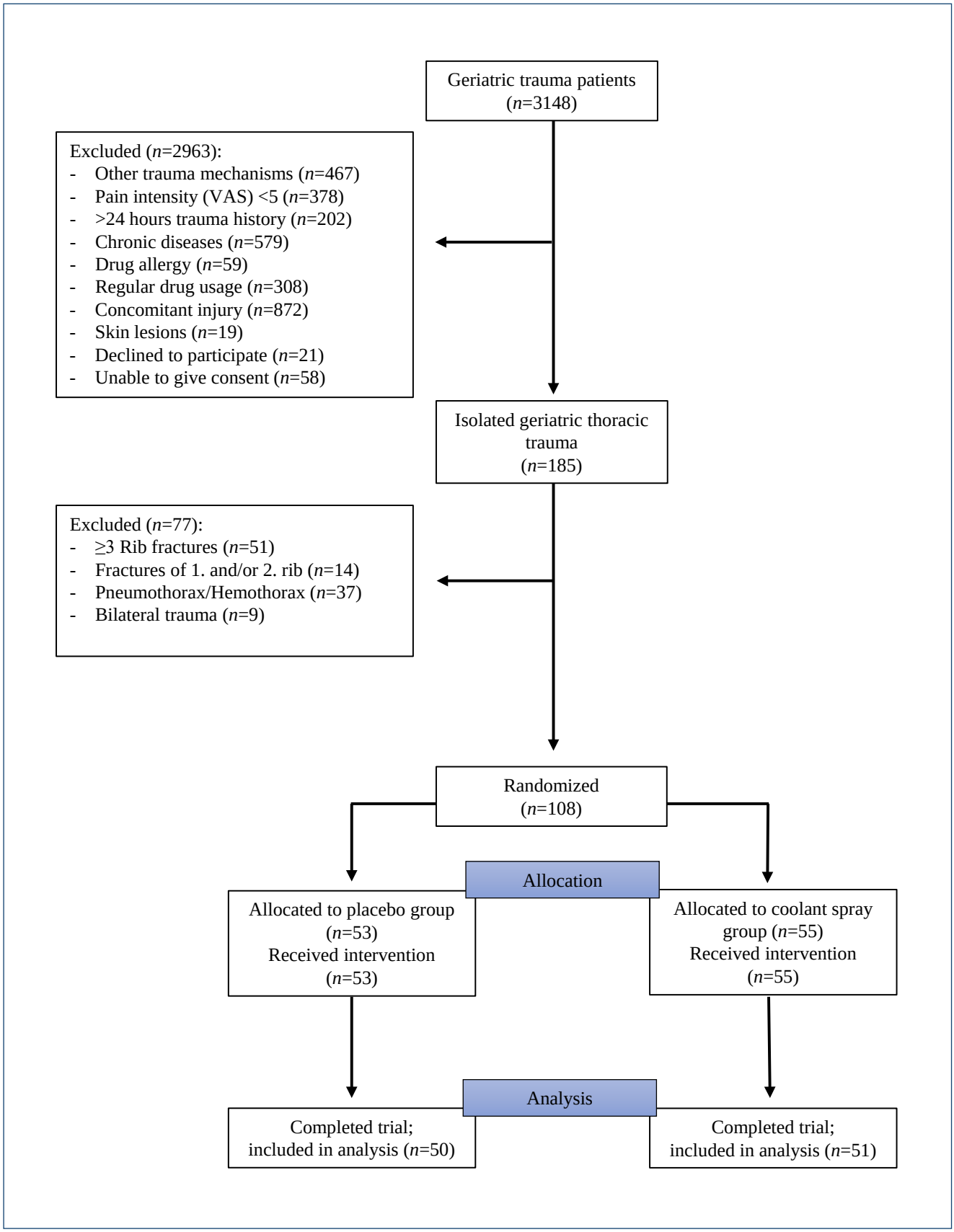


Figure 1. CONSORT diagram of the study.

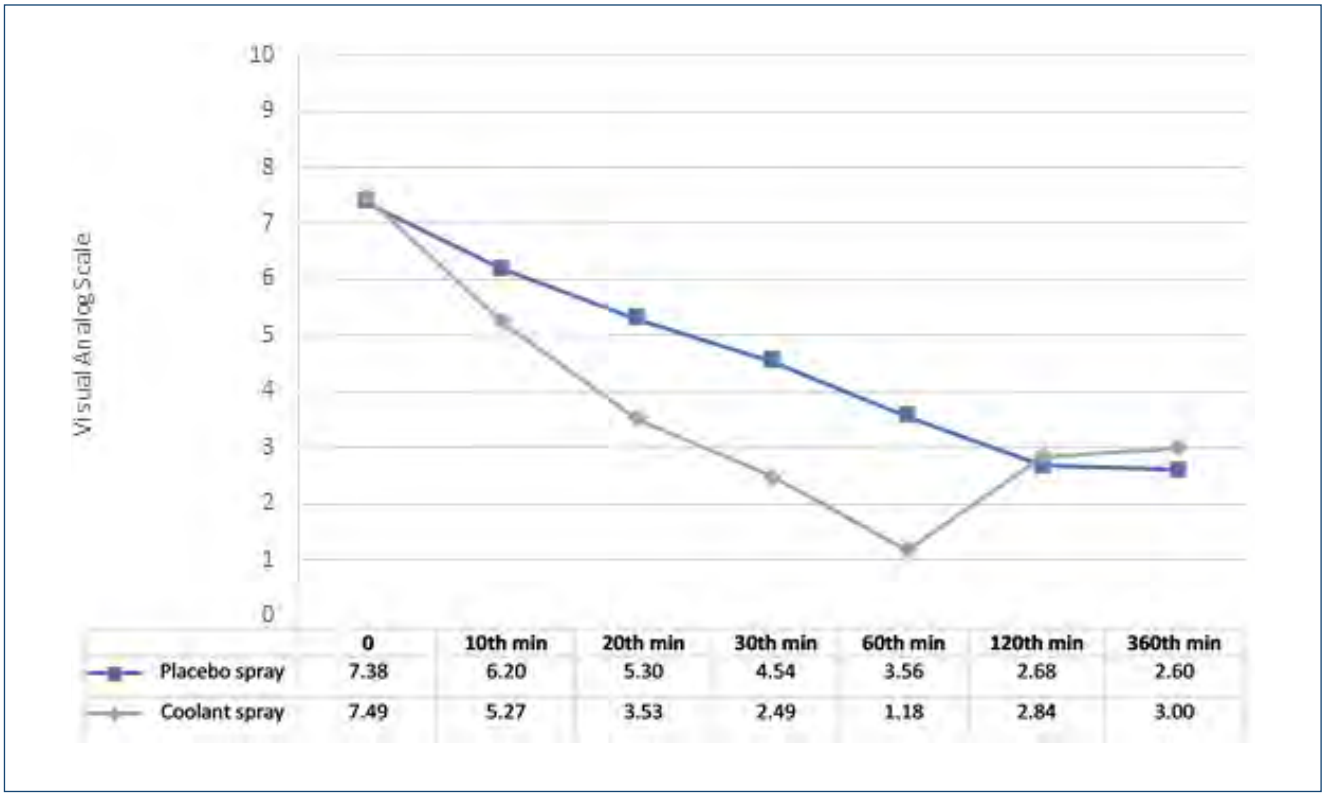
Of the 108 patients, 54 (53.3%) were male, and the mean age was 71.7±4.2. Demographics and baseline variables are shown in Table 1; no significant differences were determined between the two treatment groups.

Efficacy

The mean V0 values of the groups were 7.38 (placebo spray) and 7.49 (coolant spray), and this difference was not statistically significant (p>0.05). The mean VAS values at each measurement time and the changes over time are presented in Figure 2. The differences between V0 and V1, V0 and V2, V0 and V3, and V0 and V4 mean delta VAS values of the coolant spray group were found to be significantly higher. These scores were as follows: V0–V1: 1.04, 95%CI 0.56–1.51 (p<0.001); V0–V2: 1.88, 95%CI 1.25–2.52 (p<0.001); V0–V3: 2.16, 95%CI 1.51–2.81 (p<0.001); V0–V4: 2.49, 95%CI 1.82–3.14 (p<0.001). However, measurements between V0 and V5 and between V0 and V6 showed no significant difference between the two treatment groups (p>0.05). The mean percentage reduction in the V0 and V1, V0 and V2, V0 and V3, and V0 and V4 scores in the coolant spray group was significantly higher than that in the placebo spray group. These values were as follows: % mean for V0–V1: 14.2, 95%CI 7.6–20.5 (p<0.001); % mean for V0–V2: 24.6, 95%CI 16.8–32.3 (p<0.001); % mean for V0–V3: 28.0, 95%CI 20.3–35.7 (p<0.001); and % mean for V0–V4: 32.7, 95%CI 25.1–40.3 (p<0.001). There was no significant difference between the two treatment groups in the % mean difference measurements for V0 and V5, and V0 and V6 (p>0.05).

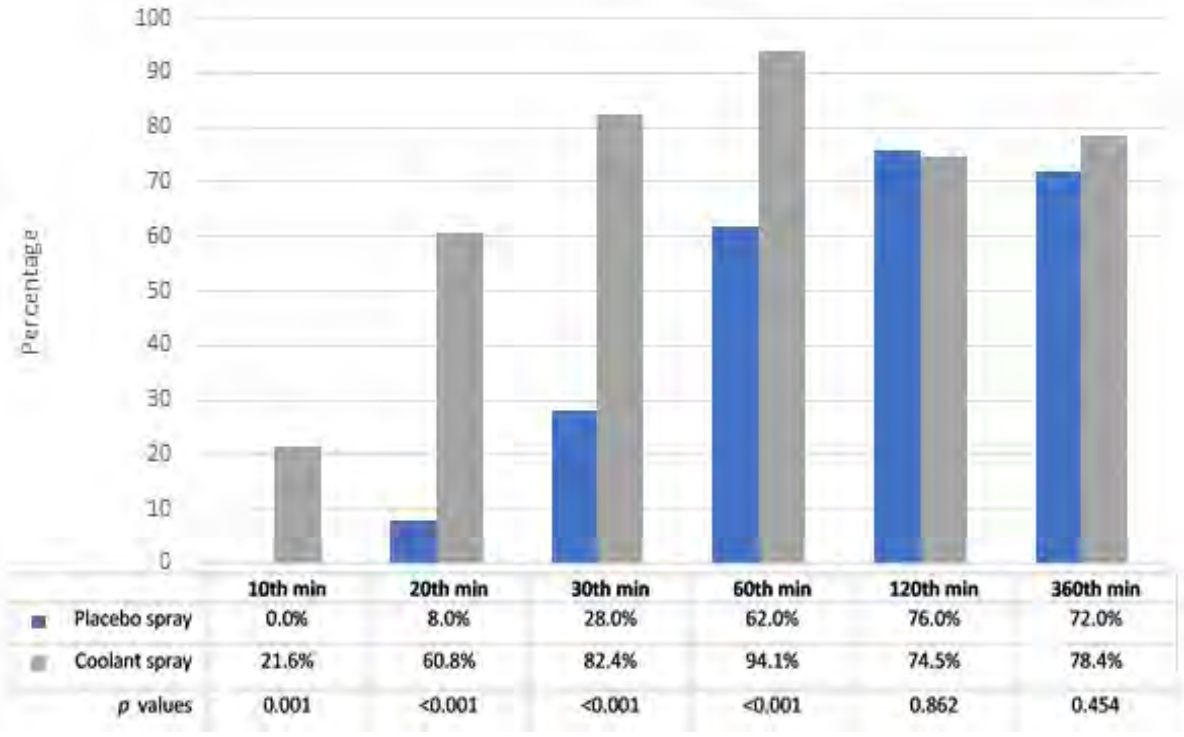
The frequency of "clinical effectiveness" was also shown in Figure 3. In V1, V2, V3, and V4 measurements, the incidence of "clinical effectiveness" in the coolant spray group was significantly higher than in the placebo group. In terms of the proportions of patients with "clinical effectiveness," placebo/coolant spray were 0 of 11 patients at V1 (p=0.001), 4 of 31 patients at V2 (p<0.001), 14 of 42 patients at V3 (p<0.001), 31 of 48 patients at V4 (p<0.001), 38 of 38 patients at V5 (p=0.862), and 36 of 40 patients at V6 (p=0.454).

Rescue analgesic medication was needed in 15 (14.9%) patients. There was no significant difference between the treatment groups in terms of rescue analgesic medication need (p=0.425). None of the patients described side effects that could be associated with coolant spray (e.g., frostbite, urticaria, and nerve damage).



	0	10th min	20th min	30th min	60th min	120th min	360th min
Placebo spray	7.38	6.20	5.30	4.54	3.56	2.68	2.60
Coolant spray	7.49	5.27	3.53	2.49	1.18	2.84	3.00

Figure 2. Efficacy of placebo spray versus coolant spray on mean VAS scores.



	10th min	20th min	30th min	60th min	120th min	360th min
Placebo spray	0.0%	8.0%	28.0%	62.0%	76.0%	72.0%
Coolant spray	21.6%	60.8%	82.4%	94.1%	74.5%	78.4%
p values	0.001	<0.001	<0.001	<0.001	0.862	0.454

Figure 3. At the time of each measurement, the frequency of detection (shown as percentage) of clinical effectiveness (decrease in VAS score by ≥50% compared to the beginning).

Retrospective power analysis

The mean VAS values were examined when 20 patients were present in both treatment groups. We found a statistically significant difference between delta VAS values at the 30th minute (mean difference: 2.85, 95%CI 1.78–3.92, p<0.001). In our power analysis based on these mean values and SDs, the power of the test was estimated at 0.91, while type I error was 0.01.

DISCUSSION

The coolant spray was preferred for cryotherapy because of its nonpharmacological nature and ease of use by health care workers. In our study, the primary outcome of "clinical effectiveness" was determined as the situation in which a reduction of ≥50% was achieved according to the initial VAS score.

Rib fractures constitute a significant proportion of thoracic trauma-related injuries[9]. An early and multimodal analgesic treatment approach applied from the moment of the first application allows these patients to breathe deeply, cough, and expectorate their secretions[5]. The effectiveness of analgesic therapies applied in treating pain due to rib fractures has been discussed in the literature for many years. The most commonly studied analgesic methods were interventional ones [10,11]. Since the physical characteristics of each traumatized patient will not be the same, it may be necessary to develop special treatment protocols for different populations. Different from the literature, we examined geriatric patients who are a trauma-sensitive and vulnerable group.

Geriatric patients become more vulnerable to falls and related injuries[12]. Kara and his colleagues found that "low-energy fall" was the most common cause of trauma-related referrals in the population aged 65 years and over[13]. In the literature, it is stated that NSAID and opioid analgesics are frequently preferred treatments for analgesia in young adults with rib fracture that develops as a result of such traumas[14]. However, age-related changes in the pharmacokinetic and pharmacodynamic properties of these drugs increase the incidence of unexpected side effects[3]. This has led us to suggest the need for new methods that are not systemic and have a low risk of side effects in providing analgesia to geriatric patients.

Cryotherapy decreases the tissue temperature in the application area, resulting in slowing the conduction velocity in peripheral sensory nerve endings. It also provides topical analgesia and anesthesia by

slowing down metabolism and suppressing inflammation[15,16]. Studies in the literature show that coolant spray is successfully applied for analgesia and topical anesthesia before injection or minor interventional procedures[15,17]. We found a significant decrease in the scores of the patients who were applied coolant spray compared to placebo. In addition, the number of patients who achieved "clinical efficacy" during the four measurement times during this period was significantly higher in the coolant spray group. In EDs, the first 60 min usually involves uncomfortable procedures such as physical examination and transfer for radiologwical examination. We believe that coolant spray application can be added to treatment as a method that can increase the comfort of both the patient and the ED doctor by reducing VAS from the first moment patients step into ED or even from triage.

It was stated that there were no serious side effects associated with the treatment in the studies in which coolant spray was applied in literature[18,19]. Similar to the literature, we did not encounter any side effects related to treatment application. Topical coolant spray application provides the possibility of use in repetitive doses thanks to its reliability and nonpharmacological structure.

Limitations

We evaluated the effectiveness of the treatment during our study by applying two doses of spray to patients in the coolant spray group. However, we think that a study in which repetitive coolant spray is used more frequently and longer term VAS measurements are monitored can contribute to the literature.

CONCLUSION

The geriatric population is a special group of patients who need alternative analgesic methods due to aging-related effects, additional diseases, and multiple drug use. Coolant spray therapy can be safely used in geriatric patients as a recovery option to reduce the uncomfortable moments that pain can cause during their time in ED.

AUTHORS' CONTRIBUTION

İA: Conceptualization, Formal analysis, Data curation, Methodology, Investigation, Resources, Validation, Writing – review & editing, Writing – original draft, Visualization, Project administration. **SD:** Conceptualization, Formal Analysis, Methodology, Validation, Writing – review & editing, Writing – original draft, Visualization. **AOK:** Formal analysis, Data curation, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **TD:** Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing. **MBK:** Conceptualization, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing. **STAG:** Data curation, Formal analysis, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **ZC:** Formal analysis, Methodology, Validation, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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The effect of positron emission tomography/computed tomography in axillary surgery approach after neoadjuvant treatment in breast cancer

Ecem Memişoğlu[1], Ramazan Sarı[1]*

SUMMARY

OBJECTIVE: The aim of this study was to determine the role of positron emission tomography/computed tomography in the decision to perform axillary surgery by comparing positron emission tomography/computed tomography findings with pathology consistency after neoadjuvant chemotherapy.

METHODS: Patients who were diagnosed for T1-4, cN1/2 breast cancer receiving neoadjuvant chemotherapy in our clinic between January 2016 and February 2021 were evaluated. Clinical and radiological responses, axillary surgery, and histopathological results after neoadjuvant chemotherapy were evaluated.

RESULTS: Axillary involvement was not detected in positron emission tomography/computed tomography after neoadjuvant chemotherapy in 140 (60.6%) of 231 node-positive patients. In total, 88 (62.8%) of these patients underwent sentinel lymph node biopsy, and axillary lymph node dissection was performed in 29 (33%) of these patients upon detection of 1 or 2 positive lymph nodes. The other 52 (37.1%) patients underwent direct axillary lymph node dissection, and no metastatic lymph nodes were detected in 33 (63.4%) patients. No metastatic lymph node was found pathologically in a total of 92 patients without involvement in positron emission tomography/computed tomography, and the negative predictive value was calculated as 65.7%. Axillary lymph node dissection was performed in 91 (39.4%) patients with axillary involvement in positron emission tomography/computed tomography after neoadjuvant chemotherapy. Metastatic lymph nodes were found pathologically in 83 of these patients, and the positive predictive value was calculated as 91.2%.

CONCLUSION: Positron emission tomography/computed tomography was found to be useful in the evaluation of clinical response, but it was not sufficient enough to predict a complete pathological response. When planning axillary surgery, axillary lymph node dissection should not be decided only with a positive positron emission tomography/computed tomography. Other radiological images should also be evaluated, and a positive sentinel lymph node biopsy should be the determinant of axillary lymph node dissection.

KEYWORDS: Axilla. Breast. Neoadjuvant therapy.

INTRODUCTION

Neoadjuvant chemotherapy (NAC) can reduce the size of the primary tumor and eliminate axillary lymph node metastasis, preventing axillary lymph node dissection (ALND) and increasing the chance of breast-conserving surgery (BCS)[1]. NAC is a component of standard treatment for locally advanced breast cancers and breast cancers with negatively impacting tumor profiles such as triple-negative and human epidermal growth factor receptor 2 (HER-2) positive diseases. In recent years, using sentinel lymph node biopsy (SLNB) to evaluate axillary involvement has increased the importance of NAC. Prospective studies such as NSABP B27, ACOSOG-Z1071, SENTINA, SN-FNAC, and GANEA 2 performed in patients with clinically lymph node-positive (cN+) breast cancer before NAC showed that SLNB could be done in patients with no clinical lymph node involvement (cN0) after NAC[2-6]. In light of these studies, the St. Gallen consensus in 2019 recommended that SLNB is sufficient if three or more sentinel lymph nodes (SLN) are negative in patients with cN0 after NAC, and axillary lymph node dissection (ALND) should be performed in patients with cN+ after NAC and macrometastasis in SLNB[7].

One of the most important prognostic factors in breast cancer is axillary lymph node metastasis. Preoperative estimation of metastatic lymph nodes is helpful in identifying patients with few lymph node metastases, the need for SLNB, and the need to avoid unnecessary ALND. Clinical examination and radiological imaging methods are used to predict preoperative lymph node metastasis. After NAC, radiological imaging methods gain more importance in evaluating the response of these lymph nodes to treatment and in the surgical decision. 18F-fluorodeoxyglucose positron emission tomography/computed tomography (18F FDG PET/CT) is a useful imaging

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method for staging, evaluating treatment response, and predicting the prognosis of breast cancer[8]. Almost all breast cancers show FDG uptake, but the intensity of FDG uptake is related to the breast cancer subtype. When compared to the ER-negative breast cancers, FDG uptake is higher in estrogen receptor-positive (ER+), triple-negative, and HER-2 expression-positive breast cancers[9].

In this study, it was aimed to evaluate the axillary involvement in PET/CT images before and after neoadjuvant treatment in breast cancer patients with axillary lymph node metastasis at the time of diagnosis, to compare the PET-CT findings and postoperative histopathology in order to evaluate its consistency as an imaging tool, and to assess the role of PET/CT in guiding the need for axillary dissection.

METHODS

Patient selection

The data of 867 patients who underwent surgery with the diagnosis of breast cancer in our clinic between January 2015 and December 2020 were analyzed retrospectively. A total of 231 cN+ patients who received NAC and underwent PET/CT imaging before and after NAC were included in the study. Patients who did not receive NAC or could not complete the treatment but had cN- disease before NAC and those diagnosed with stage 1 breast cancer were excluded from the study. All patients included in the study were evaluated and staged by clinical examination, mammography, ultrasonography, magnetic resonance imaging, and PET/CT before and after NAC. The metastatic axillary lymph nodes in the pre-NAC patient were confirmed by ultrasound-guided biopsy and/or PET/CT uptake.

Surgical technique

Surgical treatment was performed as per the standard guidelines. For the primary breast tumor, mastectomy or BCS was performed according to the patient's characteristics. Surgical management of the axilla has evolved over the years in light of studies and published guidelines. ALND was performed in 91 patients with (yc) N+ in the clinical staging performed after NAC.

In patients with 52 ycN- after NAC, in line with the prospective studies recommending SLNB before the 2019 St. Gallen consensus conference[10], SLNB was performed according to the surgeon's choice. However, ALND was performed on all of these patients. After the 2019 St. Gallen consensus conference, no additional intervention was performed in 59 ycN- patients with dual tracer mapping (radio-labeled colloid and patent blue) and removal of three or more negative lymph nodes. In some patients, in addition to the dual method, lymph nodes that were clip-marked at the time of needle biopsy were localized with wire and removed to reduce the false-negative rate. On the contrary, ALND was performed in 29 patients with one or more positive lymph nodes in the SLNB.

Clinical and pathological evaluation

Patient age, menopausal status, tumor size, lymph node involvement, the presence of metastasis, clinical tumor stage, histopathological type, histological and nuclear grade, tumor receptor (ER, the estrogen receptor; PR, progesterone receptor; and HER-2, human epidermal growth factor receptor) status, the molecular subtype of the tumor, Ki-67 level, PET/CT and radiological imaging findings before and after NAC, axillary clinical response status after NAC with ultrasonography, type of surgery, intraoperative frozen section findings, and final pathology findings were evaluated. Tumor staging was performed according to the 8th TNM staging system defined by the American Joint Committee on Cancer (AJCC). The pathological response after NAC was evaluated according to the CAP 2019 criteria determined by the College of American Pathologists (CAP).

Statistical data analysis

Data were analyzed using SPSS version 22. Frequency, percentage, mean, standard deviation, median, and interquartile range were used as descriptive statistical methods. Continuous variables were evaluated using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. One-way ANOVA test was used for normally distributed continuous variables, and Mann-Whitney U and Kruskal-Wallis tests were used for abnormally distributed continuous variables. The Chi-square test was used to evaluate categorical data. To determine the positive predictive value (PPV) and negative predictive value (NPV) of PET/CT findings after NAC, the axillary lymph node status on PET/CT after NAC was compared with the final surgical pathology result. $p<0.05$ was considered significant for all comparisons.

RESULTS

The mean age of 231 patients who received NAC was 52.5±12.1 years. According to tumor molecular subtypes, 9.5% of patients were Luminal A (ER and/or PR+, HER-2-, ki-67 ≤14%), 59.7% of them were Luminal B (ER and/or PR+, HER-2- or +, ki-67 >14%), 19.5% of them were HER-2 positive (ER-, PR-, HER-2+), and 11.3% of them were triple-negative (ER-, PR-, HER-2-). In total, 197 of the 231 (85.3%)

patients had FNA-confirmed axillary metastases. A total of 34 (14.7%) patients with inconclusive FNA findings but uptake in the PET-CT were regarded as clinically positive for axillary metastases. The clinicopathological features of the patients before NAC are summarized in Table 1.

The highest clinical and pathological complete response after NAC was observed in the HER-2 positive group. When the axillary clinical response was evaluated, it was observed that the best response was in the HER-2 positive group. While the triple-negative group had the highest SLNB negativity, the

Table 1. Clinicopathological data and clinical and pathological response after neoadjuvant chemotherapy regarding molecular tumor subtype.

	Total (n=231)	Luminal A (n=22)	Luminal B (n=138)	HER 2+ (n=45)	Triple- (n=26)	p-value
Age (years)	52.51±12.10	56.72±13.45	52.63±12.14	52.37±11.55	48.70±11.03	0.147*
Mitosis index (ki-67)	35.00 [25.00]	10.00 [5.00]	30.00 [15.00]	40.00 [26.25]	60.00 [30.00]	<0.001†
Total LN	10.00 [9.00]	11.50 [4.75]	10.00 [9.00]	10.00 [10.25]	11.00 [13.50]	0.755†
Menopausal status						0.934‡
Premenopause	59 (25.5%)	5 (22.7%)	36 (26%)	12 (26.6%)	6 (23%)	
Perimenopause	27 (11.7%)	3 (13.6%)	14 (10.2%)	5 (11.1%)	5 (19.3%)	
Postmenopause	145 (62.8%)	14 (63.6%)	88 (63.8%)	28 (62.2%)	15 (57.7%)	
Histology						0.176‡
Ductal	222 (96.1%)	21 (95.5%)	132 (95.6%)	45 (100.0%)	24 (92.3%)	
Lobular	4 (1.7%)	–	4 (2.9%)	–	–	
Other	5 (2.2%)	1 (4.5%)	2 (1.5%)	–	2 (7.7%)	
cT						0.129‡
1	47 (20.4%)	7 (31.8%)	26 (18.8%)	11 (24.4%)	3 (11.5%)	
2	131 (56.7%)	11 (50.0%)	84 (60.9%)	26 (57.8%)	10 (38.6%)	
3	30 (13%)	3 (13.6%)	14 (10.15%)	6 (13.3%)	7 (26.9%)	
4	23 (9.9%)	1 (4.5%)	14 (10.15%)	2 (4.5%)	6 (23%)	
cN						0.291‡
0	1 (0.5%)	–	1 (0.7%)	0 (0%)	0 (0%)	
1	111 (48%)	11 (50.0%)	71 (51.4%)	20 (44.4%)	9 (34.6%)	
2	89 (38.5%)	8 (36.4%)	51 (37%)	21 (46.7%)	9 (34.6%)	
3	30 (13%)	3 (13.6%)	15 (10.9%)	4 (8.9%)	8 (30.8%)	
Metastasis						0.252‡
No	220 (95.2%)	22 (100.0%)	131 (94.9%)	41 (91.1%)	26 (100.0%)	
Yes	11 (4.8%)	–	7 (5.1%)	4 (8.9%)	–	
Staging						0.130‡
2A	31 (13.4%)	5 (22.7%)	19 (13.7%)	6 (13.4%)	1 (3.8%)	
2B	61 (26.4%)	6 (27.3%)	40 (29%)	10 (22.2%)	5 (19.2%)	
3A	82 (35.5%)	7 (31.8%)	48 (34.8%)	19 (42.2%)	8 (30.8%)	
3B	18 (7.8%)	1 (4.5%)	11 (8%)	2 (4.4%)	4 (15.4%)	
3C	27 (11.7%)	3 (13.6%)	13 (9.4%)	3 (6.7%)	8(30.8%)	
4	12 (5.2%)	–	7 (5.1%)	5 (11.1%)	–	
PET breast involvement						0.007‡
Positive involvement	78 (33.8%)	4 (18.2%)	40 (29%)	23 (51.1%)	11 (42.3%)	
Negative involvement	153 (66.2%)	18 (81.8%)	98 (71%)	22 (48.9%)	15 (57.7%)	
PET axillary involvement						0.001‡
Positive involvement	143 (61.9%)	11 (50.0%)	82 (59.4%)	39 (86.6%)	11 (42.3%)	
Negative involvement	88 (38.1%)	11 (50.0%)	56 (40.6%)	6 (13.4%)	15 (57.7%)	

Continue...

Table 1. Continuation.

	Total (n=231)	Luminal A (n=22)	Luminal B (n=138)	HER 2+ (n=45)	Triple- (n=26)	p-value
Clinical response						0.033‡
No response	47 (20.3%)	6 (27.3%)	32 (23.2%)	3 (6.7%)	7 (26.9%)	
Partial response	129 (55.8%)	13 (59.1%)	77 (55.8%)	24 (53.3%)	15 (57.7%)	
Complete response	53 (22.9%)	3 (13.6%)	29 (21%)	18 (40%)	4 (15.4%)	
Pathological response (CAP)						<0.001‡
No residual tumor	63 (27.3%)	0 (0.0%)	29 (21%)	23 (51.1%)	11 (42.3%)	
Full response	29 (12.5%)	2 (9.1%)	16 (11.6%)	9 (20%)	2 (7.8%)	
Moderate response	38 (16.5%)	3 (13.6%)	25 (18.1%)	4 (0.9%)	6 (23%)	
Minimal / no response	101 (43.7%)	17 (77.3%)	68 (49.2%)	9 (20%)	7 (26.9%)	
SLNB groups						0.071‡
Negative	119 (78.8%)	10 (66.7%)	65 (73%)	32 (71.1%)	12 (92.3%)	
1–2 lymph node	19 (12.6%)	2 (13.3%)	14 (15.8%)	2 (4.4%)	1 (7.7%)	
3 lymph node	13 (8.6%)	3 (20.0%)	10 (11.2%)	–	–	
pN						<0.001‡
0	101 (43.7%)	1 (4.5%)	51 (37%)	35 (77.7%)	14 (53.9%)	
1	83 (36%)	13 (59.1%)	59 (42.7%)	6 (13.4%)	5 (19.2%)	
2	29 (12.5%)	6 (27.3%)	16 (11.6%)	3 (6.7%)	4 (15.4%)	
3	18 (7.8%)	2 (9.1%)	12 (8.7%)	1 (2.2%)	3 (11.5%)	

Data are denoted as mean±standard deviation, median [IQR], and n (%). *One-way ANOVA test. †Kruskal-Wallis test. ‡Chi-square test.

HER-2 positive group had the lowest lymph node metastases in the final pathology (Table 1).

HER-2 positivity, triple-negative subtype, and the absence of axillary lymph node involvement in PET/CT were the most critical factors in reducing pN after NAC (p<0.005). Although lymph node metastasis was higher in postmenopausal patients, this difference was not statistically significant (p=0.534) (Table 2).

SLNB was performed in 88 (62.8%) of 140 (60.6%) patients without axillary lymph node involvement in PET/CT after NAC, and ALND was performed in 29 (33%) patients who underwent SLNB after detecting one or more lymph node metastases. A total of 52 (37.1%) patients who underwent ALND without SLNB had negative PET-CT findings, and 33 (63.4%) of these 52 patients had no lymph node metastasis in the final histopathology. No metastatic lymph node was observed in 92 of the 140 patients, and the negative predictive value (NPV) was calculated as 65.7%. Direct ALND was performed in 91 (39.4%) patients with axillary involvement in PET/CT after NAC. Metastatic lymph nodes were detected in 83 of 91 patients, and the positive predictive value (PPV) was calculated as 91.2%. The sensitivity of PET/CT in detecting metastatic lymph nodes was 63.3%, and the specificity was 92%. A receiver operating characteristic analysis was performed to evaluate the overall predictive ability of PET/CT in

Table 2. Factors affecting pathological lymph node involvement after neoadjuvant chemotherapy.

	pN		p-value
	Negative	Positive	
Menopausal status			0.534†
Premenopause	27 (45.8%)	32 (54.2%)	
Perimenopause	14 (51.8%)	13 (48.2%)	
Postmenopause	60 (41.4%)	85 (58.6%)	
Grade			0.001†
Grade 1	2 (11.8%)	15 (88.2%)	
Grade 2	63 (40.9%)	91 (59.1%)	
Grade 3	36 (60.0%)	24 (40.0%)	
HER 2			<0.001*
Negative	32 (24.6%)	98 (75.4%)	
Positive	69 (68.3%)	32 (31.7%)	
Molecular subtype			<0.001*
Luminal A	1 (4.6%)	21 (95.4%)	
Luminal B	51 (36.9%)	87 (63.1%)	
HER 2+	35 (77.7%)	10 (22.3%)	
Triple-	14 (53.8%)	12 (46.2%)	
PET axillary involvement			<0.001*
Negative involvement	92 (65.7%)	48 (34.3%)	
Positive involvement	8 (8.7%)	83 (91.3%)	

*Chi-square test. †Mann-Whitney U test.

determining axillary status. The AUC (area under the curve) was 0.774 (95%CI 0.713–0.835, p<0.001) (Figure 1).

In the PET/CT evaluation of 172 patients who underwent axillary lymph node dissection after NAC, 81 (47.1%) had no involvement of the axilla. However, in the final pathology, no metastatic lymph node was detected in 40.7% of these patients. The metastatic lymph node was detected in 82.2% of 91 (52.9%) patients who had axillary involvement in PET/CT after NAC and underwent ALND.

DISCUSSION

The surgical management of the axilla after neoadjuvant therapy is still a controversial issue. Today, axillary lymph node dissection is the standard treatment for patients with an N2 pre-NAC axillary stage or an N1 with no clinical response in axillary involvement after NAC10. On the contrary, in patients who do not have clinical axillary involvement after NAC and underwent SLNB, ALND is still performed if the SLNB is positive. However, studies on the adequacy of radiotherapy instead of ALND in these patients are still ongoing[11,12].

Identifying patients who do not require ALND is difficult but essential. By meticulously evaluating the axillary clinical response after NAC, we can avoid ALND and its morbidities and improve the patient's quality of life. Lymphedema is seen in one of five patients who undergo ALND, and the incidence of lymphedema increases to one in four patients with the addition of radiotherapy to the treatment. Lymphedema significantly affects the quality of life of the patient[13]. While searching for a solution to improve the quality of life, the best treatment should be determined without ignoring the risk of recurrence and its effect on survival. NAC is recommended to avoid ALND in breast cancer patients with biopsy-proven axillary lymph node metastases.

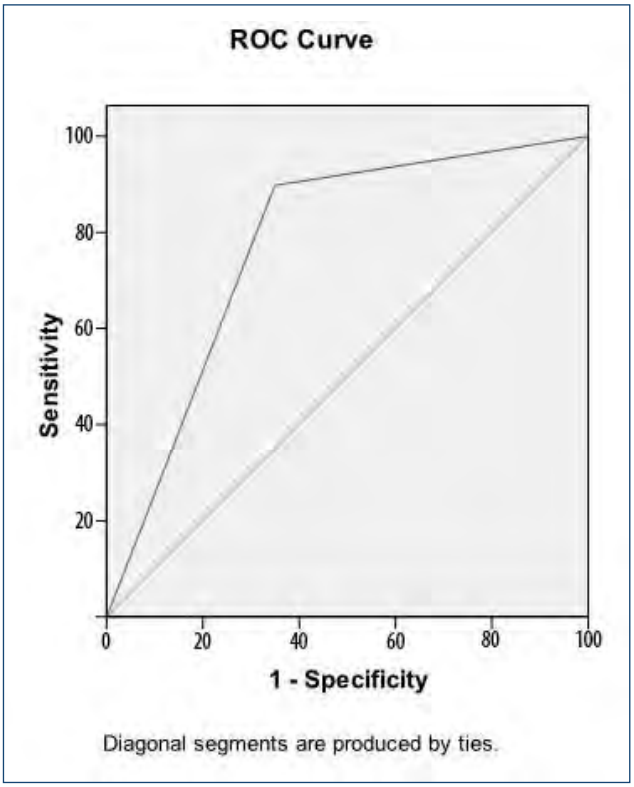

Figure 1. Receiver operating characteristic analysis for positron emission tomography/computed tomography in determining axillary status.

As it is known, the NAC response varies according to the molecular subtype of breast cancer, and the primary tumor response is not always similar to the axillary response. According to studies, the rate of no metastasis in axillary lymph nodes in the final pathology after NAC was found to be 0–29% in luminal tumors, 45–82% in HER-2 positive tumors, and 47–67% in triple-negative tumors[14-18]. In our study, these rates were 4.5% in luminal A, 37% in luminal B, 77.7% in HER-2 positive, and 53.9% in triple-negative tumors. With a detailed clinical and radiological evaluation of the axilla before surgery, surgeons can identify patients suitable for SLNB or avoid unnecessary SLNB by identifying patients who require upfront axillary lymph node dissection. Thus, the patients are properly evaluated preoperatively to avoid unnecessary procedures that lengthen the duration of the surgery. The sensitivity of ultrasonography in predicting residual axillary lymph node metastasis is higher than clinical examination and magnetic resonance imaging or PET/CT. However, PET-CT, even though not recommended in the standard guidelines, has been widely used by medical oncologists in our facility and the country in general to assess the NAC response. There are studies in the literature reporting that PET/CT imaging can change preoperative clinical staging and that the surgical procedure can be changed by avoiding unnecessary SLNB[19,20]. Orsaria et al.[21] reported the sensitivity of PET/CT for axillary lymph node staging as 87%, specificity as 90%, PPV as 93%, and NPV as 82%. The authors stated that PET/CT could guide clinical practice by predicting tumor behavior for axillary staging. In our study, the sensitivity, specificity, PPV, and NPV of PET/CT for axillary lymph node staging were 63.3, 92, 91.2, and 65.7%, respectively. PET/CT was false-negative in 34.3% and false-positive in 8.8% of patients, so ALND could have been avoided in 8.8% of these patients. Therefore, ALND should not be decided with only a positive PET/CT. Other radiological images should also be evaluated, and a positive SLNB should be the determinant of ALND.

The most important limitations of our study are its retrospective nature and the small number of patients. Another critical point is that since the data of the study were extracted from

medical records, imaging methods such as ultrasonography and magnetic resonance imaging, which were used to evaluate the clinical response, could not be compared with PET/CT data due to missing data. In addition, our clinical axillary response was low after NAC. We think most patients may not have benefited from the NAC due to the luminal nature of their disease.

CONCLUSION

As a result of the study, PET/CT was found to be useful in the evaluation of clinical response, but it was not sufficient alone to predict a complete pathological response. When planning axillary surgery according to PET/CT findings after NAC, even if there is no axillary involvement, it should be confirmed with ultrasound, and then SLNB should be performed.

AUTHORS' CONTRIBUTIONS

EM: Conceptualization, Data curation, Methodology, Project administration, Visualization, Writing – review & editing. **RS:** Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft.

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ORIGINAL ARTICLE

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Molecular epidemiology and antifungal susceptibilities of Aspergillus species isolated from patients with invasive aspergillosis

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the demographic data, molecular epidemiology, and in vitro antifungal susceptibility results of patients with *Aspergillus* isolated from various clinical specimens.

METHODS: A total of 44 *Aspergillus* strains were studied. The definition of invasive aspergillosis in patients was made according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria. Strains were phenotypically and molecularly identified. Demographic characteristics of patients and genotypes of strains were evaluated. Phylogenetic analysis was done by the The Unweighted Pair-Group Method with Arithmetic Mean (UPGMA). Antifungal susceptibility of strains was determined according to The Clinical and Laboratory Standards Institute (CLSI)-M61-Ed2 and The European Committee on Antimicrobial Susceptibility Testing (EUCAST).

RESULTS: A total of 11 patients were classified as proven and 33 as probable invasive aspergillosis. There was a statistically significant difference in age groups, subdisease, neutropenic, and receiving chemotherapy between groups. A total of 23 strains were identified as *Aspergillus fumigatus*, 12 as *Aspergillus niger*, 6 as *Aspergillus flavus*, and 3 as *Aspergillus terreus*. Phylogenetic analysis revealed five different genotypes. No statistical difference was found in the comparisons between patients groups and genotype groups. There was a statistically significant difference between genotype groups and voriconazole, posaconazole, and itraconazole Minimum Inhibition Concentration (MIC).

CONCLUSION: Accurate identification of strains and antifungal susceptibility studies should be performed due to azole and amphotericin B resistance. Genotyping studies are important in infection control due to identifying sources of infection and transmission routes.

KEYWORDS: Aspergillus. Microbial sensitivity tests. Molecular epidemiology. Sequence analysis.

INTRODUCTION

Aspergillus spores are commonly found in our environments and generally enter the body through respiration. These spores easily lead to invasive infection in immunocompromised individuals, and infections are associated with high mortality[1].

The diagnosis of *Aspergillus* infections is usually delayed due to the lack of reliable and easy-to-apply diagnostic tests, and effective treatment cannot be started in a timely manner. In order to prevent delays in the diagnosis of invasive fungal infections in patients with immunosuppressed, EORTC/MSG diagnostic criteria were established[2].

The identification of *Aspergillus* strains is performed by conventional, molecular methods, and serological tests[1]. Genotyping methods allow the epidemiological relationship between clinical and patient environmental isolates[3-6].

Antifungal resistance is increasing in clinical strains due to the intensive use of azole group pesticides as pesticides[1]. The mold should be produced from the clinical sample to detect antifungal resistance; a susceptibility test should be performed by reference methods (CLSI, EUCAST)[7-9].

The aim of this study was to retrospectively evaluate the demographic data, molecular epidemiology, and in vitro antifungal susceptibility results of patients with *Aspergillus* mold growth isolated from various clinical specimens over a period of 2 years.

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METHODS

Strains were isolated in the Erciyes University Medical Faculty Hospital, Mycology Laboratory, in Kayseri-Turkey in the past 2 years. All 44 strains were late from clinical samples of patients with suspected invasive aspergillosis (IA) infection. The galactomannan antigen (GMA) was detected in 22 patients.

The definition of IA was made according to the EORTC/MSG criteria. The data of the patients were compared with the data of 19 control patients who were negative for direct microscopy, culture growth, histopathology, and GMA.

Phenotypic identification of the strains was performed according to microscopic and macroscopic features. Molecular identifications of strains were determined by sequence analysis of the ITS1-4, D1D2-NL1-4 region. DNA sequence analysis was performed using the ABI 3130XL analyzer device (Applied Biosystems, USA). The ITS1-4 and D1/D2 nucleotide sequences of *Aspergillus* were analyzed using the BLASTN program provided on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis: ITS and D1/D2 nucleotide sequences of *Aspergillus* strains were obtained from GenBank. The sequences were aligned using the Clustal X software. Nucleotide sequences were then used for phylogenetic analysis by the UPGMA method with 1000 bootstrap replication to ensure robustness using the MEGA software (MEGA Inc., Englewood, USA).

Antifungal susceptibility testing was performed according to the CLSI-M61-Ed2 and EUCAST-v10.0[7,8]. AmphotericinB (AMB), voriconazole (VOR), and itraconazole (ITR) (Sigma, USA) were tested by the broth microdilution test. Caspofungin (CAS), anidulafungin (ANI), and posaconazole (POS) (Etest, bioMerieux, France) were tested by the gradient strip test. There were reported VOR breakpoints for only *Aspergillus fumigatus* strain in CLSI-M61-Ed2[7]. ECOFF values and susceptibilities breakpoints for AMB, ITR, POS, and VOR of *A. fumigatus*, *Aspergillus niger*, *Aspergillus flavus,* and *Aspergillus terreus* strains were evaluated according to EUCAST[8,9].

Ethics statement

Ethical approval was not considered necessary as the isolates were stock samples taken during routine laboratory activities.

Statistical analysis

Statistical analyses were performed using IBM-SPSS-Statistics V22.0. Chi-square test was used for the comparison between the characteristics of the patients and their diagnosed groups, and t-test and one-way ANOVA tests were used for the comparison between antifungal MIC means and genotypes. $p<0.05$ was considered significant.

RESULTS

A total of 44 patients (12 females, 32 males) were included in the study. Most of the patients were hospitalized for chest diseases (29.5%) and hematology-oncology services (18.1%). In total, 20 (45.45%) of the samples were found to be bronchoalveolar lavage, 10 tissue, 5 wounds, 5 sterile body fluids, and 4 sputa. Most patients have an underlying factor such as malignancy (n.24, 54.5%).

According to EORTC/MSG criteria, 11 patients were classified as proven and 33 as probable IA. The clinical samples of 11 patients with proven IA were three sterile body fluids and eight tissues, and hyphae were seen in the direct microbiological and histopathological examination of all of them. The mean age of patients with IA was higher than that of the control group. The rate of association with hematological cancer in patients with proven IA was found to be higher than in other groups. There was a statistically significant difference in subdisease, neutropenic, and receiving chemotherapy between groups. There were no significant differences in having a catheter and prophylactic antifungal drugs between groups. VOR was used most frequently as a prophylactic antifungal agent. GMA test was above 0.5 ng/mL in 12 patients (27.2%). Demographic data of patients with IA and the control group are shown in Table 1.

A total of 44 strains were identified by conventional methods, and 23 were identified as *A. fumigatus*, 12 *A. niger*, 6 *A. flavus*, AND 3 *A. terreus.* However, sequence analysis could be performed on 35 of 44 strains, and these strains were identified using the same by conventional methods (23 *A. fumigatus*, 4 *A. niger*, 5 *A. flavus*, 3 *A. terreus*). There was no statistical difference between IA patient groups and isolated strains (Table 1).

The 14 *A. fumigatus*, 8 *A. niger*, and 2 *A. flavus* were isolated from 24 respiratory tract samples. Five *A. fumigatus*, four *A. flavus*, and one *A. niger* STRAIN were isolated from tissue samples. Three *A. fumigatus*, one *A. niger*, and one *A. terreus* strain were isolated from wound samples. Two *A. niger*, two *A. terreus*, and one *A. fumigatus* strain were isolated from a sterile liquid.

Result of phylogenetic tree analysis using UPGMA method

Main genotypes were designed A, B, C, D, and E. The most common genotype was genotype A, which accounted for 23 (65.7%) of the 35 isolates. Subgenotypes were determined in main clone A (A1–A2). Genotype A contained *A. fumigatus* strains. Genotype B contained three *A. terreus* strains. Genotype C contained four *A. flavus* strains. Genotype D contained one *A. flavus* strain. Genotype E contained four *A. niger*

Table 1. Evaluation of demographic characteristics, isolated strains, and genotypes of patients diagnosed with proven and probable IA, and control groups.

	Proven IA (n=11)	Probable IA (n=33)	Control group (n=19)	χ^2	p-value
	n (%)	n (%)	n (%)		
Gender*					
Men	8 (17.8)	24 (53.3)	13 (28.9)	0.121	0.941
Women	3 (16.7)	9 (50.0)	6 (33.3)		
Age (years)*					
<18	0 (0.0)	2 (66.7)	1 (33.1)	13.033	**0.043**
18–45	2 (10.5)	6 (31.6)	11 (57.9)		
46–64	5 (18.5)	16 (59.3)	(22.2)		
>65	4 (28.6)	9 (64.3)	1 (7.1)		
Neutropenia*					
Yes	7 (26.9)	16 (61.5)	3 (11.5)	8.069	**0.018**
No	4 (10.8)	17(45.9)	16 (43.2))		
Intravenous catheter*					
Yes	9 (25.7)	17 (48.6)	9 (25.7)	3.807	0.149
No	2 (18.2)	16 (57.1)	10 (35.7)		
Chemotherapy*					
Yes	10 (25.0)	16 (40.0)	14 (35.0)	7.625	**0.022**
No	1 (4.3)	17 (73.9)	5 (21.7)		
Prophylactic antifungal*					
Yes	7 (31.8)	10 (45.5)	5 (22.7)	4.920	0.085
No	4 (9.8)	23 (56.1)	14 (34.1)		
Mortality*					
Yes	4 (26.7)	5 (33.3)	6 (40.0)	2.952	0.229
No	7 (14.6)	28 (58.3)	13 (27.1)		
Subdisease (n=63)*					
Hematological cancers	7 (41.2)	3 (17.6)	7 (41.2)	31.978	**0.000**
Other cancers	4 (22.2)	10 (55.6)	4 (22.2)		
COPD	0 (0.0)	6 (85.7)	1 (14.3)		
DM	0 (0.0)	7 (100.0)	0 (0.0)		
RA	0 (0.0)	7 (100.0)	0 (0.0)		
Others	0 (0.0)	3 (30.0)	7 (70.0)		
Strains**					
A. fumigatus	4 (36.4)	19 (57.5)	0	6.821	0.078
A. niger	2 (18.1)	10 (30.3)	0		
A. flavus	4 (36.4)	2 (6.1)	0		
A. terreus	1 (9.1)	2 (6.1)	0		
Genotypes**					
A1	4 (36.4)	16 (48.6)	0	8.214	0.223
A2	0	3 (9.1)	0		
B	1 (9.1)	2 (6.1)	0		
C	3 (27.4)	1 (3.0)	0		
D	0	1 (9.1)	0		
E1	1 (9.1)	1 (3.0)	0		
E2	0	2 (6.1)	0		

*Line percentage. **Column percentage. Bold indicate statistically significant p-values. Hematological cancers: acute myeloid leukemia, multiple myeloma, and chronic lymphocytic leukemia. Other cancers: lung, bone, nasal, skin, kidney, breast, and stomach cancers. COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; RA: rheumatological disease.

strains and consisted of two subgenotypes (E1–E2) (Figure 1). The clinical origin and isolation date of strains were evaluated, and there was no clonal relationship found among strains to determine an outbreak. No statistical difference was found in the comparisons between the patient groups and the genotypes groups. Of 35 strains, 9 belonged to patients diagnosed with proven IA. Of the nine strains, four were of the A1 genotype, and three strains in the C, B, and E1 genotypes had one strain each (Table 1).

Antifungal susceptibility testing

There was a statistically significant difference between genotypes groups and VOR, POS, and ITR MIC GO.

MIC range, MIC_{50}, MIC_{90} values of strains, and wildtype (WT) strain number and susceptibility according to EUCAST-10.0v as shown in Table 2. The only one *A. fumigatus* strain was found to be resistant to AMB (1.5 µg/mL) and non-wildtype. According to CLSI, the VOR MIC value of all *A. fumigatus* strains was found low from 0.5 µg/mL.

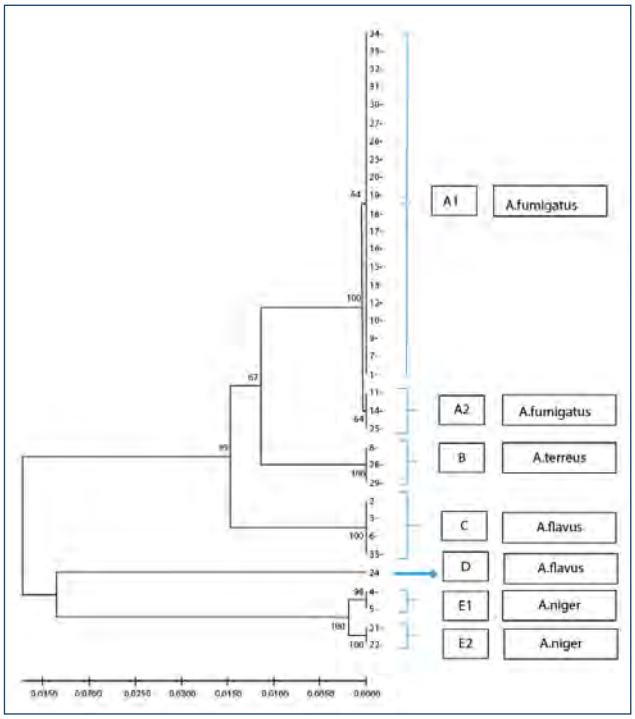

Figure 1. For phylogenetic analysis, the UPGMA of 35 *Aspergillus* strains with ITS1-4 and D1/D2 sequences available in GenBank. Strains tested were represented by isolation numbers. Strains with a similarity of 95% or high in the phylogenetic tree were considered main clones, and those in the main clone category with a similarity of 97% or above were considered subclone. Main clones were designed A, B, C, D, and E, respectively.

DISCUSSION

The incidence of IA has increased due to the increase in the number of immunocompromised patients. The incidence of IA reported in various European countries varies according to patient population and geography (0.4–23%). Patients with IA have high mortality of 30–85%. However, with early diagnosis and appropriate treatment, this rate drops below 50%[1]. The mortality rate in our patients was 20.4%.

Since the incubation period is not known precisely in IA, there is no consensus on the definition of hospital-acquired cases. In a France study, in which cases up to 10 days after hospitalization were considered hospital-acquired, it was reported that 23.8% of aspergillosis cases were hospital-acquired[10]. All the patients in our study were hospitalized for more than 10 days and had a subdisease or drug use that suppresses the immune system.

The species most commonly isolated in hospital-acquired aspergillosis are *A. fumigatus* and *A. flavus*. In a study by Atalay et al.[11] in our hospital, 12 of 24 *Aspergillus* strains were identified as *A. fumigatus*, 8 as *A. flavus*, 3 as *A. niger,* and 1 as *A. terreus,* and *A. fumigatus* was reported to be the dominant species in respiratory tract samples compared with other *Aspergillus* strains. The results obtained are similar to other results. The GMA test can also contribute significantly to the diagnosis of IA through serial follow-up[1]. In our study, there were no statistically significant differences in GMA test results between the patient groups.

Genotyping of *Aspergillus* strains allows investigation of nosocomial aspergillosis to identify outbreak-related strains, distinguish epidemic from endemic or sporadic strains, and determine the origins of infection. Refojo et al.[6] investigated whether there is a genetic relationship between the *A. flavus* strains isolated from the air of the hemato-oncology service and two patients. This study's results show that the *A. flavus* isolates recovered from the patients were not genetically related to those retrieved from the patient rooms. de Valk et al.[5] used two molecular methods for genotyping in their study and showed that the strains isolated from each patient were of different genotypes, while the strains isolated from the same patient were all of the same genotypes. Also, they also showed that there were multiple genotypes among the isolates obtained from respiratory tract samples. Similarly, Vanhee et al.[4] performed a genotypes analysis of 41 *A. fumigatus* isolates from nine patients with proven IA hospitalized in two different centers, and was shown in the identification of 11 distinct genotypes. The researchers reported the lack of a clear epidemiological link between patients, even between those hospitalized in the same center. The results of our study

Table 2. MIC range, MIC_{50}, MIC_{90} values of strains, and WT strain number and susceptibility according to EUCAST 10.0v.

	MIC (μg/mL)			Antifungal susceptibility, n (%)		
	Ranges	MIC_{50}	MIC_{90}	WT	S	R
A. fumigatus (n: 23)						
AMB	0.016–1.5	0.125	0.50	22	22	1
VOR	0.064–0.25	0.125	0.125	23	23	–
POS	0.002–0.25	0.032	0.125	23	23	–
ANI	0.002–0.032	0.002	0.032	ND	ND	ND
ITR	0.125–1	1	1	23	23	–
CAS	0.016–0.25	0.032	0.125	ND	ND	ND
A. niger (n: 12)						
AMB	0.125–0.5	0.125	0.25	12	12	–
VOR	0.036–0.25	0.125	0.25	12	ND	ND
POS	0.002–0.25	0.064	0.125	12	ND	ND
ANI	0.002–0.02	0.002	0.008	ND	ND	ND
ITR	0.125–1	1	1	12	ND	ND
CAS	0.002–0.25	0.008	0.016	ND	ND	ND
A. flavus (n: 6)						
AMB	0.25–0.5	0.5	0.5	6	ND	ND
VOR	0.064–0.5	0.125	0.125	6	ND	ND
POS	0.032–0.25	0.125	0.125	6	ND	ND
ANI	0.002–0.004	0.002	0.004	ND	ND	ND
ITR	0.25–1	0.25	0.5	6	6	–
CAS	0.008–0.064	0.016	0.016	ND	ND	ND
A. terreus (n:3)*						
AMB	0.002–0.50			3	ND	ND
VOR	0.064–0.125			3	ND	ND
POS	0.016–0.02			3	3	–
ANI	0.002–0.02			ND	ND	ND
ITR	0.25–0.25			3	3	–
CAS	0.032–0.125			ND	ND	ND

*MIK_{50} and MIK_{90} not calculated due to low number of *A. terreus* strains. ND: not done; WT: wild type; S: susceptible; R: resistant; AMB: amphotericin B; VOR: voriconazole; POS: posaconazole; ANI: anidulafungin; ITR: itraconazole; CAS: caspofungin.

were found to be similar to the studies mentioned. When the clinical origin and isolation date of strains were evaluated, there was no clonal relationship found among the *Aspergillus* strains to determine an outbreak. There was no statistically difference in the comparisons between the patient groups and the genotype groups. When we compared the geometric mean of antifungals and the genotypes, there was a statistically significant difference between the azole group antifungals (ITR, VOR, and POS) and genotypes.

In high-risk patients, prophylactic antifungal drug options are recommended by international guidelines. With the use of prophylactic antifungals, there may be a decrease in IA cases and a positive effect on the prognosis[1]. In a study conducted on patients with chronic obstructive pulmonary disease (COPD) diagnosed with IA in our hospital in 2013, researchers reported that VOR could be given as the initial treatment[12]. In a study conducted in our hospital in 2014, the MIC ranges of 26 *Aspergillus* isolates were found 0.004–2 μg/mL for AMB,

0.004–1 µg/mL for CAS, and 0.016–0.64 µg/mL for VOR[13]. These results are similar to our findings. Considering the toxic effects of AMB, we thought that the choice of VOR in prophylactic treatment in our hospital was appropriate. We can see that it had very low MIC values of azole group drugs for all strains. Although there were no statistically significant differences in receiving prophylactic antifungal between patient groups, there was no death in the receiving prophylactic antifungal drug group.

In the ESCMID guideline, VOR or isavuconazole is recommended in the treatment of IA as the first choice, but if high azole MIC values are detected, it is necessary to turn to AMB or combined treatment options[1]. According to the ESCMID, antifungal susceptibility tests should be performed[1]. In the ARTEMIS global surveillance study, the azole resistance rate was found to be 5.8% (29/497) in *A. fumigatus* isolates, which are the causative agents of IA, and it was stated that all resistant isolates were isolated in China[14]. In our study, all *A. fumigatus* strains were found to be susceptible to VOR according to CLSI. According to EUCAST 10.0 v, 43 *Aspergillus* strains were found as WT. Only one of the 23 *A. fumigatus* strains had the highest MIC value for AMB (1.5 µg/mL). Characteristics of the patient with AMB-resistant *A. fumigatus* isolated in the proven IA patient group: in genotype A1, 59 years old, male, clinical specimen sterile fluid (pleura), subdisease chronic lymphocytic leukemia, with a catheter and receiving chemotherapy, and the patient died. Also, when we look at the MIC values for echinocandin group antifungal drugs (ANI, CAS) of all strains, we saw that the MIC values are very low (0.002–0.25 µg/mL) (Table 2).

CONCLUSION

Early diagnosis of IA according to the EORT/MSG diagnostic criteria is very important, and early appropriate antifungal drug intake reduces mortality. Accurate identification of strains and antifungal susceptibility studies is required, especially due to azole and AMB resistance. Genotyping studies provide a better understanding of infection sources and transmission routes, and aid infection control.

Reference-resistant or susceptibility breakpoints for antifungal drugs for some *Aspergillus* strains are unclear. Such studies in the future will contribute to the necessary breakpoints for antifungal resistance for *Aspergillus* strains.

AUTHORS' CONTRIBUTIONS

FMS: Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **ANK:** Methodology, Supervision, Writing – original draft, Writing – review & editing. **PS:** Writing – original draft, Writing – review & editing. **MAA:** Writing – original draft, Writing – review & editing **AB:** Methodology, Writing – original draft, Writing – review & editing. **OC:** Methodology, Writing – original draft, Writing – review & editing. **BD:** Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of toxin-antitoxin genes, antibiotic resistance, and virulence genes in *Pseudomonas aeruginosa* isolates

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SUMMARY

OBJECTIVE: Toxin-antitoxin genes *RelBE* and *HigBA* are known to be involved in the formation of biofilm, which is an important virulence factor for *Pseudomonas aeruginosa*. The purpose of this study was to determine the presence of toxin-antitoxin genes and *exoenzyme S* and *exotoxin A* virulence genes in *P. aeruginosa* isolates and whether there is a relationship between toxin-antitoxin genes and virulence genes as well as antibiotic resistance.

METHODS: Identification of the isolates and antibiotic susceptibilities was determined by a VITEK 2 (bioMérieux, France) automated system. The presence of toxin-antitoxin genes, virulence genes, and transcription levels were detected by real-time polymerase chain reaction.

RESULTS: *RelBE* and *HigBA* genes were detected in 94.3% (82/87) of *P. aeruginosa* isolates, and *exoenzyme S* and *exotoxin A* genes were detected in all of the isolates (n=87). All of the isolates that harbor the toxin-antitoxin and virulence genes were transcribed. There was a significant increase in the *RelBE* gene transcription level in imipenem- and meropenem-sensitive isolates and in the *HigBA* gene transcription level in amikacin-sensitive isolates ($p<0.05$). There was a significant correlation between *RelBE* and *exoenzyme S* ($p=0.001$).

CONCLUSION: The findings suggest that antibiotic resistance may be linked to toxin-antitoxin genes. Furthermore, the relationship between *RelBE* and *exoenzyme S* indicates that toxin-antitoxin genes in *P. aeruginosa* isolates are not only related to antibiotic resistance but also play an influential role in bacterial virulence. Larger collections of comprehensive studies on this subject are required. These studies should contribute significantly to the solution of the antibiotic resistance problem.

KEYWORDS: *Pseudomonas aeruginosa.* Toxin-antitoxin systems. Virulence. Anti-bacterial Agents.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the major pathogens causing hospital-acquired infections, particularly affecting patients with immunocompromised or prolonged stay in the intensive care unit[1]. As a pathogen, *P. aeruginosa* is of growing clinical significance as a result of its inherent resistance to multiple antimicrobials and its ability to develop high-level multidrug resistance (MDR) due to the presence of a lot of virulence factors expressed in its genome[2].

These virulence factors allow *P. aeruginosa* to easily reproduce and live in both the host cell and the environment. Virulence factors can cause a number of harmful effects, including damage to tissues, the spread of infection to blood and tissue, the escape of bacteria from the host cell defense, and disease progression. In addition, they can induce antibiotic resistance in *P. aeruginosa*, making treatment difficult[3].

In recent years, studies on toxin-antitoxin (TA) genes have shown that they are associated with virulence regulation, biofilm formation, plasmid maintenance, and antibiotic resistance[4-6]. TA genes are small operons composed of both a growth-inhibitory toxin and an antitoxin that regulates toxin activity by direct inhibition. This antitoxin also plays a role in cell physiology by acting as a regulator of transcription[7-9]. Previous studies have shown that TA genes play several important physiological roles and, therefore, may be able to treat infections caused by MDR bacteria[6].

It has been indicated that TA genes are involved in the formation of biofilm, which is an important virulence factor for *P. aeruginosa*[10-12]. The purpose of this study was to determine the presence of *RelBE* and *HigBA* TA genes and *exoenzyme S* (*ExoS*) and *exotoxin A* (*ToxA*) virulence genes in *P. aeruginosa* isolates. In addition, we aimed to investigate

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whether there is a relationship between TA genes and virulence genes as well as whether TA genes are associated with antibiotic resistance.

METHODS

Bacterial isolates and antimicrobial susceptibility testing

This study included 87 *P. aeruginosa* isolates from various samples sent to the Microbiology Laboratory at Tokat Gaziosmanpasa University Training and Research Hospital between January 2016 and March 2017. Identification and antibiotic resistance profile of *P. aeruginosa* isolates were determined by a Vitek 2 (bioMérieux, France) automated system according to Clinical and Laboratory Standards Institute criteria[13]. The total number of susceptible and resistant antibiotics is not equal to all antibiotics because not all antibiotics have been studied in every isolate. Only one isolate was obtained from each patient. *P. aeruginosa* ATCC 27853 isolates were used as quality controls.

Genomic DNA isolation of *RelBE*, *HigBA*, *ExoS*, and *ToxA* genes in *Pseudomonas aeruginosa* isolates by real-time polymerase chain reaction

A volume of 10^5 McFarland bacterial suspension (1.5 µL) was centrifuged at 12,500 × *g* for 5 min. Then, 200 µL of lysozyme was added to the pellet and incubated at 37°C for 30 min. To degrade the RNA, 4 µL of RNase A (50 mg/mL) was added to the sample, which was vortexed for 10 min at room temperature. In addition, 40 µL of proteinase K was added, and the DNA isolation was completed according to the manufacturer's recommendations using a Magnesia 16 isolation device (Anatolia Geneworks, Turkey).

Total RNA isolation from *Pseudomonas aeruginosa* isolates

The prepared bacterial suspension (1.5 µL) was centrifuged at 12,500 × *g* for 5 min and 200 µL of RB buffer prepared with mercaptoethanol was added to the pellet, and RNA isolation was performed by using the Magnesia 16 Cultured Cell and Tissue Total RNA Extraction Kit. To degrade the genomic DNA, 20 µL of 10´ reaction mix, 7.5 µL of DNase I, and 172.5 µL of water were added to each sample, and pure RNA was obtained. RNA isolation was performed using a Magnesia 16 isolation device (Anatolia Geneworks, Turkey).

Preparation of cDNA from total RNA in *Pseudomonas aeruginosa* isolates

The cDNA mixture was prepared by adding 10 µL of water, 8 µL of the reaction mix, and 2 µL of reverse transcriptase (RT) to the final volume of 20 µL. The cDNA was prepared using a Montania 4896 real-time PCR device (Anatolia Geneworks, Turkey) for a total of 40 min as follows: 5 min at 22°C, 30 min at 42°C, and 5 min at 85°C. The activity of the gene region was proven by the detection of the cDNA using SYBR green dye.

Detection of *RelBE*, *HigBA*, *ExoS*, and *ToxA* genes expression in *Pseudomonas aeruginosa* by real-time polymerase chain reaction

All of the genes were prepared by mixing 12.5 µL of Super SYBR Mix, 0.5 µL of forward and reverse primers, 6.5 µL of water, and 3 µL of cDNA for a total volume of 20 µL. The gene expression levels were detected by a Montana 4896 real-time PCR device (Anatolia Geneworks, Turkey).

The amplification programs for *RelBE*, *HigBA*, *ExoS*, and *ToxA* were as follows: 3 min of denaturation at 95°C and 45 cycles of 15-s denaturation at 95°C; for the *RelBE* primer, binding at 56°C for 45 s; elongation at 72°C for 30 s, followed by a final elongation step in which the temperature was increased from 60 to 90°C; for the *HigBA*, *ExoS*, and *ToxA* primers, binding at 52°C for 45 s; elongation at 72°C for 30 s, followed by a final elongation step in which the temperature was increased from 60 to 90°C. The primers were used in the PCR step according to the previous study[12,14].

Statistical analysis

Statistical analysis was performed by using commercial software IBM SPSS Statistics version 20 (SPSS Inc., an IBM Corp., Somers, NY, USA). The differences between antibiotic resistance in the *P. aeruginosa* isolates and the transcription levels of the TA and virulence genes were investigated with independent samples t-test and Mann-Whitney U test. The relationship between *RelBE* and *HigBA* genes and *ExoS* and *ToxA* genes was investigated with the Pearson's correlation test. The values of $p \leq 0.05$ were considered significant.

Ethics

This study was approved by the Ethics Committee of Tokat Gaziosmanpasa University (number 17/KAEK/022).

RESULTS

The isolates were obtained from respiratory samples (40.3%, n=35), wound samples (26.4%, n=23), urine (20.7%, n=18),

blood (11.5%, n=10), and sterile body fluid samples (1.1%, n=1). The *P. aeruginosa* isolates had the highest rates of antibiotic resistance to aztreonam 64.4% (47/73), piperacillin-tazobactam 64% (55/86), imipenem 42.7% (35/82), and meropenem 36.8% (32/87). While the *RelBE* and *HigBA* genes were detected in 94.3% (82/87) of the *P. aeruginosa* isolates (n=87), the *ExoS* and *ToxA* genes were detected in all of the isolates (n=87). It is shown that TA genes (82/82) and virulence genes (87/87) are involved.

There was a significant increase in the *RelBE* gene transcription level in imipenem- and meropenem-sensitive isolates ($p<0.05$). There were no correlations between *RelBE* and *HigBA* gene transcription levels with any of the other antibiotics ($p>0.05$). Antibiotic susceptibility rates of isolates and the relationship between antibiotic susceptibilities and transcription levels of *RelBE* and *HigBA* TA genes are shown in Table 1. There was a significant correlation between *RelBE* and *ExoS* (p=0.001); none of the other correlations were significant.

DISCUSSION

The MDR *P. aeruginosa* caused 32,600 estimated infections among hospitalized patients and 2,700 estimated deaths in the United States. Some types of MDR *P. aeruginosa* are resistant to nearly all antibiotics, including carbapenems, which means that several classes of antibiotics including aminoglycosides, cephalosporins, fluoroquinolones, and carbapenems may not cure these infections[1]. In recent years, the increase in carbapenem-resistant frequency among *P. aeruginosa* is becoming a major challenge. The reported rates of carbapenem resistance seem to be considerably different (12–67%) in various regions[15-17]. In this study, it was observed that the carbapenem's resistance rates were consistent with the previous studies. It is seen that carbapenems still maintain their importance among the antibiotics used in the treatment of *P. aeruginosa* infections.

Pseudomonas aeruginosa have virulence factors including biofilm, *ToxA*, *ExoS*, pigments, mucoid exopolysaccharide, lipopolysaccharide, protease, leucocidin, and hemolysins. *ToxA* is secreted outside the cell and causes cell death and cell damage as well as suppression of host response by inhibiting protein synthesis[18]. Nikbin et al. indicated *ExoS* and *ToxA* genes existed in wound samples at rates of 62 and 90% and in respiratory system at rates of 47.4 and 46.6%, respectively. They indicated that the prevalence of *Tox*A gene was significantly higher in the pulmonary tract and burn isolates. In addition, the difference between *ExoS* prevalence in isolates from the pulmonary tract and burn isolates was statistically significant[19].

Table 1. Antibiotic susceptibility rates of isolates and the relationship between antibiotic susceptibilities and expression levels of *RelBE* and *HigBA* Toxin-antitoxin genes.

Antibiotic		n	%	*RelBE* gene p	*HigBA* gene p
Piperacillin-tazobactam	Sensitive	31	36	0.436[a]	0.723[a]
Seftazidime	Sensitive	62	71.3	0.154[a]	0.636[a]
Sefepime	Sensitive	55	66.3	0.331[a]	0.431[a]
Aztreonam	Sensitive	26	35.6	0.449[a]	0.664[a]
Imipenem	Sensitive	47	57.3	**0.002**[a]	0.365[a]
Meropenem	Sensitive	55	63.2	**0.043**[a]	0.367[a]
Amikacin	Sensitive	80	92.0	0.627[a]	0.050[a]
Gentamycin	Sensitive	75	86.2	0.756[a]	0.181[a]
Netilmicin	Sensitive	64	87.7	0.848[a]	0.228[a]
Tobramycin	Sensitive	67	93.1	0.406[b]	0.086[b]
Siprofloxacin	Sensitive	67	77.9	0.251[a]	0.144[a]
Levofloxacin	Sensitive	52	72.2	0.236[a]	0.323[a]
Colistine	Sensitive	82	100	-	-

TA: toxin-antitoxin. The total number of susceptible and resistant antibiotics is not equal to all antibiotics because not all antibiotics have been studied in every isolate. Differences in antibiotic resistance in *P. aeruginosa* isolates and transcription levels of TA genes were investigated with the independent samples t-test and Mann-Whitney U test. The relationship between *RelBE* and *HigBA* genes and *ExoS* and *ToxA* genes was investigated with a Pearson's correlation test. [a]Independent samples t-test. [b]Mann-Whitney U-test. Bold indicates statistically significant p-value.

Faraji et al. in 2016 reported that *ExoS* and *ToxA* genes were detected in cystic fibrosis isolates at rates of 70.8 and 63.1%, respectively[20]. Wolska et al. indicated that the *ExoS* gene was present in 78.5% of 62 *P. aeruginosa* isolates, while the *ToxA* gene was found in 88.7%[14]. In this study, all the *P. aeruginosa* isolates had *ExoS* and *ToxA* genes. Therefore, the statistical distribution of virulence genes according to the samples has not been studied. However, most isolates were isolated from respiratory tract samples. The results of this study determined that *ExoS* and *ToxA* virulence factors are found at high rates in *P. aeruginosa* isolates, which is consistent with previous studies.

Hemati et al. observed biofilm formation in 87.5% of 140 *P. aeruginosa* isolates; furthermore, the TA genes *MazEF*, *RelBE*, *HigBA*, *CcdAB*, and *MqsR* were found at rates of 85.71, 100, 1.42, 100, and 57.14%, respectively. In addition, they reported a relationship between biofilm formation and TA gene expression[9]. In 2016, Wood et al. detected the *HigBA* gene in *P. aeruginosa* PA14 isolate and investigated the biofilm formation by using crystal violet, pyocyanin production by using acetic acid, and dichloromethane and pyoverdine production by using chrome azurol S agar plate method. They indicated that the *HigBA* TA gene is effective not only on biofilm formation but also on pyoverdine production[10].

Previous studies have determined that *P. aeruginosa* isolates have *RelBE* and *HigBA* TA genes[9,13,21,22]. Guo et al. demonstrated the antitoxin *HigA* regulates virulence in *P. Aeruginosa* by binding especially to the promoter region of the *MvfR* gene that regulates pyocyanin synthesis[5]. Song et al. indicated that *HigA*-mediated transcriptional inhibition on stress stimulation could affect virulence genes and also take attention to the potential of the *HigBA* TA system as an antibacterial treatment target[6]. In 2022, Zadeh et al. determined that ciprofloxacin and colistin may induce persister cell formation by enhancing the expression of type II TA systems during stationary and exponential phases[23]. Also, it was shown that there was a strong correlation between the *mazEF* TA gene and resistance against gentamicin, meropenem, and amikacin[9].

Even though TA genes and virulence genes were considered to be associated with antibiotic resistance, we observed the level of *RelBE* gene expression was higher in imipenem- and meropenem-sensitive isolates, and the level of *HigBA* gene expression was higher in amikacin-susceptible isolates. In our previous study, we investigated the relationship between toxin genes and antibiotic resistance in a different bacterial collection consisting of 92 *P. aeruginosa* and 148 staphylococci isolates. It was found that in *P. aeruginosa*, the level of *RelBE* TA gene expression is increased in isolates sensitive to aztreonam compared to those resistant to aztreonam. Also, in staphylococci, the levels of mazEF gene expression were found to be higher in isolates sensitive to gentamicin, ciprofloxacin, levofloxacin, clindamycin, phosphomycine, nitrofurantoin, fusidic acid, and cefoxitin compared to those resistant to the above antibiotics[21]. In the present study, we observed that toxin genes were associated with antibiotic resistance, and *RelBE* TA gene expression was associated with the exoS virulence gene in *P. aeruginosa* isolates.

CONCLUSION

The fact that TA genes are expressed more in strains sensitive to carbapenems should draw attention to these strains, which may cause serious infections that are difficult to treat in the future. The relationship between *RelBE* and *ExoS* indicates that TA genes in *P. aeruginosa* isolates are not only related to antibiotic resistance but also play important roles in bacterial pathogenesis and virulence. Further studies including larger numbers of genes are necessary to illustrate the role of TA genes in the pathogenesis of *P. aeruginosa* and to elucidate their connection with antibiotic resistance. These studies should make a significant contribution to the solution of the antibiotic resistance problem.

ETHICAL APPROVAL

This study was financially supported by Tokat Gaziosmanpasa University Scientific Research and Projects Unit (project number 2015/25). This study was presented in the 4th National Clinical Microbiology Congress (Abstract Paper/Poster) (publication number: 3825136). All the protocols were performed under the supervision of the Ethics Committee of Tokat Gaziosmanpasa University (number 17/KAEK/022).

AUTHORS' CONTRIBUTIONS

USSC: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **YD:** Data curation, Methodology, and Writing – review & editing.

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ORIGINAL ARTICLE

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Diffusion-weighted imaging versus non-contrast magnetic resonance imaging in the diagnosis of acute appendicitis during pregnancy

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the diagnostic performance of diffusion-weighted imaging compared to non-contrast magnetic resonance imaging in the differential diagnosis of acute appendicitis in pregnant patients.

METHODS: A total of 72 pregnant patients with the suspicion of acute appendicitis who underwent magnetic resonance imaging combined with diffusion-weighted imaging examinations were enrolled in this retrospective study. Magnetic resonance imaging images (non-contrast and diffusion-weighted imaging sequences) were evaluated. Moreover, apparent diffusion coefficient ratios were estimated. The diagnostic performances of magnetic resonance imaging and diffusion-weighted imaging findings were statistically analyzed on the basis of surgical and follow-up results.

RESULTS: Of 72 pregnant patients, 10 (14%) had acute appendicitis on magnetic resonance imaging and diffusion-weighted imaging. Among 10 patients with acute appendicitis, three (3/10) had perforation. diffusion-weighted imaging findings had higher sensitivity (90 versus 60%), negative predictive value (98.41 versus 93.94%), and accuracy (98.61 versus 94.44%) ratios compared to non-contrast magnetic resonance imaging in the diagnosis of acute appendicitis. There was one false-negative result on diffusion-weighted imaging. Diffusion restriction facilitated the detection of appendicitis. The apparent diffusion coefficient ratios were lower in acute appendicitis than in the normal appendix (0.70±0.19 versus 0.96±0.16) ($p<0.05$).

CONCLUSION: With a shorter scan time and higher diagnostic accuracy, diffusion-weighted imaging can be useful for the early diagnosis of acute appendicitis and for planning appropriate management.

KEYWORDS: Abdomen, Acute. Appendicitis. Diffusion. Magnetic resonance imaging. Pregnant Women.

INTRODUCTION

Acute appendicitis during pregnancy is a life-threatening emergency for both the mother and the fetus. Because of pregnancy-related anatomical and physiological changes, the differential diagnosis of acute appendicitis is frequently difficult. The clinical findings can mimic other diseases that present abdominal pain. It is important to decide whether surgical management is required or not. An accurate diagnosis is necessary for early management in pregnant patients with acute abdominal pain because of maternal and fetal mortality risks[1-7].

Ultrasonography (US) is the first choice of medical imaging modality for pregnant patients[2-5]. Due to maternal anatomical changes, bowel gas, and larger patient body habitus, US examination may be insufficient for accurate diagnosis. Computed tomography (CT) is avoided in pregnant patients due to radiation risk and teratogenic and carcinogenic effects on the fetus. Alternatively, magnetic resonance imaging (MRI) is performed in conflicting situations. Several studies have reported that MRI is a problem-solving modality in pregnant patients with acute abdomen pain[1-11]. Because of gadolinium accumulation in the amniotic fluid, non-contrast sequences should be considered[10]. Diffusion-weighted imaging (DWI) needs no contrast administration and has a short scan time (approximately 2 min). DWI depicts the randomized motion of water. Hypercellular tumors, ischemia, abscess, and hemorrhage show diffusion restriction. Diffusion restriction is measured on apparent diffusion coefficient (ADC) map[12,13].

MRI may have some heating effects on the fetus due to radiofrequency pulse, especially at longer scanning time[14-18]. Optimal imaging with shorter scan times is essential in pregnant patients, especially in emergent situations. Thus, we aimed to evaluate the diagnostic performance of DWI compared to non-contrast MRI in the differential diagnosis of acute appendicitis in pregnant patients.

METHODS

Study design and setting

This is a retrospective, analytic, and cross-sectional study. The Institutional Review Board approved this retrospective

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study, and informed consent was waived. Between January 2016 and January 2019, at a single institution, MRI examinations of 78 pregnant patients with the suspicion of acute appendicitis who had in-conclusive US examinations were reviewed from the picture archiving and communications system. Pregnant patients without follow-up or histopathological results (n=4) and patients without DWI sequences (n=2) were excluded. A total of 72 pregnant patients with the suspicion of acute appendicitis who had undergone abdominal MRI combined with DWI examinations were enrolled in this retrospective study. The mean age of pregnant patients was 29±6 (SD) years (range 18–42 years).

Magnetic resonance imaging protocol

All MRI examinations were performed on a 1.5-Tesla system (Optima MR450w, GE Healthcare, Milwaukee, WI, USA). Parameters of lower abdominal MRI sequences were sagittal T2-weighted periodically rotated overlapping parallel lines with enhanced reconstruction (PROPELLER) [TR/TE: 643/90 ms, the field of view (FOV): 330 mm, image matrix: 256×256, slice thickness 5 mm], coronal fat saturated T2-weighted PROPELLER (TR/TE: 5,023/71 ms, FOV: 400 mm, image matrix: 256×256, slice thickness: 5 mm), axial T2-weighted fast relaxation fast spin echo (FR-FSE) (TR/TE: 7,773/110 ms, FOV: 430 mm, image matrix: 256×192, slice thickness 5 mm), non-contrast axial T1 spin echo (TR/TE: 744/35 ms, FOV: 430 mm, image matrix: 256×192, slice thickness 5 mm), non-contrast axial T1 3D LAVA (TR/TE: 6.6/2.1 ms, FOV: 430 mm, image matrix: 256×192, slice thickness 5 mm) sequences, and axial diffusion-weighted single-shot echo-planar imaging (TR/TE: 7,098/35 ms, NEX: 4, FOV: 430, slice thickness: 5 mm) with b values 0 and 1,000 s/mm^2.

Image evaluation

MRI and DWI examinations of patients (n=72) were reviewed by an experienced radiologist who was blinded to the clinical data of patients. There were two sets for evaluation of MRI examinations; set 1 included non-contrast conventional MRI examinations without DWI, and set 2 included only DWI without conventional MRI sequences with the calculation of ADC values. Radiological findings were divided into two groups: only non-contrast MRI and only DWI findings. The characteristics of the appendix (normal/non-visualized/appendicitis, wall thickness, and diameter), intra-abdominal free fluid, pericecal fat stranding, lymph nodes, and other abnormalities were noted. Qualitative and quantitative DWI findings were investigated.

Region of interest (ROI) measurements were performed at the appendix and paravertebral muscle in the dedicated workstation. The mean ADC value was obtained for each visible normal appendix, normal paravertebral muscle, and appendicitis. Besides, the ADC ratio (the ratio of mean ADC of normal appendix/appendicitis to mean ADC of normal paravertebral muscle) was calculated for standardization.

After two sets of image interpretation, surgical and follow-up results were noted from the hospital information system. Patients were categorized into two groups: those with and those without acute appendicitis. MRI and DWI findings were analyzed according to surgical and follow-up results. The diagnostic accuracy of MRI and DWI for acute appendicitis was estimated. Specific MRI and DWI findings were investigated.

Statistical analysis

The distribution of parameters was analyzed by the Shapiro-Wilk test. Fisher's exact test or Mann-Whitney U test was used, where appropriate, and $p<0.05$ was used to determine statistical significance. Statistical analysis was done using the MedCalc 12.1.4.0 statistical software.

RESULTS

Of 72 pregnant patients, 10 (14%) had acute appendicitis on MRI plus DWI, and 29 (40%) had normal radiological findings. The mean gestational week was 24±9 (SD). The mean follow-up period of patients was 5.7±1.7 (SD) months.

There were 10 patients (14%) with acute appendicitis. Three patients with acute appendicitis (3/10, 30%) had perforation and abscess formation (Figure 1). Acute interventional management was performed on ten patients with acute appendicitis and one with ovarian torsion. The remaining patients (n=61) were conservatively treated and underwent follow-up. Among conservatively treated patients, no surgical management was needed until the parturition. Among patients with acute appendicitis, there was one false negativity on DWI and four false negativities in the non-contrast MRI group. For diagnosis of acute appendicitis, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy ratios of non-contrast MRI were 60% (26.24–87.84), 100% (94.22–100), 100%, 93.94% (87.89–97.07), and 94.44% (86.38–98.47), respectively. Only DWI had a sensitivity of 90% (55.50–99.75), specificity of 100% (94.22–100), PPV of 100%, NPV of 98.41% (90.62–99.75), and accuracy of 98.61% (92.50–99.96). In only DWI group, higher sensitivity, negative predictive value (NPV), and accuracy ratios were obtained for the diagnosis of acute appendicitis.

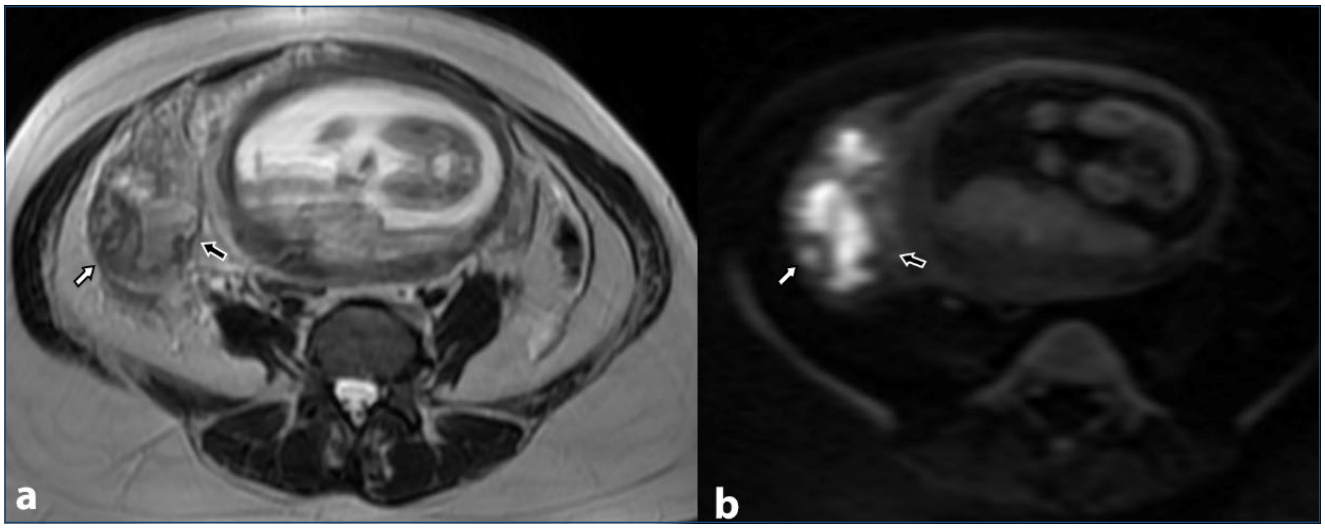

Figure 1. A 23-year-old pregnant patient in 21st week of gestation. Perforated appendicitis (white arrow) with free fluid, fat stranding, and abscess formation (black arrow) as shown on axial T2-weighted sequence (a) and on diffusion-weighted imaging (b).

Among 72 patients, seven had non-complicated acute appendicitis, three had perforated appendicitis associated with abscess, and 62 were without appendicitis.

In those patients without appendicitis, the normal appendix was shown on MRI in 33(33/62, 53%) and DWI in 14 (14/62, 22%). Non-contrast MRI sequences are more effective in the demonstration of a normal appendix. In the remaining patients, diagnosis of acute appendicitis was excluded due to non-visualization of the appendix and lack of indirect signs of acute appendicitis on MRI and DWI. Although lower mean age (27±6 versus 29±6 years old) and lower gestational week (22±8 versus 23±75 weeks) were observed in patients with acute appendicitis than those without appendicitis, there was no statistically significant difference between the two groups ($p>0.05$).

In our study, specific findings for acute appendicitis were a thick wall, increased diameter with a mean value of 9.6±2 mm (range: 8–14 mm), and pericecal fat stranding (Figure 2). Pericecal fat stranding, pericecal lymph nodes, and intra-abdominal free fluid were important clues for acute appendicitis (Table 1). Peripheral diffusion restriction was remarkable (Figure 3). Mean ADC values and ratios were significantly lower in acute appendicitis than in normal appendix ($0.99±0.29×10^{-3}$ mm^2/s and 0.70±0.19 versus $1.45±0.30×10^{-3}$ mm^2/s and 0.96±0.16) ($p<0.05$). However, anatomical details were more demonstrative on non-contrast MRI, especially on T2-weighted sequences.

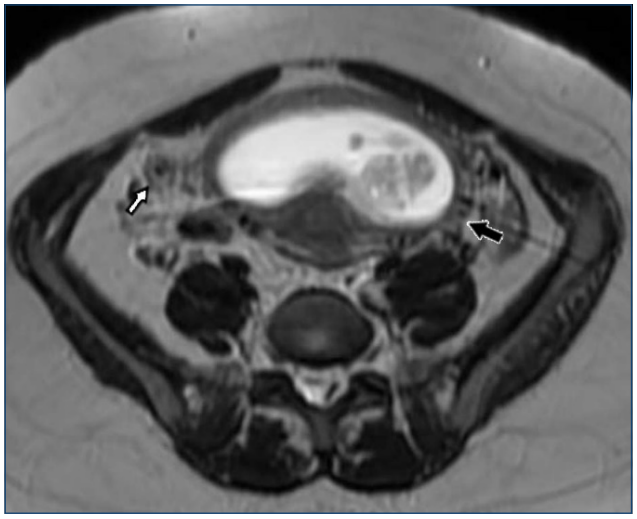

Figure 2. A 24-year-old pregnant patient in 16th week of gestation. Acute appendicitis (white arrow) with pericecal fat stranding and fetus (black arrow) are seen on axial T2-weighted sequence.

DISCUSSION

In pregnant patients with acute abdomen pain, acute appendicitis is the most common surgery-required etiology[19]. It is mostly seen in the second trimester[20]. MRI is usually performed following an inconclusive US examination. Administration of contrast agent is avoided because of transplacental passage. Sometimes, non-contrast MRI findings can be suspicious, and additional modalities can be required for accurate diagnosis. DWI has the advantages of short scanning time and no need for contrast agent[12,13].

Several studies have been conducted on the feasibility of MRI for acute appendicitis in pregnant patients. In a meta-analysis, MRI showed various sensitivity (range: 50–100%), specificity (range: 93–100%), PPV (range: 61–100%), and NPV (range: 94–100%) ratios for acute appendicitis in pregnant patients[5].

Table 1. The imaging findings of pregnant patients with acute appendicitis on magnetic resonance imaging and diffusion-weighted imaging.

Number of patients	Age	Gestational week	Diameter of appendix	Pericecal stranding*	Free fluid*	Abscess	Pericecal lymph nodes*	ADC ratio*
1	35	24	Perforated	+	+	+	+	0.40
2	24	16	8	+	-	-	+	0.85
3	27	19	Perforated	+	+	+	+	0.33
4	18	30	14	+	+	-	-	0.87
5	27	29	10	+	+	-	-	0.61
6	37	21	8	+	-	-	+	0.83
7	23	18	9	+	+	-	+	0.81
8	23	21	Perforated	+	+	+	+	0.78
9	27	31	8	+	+	-	-	0.81
10	30	6	10	+	+	-	-	0.75

*There was a statistically significant difference between patients with acute appendicitis and without appendicitis. In Fisher's exact test/Mann-Whitney U test, $p<0.05$ was used to determine statistical significance.

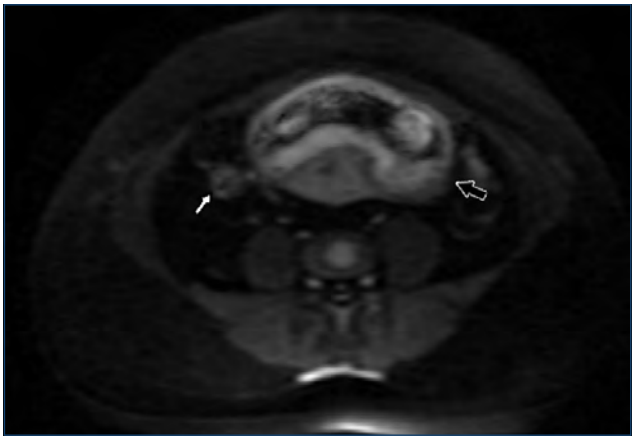

Figure 3. A 24-year-old pregnant patient in 16th week of gestation. Acute appendicitis (white arrow) with pericecal fat stranding and fetus (black arrow) are seen on diffusion-weighted imaging.

In another study, MRI showed high accuracy (88%) and specificity (92%) ratios with a sensitivity of 60% for acute appendicitis in pregnancy[17]. Tsai et al.[6] found high sensitivity (93%), specificity (95–96%), and accuracy (99.5%) ratios with one false negative (1/14) interpretation on MRI. They declared that peri-appendiceal fat stranding was an important clue. Non-visualization of the appendix and a lack of appendicitis signs can exclude acute appendicitis[6]. Similarly, none with non-visualized appendix had appendicitis in our study. Furthermore, the thick appendiceal wall and increased signal in pericecal fat were more noticeable in only DWI group with high accuracy ratios in our study.

In another study, Wi et al.[21] reported high sensitivity (100%), specificity (95%), and accuracy (96%) ratios of MRI in the diagnosis of acute appendicitis. Similar results were achieved by performing MRIs with and without DWI. They observed no significant difference in diagnostic performance with a combination of DWI[21]. Moreover, only DWI was not investigated for diagnosis of acute appendicitis.

Pedrosa et al.[18] documented that the negative laparoscopy rate was 30% in pregnant patients with suspicion of acute appendicitis. They found that the visualization of the normal appendix in patients without appendicitis was more prevalent in MRI compared to the US [87% (116/134) versus <2% (2/126)]. They recommended MRI to decrease negative laparoscopy rate[18].

In our study, we emphasized that DWI is an efficient modality for the early and accurate diagnosis of acute appendicitis in pregnant patients. Thick appendiceal wall, pericecal fat stranding, intraabdominal fluid, and peripheral diffusion restriction with a low ADC ratio were specific findings for acute appendicitis. Non-visualization of the appendix was helpful for the exclusion of appendicitis. With higher accuracy, DWI improves the notification of abnormality. Therefore, unnecessary laparoscopic procedures can be avoided.

There are some limitations to our study. First, retrospectively collected data were analyzed. Second, the sample size was small owing to the rarity of MRI with DWI examinations in pregnant patients with acute abdomen pain. Third, an experienced radiologist evaluated images into two sets. It can lead to a possible bias.

CONCLUSION

Early and accurate diagnosis of acute abdomen is important to decrease maternal and fetal mortality. In a pregnant patient with conflicting diagnosis, DWI can be useful with or without

non-contrast MRI for the diagnosis of appendicitis, with higher diagnostic accuracy and shorter scan time.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Umraniye Training and Research Hospital Institutional Clinical Research Ethics Committee (Date: 23.01.2019/No: 234).

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ORIGINAL ARTICLE

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Vitamin D deficiency in bedridden elderly people at home

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate serum 25(OH)D concentrations in the homebound elderly people and relate them to level of dementia, nutritional risk, and route of dietary administration.

METHODS: This is a cross-sectional study involving 207 bedridden elderly people assisted by the Home Care Service in the city of Santo André – SP, from June to December 2016. The following factors were evaluated: dietary intake of vitamin D, arm circumference, triceps skin fold thickness, calf circumference, nutritional risk by Mini-Nutritional Assessment, level of dementia by the adapted Clinical Dementia Rating questionnaire, and laboratory tests such as serum concentrations of 25(OH)D, ultrasensitive C-reactive protein, alkaline phosphatase, serum calcium, and parathormone.

RESULTS: The mean age of the elderly people was 81.6 (9.2) years. Deficiency of 25(OH)D was observed in 76.3% of the elderly people. There was an inverse correlation between serum concentrations of 25(OH)D: parathormone ($r=-0.418$, $p<0.001$) and alkaline phosphatase ($r=-0.188$, $p=0.006$) and a direct correlation with serum calcium ($r=-0.158$, $p=0.022$). Logistic regression showed that vitamin D deficiency was directly and independently associated with oral feeding (odds ratio 7.71; 95%CI 2.91–20.40).

CONCLUSION: Bedridden households showed high prevalence of vitamin D deficiency without association with nutritional risk and level of dementia. Oral diet was associated with vitamin D deficiency, possibly due to low consumption of source foods.

KEYWORDS: Vitamin D. Nutritional status. Micronutrient intake. Aged.

INTRODUCTION

The increase in the world population of elderly people aged 60 years and older has occurred significantly and rapidly. This population is at high risk of malnutrition and other nutritional deficiencies, such as hypovitaminosis D, due to decline in cognitive and physiological functions that compromise the consumption and metabolism of nutrients, increase in the risk of fractures, hospitalizations, and chronic diseases such as cardiovascular and neurodegenerative diseases[1].

Hypovitaminosis D is very prevalent in all age groups worldwide, including tropical countries like Brazil[2]. The elderly people are particularly at risk of this disability, since with increasing age there is a reduction in synthesis of vitamin D from exposure to sunlight through skin and absorption from foods containing vitamin D[3].

Low serum concentrations of vitamin D are also frequently reported in institutionalized elderly people. In Europe, it is estimated that serum concentrations of vitamin D below 20 ng/mL can affect from 80 to 100% of nursing home residents[4,5]. In Brazil, studies with nursing home residents or homebound people are scarce. In the southern region of Brazil, vitamin D deficiency was observed in 86.5% of the nursing home residents[6]. The Brazilian Society of Endocrinology recommends a goal of serum vitamin D [25(OH)D] above 30 ng/mL for the elderly people[7].

In this context, considering there are few studies assessing the nutritional status regarding vitamin D in representative samples of elderly people at home and there are no recommendations for prophylactic supplementation in this age group in Brazil, this study sought to assess plasma concentrations of vitamin D in bedridden elderly people and relate them to the level of dementia, nutritional status, and route of diet administration.

METHODS

This is a cross-sectional study involving 207 bedridden household elderly people, assisted by a multiprofessional team (doctors, nurses, nutritionists, physiotherapists, speech therapists,

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and psychologists), by the Home Care Service (HCS) in the city of Santo André – SP, Brazil, from June to December 2016. The service is a public assistance program that attends bedridden patients in their homes. The elderly people are admitted to the program upon spontaneous demand or by referral from the municipal hospital in the region. There are monthly visits by the multiprofessional team.

The study was approved by the Research Ethics Committee of the FMABC University Center, opinion number: 1.781.509, CAAE: 60667716.8.3001.5484.

The inclusion criteria were all bedridden elderly individuals of both genders over 60 years of age. The exclusion criteria were elderly people who were diagnosed with chronic diseases (except obesity, hypertension, diabetes, and neurodegenerative diseases) or who had acute infections or were taking antibiotics or corticosteroids at the time of the evaluation.

Of the 573 registered individuals in HCS, 185 of them were under the age of 60 years. Of the 388 eligible individuals, 25 did not consent to participate, 116 presented chronic diseases, and 40 were taking antibiotics. Thus, 207 bedridden elderly people were included.

A standardized questionnaire covering personal, economic, and health antecedents was applied to those responsible for the elderly people.

The evaluation of food intake was performed through a 24-h food recall informed by a family member or a responsible caregiver. The nutritional calculations were performed using the Avanutrionline® program and compared with the references proposed by the Institute of Medicine [Dietary Reference Intake][8].

The following anthropometric variables were gauged: arm circumference and triceps skin fold thickness, which were classified according to Burr and Phillips[9]. The calf circumference measurement was done as recommended by Guigoz et al.[10]. As the elderly people were bedridden, it was not possible to directly measure weight and height to calculate the body mass index[11].

The Mini-Nutritional Assessment (MNA) was performed as a nutritional risk screening, which is a good prognostic tool to detect malnutrition in the elderly people[12].

To evaluate the level of dementia, the Clinical Dementia Rating scale was used. This scale aims to assess cognition, behavior, and the influences of cognitive losses on the ability to adequately perform activities of daily living. The classification was made as proposed by Morris[13].

For the evaluation of laboratory tests, 15 mL of blood were collected by peripheral puncture to perform alkaline phosphatase (AP) by colorimetric kinetic method, serum calcium by Arsenazo III method, parathyroid hormone by electrochemiluminescence, ultrasensitive C-reactive protein (CRPus) by turbidimetric method, and serum calcium 25(OH)D concentrations by electrochemiluminescence. The classification based on 25(OH)D (deficiency <20 ng/mL, insufficiency 21–29 ng/mL, and sufficiency >30 ng/mL) was performed as recommended by the Endocrine Society Clinical Practice Guideline, 2011[14].

Statistical analysis

The data were analyzed using the Stata software (version 14.0). Dichotomous and qualitative variables were presented as absolute and relative frequency values and compared by the chi-square or Fischer's exact test. Continuous variables were tested using the Shapiro-Wilk test and compared by Student's t-test (parametric) or Mann-Whitney (non-parametric) U test. The independent variables such as gender, ethnicity, age group, vitamin D supplementation, MNA classification, dementia classification, CB classification, PCT classification, calf classification, CRP, alkaline phosphatase, parathormone, and calcium were analyzed according to the dependent variable, i.e., vitamin D deficiency, according to the multivariate logistic regression model being analyzed by odds ratio (OR). The significance level adopted was $p<0.05$.

RESULTS

Of the 388 eligible elderly people enrolled in the HCS during the study period, 46.6% (n=181) were excluded, thus 207 individuals participated in the study.

The mean age was 81.69 (9.24) years (range 60–103 years). The per capita income was $204.29. Regarding education, 82.6% of the sample had less than 9 years of schooling.

When evaluated in relation to vitamin D supplementation, it was observed that only 23 (11%) received supplementation with a dose of 10,000 IU/week.

None of the participants had alcoholism during the study, and 5.79% reported smoking at some point in their lives. As for the dietary intake of vitamin D, 100% of the sample had an insufficient intake according to the dietary recall. Demographic characteristics and nutritional status can be observed in Table 1.

Regarding laboratory variables, the following inadequacies were observed: 161 (77.8%) high alkaline phosphatase, 128 (61.8%) high parathormone, and 68 (32.8%) low serum calcium. Regarding 25(OH)D concentrations, it was found that 158 (76.3%), 25 (12.5%), and 24 (11.6%) of the subjects had vitamin D deficiency, insufficiency, and sufficiency, respectively. In addition, 44 patients (21.6%) had increased CRPus concentrations.

Table 1. Demographic and nutritional characteristics of the study population (n=207).

Variable		n	%
Demographic characteristics			
Gender	Female	142	68.6
	Male	65	31.4
Age (years)	60–75	48	23.2
	75–90	122	58.9
	≥90	37	17.9
Ethnicity	Caucasoid	151	72.9
	Non-Caucasoid	56	27.1
Admission diagnosis	Heart diseases	70	32.0
	Neurological diseases	64	31.0
	Diabetes	9	6.0
	Others	64	31.0
Bed is next to a window	Yes	147	71.0
	No	60	29.0
Vitamin D supplementation	Yes	23	11.0
	No	184	89.0
Feeding route	Oral	146	70.5
	Tube/ostomy	61	29.5
Dementia level	Moderate or severe dementia	149	72.0
	Mild or no dementia	58	28.0
Nutritional status			
Nutritional risk screening	Malnourished	92	44.4
	With nutritional risk	108	52.2
	Without nutritional risk	7	3.4
Arm circumference classification	Nutritional risk	82	39.6
	Eutrophics	53	25.6
	Risk for nutritional disorder	72	34.8
Triceps skin fold thickness classification	Malnourished	84	40.6
	Eutrophics	73	35.2
	Overweight	50	24.2
Calf circumference (cm)	<31	162	78.3
	≥31	45	21.7

Concentrations of 25(OH) were significantly higher in individuals using vitamin D supplementation [38.69 (35.31–45.50); p<0.001], using probes/stomies [19.43 (14.90–22.81); p<0.001], with no risk of malnutrition (MNA screening) [13.24 (11.70–17.65); p<0.001), and with moderate/severe dementia [11.9 (11.11–13.35); p=0.010]. There was no statistically significant difference between gender, age classification, ethnicity, and sunlight exposure.

Table 2 shows the comparison between the groups with and without vitamin D deficiency with respect to age and related laboratory variables.

Multivariate logistic regression with vitamin D deficiency as the dependent variable showed a direct association with oral diet administration (OR 7.71; 95%CI 2.91–20.40) and altered elevated alkaline phosphatase levels (OR 3.50; 95%CI 1.20–10.17). There was no association between ethnicity and nutritional status (Table 3).

DISCUSSION

In this study, vitamin D deficiency and insufficiency were observed in 75.2 and 11.6% of the bedridden elderly people, respectively. Oral feeding and alkaline phosphatase concentrations above the upper limit of the reference value were directly and independently associated with vitamin D deficiency.

Vitamin D deficiency is a global health problem, especially among the elderly people[15]. A study conducted in Teresina (PI), with elderly people assisted by the family health strategy, showed a prevalence of vitamin D insufficiency in 66.5% of the sample[2]. The Longitudinal Study of the Health of Elderly Brazilians (LSHE) 2020 (n=2264, mean age 62.4 years) showed prevalence of vitamin D deficiency and insufficiency of 1.7 and 16%, respectively[16].

Brazil has a geographic location that provides a good incidence of ultraviolet rays throughout the year, which allows for sunlight exposure and cutaneous synthesis of vitamin D in adequate concentrations in most seasons. The prevalence of vitamin D deficiency observed in our study was higher than that observed in other national studies with a similar population, possibly due to the higher age of the participants included, low economic condition, and being bedridden at home.

It was possible to verify a direct and independent association between vitamin D deficiency and oral feeding. It was also observed that the dietary intake of vitamin D was inadequate (less than 15 µg) in all participants. Patients using probes or stomas for food often received industrialized diets free of charge from the program. The use of industrialized enteral diets may have contributed to the better status of vitamin D. The consumption of vitamin D by oral diet in the elderly population is usually low, ranging around 100–200 IU/day[17,18].

In this study, the elderly people receiving vitamin D supplementation had higher 25(OH)D concentrations compared to those who did not. The Institute of Medicine proposes guidelines on vitamin D supplementation. The report recommends that adults up to 70 years of age consume 600 IU of vitamin D daily and those over 70 years of age consume 800 IU[19].

The multivariate analysis showed that vitamin D deficiency was not associated with nutritional risk. A randomized study of

Table 2. Comparison of age, gender, and laboratory variables between the groups with and without vitamin D deficiency of bedridden elderly people (n=207).

Variable		Vitamin D <20 ng/mL Deficiency (n=157)	Vitamin D ≥20 ng/mL Insufficiency (n=50)	p
Age	Years	81.59±9.27	82.00±9.23	0.790[a]
Gender	Female	111 (78.2%)	31 (21.8%)	0.248[c]
CRPus	mg/L	5.5 (5.5; 5.5)	5.5 (5.5; 5.5)	0.730[b]
Parathormone	pg/mL	78 (72.37; 86.63)	57.95 (47.31; 69.99)	**0.0001**[b]
Alkaline phosphatase	U/L	168.2 (152.01; 175.42)	148.35 (134.95; 165.52)	0.216[b]
Calcium	mg/dL	8.90±0.81	8.99±0.73	0.480[a]

CRPus: ultrasensitive C-reactive protein. p: level of significance of Student's t-test[a], Mann-Whitney U test[b], and chi-square test[c]. Bold value indicate statistical significance at the p<0.05 level.

Table 3. Logistic regression of variables associated with vitamin D deficiency in bedridden elderly people (n=207).

		OR	95%CI	p
Age (years)	>75	1.00	0.27–3.63	0.997
Gender	Female	2.44	0.70–8.42	0.159
Feeding route	Oral	7.71	2.91–20.40	**0.000**
Ethnicity	Non-caucasoid	2.44	0.66–9.02	0.181
Level of dementia	Moderate to severe	1.69	0.39–7.23	0.479
Calf circumference (cm)	<31	1.26	0.26–5.99	0.771
Parathormone	Altered	2.26	0.85–5.97	0.098
Alkaline phosphatase	Altered	3.50	1.20–10.17	**0.021**
Calcium	Altered	1.12	0.39–3.24	0.821

Dependent variable: Vitamin D deficiency; 95%CI: confidence interval of 95%. Bold values indicate statistical significance at the p<0.05 level.

malnourished hospitalized elderly people, contrary to what we observed, found 60% vitamin D deficiency in association with higher mortality rates. The difficulty in performing an objective assessment of the nutritional status by measuring anthropometric measurements in bedridden elderly people, such as weight and height, may explain our findings[20].

Elderly people with moderate/severe dementia showed higher concentrations of 25(OH)D compared to those with mild/no dementia. A current meta-analysis has shown additional evidence of relations between vitamin D deficiency and the risk of dementia and Alzheimer's disease. This factor may be attributed because elderly patients with moderate/severe dementia are using feeding ostomies with a partially processed diet and a higher concentration of vitamin D[21].

No statistically significant difference in vitamin D concentrations was observed among the individuals who had their beds near the windows. However, one of the ways of absorption of vitamin D is through exposure to sunlight[22], besides the fact that the study subjects were restricted to sunlight exposure due to their clinical conditions.

In this study, it was possible to observe an association between vitamin D deficiency and elevated alkaline phosphatase levels (OR 3.50; 95%CI 1.20–10.17); similar results were seen in an Italian study of 230 patients, which observed a negative correlation between insufficient levels of 25(OH)D and alkaline phosphatase (r=-0.2; p=0.0008)[23].

This study has relevant aspects, such as including very elderly participants (with average age of 81 years), bedridden households, and those with low economic status. Limitations are the cross-sectional design, lack of objective assessment of nutritional status, and assessment of vitamin D intake based on a single 24-h recall.

CONCLUSION

This study found a high prevalence of vitamin D deficiency (75.2%) and insufficiency (11.6%) in the bedridden elderly people. There was no association between the level of dementia and nutritional status. There was a direct and independent association between vitamin D deficiency with dietary route and high concentrations of alkaline phosphatase. Given the importance of vitamin D, it is important to evaluate its concentrations for proper monitoring and indication of prophylaxis or early treatment of the deficiency when necessary.

AUTHORS' CONTRIBUTIONS

NPL: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Writing – original draft. **TSA**: Conceptualization, Investigation, Methodology, Writing – original draft. **FLAF**: Data curation, Investigation, Writing – review & editing. **SH**: Data curation, Formal Analysis, Writing – review & editing. **ROSS**: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Analgesic efficacy of Intraoperative lidocaine infusion in patients undergoing thyroidectomy

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SUMMARY

OBJECTIVE: A significant proportion of patients may experience moderate pain requiring treatment in the postoperative first 24 h following thyroidectomy. The aim of this study was to investigate the evaluation of postoperative patient-reported pain from intraoperative intravenous infusion of lidocaine in patients undergoing thyroidectomy surgery.

METHODS: A total of 40 patients with American Society of Anesthesiologists physical status classifications I and II, aged 18–65 years, who were scheduled for elective thyroidectomy with the same indications under general anesthesia at the Ataturk University Medical Faculty's Ear, Nose, and Throat Clinic between November 2019 and February 2020, were divided into two equal groups as randomized and double-blind. Before induction of anesthesia, patients in the lidocaine group were given 1.5 mg/kg lidocaine IV bolus infusion during the operation and until the end of the first postoperative hour, followed by a continuous infusion of 1.5 mg/kg/h. Patients in the control group were given 0.9% isotonic solution according to the same protocol. In the postoperative period, 50 mg of dexketoprofen trometamol was administered and repeated every 12 h. Postoperative pain scores, additional analgesia, and side effects were recorded.

RESULTS: Postoperative pain scores were significantly lower in the lidocaine group (n=20) compared to the control group (n=20) at 30 min and 1st, 2nd, 4th, 8th, and 12th h postoperatively ($p < 0.05$). Additional analgesia requirements were also significantly lower in the lidocaine group than in the control group ($p<0.05$).

CONCLUSION: We recommended the use of intravenous lidocaine infusion intraoperatively in thyroidectomy surgery as it reduces pain scores.

KEYWORDS: Lidocaine. Thyroidectomy. Pain. Analgesics.

INTRODUCTION

Surgical treatment is of considerable importance in diseases of the thyroid gland, particularly thyroid gland malignancies[1]. The principal causes of post-thyroidectomy pain include skin incisions to the neck region, cervical hyperextension, damage associated with orotracheal intubation, and drains inserted into the surgical area. Studies have reported that 90% require opioids in the first 24 h after thyroid surgery[2].

The multimodal analgesia technique, in which analgesics with different effect mechanisms are combined, is becoming increasingly used in the management of postoperative pain. With this technique, analgesia dosages are reduced through the additive and synergistic effects of the analgesic agents, fewer side effects occur, and more effective analgesia is provided[3].

Lidocaine, one of the most commonly used anesthetic drugs, was first synthesized in 1942 under the name Xylocaine® and was later approved for use in Sweden in 1948[4]. Lidocaine can be used clinically in different ways and by different routes of administration (epidural, subarachnoid, intrapleural, intravenous, intramuscular, intraarticular, and topical). It is also used in central and peripheral nerve blocks, in regional intravenous anesthesia applications, in the treatment and prophylaxis of life-threatening ventricular arrhythmias, in the treatment of chronic and neuropathic pain, and recently for postoperative pain control with IV infusion[5,6].

Lidocaine has analgesic, antihyperalgesic, and anti-inflammatory properties and thus has many beneficial effects in many surgeries[7,8]. Several studies have shown that intravenous IV lidocaine use in the intraoperative period reduces postoperative pain[9-13].

The aim of this study was to investigate the effect of intraoperative IV infusion of lidocaine on pain scores in patients undergoing thyroidectomy.

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METHODS

This study was carried out after obtaining approval numbered B.30.2.ATA.0.01.00/552 from the Ethics Committee of Ataturk University Faculty of Medicine, which convened on November 07, 2019, at Ataturk University Faculty of Medicine, Department of Otorhinolaryngology. The study included patients with American Society of Anesthesiologists (ASA) physical status classifications I and II, aged 18–65 years, and who were scheduled for elective thyroidectomy surgery with the same indications under general anesthesia at the Ataturk University Medical Faculty's Ear, Nose, and Throat Clinic in Turkey between November 2019 and February 2020. All advantages and disadvantages were explained to all patients both verbally and in writing one day before surgery, after which informed consent was obtained. Patients with an ASA score of 3 and above, patients allergic to lidocaine, patients with severe hepatic and renal impairment, patients with a long history of opioid and non-opioid analgesic use, history of gastrointestinal bleeding, peptic ulcer and inflammatory patients, body weight less than 50 kg, patients who had to discontinue drug therapy for any reason during the study, did not volunteer to participate in the study, could not cooperate, and were inadequate in evaluating the postoperative pain score were excluded from the study.

The present research was planned as a prospective, randomized, placebo-controlled, double-blinded clinical study. The patients were randomly divided into two groups using a computer program at a ratio of 1:1. Thus, lidocaine group (n=20) and control group (n=20) were formed. Practitioners, patients, and postoperative pain evaluators did not know which drug was administered to the group. The volumes of preoperative, intraoperative, and postoperative infusion solutions prepared for the lidocaine group were prepared with the same volume of 0.9% isotonic solution as those for the control group to ensure a double-blind study. All patients were taken to the operating room after administration of 6 mL/kg crystalloid and crystalloid infusion (8 mL/kg/h) was continued during the surgery. All personnel in the operating room were unaware of randomization. All patients underwent standard ECG, peripheral oxygen saturation (SpO_2), and noninvasive blood pressure monitoring, and all measurements were recorded at 5-min intervals during surgery. Anesthesia in both groups was established by a specialist anesthetist, with anesthesia induction being performed with a lidocaine 1.5 mg/kg iv bolus (lidocaine group) or 0.9% isotonic solution 1.5 mg/kg iv bolus (control group), 2–3 mg/kg propofol, 0.25–1 μg/kg/min remifentanil, and 0.6 mg/kg rocuronium. Lidocaine infusion at 1.5 mg/kg/h was maintained during surgery and in the first hour in the postanesthetic care unit. Postoperative pain scores were evaluated at postoperative 30 min and 1, 2, 4, 8, 12, and 24 h. All patients were given 50 mg iv dexketoprofen 30 min before the end of the surgery and was repeated every 12 h after the surgery. An anesthetist, unaware of the drugs used for analgesia and the grouping, made the postoperative evaluation of the patients. Postoperative analgesia was assessed using the visual analog scale (VAS) (VAS 0=no pain, VAS 10=the most severe pain that can be felt). Patients with a VAS score of 4 and above were administered 1 mg/kg tramadol iv as additional analgesics and recorded. Toxicity symptoms such as tongue numbness, arrhythmia, metallic taste, tinnitus, anaphylaxis, nausea, and vomiting were recorded during the 24-h follow-up.

Thyroidectomy was performed in all patients with the same technique, the same indication, and the same surgical team.

Age (years), sex, height (cm), weight (kg), BMI (kg/m^2), VAS scores at rest (at 1, 2, 4, 8, 12, and 24 h), time of the first analgesia (min), side effects such as nausea, vomiting, arrhythmia, metallic taste, tinnitus, and anaphylaxis, total surgery time and duration of anesthesia (min), and total analgesic consumption were evaluated.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 20.0 software. Categorical variables were recorded as number and percentage, and numerical variables as mean±standard deviation. The compatibility of normal distribution for numerical variables was evaluated using the Kolmogorov-Smirnov test, while z-values calculated for skewness and kurtosis were assessed using graphs. The Kruskal-Wallis and Friedman tests were applied to non-normally distributed numerical variables, while the Bonferroni-corrected Mann-Whitney U test and Bonferroni-corrected Wilcoxon test were employed in post-hoc analyses. The χ^2 test was applied in the analysis of categorical variables. Correlations between non-normally distributed constant variables were evaluated using Spearman's rho correlation analysis. $P<0.05$ were regarded as statistically significant.

RESULTS

A total of 40 patients undergoing thyroid surgery under general anesthesia completed this study. Patients were randomly divided into two equal groups (20 patients in each group).

Demographic data such as age (years), sex, height (cm), body weight (kg), and body mass index (BMI, kg/m^2) are shown in Table 1.

Operation data of the groups (duration of surgery, duration of anesthesia, and the average gland weight) are shown in Table 2.

Time-dependent changes in VAS scores between the groups are shown in Table 3 and Figure 1. VAS scores at postoperative 30 min and 1, 2, 4, 8, and 12 h were statistically significantly lower in the lidocaine group than in the control group ($p<0.05$). No significant difference was determined between the VAS scores in two groups at 24 h postoperatively ($p=0.060$). The highest VAS scores were observed at the postoperative 30th minute in both groups, and the VAS scores decreased gradually in both groups except at the 4th hour in the lidocaine group.

Additional analgesic requirements (tramadol 1 mg/kg) were significantly lower in the lidocaine group compared to the control group ($p=0.027$). Two of the 14 cases in the control group with additional analgesic requirements also needed a second dose of tramadol. No second tramadol requirement occurred in the lidocaine group.

The additional analgesic requirement occurred within the first 4 h postoperatively in all cases with such requirements. In terms of time of first analgesic requirement, first tramadol use occurred significantly later in the lidocaine group compared to the control group ($p=0.042$). A comparison of the two groups in terms of total tramadol consumption in the first 24 h postoperatively revealed that the total analgesic requirement was significantly lower in the lidocaine group than in the control group ($p=0.019$).

No nausea, vomiting, anaphylaxis, or arrhythmia occurred as lidocaine-related side effects in any case in the lidocaine group. No cases of lidocaine toxicity were also observed. No intraoperative excessive hemorrhage, postoperative hematoma, or vocal cord paralysis occurred in any case.

DISCUSSION

Although pain occurring following thyroidectomy is not as severe as that developing after major surgery, such postoperative pain must still not be overlooked[14]. Pain severity is subjective and highly variable, and it is important to ameliorate pain. The

Table 3. Pain scores.

	Lidocaine group (n=20, Mean±SD)	Control group (n=20, Mean±SD)	p-value
VAS 30. min	3.05±0.6	4.25±1.37	0.001
VAS 1. h	2.55±0.6	3.35±0.88	0.002
VAS 2. h	2.45±0.83	3.05±0.69	0.008
VAS 4. h	2.5±0.51	2.95±0.6	0.021
VAS 8. h	2.1±0.55	2.65±0.59	0.006
VAS 12. h	1.95±0.51	2.4±0.6	0.018
VAS 24. h	1.30±0.47	1.6±0.5	0.060

n: number of patients; min: minutes; $p<0.05$: statistically significant; SD: standart deviation; VAS: visual analog scale.

Table 1. Patients demographic data (mean±std. deviation).

	Lidocaine group (n=20, Mean±SD)	Control group (n=20, Mean±SD)	p-value
Gender (F/M)	15/5	17/3	0.695
Age (year)	46.5±10.15	49.8±11	0.285
Height (cm)	164.7±6.53	163.05±5.34	0.4
Weight (kg)	75.2±7.52	73.4±7.24	0.724
BMI (kg/m^2)	27.79±3.17	27.7±3.39	0.818

n: number of patients; F: female; M: male; $p<0.05$: statistically significant; SD: standart deviation; BMI: body mass index.

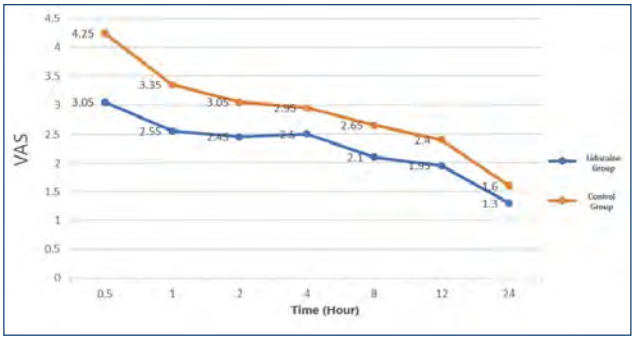

Figure 1. The relation between pain scores and time in the groups in postoperative period.

Table 2. Operation data (mean±std. deviation).

	Lidocaine group (n=20, Mean±SD)	Control group (n=20, Mean±SD)	p-value
Duration of surgery (min)	119.75±29.4	130.25±24.79	>0.05
Duration of anesthesia (min)	146±29.54	158.25±23.91	>0.05
The average gland weight (g)	43.2 ±1.361	43.05 ±1.495	>0.05
Recurrent laryngeal nerve injury	0	0	

n: number of patients; min: minutes; $p<0.05$: statistically significant; g: gram.

aim of postoperative pain control is to reduce the pain felt by the patient, and various studies have been performed for the reduction of post-thyroidectomy pain[2,10,15,16].

The principal causes of post-thyroidectomy pain include the skin incision to the neck region, cervical hyperextension, damage associated with orotracheal intubation, and drains inserted in the surgical site[2].

"Multimodal analgesia" methods, in which more than one drug and technique are combined, have begun being employed in the management of postoperative pain. Analgesia dosages are thus restricted by making use of the additive and synergistic effects of the analgesic agents, and more effective analgesia is provided with fewer side effects[3,17]. The present study investigated the effects of iv lidocaine administered intraoperatively and in the postoperative first hour on postoperative pain and analgesic consumption in patients undergoing thyroidectomy.

Choi et al. investigated the clinical effect of perioperative lidocaine infusion by dividing 56 patients scheduled for thyroidectomy into two groups. The patients in the lidocaine group received 1.5 mg/kg lidocaine iv bolus infusion immediately prior to surgery, followed by continuous 2 mg/kg/h lidocaine infusion. The patients in the control group received saline solution by using the same method. Postoperative VAS scores were significantly lower in the first 4 h after surgery in the lidocaine group compared to the control group. The highest VAS scores in both groups were registered in the postoperative care unit, decreasing in a time-dependent manner in the lidocaine group with the exception of the 12th postoperative hour. Total fentanil consumption and the total number of analgesia requirements were significantly higher in the control group than in the lidocaine group[11]. Similarly, in the present study, the highest VAS scores in both groups were observed in the postoperative care unit. VAS scores in the lidocaine group were significantly lower than those in the control group, with the exception of the postoperative 24th hour.

Statistically significantly lower VAS scores have been reported in patients receiving lidocaine infusion compared to opioid analgesia in various types of surgery[12,18]. Terkawi et al. showed that intraoperative lidocaine infusion in major abdominal surgery reduced postoperative pain and accelerated the return of bowel functions[19]. Wang et al. investigated the effects of postoperative pain of lidocaine infusion in gynecological surgeries, applying 2 mg/kg maintenance following a 1.5 mg/kg bolus dose, and observed significantly lower VAS scores with lidocaine infusion[20].

McCarthy et al. published a meta-analysis consisting of 16 studies comparing iv lidocaine infusion with placebo in abdominal surgery, cardiac surgery, orthopedic surgery, and tonsillectomy cases. Decreases were determined in intraoperative anesthetic requirements, postoperative pain, and additional analgesia requirements in the groups receiving iv lidocaine infusion in the abdominal surgery cases. In contrast, no postoperative analgesic efficacy of lidocaine infusion was observed in the tonsillectomy, total knee arthroplasty, and coronary bypass surgery cases[13].

Additionally, in a study of patients undergoing hysterectomy, De Oliveira et al. reported no significant difference between the patients in the lidocaine group (2 mg/kg/h lidocaine) and the control group (0.9% saline solution) in terms of pain severity of additional morphine consumption[21].

The inconsistencies between these findings in the previous literature and the present study may be due to variations in the dosages of lidocaine infused or to lidocaine being administered at different times. In addition, different types of surgery and different areas capable of affecting peripheral and central sensitization patterns may also have resulted in these discrepancies. Another explanation for these inconsistencies may involve individual pain threshold variations and the different responses to analgesic drugs of different patient groups.

Thyroid surgery generally involves a high incidence of postoperative nausea and vomiting. The mechanisms involved in postoperative nausea and vomiting after thyroidectomy are still unclear, although they may be associated with surgical inflammatory responses caused by surgical injury to the structures of the neck and the stimulation of vagal afferents[22]. In the present study, the incidence of nausea and vomiting in the lidocaine group was significantly lower than in the control group. In one meta-analysis, Marret et al. evaluated 170 patients from five different randomized controlled studies and reported 20% less nausea and vomiting in patients receiving lidocaine infusions compared to the control (saline) groups[23].

Some previous studies have avoided administering non-steroid anti-inflammatory agents in the postoperative period since these increase the risk of postoperative hemorrhage and hematoma[15,24,25]. Despite our use of dexketoprofen as an analgesic agent in the postoperative period in the present study, no postoperative hemorrhage or hematoma developed in any patient.

Examination of the data for patients receiving a 1.5 mg/kg bolus dose followed by a 1.5 mg/kg/h lidocaine infusion in this study revealed no toxicity findings in any case. There are a number of limitations to the present study. The most important limitation of our study was the small sample size. The fact that plasma lidocaine levels were not investigated may be a limitation of this research. However, the lidocaine dosage applied in this study was lower than that in previous studies in which

toxic lidocaine levels were not observed and in which no side effects were reported. In addition, patients were followed up until the postoperative 24th hour. A longer monitoring period might have revealed the duration of the decreases observed in VAS scores.

CONCLUSION

We conclude that intraoperative IV lidocaine infusion is an effective alternative method that can be used in thyroidectomy operations as a component of multimodal analgesia.

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AUTHORS' CONTRIBUTIONS

EA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MSG:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AK:** Data curation, Formal Analysis, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **AS:** Conceptualization, Data curation, Formal Analysis, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **IA:** Data curation, Formal Analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Is the length of time between endometrial scratching and embryo transfer important for pregnancy success? An observational study

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SUMMARY

OBJECTIVE: This study sought to evaluate the influence of time (early <90 days and late >90 days) and endometrial injury on pregnancy success.

METHODS: This is a retrospective study in which all infertile women who underwent at least one in vitro fertilization cycle at Clinica Gera between 2010 and 2015 were considered for inclusion. We included patients with a normal ovarian reserve and regular menses at intervals of up to 30 days. A total of 315 patient files were reviewed, and the study group was composed of patients who faced fertility issues and had male-caused infertility or idiopathic infertility. Also, women with male or unknown cause of infertility who have performed endometrial biopsy and have undergone embryo transfer up to 180 days after this procedure between 2010 and 2015 were included. The patients were divided into two groups according to the interval between biopsy and embryo transfer: group 1 (early—an interval of <90 days) and group 2 (late—an interval of >90 days and up to 180 days).

RESULTS: The results were superior for the group with an interval of less than 90 days relative to the group with an interval of more than 90 days ($p<0.04$). The pregnancy rates for group 1 and group 2 were 58.5% and 43.4%, respectively. The odds ratio for pregnancy success was 1.63 (95% confidence interval: 1.04 to 2.55).

CONCLUSION: The early transfer of embryos (<90 days) may produce better results with a high rate of pregnancy. Further studies are necessary to identify the mechanism involved in this phenomenon.

KEYWORDS: Endometrial cycle. Embryo transfer. Pregnancy rate. Pregnancy outcome. In vitro fertilization.

INTRODUCTION

Embryo implantation is a process that involves the apposition and adhesion of a blastocyst to the endometrium, followed by trophoblast invasion into endometrial epithelial cells. Such events occur in a receptive endometrium that has been stimulated by the ovarian steroids estrogen and progesterone[1]. Embryo implantation, which is an important requirement for a successful pregnancy, can only occur in a receptive uterus. In humans, the uterus becomes favorable to embryo implantation between days 19 and 23 of the menstrual cycle, a period known as the implantation window[2]. At present, implantation is the critical step that limits the success of in vitro fertilization (IVF) techniques[3].

In 2003, Ejzenberg et al.[4] explored the possibility that local injury to the endometrium may increase implantation rates and, therefore, improve pregnancy success. A total of 134 "good responder" patients were studied, 45 of whom underwent repeated endometrial biopsies before undergoing an IVF cycle. The pregnancy rate was approximately two times higher in the endometrial biopsy group than that in the control group, indicating that local damage induced by the biopsy may have beneficially affected the outcome of the IVF cycle; however, the mechanisms involved in this effect are unclear[4].

Narvekar et al.[5] suggested that biopsy during the cycle preceding an IVF cycle was more effective than biopsy during the conventional fertilization cycle. In addition, Gnainsky et al.[6] suggested that endometrial biopsy may promote inflammatory responses that attract pro-inflammatory cytokines, which are important to the implantation process. In particular, these substances cause the endometrial epithelium to produce molecules that favor interactions with blastocyst apposition and improve adhesion to the uterine wall[2]. This phenomenon may partially explain the effect of endometrial injury on pregnancy success. However, this effect may be temporary. Therefore, the delay on the embryo transfer might influence the results of pregnancy outcome.

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In investigations after an unsuccessful IVF cycle, for cases involving a good-quality embryo, evaluation of the endometrium[7] is performed prior to the subsequent embryo transfer, which is dependent on the endometrial biopsy results, laboratory conditions, and patients' desires. Embryos remain cryopreserved while scheduling is determined, and the duration of the interval between endometrial biopsy and embryo transfer may vary greatly in some cases. Consequently, the frozen and thaw process might influence the oocyte quality. However, the data of the length between endometrial injury and embryo transfer may affect the pregnancy rate. The aim of this study was to assess whether the length of frozen embryo may interfere with pregnancy success in assisted human reproduction after endometrial injury.

METHODS

Study design and setting

The study was retrospective observational. All infertile women who underwent at least one cycle at Clinica Gera located in the city of São Paulo, Brazil, between 2010 and 2015 were considered for inclusion. In addition, the Disciplina de Ginecologia do Departamento de Obstetrícia e Ginecologia of the Faculdade de Medicina da Universidade de São Paulo analyzed study data and validated the data of medical chart. A total of 455 patient files were initially reviewed, and the study group was composed of patients who faced fertility issues and had male-caused infertility or idiopathic infertility. The research ethics committee of IRB of Medical School USP approved this study (number 070/14, dated: April 2, 2014).

Participants

Eligibility criteria

Women with regular menses at intervals of up to 30 days with male or unknown cause of infertility who have performed endometrial biopsy and have undergone embryo transfer up to 180 days after this procedure between 2010 and 2015 were included. We excluded patients with an abnormal ovarian reserve (follicle-stimulating hormone [FSH] >12, estradiol >80 pg/mL, and less than eight antral follicles throughout the 3-day cycle of pelvic ultrasound exposure), diabetes mellitus, systemic arterial hypertension, ovarian failure, the chronic use of any medicine, any type of endocrinopathy, rheumatologic disease, chronic anovulation, or other conditions that may interfere with the endometrium, such as a sexually transmitted disease. After biopsy, the women with endometritis or functional micropolyps were excluded.

Procedures (data and sources)

All patients underwent a physical examination and routine laboratory tests to exclude female causes of infertility after failure of an IVF cycle or ovarian stimulation. Endometrial biopsy was conducted in a superior-to-inferior direction; a silicone urethral catheter (#8) coupled to a 10-mL syringe was used to create a vacuum in the entire endometrial cavity. Prior to the performance of any endometrial biopsies or sampling, a diagnostic hysteroscopy was used to examine the patient's uterine cavity, with saline solution as the distension medium and no anesthesia. Patients with endometrial polyps, submucosal myomas, or synechiae revealed by hysteroscopy were not included in the final analysis.

Samples were fixed with 4% formaldehyde in Tris-buffered saline for 24 h and then dehydrated with serially increasing concentrations of graded ethyl alcohol (EtOH) (30, 50, 70, 80, and 90%). Subsequently, they were diluted in TBS and finally in 100% EtOH. EtOH was replaced with isopropyl alcohol before samples were embedded in paraffin wax and mounted. The paraffin block was cut into thin, 5-µm-thick sections using a sledge microtome (Leica Microsystems, Wetzlar, Germany)[7]. Two independent pathologists received only histological sections of the endometrium and were blinded to patient information. Patients with endometritis or functional micropolyps were not included.

Embryo quality

Good-quality embryos with 4 to 8 cells, morulae, or blastocysts were washed twice in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered HTF medium supplemented with 10% SSS. These embryos were placed in an equilibration solution (1 mL) containing 20% (v/v) ethylene glycol (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 24% (w/v) Ficoll 70 (Pharmacia Biotech, Uppsala, Sweden), and 0.4 mol/L trehalose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) for approximately 3 min at room temperature (25°C) under a dissecting microscope. After equilibration, embryos were placed into a vitrification solution (1 mL) containing 40% (v/v) ethylene glycol, 18% (w/v) Ficoll 70, and 0.3 mol/L trehalose for30 s at room temperature (25°C). Embryos were then placed into a 0.25 mL plastic straw (IMVTechnologies, L'Aigle, Basse-Normandie, France) that was loaded with the vitrification solution using a fine pipette, and the end of the

straw was heat sealed. The straw was positioned vertically in liquid nitrogen vapor for 30 s and was then plunged into the liquid nitrogen.

The straw was taken out of the liquid nitrogen, remained in air for 10 s, and was then immersed into a 37°C water bath for 10 s. After the sealed end of the straw was cut off, embryos were expelled into a warming solution composed of HEPES-buffered HTF medium (1 mL) containing 5% SSS and 1.0 mol/L trehalose. The embryos were kept on a heated plate at 37°C for approximately 5 min under a dissecting microscope. The cryoprotectant was removed by placing embryos for 2 min each in serial dilutions of trehalose (0.5, 0.25,0.125, 0.0625, and 0 M) in HEPES-buffered HTF medium containing 5% SSS that were on a heated plate at 37°C. The embryos were then washed and incubated at 37°C in an atmosphere of 5% O_2, 5% CO_2, and 95% N_2 until they were transferred in Global medium.

An inverted microscope at 400× magnification was used to examine embryos 1–2 h after warming, and degrees of damage were calculated. The levels used to classify embryo damage were 0, 1–25, 26–50, and >50%. We selected embryos with <26% damage. Selected embryos were cultured for an additional 12–18 h. Embryos with equal blastomeres and no detectable fragmentation on the day of embryo transfer were referred to as good-quality embryos. For blastocyst-stage embryos, good quality was characterized by the presence of many tightly packed cells in the inner cell mass. We transferred two embryos per patient.

After embryo transfer, patients were monitored. A urine β-hCG test was performed 14 days after embryo transfer, and clinical pregnancy was verified when a gestational sac was detected via pelvic ultrasound. Both of these parameters were used to confirm pregnancy success.

Groups

After eligibility criteria were applied, 315 patients were divided into two groups according to the interval between biopsy and embryo transfer: group 1 (early, n=134—an interval of <90 days) and group 2 (late, n=181—an interval of >90 days and up to 180 days).

Variables

The main variable included the pregnancy success rate after embryo transfer in two moments: early (<90 days) and late (90–180 days). Also, we analyzed the other variables such as age (years), body mass index (BMI), type of assisted reproductive technology (ART), and endometrial preparation through the clinical chart in the medical records of Clinica Gera.

Bias

This study is retrospective based on the medical records. Also, we did not include a group without endometrial scratching. Other bias was the lack of live pregnancy.

Statistical analysis

A power analysis was performed between the early and late groups with 240 patients based on pregnancy success rates and found a difference of 50 and 30% between the groups (α=0.40). For each group with 80% power (1-β), there was a minimum of 120 females per group. Parameters were evaluated using χ^2 tests, and Pearson's coefficients (r) were calculated to determine correlations; Student's t-test was also used for statistical analysis. We analyzed the time between endometrial biopsy and embryo transfer. We considered the assessed outcome (pregnancy success or failure). We also used multilevel multivariate regression analysis to evaluate the confounding effects of various variables, such as age, BMI, type of ART, and endometrial preparation, on the results.

RESULTS

Participants

A flowchart of the study patients is shown in Figure 1. Initially, 450 patients were included. Later, 135 patients who did not meet the inclusion criteria were excluded: patients with endometritis or functional micropolyps (n=40), systematic arterial hypertension (n=33), psychotropic drugs (n=28), hyperprolactinemia (n=12), systemic erythematosus lupus (n=9), thyroid dysfunction (n=8), and diabetes mellitus (n=5). The final number of patients (315 women) was divided into two groups as follows: (1) early group (n=134) and (2) late group (n=181).

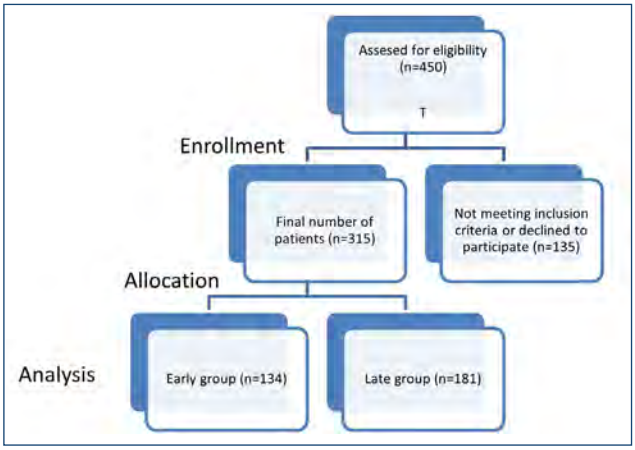

Figure 1. Flowchart of the study.

The clinical characteristics of the patients are summarized in Table 1. The two groups were similar with respect to age, BMI, type of ART, and endometrial preparation.

Main results

Data regarding pregnancy success rates in the two groups are presented in Figure 2. The overall positive pregnancy rate regardless of the interval between biopsy and embryo transfer was 49.5% through positive pregnancy test. When the two groups were analyzed independently, it became evident that pregnancy rate was influenced by the number of days between endometrial scratching and embryo transfer.

The mean number of oocytes retrieved was 6.85±5.41 and 7.02±4.52 in early and late groups, respectively (p=0.57). The mean number of produced embryos available was 4.25±0.78 and 3.92±0.94 in early and late groups, respectively (p=0.35). The number of embryos transferred was fixed in two for each group.

Superior results were obtained for the group with an interval between biopsy and embryo transfer of less than 90 days relative to the group with an interval of more than 90 days (p=0.04). Positive pregnancy test rates of 58.5 and 43.4% were observed in late and early groups, respectively. The odds ratio for positive test and clinical pregnancy success was 1.63 (95% confidence interval: 1.04 to 2.55) and 2.48 (95% confidence interval: 1.46–3.50), respectively. The number of patients with clinical pregnancy for early and late groups was 52 (134) and 48 (181), respectively.

Other analyses

The results of the multivariate regression analysis indicated that age, BMI, type of ART, and endometrial preparation did not significantly influence the study results.

DISCUSSION

Key results

The best time for embryo transfer is a dilemma in the reproductive studies, but the endometrial scratching is considered to enhance the reproductive outcomes of embryo implantation[8]. In fact, our main result was that the pregnancy rate when analyzed for the group with an interval between biopsy and embryo transfer of less than 90 days was significantly higher compared to the group with an interval of more than 90 days. This finding has clinical application and relevance for deciding the best moment for embryo transfer. Also, the influence of clinical characteristics such as age, BMI, type of ART, and endometrial preparation on the results was similar between the groups analyzed.

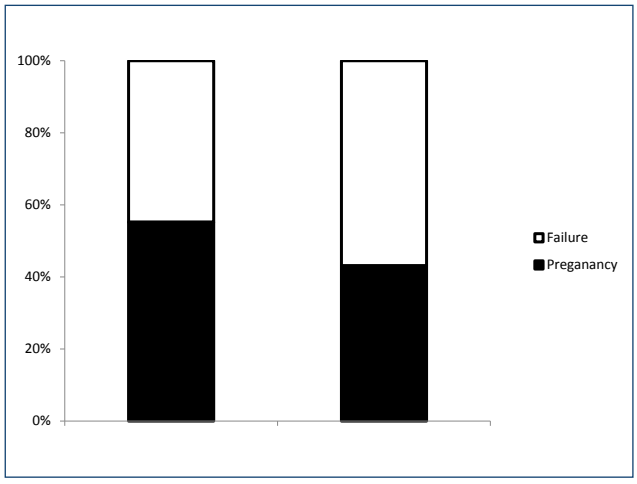

Figure 2. Evaluations of pregnancy success for the two groups: group 1 (n=134) (early—<90 days and group 2 (n=181) (late—>90 days and up to 180 days). p=0.04. The χ^2 test was applied.

Table 1. Clinical features of patients in the two groups.

Endometrial biopsy	<90 days	>90 days and up to 180 days	P
Number of patients	134	181	
Clinical aspects			
Age (years)	36.9±0.1	37.1±0.9	ns
BMI (years)	23.4±0.8	23.2±0.5	ns
Type of ART, n (%)			
ICSI	134 (100)	181 (100)	ns
Type of endometrial preparation, n (%)			
Natural cycle	22 (16.4)	27 (14.9)	ns
Progesterone supplementation	112 (83.6)	154 (85.1)	
Pregnancy rate, %	58.5	43.4	0.04

BMI: body mass index (km/m^2); ART: assisted reproductive technology; ICSI: intracytoplasmic sperm injection; ns: nonsignificant. Student's t-test was applied for age and BMI variables and the other variables were analyzed by χ^2 test.

Interpretation

Our results may enforce this idea by indicating that early embryo transfer (<90 days) is better than late embryo transfer for pregnancy success. Amaral et al.[9] reviewed the influencing factors of pregnancy loss and survival probability of clinical pregnancies through ART and some factors such as maternal age, controlled ovarian hyperstimulation protocol, cycle type, and serum hCG level 14 days after transfer. Bashiri et al.[10], who reviewed on recurrent implantation failure (RIF), suggested a new initial step in approach to patients with RIF, as in this study. A 2022 Cochrane review[11] calls for more trials, suggesting that there is only moderate-quality evidence that endometrial injury done between day 7 of the previous cycle and day 7 of the embryo transfer cycle can lead to increased clinical pregnancy and live birth rates in women with previous embryo transfer[12,13].

Our results of clinical characteristics in endometrial biopsy between the groups less than 90 days and the time between 90 and 180 days did not show differences between the groups with the characteristics of mother's age, BMI, type of endometrial preparation, natural cycle, and progesterone supplementation. Our results are not in agreement with some clinical characteristics in the literature, such as maternal age, that the older the mother, the lower the pregnancy success rate and the higher the BMI, and hence the lower the pregnancy success rate[9,14]. Another study concluded that infertility duration, endometrial thickness, and number of embryos transferred might affect the live birth rate after frozen embryo transfer among young women[15].

Certain investigators have hypothesized that the expression of inflammatory genes after mechanical damage may be responsible for the observed increase in endometrial receptivity, which is a key factor regulating blastocyst implantation[6-9]. In fact, mechanical trauma to the endometrium alters gene expression and the local immune system (via monocyte recruitment), enhances the secretion of growth factors, and makes the endometrium more receptive to implantation[6]. However, other investigators have concerns regarding these effects due to certain divergent results[10]. A possible explanation may be that the aforementioned process is time dependent and transient. In fact, it is not only time of frozen oocyte as important factor, but the endometrial preparation may be other that influences the final results.

Nastri et al.[13] evaluated the effectiveness and safety of endometrial injury prior to embryo transfer in women undergoing treatment with ART. These authors included 591 patients from 5 different trials and concluded that endometrial injury prior to the embryo transfer cycle improves clinical pregnancy and live birth rates in women undergoing ART but that inflicting endometrial injury on the day of oocyte retrieval is not advised since that approach appears to significantly reduce clinical and ongoing pregnancy rates. However, Potdar et al.[8] and Nastri et al.[13] did not describe how type of endometrial injury or the length of time between endometrial injury and embryo transfer may influence blastocyst implantation. We believe that endometrial biopsy is the preferred approach because a biopsy helps identify certain microscopic endometrial causes of infertility, such as chronic endometritis (CE), which is a condition involving the breakdown of the peaceful coexistence between microorganisms and the host immune system in the endometrium[16,17]. Unfortunately, in most cases, CE produces no noticeable signs or only mild symptoms. Therefore, this entity may be neglected by gynecologists and pathologists due to its mild clinical manifestations and the time-consuming microscopic examinations necessary for its diagnosis. Based on diagnostic criteria for CE, the prevalence of this condition is approximately 11.1% in the general population[18], and it is highly prevalent among infertile women[14-18].

Generalizability

Although two independent pathologists examined our biopsy samples for endometrial quality, this procedure was not a primary outcome of our study. Also two independent embryologists analyzed the quality of embryo after the frozen procedures.

Limitations

Our study design was retrospective and observational. In addition, our study is neither prospective nor randomized, and these aspects of our investigation may have influenced our results. Also, the question is about the influence of endometrial quality on the results was not possible with our protocol.

CONCLUSION

The early transfer of embryos (<90 days) may produce better results with a high rate of pregnancy. Further studies are necessary to prove that the length of time between endometrial injury and embryo transfer has a critical influence on pregnancy success and to identify the mechanism involved in this effect.

AUTHORS' CONTRIBUTIONS

JU: Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **RMS:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **DE:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing

– review & editing. **FMHC:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **ECAV:** Writing – original draft, Writing – review & editing. **JMSJ:** Data curation, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **ECB:** Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of candesartan treatment on echocardiographic indices of cardiac remodeling in post-myocardial infarction patients

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SUMMARY

Objective: Myocardial infarction has unfavorable effect on structural and functional properties of the myocardium, referred to as cardiac remodeling. Left ventricular mass, left ventricular mass index, and relative wall thickness are important predictors of cardiac remodeling. In this study, we investigated the effect of candesartan treatment in comparison with zofenopril treatment on echocardiographic indices of cardiac remodeling in post myocardial infarction patients.

Material and Methods: In this prospective study, patients who underwent successful percutaneous coronary intervention were randomly assigned to a candesartan or zofenopril treatment. After randomization, echocardiographic indices of cardiac remodeling including left ventricular mass, left ventricular mass index, and relative wall thickness were evaluated before the start of treatment along with 1- and 6-month follow-ups.

Results: According to our study, candesartan group showed significant reduction of estimated left ventricular mass and left ventricular mass index at 6-month follow-up visit compared to baseline values (199.53±38.51 g vs. 212.69±40.82 g; 99.05 g/m^2 (90.00–116.5) vs. 106.0 g/m^2 (96.0~123.00), p<0.05, respectively). This trend was also observed in zofenopril group during the 6-month period (201.22±40.07 g vs. 207.52±41.61 g; 101.0 g/m^2 (92.25–111.75.0) vs. 104.50 g/m^2 (95.0~116.75), p<0.05, respectively). Although both classes of drugs had favorable effects on post-myocardial infarction cardiac remodeling, the absolute benefit was more prominent in candesartan group as compared to zofenopril group (p<0.05).

Conclusion: Our results suggest that candesartan treatment following myocardial infarction may potentially be useful in terms of improving post-myocardial infarction cardiac remodeling.

Keywords: Myocardial infarction. Cardiac remodeling. Candesartan treatment.

INTRODUCTION

Myocardial infarction (MI) has unfavorable effect on structural and functional properties of the myocardium, referred to as cardiac remodeling. This pathological condition is associated with deterioration in ventricular performance and adverse cardiac events, including heart failure and ventricular arrhythmias[1]. Despite significant improvements in coronary interventions, coronary care, and novel medical therapies, patients presenting MI still develop ventricular dysfunction in the chronic stage of the disease as a result of cardiac remodeling[2].

Left ventricular (LV) mass, LV mass index (LVMI), and relative wall thickness (RWT) are important predictors of cardiac remodeling and are associated with cardiovascular morbidity and mortality. It has been observed that these geometrical indices may provide considerable benefits for assessment of different patterns of cardiac remodeling, such as concentric remodeling, concentric hypertrophy, and eccentric LV hypertrophy[3,4].

According to previous studies, angiotensin II type 1 receptor blockers (ARBs) may reverse cardiac hypertrophy and structural remodeling and reduce the risk of ventricular arrhythmias in patients with prior history of cardiac injury[5-7]. However, their effects on LV mass, LVMI, and RWT are not well known. In this study, we investigated the effect of candesartan treatment in comparison with zofenopril, an inhibitor of the angiotensin-converting enzyme (ACE), on LV mass, LVMI, and RWT in post-MI patients.

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METHODS

Study design and population

In this prospective study, patients aged ≥18 years presenting with acute MI who underwent successful percutaneous coronary intervention (PCI) between January 2018 and January 2020 were recruited. Diagnosis of acute MI was defined based on criteria by the European Society of Cardiology[8]. Patients with prior history of coronary artery disease, end-stage renal disease, liver failure, coagulopathy, cardiogenic shock, and pregnancy were excluded from the study. Patients taking an ACE inhibitor or ARB at presentation or patients intolerant to ACE inhibitor or ARB treatment were also excluded. After successful PCI, patients were randomly assigned to a candesartan group or zofenopril group by using a sealed envelope system. The candesartan or zofenopril therapy started within 24 h following hospital admission. The initial doses of candesartan and zofenopril therapies were 4 and 7.5 mg, respectively. According to our study protocol, the initially given doses of candesartan and zofenopril were doubled every 2 weeks up to maximum doses of 32 and 60 mg, respectively. Whenever a sign of drug intolerance was observed, the dose was decreased to the previous level at which the subject was confirmed to be free of drug-related symptoms. After randomization, all subjects were evaluated before the start of treatment along with 1- and 6-month follow-ups. Patients' demographics, medical history, anthropometric measurements, medications, and electrocardiographic and echocardiographic measurements were recorded. Informed consent was obtained from all patients in accordance with the ethical guidelines of the 1975 Declaration of Helsinki protocol and approved by the Ethics Committee of Konya Selçuk University (approval number: 2019/138, date: May 22, 2019).

Coronary angiograms

PCI procedures were performed through the femoral or radial artery using 6 or 7 Fr sheaths. All patients were treated with dual antiplatelet therapy including aspirin (162–325 mg) and clopidogrel (300 mg for patients <75 years of age and 75 mg for patients >75 years of age) loading dose or ticagrelor (180 mg) loading dose prior to the procedure. Aspirin was continued indefinitely, and clopidogrel or ticagrelor was recommended for 12 months. Other medications, including beta-blockers, nitrates, and statins, were prescribed according to standardized protocols. Intravenous heparin was administered to achieve an activated clotting time of 300 s. Adjunctive pharmacotherapies, the type of stent, and the use of predilatation and postdilatation were at the discretion of the interventional cardiologist. Epicardial coronary blood flow was quantified visually using the Thrombolysis in Myocardial Infarction (TIMI) flow grade classification[9]. Procedural success was defined as residual stenosis <20% and TIMI flow grade 3.

Echocardiographic evaluation

During the echocardiographic examination, parasternal long-axis, short-axis, and apical four-chamber and two-chamber images were obtained and evaluated using M-mode, two-dimensional (2D), continuous-wave Doppler, pulse-wave Doppler, and tissue Doppler methods according to the American Echocardiography Society criteria[10]. M-mode and standard 2D echocardiographic evaluation were performed on all patients with transthoracic echocardiography using Vivid S5 (GE Healthcare, Horten, Norway) 1–3 MHz transducer. All measurements were performed by a cardiologist who was blind to the patient data and study protocol and verified by a second physician to avoid error in measurements. In our study, the Devereux equation, i.e., LV mass=0.8´[1.04´(interventricular septal thickness+LV end-diastolic diameter+posterior wall thickness)^3–(LV end-diastolic diameter)^3]+0.6 (g), was used to calculate LV mass[11]. LVMI was calculated by dividing an individual's LV mass by body surface area (body weight$^{0.425}$´height$^{0.725}$´0.007184)[12]. RWT was also calculated by using the following formula: 2´(posterior wall thickness/LV end-diastolic diameter).

Statistical analysis

Data were analyzed using the SPSS version 21.0 software for Windows (IBM SPSS Statistics for Windows, version 21.0.; IBM Corp., Armonk, NY, USA). In this study, data are expressed as mean±SD and median (interquartile ranges at the 25–75th percentiles, IQR) for continuous variables and as counts and percentages for categorical variables. The Kolmogorov-Smirnov test and Shapiro-Wilk test were used to evaluate the distribution of continuous variables. The χ^2 test and Fisher's exact test were used to analyze categorical variables. Student's t-test was used for continuous variables with normal distribution and the values were presented as mean±SD. A comparison of intergroup continuous variables without normal distribution was analyzed using Mann-Whitney U test. In all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Initially, 246 patients were invited to participate in the study, of whom 217 gave their consent. Notably, 17 participants were excluded from the study due to treatment discontinuation. Therefore, 200 patients were finally included in the study. All patients were to be randomized in a 1:1 ratio to candesartan or zofenopril treatment. The baseline demographic and clinical characteristics of the study population are summarized in Table 1.

Table 1. Comparison of the baseline clinical characteristics and laboratory parameters of the groups.

Variable	Zofenopril (n=100)	Candesartan (n=100)	p-value
Age, years	56.74±10.58	59.08±12.38	0.153
Male gender, n %	84	77	0.212
Body mass index (kg/m^2)	27.83±3.35	28.60±4.32	0.162
Hypertension, n %	36	48	0.086
Smoking, n %	62	59	0.664
STEMI, n %	47	46	0.887
Systolic blood pressure (mmHg)	119.35±13.88	122.80±21.09	0.173
Diastolic blood pressure (mmHg)	73.25±9.62	74.80±13.16	0.343
Medicine			
Acetylsalicylic acid, n %	100	100	
ADP receptor antagonists, n %	100	100	
Beta-blocker (metoprolol), n %	100	100	
Metoprolol dose (mg)	55.0±21.61	57.75±18.69	0.337
Mineralocorticoid receptor antagonists, n %	7	8	0.788
Statins (atorvastatin/rosuvastatin)	29/71	86/14	0.000
Zofenopril dose (mg)	32.21±7.87		
Candesartan dose (mg)		14.28±5.53	
Blood parameters			
Hb, g/dL	15.06±1.59	14.49±1.93	0.023
WBC, $10^3/\mu L$	11.40±3.39	11.26±4.80	0.823
Platelet, $10^3/\mu L$	260.52±89.76	259.76±73.13	0.948
Glucose, (mg/dL)	144.47±70.42	158.09±79.82	0.202
e-GFR (mL/min/1.73 m^2)	91.42±23.13	89.52±22.00	0.552
Creatinine (mg/dL)	0.83 (0.74–1.03)	0.89 (0.73–1.02)	0.984
Total cholesterol (mg/dL)	194.60±50.32	194.01±50.53	0.934
HDL-C (mg/dL)	39.67±10.26	39.08±10.30	0.685
LDL-C (mg/dL)	120.43±43.11	123.28±42.50	0.638
Triglyceride (mg/dL)	145.00 (105.25–224.75)	148.0 (91.50–203.5)	0.475
Echo parameters			
LVEF (%)	50.12±8.87	48.16±8.51	0.113
LVIDd (mm)	51.57±4.89	50.68±3.99	0.161
LVIDs (mm)	34.44±6.64	34.09±5.59	0.688
PWD (mm)	10.40±1.09	10.64±1.26	0.153
IVS (mm)	10.77±1.30	11.33±1.49	0.005
LAD (cm)	3.85±0.40	3.90±0.47	0.443
LWM (g)	207.52±41.61	212.69±40.82	0.779
LWMI (g/m^2)	104.50 (95.0–116.75)	106.0 (96.0–123.00)	0.625
RWT (cm)	0.39±0.05	0.41±0.06	0.017

HDL-C: high-density lipoprotein cholesterol; Hb: hemoglobin; LDL-C: low-density lipoprotein cholesterol; LVEF: left ventricular ejection fraction; STEMI: ST-segment elevation myocardial infarction; WBC: white blood cells; e-GFR: estimated glomerular filtration rate; LVIDd: left ventricular internal diameter end diastole; LVIDs: left ventricular internal diameter end systole; PWD: posterior wall thickness at end diastole; IVS: interventricular septal thickness at end diastole; LAD: left atrium diameter; LWM: LV mass; LWMI: LV mass index; RWT: relative wall thickness.

The mean age of the study population was 57.91±11.55 years, and 80.5% were male. There were statistically no significant differences between the two groups in terms of age, sex, body mass index (BMI), and clinical features (p>0.05). The mean maintenance dose was 14.8 mg for candesartan and 32.2 mg for zofenopril. Although cardiac medications were comparable between the two groups, the use of lipid-lowering medications was significantly different. According to our data, the majority of patients in the candesartan group received rosuvastatin therapy, while the majority of patients in the zofenopril group received atorvastatin therapy. As a result of the sealed envelope system that used for patient randomization, this difference occurred (Table 1).

With respect to echocardiographic measurements, baseline LV systolic functions were similar in both groups (p>0.05). Similarly, estimated LV dimension, LV mass, and LVMI were comparable between the two groups (p>0.05) (Table 1). Follow-up echocardiographic measurements revealed significant improvements in the candesartan group in terms of changes in LV end-systolic diameter, interventricular septum thickness, posterior wall thickness, and left atrium diameter (p<0.05). Similar changes in the abovementioned echocardiographic parameters were also obtained in the zofenopril group (p<0.05). Regarding echocardiographic indices of cardiac remodeling, candesartan group showed a significant reduction of estimated LV mass and LVMI at 6-month follow-up visit compared to baseline values [199.53±38.51 g vs. 212.69±40.82 g; 99.05 g/m^2 (90.00–116.5) vs. 106.0 g/m^2 (96.0–123.00), p<0.05]. This trend was also observed in the zofenopril group during the 6-month follow-up period [201.22±40.07 g vs. 207.52±41.61 g; 101.0 g/m^2 (92.25–111.75.0) vs. 104.50 g/m^2 (95.0–116.75), p<0.05]. Follow-up outcomes of echocardiographic measurements in both groups are summarized in Tables 2 and 3. According to our study, both classes of drugs had favorable effects on post-MI cardiac remodeling. However, the absolute benefit was more prominent in the candesartan group as compared to the zofenopril group. In our study, observed reduction in LV mass and LVMI during the 6-month follow-up period was significantly higher in the candesartan group as compared to the zofenopril group (13.16±2.63 g vs. 6.30±2.87 g; 6.47±1.38 g/m^2 vs. 1.94±1.29 g/m^2, p<0.05). In addition, the percent reduction in LV mass and LVMI was found to be significantly higher in the candesartan group than in the zofenopril group (5.35±1.17% vs. 2.01±1.38%; 5.15±1.16% vs. 1.07±1.36%, p<0.05). However, these reductions were not accompanied by any significant reduction of RWT (p>0.05).

DISCUSSION

In the present study, we investigated the effects of candesartan treatment in comparison with zofenopril treatment in patients with acute MI by using echocardiographic indices of cardiac remodeling. Our results indicate that favorable effects on post-MI cardiac remodeling were more prominent in candesartan treatment as compared to zofenopril treatment.

Table 2. Effects of candesartan on echocardiography parameters after 1 and 6 months treatment.

	Baseline candesartan	1 month candesartan	6 months candesartan	p-value*	p-value**	p-value***
LVIDd (mm)	50.68±3.99	50.37±3.84	50.25±4.16	0.133	0.125	0.519
LVIDs (mm)	34.09±5.59	33.31±5.59	33.14±6.26	0.007	0.016	0.516
PWD (mm)	10.64±1.26	10.54±1.20	10.46±1.08	0.025	0.017	0.171
IVS (mm)	11.33±1.49	11.09±1.40	10.77±1.39	0.001	0.000	0.000
LVEF %	48.16±8.51	49.51±8.36	50.35±8.35	0.000	0.000	0.006
LAD (cm)	3.90±0.47	3.89±0.49	3.92±0.48	0.793	0.541	0.185
LWM (g)	212.69±40.82	205.52±42.76	199.53±38.51	0.001	0.000	0.008
LWMI (g/m^2)	106.0 (96.0–123.00)	100.5 (93.250–117.0)	99.05 (90.00–116.5)	0.000	0.000	0.030
RWT (cm)	0.41±0.06	0.41±0.06	0.41±0.05	0.568	0.374	0.882
Height (cm)	167.74±7.43	167.88±7.44	167.69±7.40	0.332	0.320	0.159
Weight (kg)	80.60±12.30	80.56±12.09	80.94±12.37	0.370	0.224	0.347
Body mass index (kg/m^2)	28.60±4.32	28.53±4.20	28.79±4.33	0.235	0.165	0.421
Metoprolol dose (mg)	57.75±18.69	67.50±26.94	77.75±33.12	0.000	0.000	0.064
Candesartan dose (mg)	14.41±5.52	15.50±5.66	18.16±7.65	0.006	0.000	0.000

*Baseline vs. 1 month. **Baseline vs. 6 months. ***1 month vs. 6 months. LVIDd: left ventricular internal diameter end diastole; LVIDs: left ventricular internal diameter end systole ; PWD: posterior wall thickness at end diastole; IVS: interventricular septal thickness at end diastole; LAD: left atrium diameter; LVEF: left ventricular ejection fraction; LWM: LV mass; LWMI: LV mass index; RWT: relative wall thickness.

Table 3. Effects of zofenopril on echocardiography parameters after 1 and 6 months treatment.

	Baseline zofenopril	1 month zofenopril	6 months zofenopril	p-value*	p-value**	p-value***
LVIDd (mm)	51.57±4.89	51.40±5.08	51.28±5.37	0.393	0.255	0.493
LVIDs (mm)	34.44±6.64	33.94±6.78	33.64±7.22	0.095	0.109	0.478
PWD (mm)	10.40±1.09	10.33±1.12	10.33±1.11	0.109	0.264	0.989
IVS (mm)	10.77±1.30	10.66±1.31	10.53±1.21	0.139	0.024	0.107
LAD(cm)	3.85±0.40	3.82 ±0.43	3.83±0.44	0.225	0.458	0.697
LVEF %	50.12±8.87	50.72±8.99	50.99±9.27	0.041	0.022	0.320
LWM (g)	207.52±41.61	206.12±48.67	201.22±40.07	0.706	0.031	0.148
LWMI (g/m^2)	104.50 (95.0–116.75)	100.00 (94.0–115.75)	101.0 (92.25–111.75.0)	0.030	0.007	0.177
RWT (cm)	0.39±0.05	0.39±0.058	0.39±0.06	0.933	0.699	0.661
Height (cm)	168.98±7.22	168.89±7.29	169.04±7.32	0.181	0.622	0.170
Weight (kg)	79.64±11.66	79.72±11.66	79.92±11.62	0.545	0.405	0.585
Body mass index (kg/m^2)	27.83±3.35	27.86±3.34	27.97±3.53	0.159	0.205	0.314
Metoprolol dose (mg)	55.00±21.61	67.25±27.22	74.25±30.86	0.000	0.000	0.001
Zofenopril dose (mg)	32.23±7.91	33.82±10.06	38.29±13.49	0.025	0.000	0.000

*Baseline vs. 1 month. **Baseline vs. 6 months. ***1 month vs. 6 months. LVIDd: left ventricular internal diameter end diastole; LVIDs: left ventricular internal diameter end systole; PWD: posterior wall thickness at end diastole; IVS: interventricular septal thickness at end diastole; LAD: left atrium diameter; LVEF: left ventricular ejection fraction; LWM: LV mass; LWMI: LV mass index; RWT: relative wall thickness.

It has been well established that cardiac remodeling is associated with pathophysiological changes in cardiac myocytes and may contribute to the development of adverse cardiac events[13]. Despite various known factors, MI is the most common etiologic factor associated with cardiac remodeling. Myocardial injury secondary to MI not only induces morphological changes in the infarcted area but also causes LV eccentric hypertrophy[14,15]. In response to cardiac injury following MI, cellular and molecular alterations occurring in the infarcted area yield ventricular dysfunction and malignant ventricular arrhythmias[16-18]. According to previous reports, up to 50% of patients who suffer from ventricular dysfunction will die within 5 years following MI. Besides, the mortality rate could be higher among those who were hospitalized for cardiac failure following MI. The underlying mechanism is cardiac remodeling associated with malignant ventricular arrhythmias and sudden cardiac death[19,20]. Therefore, a better understanding of the factors associated with cardiac remodeling and administrating medical therapies that reverse this pathological condition and improve ventricular functions in post-MI patients are mandatory.

Randomized trials have shown the detrimental effects of angiotensin II on ventricular functions and have proved that inhibition of angiotensin II via a non-ACE-dependent pathway may ameliorate cardiac remodeling[21,22]. Therefore, the use of ARB not only reverses cardiac remodeling but also prevents adverse cardiac events. These favorable effects were confirmed by the Valsartan in Acute Myocardial Infarction (VALIANT) trial, which investigated the effects of valsartan administration in comparison with captopril treatment in post-MI patients experiencing LV systolic dysfunction[23]. In another study, Suzuki et al. found candesartan treatment to be more efficacious than ACE inhibitors in terms of preventing cardiac remodeling in patients presenting with MI[2]. Outcomes of our study consistent with previous reports revealed that candesartan treatment was more efficacious than ACE inhibitor treatment in terms of improving echocardiographic indices of cardiac remodeling after MI. With regard to echocardiographic indices of cardiac remodeling, we preferred LV mass, LVMI, and RWT in order to assess the effects of candesartan treatment on cardiac remodeling. Among the well-known parameters for the assessment of cardiac remodeling, LV mass, LVMI, and RWT have been well-established echocardiographic parameters to characterize cardiac remodeling and have been extensively validated in clinical practice. In addition, these variables not only give the most precise results but are also confirmed by various cardiac imaging modalities[3]. Furthermore, these geometrical indices are strongly associated with adverse cardiac events in various clinical conditions[24].

According to our study, the absolute reduction in echocardiographic indices of cardiac remodeling including LV mass and LVMI was more prominent in patients receiving candesartan treatment as compared to patients receiving

zofenopril treatment following MI ($p<0.05$). To the best of our knowledge, this is the first study demonstrating the inhibitory effects of candesartan on LV mass and LVMI in patients presenting with MI. Although both classes of drugs showed a decrease in RWT, this reduction did not reach a statistical significance ($p>0.05$).

Limitations

A major limitation of this study is that participants were observed over a relatively short period of time. Randomized trials with long-term follow-up can provide more detailed information about the long-term effects of candesartan treatment in patients presenting with MI. Although it is the largest study to date investigating the association between candesartan treatment and changes in echocardiographic indices of cardiac remodeling, it is nonetheless a relatively small, single-center study. Finally, the determination of LV mass, LVMI, and RWT was limited by the availability and interpretability of conventional echocardiographic measurements. Assessment of those parameters by cardiac magnetic resonance imaging (MRI) or computed tomography (CT) will provide more accurate results.

CONCLUSION

The present study highlights that both zofenopril and candesartan treatments have favorable effects on LV geometry. However, the observed reduction in LV mass and LVMI during the 6-month follow-up period was significantly higher in the candesartan group than in the zofenopril group. Our results suggest that early candesartan treatment following MI may potentially be useful in terms of improving post-MI cardiac remodeling.

AUTHORS' CONTRIBUTIONS

HT: Conceptualization, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. **AT**: Conceptualization, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. **KD**: Investigation, Resources, Supervision, Visualization. **BBA**: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. **NA**: Investigation, Resources, Supervision, Visualization. **MUY**: Investigation, Resources, Supervision, Visualization. **MSA**: Data curation, Validation. **CA**: Data curation, Software, Validation. **OCP**: Data curation, Software, Validation. **AMT**: Data curation, Software, Validation.

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ORIGINAL ARTICLE

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Comparison of pain levels developed during intramuscular injections to laterofemoral and ventrogluteal regions in children: a randomized controlled study

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SUMMARY

OBJECTIVE: The aim of this study was to compare the levels of pain developed during intramuscular injections to the laterofemoral and ventrogluteal regions in children.

METHODS: The study population consisted of all children aged between 7 and 12 years who presented to the pediatric emergency clinic of a hospital. The sample consisted of 62 children who met the inclusion criteria and agreed to participate in the study, and the children were randomly assigned to each group (laterofemoral n=31, ventrogluteal n=31). "Buzzy" and "deep breathing" were applied to children in both groups to relieve pain during the procedure. The data were obtained using an Information Form, a visual analog scale, and the Facial Pain Scale-Revised.

RESULTS: It was determined that the children in the ventrogluteal group during the intramuscular injections had lower visual analog scale and faces pain scale-revised scores immediately after the procedure compared with the vastus lateralis group, that is, they experienced less pain, and the difference between the two groups was significant (p<0.001).

CONCLUSION: In children, it is recommended to choose the less painful ventrogluteal region for intramuscular injection and to inform health professionals about it.

KEYWORDS: Pain. Child. Nurses. Injections, intramuscular.

INTRODUCTION

The regions used for intramuscular (IM) injection in children are the deltoid, ventrogluteal, and laterofemoral regions. Although the laterofemoral (vastus lateralis muscle) region is the most frequently preferred region in children, it is emphasized that the safest region for IM injection is the ventrogluteal region[1-4].

It is recommended to use the laterofemoral region in newborns and children younger than 3 years of age, and the ventrogluteal or deltoid region in children aged 3 years and over[1]. In addition, it is reported that the laterofemoral region can be used safely in children aged 3–18 years, if large amounts of drugs are to be injected[1,5,6].

The vastus lateralis muscle in the laterofemoral region is a safe region for IM injections due to its distance from nerves and blood vessels, the low risk of administering the drug to the subcutaneous tissue, multiple injections, easy access, and easy location[4,7]. In contrast, the ventrogluteal region is a region that does not contain large blood vessels and nerves, has a thick muscle density, is preferred for the application of irritating and oily solutions, and is less painful during injection[1]. Since this region is the thickest, consisting of both gluteus medius and gluteus minimus muscles, it can be used safely in all adults and small children who can walk[8].

Ensuring timely and effective pain control during procedures that cause pain and discomfort, such as IM injections applied to children, will increase the tolerance to pain in later applications[1,9,10]. It is estimated that the fear of injections, which is present in approximately 25% of adults, develops during childhood. Reducing injection-related pain in childhood can prevent stress and avoidance of healthcare-seeking behavior in later periods[10]. For this reason, in IM injections, it is very important to choose the right application and the right region in children to experience less pain.

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METHODS

Objective

This research was conducted to compare the levels of pain developed during IM injections to the laterofemoral and ventrogluteal regions in children.

Design

This is an experimental randomized controlled study.

Research hypothesis

Hypothesis 1 (H1): There would be a difference between laterofemoral and ventrogluteal regions in terms of pain during IM injections in children.

Participants

The population of the study consisted of children aged between 7 and 12 years who presented to the pediatric emergency clinic-injection room of a state hospital in Turkey. To use parametric tests in statistical evaluation and to obtain safer results, it was planned to include a total of 60 children, at least 30 in each group, as the sample of the study. A total of 70 children were included in the study by adding 5 more children to each group, considering that there might be losses. To determine which patient would be in which group, numbers from 1 to 70 were randomly distributed to two groups through a computer program without repeating any numbers. During the study, a total of eight children, four from each group, stated that they wanted to leave immediately after the procedure and did not evaluate their pain. Thus, the study was conducted with a total of 62 children (*laterofemoral* n=31, *ventrogluteal* n=31). Power analysis was performed using the Power (v3.1.7) program to determine the adequacy of the sample size of the study. With a significance level of 0.05, a confidence interval of 0.98, and a high effect size of 0.40, the power of the study was determined as 0.98.

The inclusion criteria were as follows: children aged between 7 and 12 years, being admitted to the emergency unit for penicillin (procaine) administration, absence of a disease causing chronic pain, absence of a neurodevelopmental disorder, not taking analgesics in the last 6 h, no history of fainting during injections, absence of mental retardation, families, and children agreeing to participate in the research.

Data collection

Information form

The information consisted of a total of nine open- and closed-ended questions about the introductory characteristics of the child and their family and the injection procedure.

Visual analog scale

This scale consisted of a 10-cm line drawn either vertically or horizontally (0–10 cm or 0–100 mm). A line of 0 on the scale indicates "no pain," and a line of 10 indicates "unbearable pain[11]." The child is asked to put a mark on the line to indicate the intensity of pain.

Faces pain scale-revised

The faces pain scale-revised (FPS-R) consists of six facial expressions graded from 0 to 10 according to the presence and severity of pain. It is a valid and reliable self-report scale for painful situations in children[11].

Buzzy®

Buzzy is an 8×5×2.5 cm, 8×5×2.5-cm, noninvasive, plastic pain control device with a battery and vibration motor, developed by pediatrician Ammy Baxter. A cold ice pack is placed under Buzzy. It is effective in reducing pain with local cold application and vibration effect (Figure 1) (http://www.buzzy4shots.com/).

Application

The procedure was explained to the parents and children, and verbal and written consent was obtained from the families and children before the application. The Information Form was completed by the researcher. According to the randomization, the group of children was determined and the children who came every day were treated accordingly. During this period, the nurse practitioner prepared the materials. Injectors with a needle size of 25 gauge[6] were used in children and adolescents. The mothers of all the children participating in the study stayed with the children throughout the injection procedure, which was administered by the researcher in all groups.

Figure 1. Buzzy®.

Ventrogluteal region injections

The child was placed in the lateral position, the left hand on the right hip, and the right hand on the left hip were used to determine the injection site. The lower part of the palm was placed in the greater trochanter. The index finger was placed on the anterior superior iliac spine, the middle finger was extended dorsally to reach the iliac crest, and the thumb was positioned to point to the inguinal region. The injection was performed in the triangle formed by the index finger, middle finger, and iliac crest[2,7].

Laterofemoral region injections

The child was placed in the supine position. The vastus lateralis muscle, located on the anterior lateral aspect of the thigh, is the best-developed muscle in children. The distance between the knee and the greater trochanter was divided into three, and an injection was performed in the middle third[7].

To alleviate the pain during the procedure, children in both groups were given "deep breathing" and "Buzzy." Buzzy was placed 3–5 cm above the region for 60 s before and during the procedure. After penicillin was administered to the children in both groups (at 1 min), pain conditions were evaluated and recorded using the VAS and FPS-R. All these processes took approximately 15–20 min for each child.

Statistical analysis

Data were evaluated using the χ^2 test, Kruskal-Wallis test, Mann-Whitney U test, one-way analysis of variance, and Spearman's correlation analysis. The statistical significance level was set at $p<0.05$.

Ethical considerations

The study was approved by the University Clinical Research Ethics Committee (2015,21347889-774.991), and written permission was obtained from the institution where the study was conducted (2015,26857650-047).

RESULTS

The comparison of demographic characteristics by groups is presented in Table 1.

DISCUSSION

Pain in IM injections can be reduced by a good injection technique and by administering the drug to the correct injection site, which is determined by considering the characteristics of the drug and the individual[12]. In the literature, studies are comparing the dorsogluteal and ventrogluteal regions for IM injections, and there are a few comparative studies on the laterofemoral region, which we use frequently in children. For this reason, evaluating the effectiveness of these two regions, which we use safely in children, on pain will contribute to this field.

It was emphasized that it was important to evaluate the body mass index (BMI), weight, and age of the child in the

Table 1. Comparison of demographic characteristics by groups (n=62).

		Ventrogluteal (n=31)	Vastus Lateralis (n=31)	Test	p
Age	Mean±SD	9.39±2.12	7.87±1.48	**z=-2.785**	[a] ***0.005***
	Min-Max (Median)	7–12 (9.0)	7–12 (7.0)		
BMI	Mean±SD	16.70±2.83	15.53±2.33	**t=1.779**	[b] ***0.080***
		n (%)	**n (%)**		
Sex	Girl	14 (45.2)	14 (45.2)	**χ^2=0.001**	[c] ***0.999***
	Boy	17 (54.8)	17 (54.8)		
How the child felt after the procedure	Very good	10 (32.3)	9 (29.0)	χ^2=0.305	[d] ***0.999***
	Good	13 (41.9)	13 (41.9)		
	Neutral	4 (12.9)	4 (12.9)		
	Bad	4 (12.9)	5 (16.1)		
Child's reaction	Very positive	6 (19.4)	1 (3.2)	χ^2=5.061	[d] ***0.164***
	Positive	19 (61.3)	20 (64.5)		
	No reaction	3 (9.7)	3 (9.7)		
	Negative	3 (9.7)	7 (22.6)		

[a]Mann-Whitney U test; [b]Student's t-test; [c]Yates's continuity correction test; [d]Fisher-Freeman-Halton test. Bold values indicate statistical significance at the $p<0.05$ level. The comparison of the mean VAS and faces pain scale-revised scores of the groups is presented in Table 2.

selection of the region for IM injections because subcutaneous tissue and adipose tissue vary according to age. It is very important to know the BMI value because it affects the quality of the IM injection and the delivery of the drug to the tissue[3]. In the research, it was seen that the BMI values were similar in both groups. This would minimize the risk of being affected by pain that might result from BMI in children.

In the study, very painful IM injections of penicillin were performed in different regions in the two groups, and it was seen that the children in the ventrogluteal region group felt less pain immediately after the penicillin injection than the laterofemoral group, and this difference was statistically significant. In addition, in the study, two pain scales based on two different personal expressions with high validity and reliability and were easy to understand were used in determining the severity of pain, and similar results were obtained with both scales ($p<0.05$; Table 2). The children in both groups were asked how they felt about the injection immediately after the procedure, and it was found that the children mostly felt good, and according to the mothers' statements, the children showed similar positive reactions during the procedure ($p>0.05$; Table 1). This result showed that the children were mostly positive about both methods, but the pain experienced in the ventrogluteal region was less. In the literature[1,13], it was stated that this region was less painful because it did not contain large blood vessels or nerves and was far from bone tissue. The results of our research show parallelism with the literature and *Hypothesis 1* is supported.

In a study by Moharreri et al. (2007), when pain severity and bleeding status were evaluated after the IM injections in the dorsogluteal and ventrogluteal regions, it was found that patients who were injected into the ventrogluteal region felt less pain and had less bleeding compared with the dorsogluteal region[14].

In studies conducted in adults, pain and bleeding occurring in injections performed in the ventrogluteal region[5,7,13] were less than those performed in the dorsogluteal region, and the ventrogluteal region was preferred for IM injections[13].

In a study, nurses expressed that the dorsogluteal region was the most frequently used IM injection region; they did not use the ventrogluteal region although, they defined it as the safest injection region, and their knowledge about injections in the ventrogluteal region was insufficient[15]. Isseven et al. (2020) compared the dorsogluteal and ventrogluteal regions in their study of adults[7]. They found the satisfaction level of the patients from the ventrogluteal region to be higher than in the dorsogluteal region. In our study, it was observed that children were satisfied at similar rates in both regions (Table 1). The ventrogluteal region is safer for injections and causes less pain because there are no large blood vessels and nerves. This region has advantages such as the low possibility of transferring the drug to the subcutaneous tissue due to the thin subcutaneous layer and easier positioning of the patient[2,5]. In addition, as a painful procedure was performed in the study, "deep breathing" and "Buzzy" were performed on the children in both groups to experience less pain. It has been reported that these methods reduce pain in children during painful procedures[9,16]. In addition to these methods, it is seen that regional preference also further reduces pain. However, although the ventrogluteal region is defined as the safest area for IM injections in the literature, it has been found that the majority of nurses do not use this area and are reluctant to change[15].

In our study and other studies, it was determined that the ventrogluteal region was less painful[12,17]. For this reason, health professionals should be informed in this direction and the importance of using this region more frequently, which causes children to experience less pain in practice, should be emphasized.

CONCLUSION

It is seen that both sites can be used in IM injections in older children, but IM injections performed in the ventrogluteal region are less painful than those performed in the vastus lateralis region.

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Table 2. Comparison of visual analog scale and faces pain scale-revised mean scores of the groups immediately after the procedure (n=62).

		Ventrogluteal (n=31)	Laterofemoral (n=31)	Test	[a]p
VAS	Mean±SD	2.39±2.01	4.58±3.56	z=-2.500	**0.012**
	Min-Max (Median)	0–8 (2.0)	0–10 (4.0)		
FPS-R	Mean±SD	2.45±1.98	4.58±3.59	z=-2.419	**0.016**
	Min-Max (Median)	0–8 (2.0)	0–10 (4.0)		

[a]Mann-Whitney U test. Bold values indicate statistical significance at the $p<0.05$ level.

AUTHORS' CONTRIBUTIONS

SB: Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **BBS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft.

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ORIGINAL ARTICLE

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Immunoadsorption therapy in refractory heart failure patients with dilated cardiomyopathy: a potential therapeutic option*

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SUMMARY

OBJECTIVE: Removal of cardiac autoantibodies by immunoadsorption might confer clinical improvement in dilated cardiomyopathy. In this pilot study, we investigated the efficacy and safety of immunoadsorption therapy in refractory heart failure patients with dilated cardiomyopathy.

METHODS: This study consisted of 9 heart failure patients with dilated cardiomyopathy, NYHA III-IV, left ventricular ejection fraction <30%, unresponsive to heart failure therapy, and with cardiac autoantibodies. Patients underwent immunoadsorption therapy for five consecutive days using a tryptophan column. Changes in cardiac function (left ventricular ejection fraction, left ventricular end-diastolic diameter, left ventricular end-systolic diameter), exercise capacity (6-minute walk distance), neurohormonal (N-terminal pro-brain natriuretic peptide), proinflammatory (high-sensitive C-reactive protein), and myocardial (cardiac troponin-I), biochemical, and hematological variables were obtained at baseline and after 3 and 6 months of immunoadsorption therapy.

RESULTS: Mean left ventricular ejection fraction and 6-minute walk distance significantly increased at 3 months (from 23.27±5.09 to 32.1±1.7%, p=0.01 for left ventricular ejection fraction and from 353±118 to 434±159 m, p=0.04 for 6-minute walk distance) and further increased at 6 months after immunoadsorption therapy (to 34.5±7.7%, p=0.02 for ejection fraction and to 441±136 m, p=0.04 for 6-minute walk distance). NT-proBNP level reduced from 1161(392.8–3034) to 385(116.1–656.5) ng/L (p=0.04), and high-sensitive C-reactive protein decreased from 9.74±0.96 to 4.3±5.8 mg/L (p=0.04) at 6 months. Left ventricular end-diastolic diameter (66.1±5.8 vs. 64.7±8.9 mm) and left ventricular end-systolic diameter (56.1±8.6 vs. 52.3±10.8 mm) tended to decrease but did not reach statistical significance. No significant worsening was observed in creatinine, cardiac troponin-I, and hemoglobin levels after the immunoadsorption procedure.

CONCLUSION: In dilated cardiomyopathy patients with refractory heart failure, immunoadsorption may be considered a potentially useful therapeutic option to improve a patient's clinical status.

KEYWORDS: Plasmapheresis. Heart failure. Cardiomyopathy, dilated.

INTRODUCTION

Dilated cardiomyopathy (DCM) is a progressive myocardial disease characterized by systolic dysfunction and ventricular dilatation[1]. Genetic and autoimmune abnormalities as well as viral infections are thought to be involved in the underlying pathophysiological mechanisms of DCM. A number of autoantibodies (AAB) against various cardiac cell proteins including contractile proteins, mitochondrial proteins, sarcolemmal Na-K-ATPase, cardiac beta-1 adrenergic receptors (ARs), and muscarinic receptors have been identified in patients with DCM[2-9]. Accumulating evidence suggests that these AABs are able to disturb the normal physiological activity of the cardiomyocytes, may contribute to cardiac dysfunction, and play an active role in the pathogenesis of DCM[6-9]. Recent controlled small studies with a limited number of patients indicate that removal of these cardiac AABs by immunoadsorption (IA) therapy may decrease myocardial inflammation and induce improvements in cardiac function and quality of life in heart failure (HF) patients with DCM[10-14]. Therefore, in selected symptomatic HF patients with idiopathic DCM who do not respond to optimal standard medical therapy, IA therapy may be considered a potentially useful method for the improvement of the patient's clinical status. In this pilot study, we investigated the efficacy and safety of IA therapy in refractory HF patients with DCM.

METHODS

Study population

The present study included symptomatic HF patients with DCM, New York Heart Association (NYHA) functional class III-IV, left ventricular (LV) ejection fraction (EF) <30%,

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refractory to optimal evidence-based guidelines-recommended HF therapy including angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB), beta blocker, diuretics, mineralocorticoid receptor antagonist (MRA), ivabradine, or digoxin treatment for at least 6 months. Coronary artery disease was excluded by coronary angiography. Patients with HF due to known origins such as primary valvular disease, congenital heart disease, or other cardiomyopathies and also those with severe chronic obstructive pulmonary disease, severe chronic kidney or liver disease, connective tissue disease, active infectious disease, chronic alcoholism, or malignancy were excluded from the study.

A total of 38 HF patients with DCM were screened for AABs directed against beta1-AR and M2-muscarinic receptors. Notably, 17 patients (44%) were positive for cardiac AABs, in which 16 patients have had AABs against beta1-AR and 3 patients have had AABs for M2-muscarinic receptors (2 of them were also positive for beta1-AR AAB). Nine patients with cardiac AABs who gave written informed consent were included in the study and underwent IA therapy (Figure 1). This study has been conducted between 2014 and 2018 in a university hospital, outpatient Heart Failure Unit with the capability of implantation of cardiac resynchronization therapy or implantable cardiac defibrillator and short-term mechanical circulatory support, which is affiliated with an institution with the availability of long-term ventricular assist devices or heart transplantation. The study protocol was approved by the ethics committee, and the study was performed in accordance with the guidelines of the Declaration of Helsinki.

Measurement of cardiac autoantibodies

The blood samples were tested by E.R.D.E – AAK – Diagnostik GmbH in Berlin, Germany. The test system is a bioassay of spontaneously beating neonatal rat cardiomyocytes. The presence of cardioactive receptor-AABs can be detected as a positive or negative reaction after the addition of the patient's immunoglobulin. The kind of ABB is verified using specific receptor antagonists. The blood samples of patients were sent to Berlin, Germany and it has taken 2–3 months to get the test results.

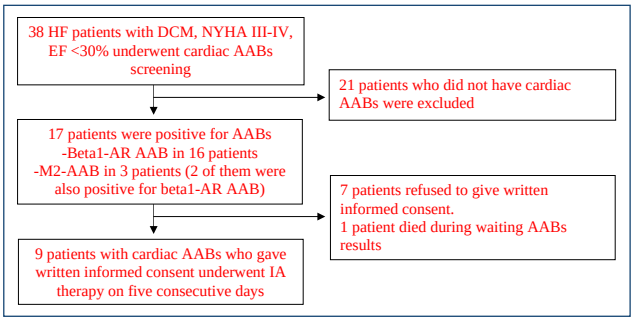

Figure 1. Flowchart of study design. AABs: autoantibodies; AR: adrenoreceptor; DCM: dilated cardiomyopathy; EF: ejection fraction; HF: heart failure; IA: immunoadsorption therapy; M2: muscarinic receptor2; NYHA: New York Heart Association.

Immunoadsorption protocol

All patients underwent IA therapy on five consecutive days in a total of five sessions and in one course. A catheter for blood access was inserted via the internal jugular vein. ACEI was switched to ARB (candesartan or valsartan in dose equivalence or maximally tolerated doses) at least 2 weeks before admission because ACEI inhibits the breakdown of the bradykinin, which is activated during IA. The patients received IA for 2–3 h in each session, and 1500–2000 mL of plasma was treated, using PLASAUTO Σ (Asahi Kasei Kuraray Medical, Tokyo, Japan). Immusorba TR-350 (Asahi Kasei Kuraray Medical, Tokyo, Japan) as IgG3-specific tryptophan column and Plasmaflo OP-05W (Asahi Kasei Kuraray Medical, Tokyo, Japan) to separate the plasma from whole blood were used. All patients received heparin during the IA procedure as an anticoagulant. After the final IA session, all patients also received intravenous Ig (IVIG) substitution (0.5 g/kg polyclonal IgG) to restore IgG plasma levels. All patients were admitted to the hospital for the entire course of therapy to avoid the risk of infection and bleeding. After IA therapy, changes in medication dosage were performed only for diuretics according to symptoms and signs of congestion. All other HF medications were continued without any change in dosages during the 6-month follow-up.

Measurements of cardiac function and exercise capacity

Transthoracic echocardiography was performed at baseline and after 3 and 6 months of IA therapy. left ventricular ejection fraction (LVEF), LV end-diastolic diameter (LVEDD), and LV end-systolic diameter (LVESD) have been determined. LVEF was measured according to the Simpson rule. Six-minute walk distance (6-MWD) was measured at baseline and after 3 and 6 months of IA therapy for the assessment of exercise capacity.

Neurohormonal, myocardial, inflammatory, and biochemical variables

N-terminal pro-brain natriuretic peptide (NTproBNP) as a neurohormonal biomarker of HF, high sensitive cardiac troponin-I (cTnI), and creatine kinase-MB isoenzyme (CK-MB) as myocardial markers, high sensitive C-reactive protein (hsCRP) as a marker of inflammation, serum creatinine as the markers of kidney function, serum electrolytes,

hemoglobin (Hgb) level, white blood cell (WBC) count, and platelet count were obtained at baseline and after 3 and 6 months of IA therapy.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences software 20.0 (IBM SPSS 20, SPSS Inc., Chicago, US). Differences in parameters before and after IA were compared using an independent sample *t*-test or paired sample *t*-test for the analysis of normally distributed variables; otherwise, the Wilcoxon signed rank test was used for the analysis of non-normally distributed variables and described using medians (interquartile ranges [IQRs]). Normally distributed variables were expressed as mean ± standard deviation. Categorical data were presented as frequencies and percentages and were analyzed by Pearson chi-square or continuity correction chi-square. Changes in clinical parameters were compared over time (baseline, 3 months, and 6 months) using a linear mixed model. $p < 0.05$ were considered statistically significant.

RESULTS

Baseline clinical characteristics of the study population including HF medication are shown in Table 1. The mean age of the study population was 44.1±7.8 years, and 55.5% were male. Four (44.4%) patients had a history of hypertension, and 2 (22.2%) had diabetes mellitus. Baseline mean EF was 23.27±5.09%, NT-proBNP was 1192±1015 pg/mL, 6-MWD was 353±118 m, creatinine level was 0.82±0.17 mg/dL, and Hgb level was 13.4±2 g/dL. At the time of screening, all patients were receiving ACEI or ARB, beta-blockers, and loop diuretics, and 88.9% were using MRA.

Changes in cardiac function, exercise capacity, neurohormonal, myocardial, proinflammatory, biochemical, and hematological variables after 3 and 6 months of IA therapy are shown in Table 2. Mean LVEF and 6MWD significantly increased at 3 months (from 23.27±5.09 to 32.1±1.7%, p=0.01 for LVEF and from 353±118 to 434±159 m, p=0.04 for 6MWD) and further increased at 6 months after IA therapy (to 34.5±7.7%, p=0.02 for LVEF and to 441±136 m, p=0.02 for 6MWD). Although LV end-diastolic (66.1±5.8 vs. 64.7±8.9 mm) and end-systolic diameters (56.1±8.6 vs. 52.3±10.8 mm) tended to decrease at 6 months, these decreases did not reach statistical significance. Reduction in NTproBNP and high-sensitive C-reactive protein (hs-CRP) was not significant at 3 months, but at the end of the 6-month follow-up, the NT-proBNP level reduced from 1161(392.8–3034) at baseline to 385(116.1–656.5) ng/L (p=0.04), and hsCRP decreased from 9.74±0.96 at baseline to 4.3±5.8 mg/L (p=0.04). Myocardial markers of hs-cTnI and CK-MB did not change significantly during the 6-month follow-up of IA therapy.

Table 1. Clinical characteristics of the patients.

Age, years	44.1±7.8
Male gender, n (%)	5 (55.5)
Body mass index, kg/m^2	32.2±9.6
Heart rate, bpm	74.2±15
Systolic BP, mm Hg	108.7±14.6
Diastolic BP, mm Hg	67.5±8.2
Diabetes, n (%)	2 (22.2)
Hypertension, n (%)	4 (44.4)
Smoking, n (%)	2 (22.2)
Atrial fibrillation, n (%)	3 (33.3)
LBBB/RBBB, n (%)	0 (0)
Anemia, n (%)	3 (33.3)
ASA, n (%)	1 (11.1)
Beta blocker, n (%)	9 (100)
RAAS inhibitors, n (%)	9 (100)
Spironolactone, n (%)	8 (88.9)
Loop diuretics, n (%)	9 (100)
Ivabradine, n (%)	4 (44.4)
Digoxin, n (%)	2 (22.2)
CRT, n (%)	0 (0)
ICD, n (%)	3 (33.3)

ASA: acetylsalicylic acid; BP: blood pressure; CRT: cardiac resynchronization therapy; ICD: implantable cardioverter-defibrillator; LBBB: left bundle branch block; RAAS: Renin-Angiotensin-Aldosterone System; RBBB: right bundle branch block.

All patients had completed IA without any major complications. No adverse event was observed during and after IA therapy. Overall, patients did not experience hypotension, tachycardia, bleeding, fever, or signs of infection during and after the procedure (Table 3). No significant worsening in renal function was found, and Hgb level or WBC count remained stable after IA (Table 2).

During 6-month follow-up, 1 patient was hospitalized with acute decompensated HF at 4 months, experienced progressive clinical deterioration, and died despite optimal HF management. Rest of the patients did not need hospital admission, implantation of ventricular assist devices, or heart transplantation during the 6-month follow-up period.

Table 2. Changes in clinical parameters after 3 and 6 months of immunoadsorption therapy.

	Baseline	3-month FU	6-month FU	P
LVEF, %	23.27±5.09	32.1±1.7	34.5±7.7	0.02
LVEDD, mm	66.1±5.8	65.7±3.2	64.7±8.9	0.71
LVESD, mm	56.1±8.6	55.7±3.5	52.3±10.8	0.92
6MWD, m	353±118	434±159	441±136	0.04
NTproBNP, pg/mL	1161(392.8–3034)	846(399.5–883.5)	385(116.1–656.5)	0.04
hs-CRP, mg/L	10.1(1.85–14.7)	3.15(0.90–21.1)	1.82(0.82–7.85)	0.04
Troponin-I, ng/mL	0.02±0.03	0.008±0.003	0.005±0.001	0.36
CK-MB, ng/mL	1.62±0.63	2.1±1.06	1.84±0.41	0.20
Na, mEq/L	138±2.86	141±2.7	138±2.8	0.75
K, mEq/L	4.48±0.4	4.6±0.28	4.45±0.22	0.90
Creatinine, mg/dL	0.82±0.17	0.84±0.14	0.86±0.18	0.31
Hgb, g/dL	13.7±1.37	14.4±2	14.1±1.6	0.38
WBC, $10^3/\mu L$	8.30 (7.7–9.6)	7.8(7.4–9.15)	9.05(7.37–10.05)	0.32
Platelet count, $10^3/\mu L$	222 .0(193.0–235.5)	229.0±60.2	247.6±55.1	0.31

*Data are presented as median (IQR) or mean±SD. CK-MB: creatine kinase myocardial band; Hgb: hemoglobin; hs-CRP: high sensitive c-reactive protein; K: potassium; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular end-systolic diameter; 6MWD: 6-minute walk test; Na: sodium; NTproBNP: N-terminal pro-b-type natriuretic peptide; WBC: white blood count.

Table 3. Fever, heart rate, and systolic and diastolic blood pressure during immunoadsorption sessions.

	Before the procedure	After the procedure	p
Body temperature, °C			
First session	36.2±0.27	36.3±0.55	0.8
Second session	36.1±0.15	36.1±0.1	0.7
Third session	36.5±0.41	36.3±0.45	0.02
Fourth session	36.2±0.49	36.1±0.32	0.34
Fifth session	36.3±0.5	36.3±0.44	1
Heart rate, bpm			
First session	77±15	79±8.5	0.66
Second session	77±18	79±16	0.39
Third session	77±16	77±16	0.91
Fourth session	76±13	77±13	0.69
Fifth session	86±18	83±15	0.7
Systolic BP, mm Hg			
First session	119±11.7	110±14.5	0.02
Second session	113±13.8	116±9	0.63
Third session	119±13.3	117±11.3	0.74
Fourth session	114±18	119±19.2	0.48
Fifth session	113±14.6	119±18.2	0.33
Diastolic BP, mm Hg			
First session	73.1±7.8	63.5±10.6	0.1
Second session	66.5±8.6	66.6±10.7	0.94
Third session	72.5±10.6	71.5±9.1	0.82
Fourth session	67.6±10.9	74±12.3	0.28
Fifth session	74±11	70.6±7.8	0.27

BP: blood pressure.

DISCUSSION

The results of this study showed that IA therapy in DCM patients with refractory HF significantly improves cardiac function and exercise capacity through increases in LVEF and 6MWD. In addition, NTproBNP and hs-CRP levels were found to significantly decrease during the 6-month follow-up. Furthermore, IA was well tolerated and appeared to be feasible in patients with DCM as no adverse event was observed during and after the procedure.

Removal of AABs by IA has been shown to be a new potential therapeutic approach in treating DCM. In the first uncontrolled pilot study in 8 patients with DCM, Wallukat et al. reported that this technique efficiently removed cardiac beta-1 AR AABs and improved symptoms and cardiac function in this group of patients[10]. In 9 patients with DCM and LVEF <25%, Felix et al. randomized 18 DCM patients with LVEF <30% who have cardiac beta-1 AR AABs to IA or conventional therapy and reported that initially, 3 consecutive days and then monthly 2 consecutive days of IA therapy with IVIG substitution for 3 months resulted in a significant increase in LVEF, cardiac index, and stroke volume index, a significant decrease in systemic vascular resistance and beta-1 AR AAB level, and significant improvement in NYHA functional capacity[11]. In the other small randomized study by Muller et al. in 34 DCM patients with NYHA II-IV, LVEF <29% who have cardiac beta-1 receptor autoantibodies, and 17 patients who underwent IA on 5 consecutive days without IVIG substitution showed a significant increase in LVEF (from 22.3±3.3 to 37.9±7.9%, p<0.0001), and improvement in NYHA functional class compared showed no significant changes in the control group[12]. These single-center small studies suggested hemodynamic and clinical improvements of IA therapy in DCM patients with HF. The results of our study are consistent with the findings of previously published small studies in improving LVEF and functional capacity and reducing NTproBNP levels. 6MWD that we used in our study is a much more objective measure than the NYHA classification for the assessment of functional capacity. Different from other studies, we were also able to show a beneficial effect of IA on inflammatory state with a significant reduction in hs-CRP levels.

The removal of AABs against cardiac beta-1 AR by IA therapy has been proposed as a potential mechanism for the improvement of cardiac functions in patients with DCM. The presence of specific cardiodepressant AABs in plasma prior to IA is referred to as a predictor of the possible efficacy of this therapeutic approach. Staudt et al. reported that DCM patients with cardiodepressant AABs demonstrated significant hemodynamic benefits from IA therapy, whereas, in the non-cardiodepressant group, hemodynamics did not change significantly throughout 3 months of repetitive IA courses[15]. These data suggested that the presence of cardiodepressant AABs predicts the hemodynamic benefits of IA therapy. Most studies enrolled beta1-AR AAB-positive patients. We, therefore, enrolled patients with DCM who had either AAB against beta1-AR or muscarinic receptors in our study.

There has been no consensus on the ideal protocol for IA therapy. In various studies, patients underwent repeated IA treatment courses at monthly intervals for 3 months, while in some studies, IA therapy has been performed as a single treatment course on 5 consecutive days. Both treatment regimens are comparable, provide acute and prolonged hemodynamic and clinical benefits, and also result in a prolonged reduction in cardiac AABs[10-17]. Since IA is an invasive and expensive treatment, a single treatment course on 5 consecutive days might be thought to be a more suitable option. So, in our study, we used the protocol of a single IA course on 5 consecutive days and were able to show the beneficial effects of one course IA protocol over 6-month follow-up period. In addition, IVIG therapy following each IA course has been given as a part of the protocol in most studies in order to reduce the risk of infection; however, in some studies, IA therapy has been performed without IVIG substitution[13]. We preferred to use IVIG substitution after the final IA session in order to avoid the risk of infection that may arise from inappropriate lowering circulating IgG levels.

Autoantibodies that are most likely to be involved in immunoregulatory activity are IgG-3 and IgG-1 subclasses. IgG-3 is referred to as the most active IgG subclass and is thought to play a key role in cardiac dysfunction as a mediator of antibody-dependent cellular cytotoxicity[18]. It is considered that AABs belonging to the IgG-3 subclass play a pivotal role in the therapeutic efficacy of IA. IA with tryptophan columns causes a marked reduction of plasma levels of IgG-3 with a low immunogenicity and a high affinity for the IgG-3 subclass[19]. In 16 patients with DCM who have refractory HF, Nagatomo et al. showed that IA therapy using IgG-3 specific tryptophan column significantly increased LVEF and 6MWD and significantly decreased NTproBNP levels and autoantibody titers against beta-1 AR and M2 muscarinic receptors with a greater extent removal of IgG3 subclass than the other IgG subclasses[19]. Due to low immunogenicity and a high affinity for the IgG-3 subclass, we used the tryptophan column in the IA procedure. Although substitution of IVIG is considered not to be required after

IgG-3 specific IA, IVIG substitution has been performed in our patients because antibody and IgG3 titers have not been obtained in our study.

Limitations

This was a single-center pilot study including only a limited number of patients. However, previously published studies with IA in DCM also included small samples with 8 to 34 patients. Furthermore, the present study was not a randomized, controlled study. However, in many studies, a repeated IA protocol at monthly intervals had been performed, and a nonspecific protein-A column or anti-IgG column had been used. Different from previous studies, the present study also provides complementary information about the protocol of a single IA course on 5 consecutive days and the IgG-3-specific tryptophan column that we used in our study. Considering that IA is an emerging, invasive, and expensive therapy, findings that come from even small-scale studies would contribute to further studies.

CONCLUSION

In idiopathic DCM patients with refractory HF who do not respond to optimal standard medical therapy, IA therapy may be considered a potentially useful method to improve the patient's clinical status. Although IA therapy is a new and promising therapeutic option in the treatment of DCM, more data from larger, randomized, prospective, multicenter studies are needed before the routine use of this therapy in this patient population.

AUTHORS' CONTRIBUTIONS

YC: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. **SM:** Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. **ST:** Conceptualization, Data curation, Formal Analysis, Investigation, Software, Validation, Visualization, Writing – original draft, **OMA:** Investigation, Methodology, Validation, Visualization.

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ORIGINAL ARTICLE

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Clinical efficacy evaluation of crisaborole ointment in the treatment of vulvar leukoplakia

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the clinical efficacy of crisaborole ointment in the treatment of vulvar leukoplakia.
METHODS: A prospective, randomized controlled clinical trial was conducted, and a total of 100 patients with vulvar leukoplakia were divided into the observation group (n=50) treated with crisaborole ointment and the control group (n=50) treated with vitamin E. The symptom improvement and vulvar leukoplakia score after 2 weeks of treatment were analyzed, and the clinical efficacy of vulvar leukoplakia was evaluated by referring to the Guidelines for Clinical Research of New Drugs of Traditional Chinese Medicine (2018 Edition).
RESULTS: After 2 weeks of treatment, the overall score of lesions in the observation group decreased, and the total treatment efficiency of patients in the observation group was 92% (46/50), which was significantly higher than that of 52% (26/50) in the control group P<0.05).
CONCLUSION: Crisaborole ointment can effectively treat vulvar leukoplakia, improving the symptoms and pathological changes of the vulvar skin.
KEYWORDS: Leukoplakia. Nutrition disorders. Crisaborole. Clinical trial.

INTRODUCTION

Vulvar leukoplakia, as a common disease in women, refers to a localized chronic lesion of the vulvar mucosa caused by pigment changes and tissue degeneration due to nutritional disorders of the vulvar skin mucosa. The etiology of this disease is still unclear, and a large number of clinical studies suggest that this disease may be related to local humid stimulation of the vulva, autoimmune disorders (cellular immunity and humoral immunity), genetics, low hormone level, infection, and metabolic disorders. The mainstay of treatment includes topical drug therapy and physiotherapy[1]. The long-term efficacy and optimization parameters of physiotherapy require further observational studies. In comparison, topical drug therapy is more psychologically acceptable to patients. The active ingredient of crisaborole ointment is crisaborole, which is FDA-approved for atopic dermatitis in patients over 2 years old[2,3]. Crisaborole is a boron-based phosphodiesterase 4 (PDE-4) inhibitor that inhibits the PDE-4 enzyme, which is a key regulator of inflammatory cytokine production in the skin. Overactive PDE-4 has been shown to contribute to the signs and symptoms of atopic dermatitis. As a non-steroidal topical monotherapy, crisaborole mediates an anti-inflammatory effect on almost all inflammatory cells[4]. This study was conducted to evaluate the clinical efficacy of crisaborole ointment in the treatment of vulvar leukoplakia, and the results are reported as follows.

DATA AND METHODS

General data

A total of 100 patients with vulvar intraepithelial non-neoplastic lesions confirmed by vulvar biopsy sections who visited our gynecology clinic from September 2020 to September 2021 were selected. The pathological results of the vulvar biopsy were vulvar lichen simplex chronicus or vulvar lichen sclerosus. They were randomly divided into the control group (n=50) and the observation group (n=50). There were no statistical differences in age and disease duration between the two groups of patients participating in this study (p>0.05), which were comparable.

Inclusion and exclusion criteria

Inclusion criteria were as follows: ①symptoms: vulvar pruritus; ②signs: vulvar hypopigmentation; and ③pathological biopsy: pathological diagnosis of vulvar lichen simplex chronicus or vulvar lichen sclerosus. Exclusion criteria were as follows: ① combined malignant tumors; ② combined lactating and pregnant women; and ③ drug allergy.

Treatment

The observation group was given 2% crisaborole ointment (produced by Pharmacia and Upjohn Company LLC, specification

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30g/branch/box, HJ20200022) to be applied to the vulvar lesions twice a day. The control group was a placebo group, and vitamin E was given to be applied externally to the vulvar lesions by cutting small openings in the vitamin E and squeezing out the liquid from it to be applied externally to the vulvar lesions twice a day. The drug was discontinued during menstruation and applied after menstruation was cleared. Follow-up was conducted after 2 weeks of treatment. The patient was also instructed to keep the vulva clean and dry and to avoid eating allergic and spicy foods.

Evaluation indicators

Indicators for determining the treatment effect: With reference to the Guidelines for Clinical Research on New Chinese Medicines (2018 edition), the scores were based on skin color, lesion area, and itching scoring method. (1) Skin color: 0 points: normal color; 1 point: red skin; 2 points: pink skin; 3 points: white skin. (2) Lesion area: 0 points: no lesion area; 1 point: lesion area less than 30% of the vulva; 2 points: lesion area accounts for 30–50% of the vulva; 3 points: lesion area greater than 50% of the vulva. (3) Itching scoring method: the itching visual analog scale (VAS) evaluation method was used, which integrates the degree of itching, scratching behavior, and the impact on sleep for evaluation; scoring method: 1 point: no itching; 2 points: occasional slight itching; 3 points: itching, need to scratch gently to stop itching, does not affect sleep; 4 points: very itchy, cannot help scratching hard many times to stop itching, waking up itching at night, but can fall asleep after scratching hard; 5 points: intense itching, requiring a burst of non-stop scratching, even scratching the skin, difficult to fall asleep habitually, or waking up after sleep with repeated scratching and itching. Overall score=skin color score×lesion extent score+itching score.

Judgment of clinical efficacy

Clinical cure: ① the area of vulvar leukoplakia is reduced by more than 80%; ② the symptoms of vulvar itching disappear; and ③ the skin color and texture of the lesion return to the level of the surrounding normal skin. Significant effect: ① the area of vulvar leukoplakia is reduced by 50–80%; ② occasional vulvar itching; and ③ the skin color of the lesion turns pink or light brown. Improvement: ① 30–50% reduction in the area of vulvar leukoplakia; ② reduction in the degree of vulvar itching; and ③ improvement in the color and texture of the lesion area. Ineffective: ① the area of vulvar leukoplakia is not significantly reduced; ② the degree of vulvar itching is not reduced. Cure, significant effect, and improvement are all considered effective.

Statistical methods

The SPSS19.00 statistical software was selected to collate the relevant experimental data, and the measurement data were expressed as percentages (%), using χ^2 validation; a t-test was used for the comparison between the two groups with normal distribution, a non-parametric test was used for those with non-normal distribution, and a chi-square test was used for the count data. P<0.05 indicates a statistically significant difference.

RESULTS

Comprehensive score of two groups of patients after 2 weeks of treatment: As shown in Table 1, the lesion overall score before treatment was 2.29±0.85 in the observation group and 2.28±1.14 in the control group. There was a 0.45 reduction in lesion overall scale score from baseline in the observation group (p<0.05) and a 0.25 decrease from baseline in the control group (p>0.05). The difference in the observation group statistically indicates that the crisaborole treatment was effective. Comparison of adverse reactions between the two groups: No serious side effects were observed during and after treatment in both groups.

Comparison of the efficiency of the two groups: The effective rate was 92% in the observation group and 52% in the control group. After one course of treatment, the efficiency of the observation group was higher than that of the control group, and the difference was statistically significant (p<0.001).

Comparison of adverse reactions and recurrence conditions: Common adverse reactions of external drugs outside the vulva include local allergy, redness, pain, and even ulceration. There are two cases in the observation group with local pain and ulceration, and the local adverse reactions subsided after timely withdrawal, with an incidence of 2/50 (4%). The control group had no adverse effects at 0%. The incidence of adverse effects between the control and observation groups was statistically significant (P<0.05), as shown in Table 2.

DISCUSSION

Vulvar leukoplakia is a vulvar skin disease with pruritus as the main symptom and vulvar skin hypopigmentation as the main sign, including vulvar lichen simplex chronicus and vulvar lichen sclerosus. The common pathology is hyperkeratosis of the epidermis with inflammatory cell infiltration in the dermis[4-6].

At present, the main treatments for vulvar leukoplakia include topical drug therapy[7] and physiotherapy, and physiotherapy

Table 1. Comparison of skin color scores before and after treatment between two groups of patients.

		Skin color score (points)	Lesion extent score (points)	Itching score (points)	Overall score (points)
Observation group (50n=case)	Before treatment	2.45±0.76	2.29±0.85	2.76±0.73	11.19±6.46
	After treatment	1.62±0.16 D*	1.84±0.41 D*	0.64±0.56 D*	4.94±6.45 D*
Control group (50n=case)	Before treatment	2.44±0.69	2.28±1.14	2.75±0.84	11.89±4.35
	After treatment	2.24±0.28Δ	2.03 ±1.36D	2.45±0.11 D	10.44±4.86 D

Comparison between the observation group and the control group, DP>0.05; compared with the same group before treatment, DP<0.05; compared with the control group after treatment, *P<0.05.

Table 2. Comparison of clinical efficacy between the two groups.

Group	Number of cases	Cure	Significant effect	Improvement	Ineffective	Effective rate	χ^2	p
Observation group	50	21	15	10	4	92%	27.71	<0.001
Control group	50	5	7	14	24	52%		

includes focused ultrasound[8], photodynamic therapy[9], and microablative fractional radiofrequency (MFR), of which MFR was an innovative, easy-to-use intervention to be considered the first therapeutic option or complement medical treatments for vulvar leukoplakia[10].

Topical drug therapy mainly includes glucocorticoids, testosterone propionate cream, progesterone cream, and topical immunosuppressants[11]. However, previous studies on drug treatment for vulvar leukoplakia are inadequate. Among them, glucocorticoids are effective in improving pruritus, but the recurrence rate is high after stopping the drug, and long-term use has the side effects of skin atrophy, skin pigmentation changes, and easy infection[12], so they are only used as short-term drugs. Testosterone propionate cream is not effective in improving pruritus, and long-term use has the side effect of causing masculinization[13,14]. Progesterone cream has few side effects but not effective. Some studies found that the efficacy of testosterone propionate ointment and progesterone ointment in the treatment of vulvar leukoplakia was similar to that of placebo[14]. Long-term use of topical immunosuppressants has the potential to induce vulvar cancer[15]. These drawbacks reduce patients' compliance and limit the long-term use of these drugs. In addition, the irregular treatment causes some patients to have poor results with the above drugs, leading to chronic prolongation of the disease. Therefore, clinicians need to find a drug that is effective and can be used safely for a longer period of time.

This study showed that there was a meaningful improvement in the histopathological changes of vulvar leukoplakia with crisaborole therapy compared with vitamin E treatment, indicating that crisaborole is an effective therapy for vulvar leukoplakia. In contrast to the aforementioned drugs used to treat vulvar leukoplakia, our administration is safer and more effective. Clenbuterol ointment is a small-molecule topical PDE-4 inhibitor approved in the United States for the treatment of patients aged 2 years and older with mild to moderate atopic dermatitis[16]. We decided to use crisaborole ointment for topical treatment due to its mechanism of action:

1. intramolecularly targets and selectively acts on the PDE-4 enzyme in the degradation of cyclic adenosine monophosphate (CAMP);
2. inhibits the release of inflammatory cytokines by inhibiting PDE-4 enzyme, which in turn increases CAMP-dependent protein kinase A activity and inhibits NFAT and NFKB signaling pathways downstream;
3. inhibits the release of various cytokines, including tumor necrosis factor-α, interferon-γ, and interleukin-2.

In addition, due to its small molecular weight, crisaborole facilitates transdermal penetration[17].

In addition, data from pivotal studies and long-term safety studies suggest that patients can well tolerate long-term treatment (>48 weeks) with crisaborole ointment[18]. Therefore, crisaborole ointment meets the safety needs of patients with vulvar leukoplakia who require long-term maintenance therapy.

The weakness of this study was its relatively small sample size. However, an a priori sample size calculation determined that the selected sample was large enough to demonstrate a

clinically meaningful difference in the histopathological changes of vulvar leukoplakia.

Vulvar leukoplakia includes a range of disorders that compromise the quality of life of patients. Standard forms of traditional treatment have their limitations, side effects, and complications. Hence, alternative methods are needed to influence them, such as the use of crisaborole ointment, which can effectively treat vulvar leukoplakia and improve the symptoms and pathological changes of the vulvar skin. Moreover, the adverse reactions of crisaborole were less, so it is well prescribed by clinicians. Continuous monitoring these patients will be helpful to better understand the role crisaborole could play in the treatment of vulvar leukoplakia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki and approved by the Jiangxi maternal and Child Health Hospital. All participants signed an informed consent form for inclusion in the study.

AUTHOR'S CONTRIBUTIONS

LL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of malnutrition frequency and related factors of geriatric patients in need of home healthcare

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SUMMARY

OBJECTIVE: The nutritional status of frail elderly people receiving home health services should be evaluated. This study aimed to determine the nutritional status of patients aged ≥65 years registered in the Home Healthcare Services unit and investigate the factors that may be associated with malnutrition.

METHODS: This cross-sectional descriptive study was conducted during routine visits to patients and their caregivers. A total of 161 patients were asked to fill in surveys asking about sociodemographic characteristics, patient history, and clinical status. Anthropometric measurements were taken from all patients. The Mini Nutritional Assessment Short Form was applied to the patients for screening purposes. Patients who scored ≤11 on the Mini Nutritional Assessment Short Form were then asked to complete the full Mini Nutritional Assessment form.

RESULTS: According to the Mini Nutritional Assessment Short Form and Mini Nutritional Assessment tests, almost half of the elderly patients included in the study (49.7%, n=161) were malnourished or at risk of malnutrition. Analyses showed that those who had COVID-19 [odds ratio (OR): 9.423, 95%CI 2.448–36.273) and those diagnosed with dementia/depression (OR: 8.688, 95%CI 3.246–23.255) were more likely to be malnourished, whereas those with diabetes (OR: 0.235, 95%CI 0.084–0.657) were less likely to have malnutrition. Strikingly, those who were fed by caregivers (OR: 15.061, 95%CI 3.617–62.710) were also more likely to be malnourished than those with self-feeding ability.

CONCLUSION: Malnutrition or the risk of malnutrition is common in elderly patients receiving home care services. Many factors can have an impact on malnutrition.

KEYWORDS: Home care agencies. Malnutrition. Aged. Nutrition assessment.

INTRODUCTION

With the increase in human life span and the decrease in fertility, the number of elderly people in the total population is increasing. In 2020, the number of elderly people aged ≥65 years was 727 million worldwide, which is estimated to exceed 1.5 billion by 2050[1]. In Turkey, in 2015, the ratio of the elderly population to the total population was 8.2%, which rose to 9.5% in 2020 and is expected to continue rising[2].

Malnutrition is more common in elderly patients than in younger adults, with a greater impact on many outcomes, such as physical function, healthcare use, length of hospital stay, length of postoperative hospital stay, and healthcare expenditures[3-7]. Therefore, determining the factors causing malnutrition in the elderly people will facilitate taking preventive measures and providing relevant treatment plans, which will reduce cost.

Most of the studies on malnutrition so far have collected data from outpatient clinics, inpatient services, and nursing homes[3,5,7-9]. Yet, receiving home health services has also been identified as a risk factor for malnutrition[10]. Home health service is a care system established in Turkey to strengthen and support the primary healthcare system by providing preventive, therapeutic, and rehabilitative care in the home environment[11]. There are only a few studies in the literature on malnutrition and related factors in home healthcare units[12,13]. Besides, these studies generally excluded frail elderly people who are bedridden, have mental status disorders, and are at major risk of malnutrition. Furthermore, these studies often neglected the effects on the caregiver.

This is one of the most comprehensive studies conducted during the pandemic in a developing country where the elderly population is rapidly increasing. This study aims to determine the nutritional status of elderly patients aged ≥65 years registered in the Home Healthcare Services unit and investigate the factors that may be associated with malnutrition.

METHODS

Study design and pilot implementation

This study uses a cross-sectional and descriptive research design to examine the malnutrition status and related factors of patients aged ≥65 years registered in the home health unit.

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Before starting the research, a pilot study was conducted with five elderly patients who received home care services in November 2020. Participants in the pilot implementation were excluded from the study.

During the routine visits, questionnaires and nutritional scales were administered to participants meeting the study criteria, and anthropometric measurement of each patient was recorded.

Participants' characteristics

The study included elderly people aged ≥65 years who were registered in the Home Healthcare Unit and agreed to participate in the study. Those who used tube-feeding methods, such as nasogastric tube, percutaneous endoscopic gastrostomy, percutaneous endoscopic jejunostomy, and those with bilateral limb amputation and elephantiasis were excluded from the study.

Nutritional status screening and evaluation

First, the Mini Nutritional Assessment Short Form (MNA-SF) was applied to screen the nutritional status of the patients. MNA-SF is an initial screening scale consisting of a total of six questions on food intake, weight loss status, mobility, acute illness/stress, neuropsychological status, and body mass index (BMI). An MNA-SF score of 14–12 indicates a normal nutritional status, 11–8 suggests a risk of malnutrition, and 0–7 reveals malnutrition. If the screening score is ≤11 points, completing the full evaluation scale is recommended to obtain the malnutrition indicator score. The Mini Nutritional Assessment (MNA) evaluation scale consists of 12 questions concerning dependency, medication usage, pressure sores, food and drink intake, number of meals, nutritional autonomy, self-perception of health and nutrition, and arm and calf circumference. The total MNA-SF and MNA evaluation scores determine the allocation of the subjects into the following groups: 30–24 normal nutritional status, 23.5–17 risk of malnutrition, and <17 as malnourished[14].

Data survey forms

The surveys were completed by the researcher during the face-to-face interview with the participant. First, a sociodemographic data questionnaire consisting of nine questions on the patient's age, gender, place of residence, marital status, educational status, perceived income level, and habits, and a questionnaire consisting of 10 questions on the patient's past or chronic diseases, history, and clinical status were obtained from the patient. Information was obtained from the caregivers of patients who could not communicate verbally. To determine the effects of the caregivers and their numbers on the patient's nutritional status, a questionnaire comprising six questions about the number of caregivers, their relation to the patient, age, gender, education level, and duration of care service was applied to the caregivers.

Anthropometric measurements

After completing the questionnaire forms, the patients' height (cm), weight (kg), and calf and arm circumferences (cm) were measured based on the measurement principles. Height measurements of wheelchair-bound or immobile patients were taken while lying flat. In weight measurements, the last known weight values were recorded. The BMI value (kg/m^2) was calculated by dividing the patient's weight (kg) to the square of the patient's height in meters (m^2).

Ethical approvals

Necessary written approvals were obtained from the local ethics committee (dated January 21, 2021, decision number 2021/11). Informed consent forms providing all details of the study were given to the patients, their relatives were informed in detail about the study, and their written consent was obtained.

Statistical analysis

Data were analyzed using the SPSS 25.0 (SPSS Inc., Chicago, IL, USA) statistical software package. Findings were presented as numerical values, percentages, median, quartile ranges, arithmetic mean, and standard deviation. The conformity of the numerical variables to the normal distribution was examined using the Shapiro-Wilk test. Categorical variables were compared using χ^2 and Fisher's exact tests when necessary. Numerical variables were compared using the Mann-Whitney U test or Kruskal-Wallis test. Correlations between numerical variables were examined with the Spearman's correlation test. Logistic regression models were used to examine the relationships between the nutritional categories of the patients and the various variables in the study. For this purpose, a two-category nutrition-dependent variable was created by combining the risky MNA-SF nutritional category group with the malnourished group. $p<0.05$ was considered statistically significant.

RESULTS

Participants

A total of 161 people, 111 (68.9%) women and 50 (31.1%) men, participated in the study. The mean age of the patients was 80.7 years (±7.7). The youngest patient was 65 years old, and the oldest patient was 102 years old. Of the patients, 59.1% were illiterate, 75.2% were living in the city, and 57.1% had

lost their spouses. Most of the caregivers were female (71.4%), and first-degree relatives/spouses (68.3%), and the majority were literate (87%).

Examination of the participants' disease status revealed that the most common diseases were hypertension (77%) and cardiovascular disease (55.3%). Also, dementia/depression (44.7%) and diabetes mellitus (34.8%) were prevalent. Of the patients, 23% were hospitalized in the last 3 months, 17.4% had a history of COVID-19, and 13% had a decubitus ulcer.

Distribution of patients' nutritional status

The mean MNA-SF score of 161 patients who took the MNA-SF test was 9.9 (±3.2), and the median value was 11. The full MNA evaluation test was applied to 91 patients who scored ≤11 on the MNA-SF. The mean MNA evaluation total score of these patients was 17.3 (±5.5), and the median value was 18.5. The initial MNA-SF screening test determined that 43.5% of 161 patients had normal nutritional status. Of the 91 patients who could not pass the MNA-SF test and underwent the MNA evaluation test, 49.4% were at risk of malnutrition and 38.5% were malnourished. Collectively, the MNA-SF and MNA evaluation tests determined that 21.7% of 161 patients were malnourished, 28% were at risk of malnutrition, and 50.3% had normal nutrition.

Bivariate comparisons

Mini Nutritional Assessment Short Form scores of male patients were significantly lower than female patients (p=0.001). Likewise, categorization of nutritional status showed that the MNA-SF categories were associated with gender (p=0.018).

The mean BMI and calf and arm circumferences were 31.4 kg/m^2, 34.6 cm, and 28.3 cm, respectively. According to MNA-SF nutrition categories, the measurements of BMI (p<0.001), calf circumference (p<0.001), and arm circumference (p<0.001) of the patients were significantly lower in the malnourished group than those in the other groups.

According to MNA-SF, patients' nutritional categories were associated with having COVID-19 (p<0.001), hypertension (p=0.003), diabetes mellitus (p=0.014), decubitus sores (p=0.006), dementia/depression (p=0.001), history of hospitalization in the last 3 months (p=0.013), dietary patterns (self-feeding/with the caregiver's intermittent assistance/continuously by the caregiver) (p=0.001), presence of mouth/dental problems (p=0.016), and being bedridden (p=0.001) (Table 1).

Multivariate comparisons

The logistic regression model created to examine the association of the MNA-SF nutritional categories with the patients' age, gender, and anthropometric characteristics revealed that the probability of being malnourished increased with age and decreased with increasing calf circumference (Table 2).

In addition, the model created to examine the association of MNA-SF nutritional categories with patients' comorbidities determined the variables of having COVID-19, diabetes mellitus, decubitus sores, dementia/depression, and hospitalization in the last 3 months to be significant. Also, patients fed with intermittent caregiver assistance and patients constantly fed by caregivers were also more likely to be malnourished than self-feeding patients (p<0.001) (Table 3).

DISCUSSION

Malnutrition is multifactorial, and many studies have shown that chronic diseases are one among them[8,10,15]. This study determined that decubitus sores, dementia/depression, hospitalization in the previous 3 months, and COVID-19 infection were associated with poor nutritional status in the elderly people.

The probability of malnutrition was found to be higher in the elderly people with a history of COVID-19. Other studies have also reported malnutrition and the risk of malnutrition to be higher in elderly patients with COVID-19 infection[9]. In addition to respiratory tract symptoms, gastrointestinal symptoms such as diarrhea, mild abdominal pain, nausea, vomiting, loss of appetite, and loss of taste and smell are also common in elderly patients with COVID-19. These symptoms may be more severe in frail elderly people. Besides, inflammation indicators such as C-reactive protein, ferritin, TNF-alpha, and interleukin family factors increase in response to the acute inflammatory state that occurs during COVID-19 infection. The increase in these acute-phase proteins accelerates the destruction of albumin and proteins in the muscles[16]. All these factors increase malnutrition and the risk of malnutrition in patients with COVID-19.

This study revealed that the incidence of malnutrition was lower in patients with diabetes (p=0.006). However, a case-control study by Turnbull and Sinclair determined that the MNA scores of elderly individuals aged ≥65 years with diabetes were significantly lower than those without diabetes[15]. Similarly, Li et al. determined diabetes mellitus as a risk factor for malnutrition[9]. In addition to the increased adipose tissue and inactivity in the elderly constituting risk factors for diabetes, oral antidiabetic drugs and insulins used may also be factors for obesity. Indeed, some oral antidiabetic drugs have side effects that cause edema (such as thiazolidinediones) and weight gain (such as sulfonylureas). Again, since insulin is an anabolic hormone, patients with type 1 and type 2 diabetes who use insulin may start to gain weight later. Moreover, hypoglycemia experienced in insulin users may be another reason for weight gain.

It was determined that patients diagnosed with dementia/depression have a higher risk of malnutrition as dementia in the elderly people is a significant factor of dependence regarding daily living activities and nutrition. Depression is not just an emotional breakdown. At the same time, it can have physical consequences such as sleep and appetite impairment. These factors can lead to weight loss and malnutrition in the elderly people. Saka et al. determined that the elderly people

Table 1. The relationship of patients' Mini Nutritional Assessment Short Form nutritional categories and accompanying/past diseases with nutritional and caregiver status distributions.

Variable		MNA-SF category			Test statistic	p
		Normal nutritional status, n(%)	At risk of malnutrition, n(%)	Malnourished, n(%)		
Having COVID-19	No	65(92.8)[a]	47(83,9)[a]	21(60)[b]	17.638*	**<0.001**
	Yes	5 (7.2)[a]	9(16.1)[a]	14(40)[b]		
Hypertension Table 1 (HT)	No	8(11.4)[a]	15(26.8)[a,b]	14(40)[b]	11.464	**0.003**
	Yes	62(88.6)[a]	41(73.2)[a,b]	21(60)[b]		
Diabetes mellitus (DM)	No	37(52.8)[a]	41(73.2)[a,b]	27(77.1)[b]	8.487	**0.014**
	Yes	33(47.2)[a]	15(26.8)[a,b]	8(22.9)[b]		
Cardiovascular disease (CVD)	No	28(40)[a]	28(50)[a]	16(45.7)[a]	1.276	0.528
	Yes	42(60)[a]	28(50)[a]	19(54.3)[a]		
Malignancy	No	60(85.7)[a]	47(83.9)[a]	27(77.1)[a]	1.258	0.533
	Yes	10(14.3)[a]	9(16.1)[a]	8(22.9)[a]		
Decubitus ulcer	No	66(94.3)[a]	49(87.5)[a,b]	25(71.4)[b]	10.770	**0.006**
	Yes	4(5.7)[a]	7(12.5)[a,b]	10(28.6)[b]		
Dementia/depression	No	54(77.1)[a]	20(35.7)[b]	15(42.9)[b]	24.391	**<0.001**
	Yes	16(22.9)[a]	36(64.3)[b]	20(57.1)[b]		
Other chronic diseases	No	25(35.7)[a]	28(50)[a]	19(54.3)[a]	4.223	0.121
	Yes	45(64.3)[a]	28(50)[a]	16(45.7)[a]		
History of hospitalization in intensive care	No	53(75.7)[a]	33(58.9)[a]	23(65.7)[a]	4.090	0.129
	Yes	17(24.3)[a]	23(41.1)[a]	12(34.3)[a]		
History of hospitalization in the last 3 months	No	60(85.7)[a]	43(76.8)[a,b]	21(60)[b]	8.719	**0.013**
	Yes	10(14.3)[a]	13(23.2)[a,b]	14(40)[b]		
Nutrition	Self	61(87.1)[a]	23(41.1)[b]	10(28.6)[b]	47.022*	**<0.001**
	Intermittent assistance	5(7.2)[a]	15(26.8)[b]	7(20)[a,b]		
	Total caregiver dependence	4(5.7)[a]	18(32.1)[b]	18(51.4)[b]		
Mouth or dental problem	No	52(74.3)[a]	36(64.3)[a,b]	16(45.7)[b]	8.332*	**0.016**
	Yes	18(25.7)[a]	20(35.7)[a,b]	19(54.3)[b]		
Live bedridden	No	60(85.7)[a]	34(60.7)[b]	14(40)[b]	23.658*	**<0.001**
	Yes	10(14.3)[a]	22(39.3)[b]	21(60)[b]		
Number of people caring for the patient	1 caregiver	30(42.8)[a]	19(33.9)[a]	15(42.8)[a]	3.655*	0.727
	2 caregivers	30(42.8)[a]	23(41.1)[a]	14(40)[a]		
	3 caregivers	7(10)[a]	12(21.4)[a]	5(14.3)[a]		
	4 and over caregivers	3(4.3)[a]	2(3.6)[a]	1(2.9)[a]		
Caregiver intimacy	First-degree relative/spouse	46(65.7)[a]	37(66.1)[a]	27(77.1)[a]	2.416*	0.653
	Second-degree relative	4(5.7)[a]	2(3.6)[a]	2(5.7)[a]		
	Three-degree relative/not a relative	20(28.6)[a]	17(30.3)[a]	6(17.1)[a]		
Gender of the caregiver	Female	46(65.7)[a]	45(80.4)[a]	24(68.6)[a]	3.448*	0.178
	Male	24(34.3)[a]	11(19.6)[a]	11(31.4)[a]		
Educational status of the caregiver	Illiterate	7(10)[a]	9(16.1)[a]	5(14.3)[a]	5.335*	0.493
	Primary school graduate	50(71.4)[a]	30(53.6)[a]	23(65.7)[a]		
	High school graduate	8(11.4)[a]	8(14.2)[a]	38(8.6)[a]		
	Graduated from a university	5(7.1)[a]	9(16.1)[a]	4(11.4)[a]		

*χ^2 test statistical value. The presence of the same superscript letters in a row indicates that there is no statistical difference between the cells. Bold indicates statistically significant p-values.

Table 2. Logistic regression model including anthropometric features affecting the Mini Nutritional Assessment Short Form nutritional category.

	OR	95%CI		p
Gender (male to female)	1.411	0.292	1.720	0.446
Age (years)	1.057	1.006	1.111	**0.029**
BMI (kg/m^2)	0.975	0.893	1.065	0.578
Calf circumference (cm)	0.818	0.737	0.909	**<0.001**
Mid–upper arm circumference (cm)	1.044	0.955	1.141	0.344

Dependent variable: MNA-SF category (normal nutritional status/malnourished). OR: odds ratio; CI: confidence interval. Bold indicates statistically significant p-values.

with dementia and depression, as well as fecal incontinence, insomnia, and neurological diseases, had a higher risk of malnutrition, and those with poor nutritional status needed more caregivers[8]. The individuals themselves can best understand their bodily needs, such as hunger and thirst. However, the elderly people experiencing problems such as dementia, weakness, and illness need the care and guidance of their caregivers. In this study, the elderly people who were fed with caregiver assistance were more likely to have malnutrition than the elderly people with self-feeding ability. Therefore, the influence of the caregiver should not be ignored in the elderly people, who often need the support of a caregiver.

Table 3. Logistic regression model including comorbid/previous disease, and nutritional and caregiver status variables affecting the Mini Nutritional Assessment Short Form nutritional category.

	OR	95%CI		p
Having had COVID-19	9.423	2.448	36.273	**0.001**
Being diagnosed with HT	0.345	0.107	1.118	0.076
Being diagnosed with DM	0.235	0.084	0.657	**0.006**
Being diagnosed with CVD	0.682	0.251	1.851	0.452
Being diagnosed with malignancy	1.342	0.392	4.595	0.639
Having a decubitus ulcer	5.852	1.096	31.251	**0.039**
Being diagnosed with dementia/depression	8.688	3.246	23.255	**<0.001**
Other diseases	0.331	0.131	0.836	0.019
Having a history of hospitalization in intensive care	3.050	0.813	11.447	0.098
History of hospitalization in the last 3 months	4.452	1.063	18.644	**0.041**
Nutrition (rc: *self*)				**<0.001**
Nutrition (*intermittent assistance*)	11.889	3.186	44.368	**<0.001**
Nutrition (*total caregiver dependence*)	15.061	3.617	62.710	**<0.001**
Having a mouth or dental problem	1.557	0.631	3.845	0.337
Live bedridden	1.131	0.261	4.907	0.869
Number of people caring for the patient (rc: 1 person)				0.789
Number of people caring for the patient (2 persons)	0.651	0.250	1.696	0.380
Number of people caring for the patient (3 persons)	1.101	0.306	3.965	0.883
Number of people caring for the patient (>3 persons)	0.980	0.127	7.540	0.984
Caregiver intimacy (rc: First-degree relative/spouse)				0.972
Caregiver intimacy (Second-degree relative)	1.268	0.150	10.725	0.827
The caregiver is male	0.290	0.102	0.821	0.020
Educational status of the caregiver (rc: illiterate)				0.286
Educational status of the caregiver (primary school)	0.966	0.245	3.811	0.961
Educational status of the caregiver (high school)	1.399	0.225	8.677	0.719
Educational status of the caregiver (university)	4.116	0.616	27.516	0.144

Dependent category: MNA-SF nutrition category. HT: hypertension; DM: diabetes mellitus; CVD: coronary vascular disease; rc: reference category. Bold indicates statistically significant p-values.

This study is a comprehensive research that examines many factors that may be related to malnutrition in Home Healthcare Units in a city. The strengths of this study include a pilot study implementation before the study, face-to-face interviews in the person's living environment, and the measurements made by a single researcher. However, it also has some limitations. First, this study was conducted in a single center and cannot be generalized to the entire population. Appropriate conditions for weight and height measurements could not be provided as some of the elderly patients were immobilized or had difficulty standing. Another limitation of the study is the inclusion of the caregivers' answers to the questions about the patient's nutrition and health perception in the MNA evaluation test instead of the patients who could not be contacted.

CONCLUSION

In this study, it was determined that malnutrition and the risk of malnutrition are high in elderly patients receiving home healthcare services. In addition, factors such as age, COVID-19, DM, decubitus, dementia/depression, hospitalization history in the last 3 months, calf circumference, and eating with the help of a caregiver were found to have a (negative) impact on the nutritional deficiency. Studies with a larger patient population should be conducted on patients receiving services from home healthcare units who are at high risk of malnutrition.

ETHICS COMMITTEE APPROVAL

Ethics committee approval for this study was taken from the Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine with protocol number 2021/11. Date: **January 21, 2021.** Number: 2021/11.

AUTHORS' CONTRIBUTIONS

MNSÖ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft. **CA:** Conceptualization, Formal Analysis, Methodology, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Syndecan-1 as a marker to predict acute kidney injury after isolated coronary artery bypass graft operations

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SUMMARY

OBJECTIVE: Postoperative acute kidney injury is an important problem that can occur after coronary artery bypass graft operations, and it is important to identify risky patient groups preoperatively. This study aimed to investigate the importance of preoperative syndecan-1 levels in predicting acute kidney injury after elective coronary artery bypass graft operations accompanied by cardiopulmonary bypass.

METHODS: Patients who underwent coronary artery bypass graft operation in our clinic between March 1 and May 10, 2022, were included in this prospective study. Patients who did not develop acute kidney injury in the postoperative period were recorded as group 1 and patients who developed it were recorded as group 2.

RESULTS: A total of 79 patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass were included in the study. There were 55 patients in group 1 and 24 patients in group 2. There was no difference between the groups in terms of age, gender, diabetes mellitus, body mass index, smoking, and hyperlipidemia rates. In multivariate logistic regression analysis, increased blood product use (odds ratio 1.634; 95%CI 1.036–2.579; p=0.035), preoperative high creatinine (odds ratio 59.387; 95%CI 3.034–1162.496; p=0.007), and high syndecan-1 (odds ratio 1.015; 95%CI 1.002–1.028; p=0.025) were independent predictors of acute kidney injury.

CONCLUSION: This study revealed that elevated preoperative syndecan-1 is associated with acute kidney injury after isolated coronary artery bypass graft accompanied by cardiopulmonary bypass and has prognostic utility independent of other recognized risk factors.

KEYWORDS: Cardiopulmonary bypass. Inflammation. Acute kidney injury. Coronary artery bypass.

INTRODUCTION

Coronary artery bypass graft (CABG) operations are one of the most significant treatment modalities in the treatment of atherosclerotic heart disease. These operations are frequently performed with cardiopulmonary bypass (CPB)[1]. In the early period after these operations, undesirable problems such as heart failure, rhythm problems, cerebrovascular events, and acute kidney injury (AKI) may occur. AKI occurs in up to 25–40% of cases after CABG operations, and the use of CPB systems can raise these rates[2]. It is important to identify risky patient groups preoperatively for taking necessary precautions.

Some parameters obtained from preoperative routine blood tests have been investigated to predict the risk of AKI after cardiac surgery. Neutrophil lymphocyte ratio (NLR) is one of the most commonly used parameters. In a recent study, it was shown that a preoperative high NLR value could predict the risk of AKI after CABG operations accompanied by CPB[3]. Syndecan-1 (SDC-1) is an important indicator of endothelial glycocalyx damage, which plays an important role in the pathophysiology of AKI[4]. In a study, it was shown that it may be a predictor of AKI developing after pediatric cardiac surgery[5].

This study aimed to investigate the importance of preoperative SDC-1 levels in predicting AKI after elective CABG operations accompanied by CPB.

METHODS

Patients who underwent CABG operation with CPB in our clinic between March 1 and May 10, 2022, were included in this prospective study. The study was started after the approval of the local ethics committee. The study was carried out in accordance with the Helsinki Declaration criteria. Demographic data of the patients (age, gender, etc.), preoperative blood values (hemoglobin, white blood cell, neutrophil, lymphocyte, albumin, C-reactive protein, SDC-1, etc.), operation data (CPB and aortic cross-clamp [ACC] duration), and postoperative

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characteristics (AKI status, blood product usage status, etc.) were recorded.

Patients with concomitant cardiac surgery disease, serum creatinine values above 1.5 mg/dL in the preoperative period, liver failure, active infection or malignancy, preoperative hemoglobin values of 10 g/dL and below, and patients with congestive heart failure were excluded from the study. In all, 82 consecutive patients were included in the study. Due to bleeding diathesis in 1 patient in the early postoperative period, development of early cardiopulmonary failure in 1 patient, and operative mortality in 1 patient, they could not be included in the postoperative AKI evaluation and were excluded from the study. After applying the exclusion criteria, a total of 79 patients were included in the study.

Blood samples were taken from antecubital veins before the operation. Hemogram parameters were measured by using an automated hematological analyzer (Coulter LH 780 Analyzer, CA, USA). The complete blood cell parameters were measured through Coulter Erythrolyse II Reagent Kit (Beckman Coulter, Ireland). EDTA tubes were used to collect blood samples that were immediately centrifuged for 20 min at 2,000–3,000 rpm and frozen at -80°C for later measurement of SDC-1. SDC-1 was measured as a biomarker of endothelial glycocalyx injury (Abcam, Cambridge, MA, USA). The detection range for SDC-1 is 8–256 ng/mL and the intra-assay coefficient of variation is 6.2%[6].

Diagnosis of postoperative AKI was determined according to the Kidney Disease Improving Global Outcomes (KDIGO) classification[7]. According to the results of this evaluation, patients who did not develop AKI in the postoperative period were recorded as group 1 (n=55), and patients who developed any of the KDIGO stages were recorded as the renal failure group (group 2, n=24).

Surgical technique

General anesthesia and median sternotomy technique were used in all patients. Aortic-venous two-stage cannulation was performed in all CABG operations, and standard CPB systems were used in mild hypothermia (32°C). In all patients, cardiac arrest was achieved with an initial blood cardioplegia of approximately 1,000 mL (10–15 mL/kg). Continuation of cardiac arrest was maintained with approximately 300 mL of blood cardioplegia at 15–20-min intervals. CPB was provided by roller pumps and membrane oxygenator (Maquet, Getinge Group, Rastatt, Germany) at a flow rate of 2–2.4 L/min/m^2, and arterial filters were used in all patients. Distal and proximal anastomoses were performed under ACC. Hot-shot blood cardioplegia without hot potassium was given to all patients before the ACC was removed. After the operation was completed, all patients were transferred to the intensive care unit with vital monitoring.

Statistical analysis

Statistical analysis was utilized using SPSS 21.0 (IBM Statistical Package for the Social Sciences Statistic Inc., version 21.0, Chicago, IL, USA). Normality distribution of data was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. The Student's t-test was used for normally distributed data (mean and standard deviation), and the Mann-Whitney U test was used for non-normally distributed data (median and minimum-maximum). Nominal variables were expressed in frequency and percentage, and the chi-square test was used for analysis. Postoperative AKI predictors were analyzed using binary logistic regression analysis. The predictive value of SDC-1 was determined using receiver operating characteristic (ROC) curve analysis and calculation of area under curve. A $p<0.05$ was considered significant.

RESULTS

A total of 79 patients undergoing CABG surgery with CPB were included in the study. There were 55 patients in group 1 (without kidney injury) and 24 patients in group 2 (with kidney injury). There was no difference between the groups in terms of age, gender, DM, BMI, smoking, and hyperlipidemia rates. In addition, preoperative ejection fraction and use of angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, and acetylsalicylic acid were similar in both groups (Table 1).

Preoperative blood parameters and operative and postoperative features of all patients are presented in Table 1. There was no difference between the groups in terms of white blood cells, hemoglobin, platelet counts, neutrophil, lymphocyte, blood urea nitrogen, and C-reactive protein value. While cross-clamp times and the number of distal anastomoses were similar between the two groups, total perfusion times and the use of packed blood products were significantly higher in group 2 ($p=0.038$ and $p<0.001$, respectively). Creatinine, SDC-1, and NLR values were significantly higher in group 2 ($p<0.001$, $p<0.001$, and $p=0.037$, respectively).

To analyze the factors affecting the development of AKI in the postoperative period, binary logistic regression analysis was utilized (Table 2). In univariate analysis, total perfusion time (OR [odds ratio] 1.021; 95%CI [confidence interval] 1.001–1.043; $p=0.045$), need of inotropic support (OR 4.523; 95%CI 1.627–12.575; $p=0.004$), increased blood product use (OR 1.971; 95%CI 1.358–2.861; $p<0.001$), preoperative high creatinine (OR 125.742; 95%CI 10.773–1467.616; $p<0.001$), NLR (OR 0.826; 95%CI 0.678–0.982; $p=0.039$), and high SDC-1 (OR 1.016; 95%CI 1.004–1.027; $p=0.008$) were correlated with the development of AKI. In multivariate logistic regression analysis, increased blood product use (OR 1.634; 95%CI 1.036–2.579;

Table 1. Demographic data, preoperative laboratory variables, and perioperative features of the patients.

Variables	Group 1 (n=55)	Group 2 (n=24)	p-value
Age (years)	60.8±8.7	62.2±10.3	0.237
Male gender, n (%)	44 (80%)	21 (87.5%)	0.422
Diabetes mellitus, n (%)	16 (29.1%)	9 (37.5%)	0.460
Hypertension, n (%)	30 (54.5%)	17 (70.8%)	0.175
BMI, kg/m^2	27.9±3.4	28.3±3.3	0.934
Smoking, n (%)	31 (56.4%)	16 (66.7%)	0.391
Hyperlipidemia, n (%)	33 (60%)	19 (79.2%)	0.163
Ejection fraction (%)	55 (30–67)	55 (35–65)	0.338
ASA use, n (%)	30 (54.5%)	16 (66.6%)	0.315
ACEI/ARB use, n (%)	33 (67.3%)	16 (66.6%)	0.574
White blood cell (10^3/μL)	8.5 (4.4–14.8)	8.8 (4.8–16.6)	0.498
Hemoglobin (mg/dL)	13.5±1.9	13.7±1.4	0.143
Platelet (10^3/μL)	259.9±62.6	261±72.7	0.216
Neutrophil (10^3/μL)	4.1 (1.3–15.1)	4.8 (1.3–18.8)	0.085
Lymphocyte (10^3/μL)	1.5±0.5	1.3±0.8	0.122
NLR	2.5 (0.9–9.3)	4.2 (1.4–10.9)	0.037
BUN	16 (9–37)	18 (11–35)	0.217
Creatinine (mg/dL)	0.92±0.2	1.18±0.24	<0.001
CRP (mg/dL)	14.4±3.9	16.7±4.5	0.290
Syndecan-1	63.7 (7.4–253.5)	91.1 (42.8–620.6)	0.001
Total perfusion time	96.7±20.9	109±29	0.038
Cross-clamp time	72.1±20.5	70.6±23.2	0.287
Number of distal anastomoses	3 (16)	3 (2–6)	0.935
Packed blood products (units)	7 (4–9)	9 (4–13)	<0.001
Inotropic support, n (%)	13 (23.6%)	14 (58.3%)	0.003

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; ASA: acetylsalicylic acid; BMI: body mass index; CRP: C-reactive protein; BUN: blood urea nitrogen; NLR: neutrophil to lymphocyte ratio.

Table 2. Logistic regression analysis to identify factors affecting postoperative acute kidney injury.

Variables	Univariate analysis			Multivariate analysis		
	p-value	Exp(B) odds ratio	95%CI Lower-upper	p-value	Exp(B) odds ratio	95%CI Lower-upper
Age	0.527	1.017	0.9656–1.073	–	–	–
Hypertension	0.179	2.024	0.724–5.657	–	–	–
Total perfusion time	0.045	1.021	1.001–1.043	–	–	–
Inotropic support	0.004	4.523	1.627–12.575	0.453	1.707	0.422–6.903
Blood product use	<0.001	1.971	1.358–2.861	0.035	1.634	1.036–2.579
Pre-creatinine	<0.001	125.742	10.773–1467.616	0.007	59.387	3.034–1162.496
NLR	0.039	0.826	0.678–0.982	–	–	–
Syndecan-1	0.008	1.016	1.004–1.027	0.025	1.015	1.002–1.028

NLR: neutrophil to lymphocyte ratio.

p=0.035), preoperative high creatinine (OR 59.387; 95%CI 3.034–1162.496; p=0.007), and high SDC-1 (OR 1.015; 95%CI 1.002–1.028; p=0.025) were independent predictors of AKI.

ROC analysis was performed to evaluate SDC-1 in predicting AKI after CABG operations performed with CPB. The cutoff value was 79.4 (area under the curve: 0.739; 95%CI 0.621–0.857; p=0.001, with 58.3% sensitivity and 46.8% specificity) (Figure 1).

DISCUSSION

CABG operation is an effective and important treatment modality in coronary artery disease; AKI is an important complication that can occur after these operations and can lead to increased mortality and morbidity. Therefore, it is very important to reveal the risk factors for AKI. For the first time in the literature, in this prospective study, we demonstrated that preoperative high SDC-1 serum levels were an independent predictor of postoperative AKI risk in patients undergoing isolated CABG operation accompanied by CPB.

Endothelial surface proteins act as a barrier between the blood and the endothelium, preventing the extravasation of electrolytes, water, and proteins. These proteins are also known as endothelial glycocalyx structures[8]. SDC-1 is a family of protein groups. It has been shown that high serum SDC-1 levels were associated with impaired endothelial glycocalyx structures and elevated serum catecholamine levels in cases with acute myocardial infarction[9]. In a study conducted on patients with acute decompensated heart failure, a significant relationship was revealed between high serum SDC-1 levels (at the time of admission) with AKI and 6-month mortality[10].

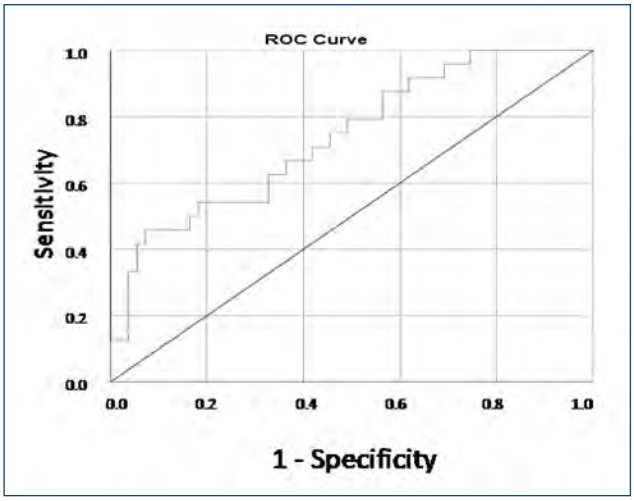

Figure 1. Data figure of the area under the curve, confidence interval, and cutoff value in receiver operating characteristic curve analysis for syndecan-1 to predict acute kidney injury (cutoff: 79.4; AUC: 0.739; 95%CI 0.621–0.857, p=0.001, with 58.3% sensitivity and 46.8% specificity).

In another study conducted in the following years, the importance of SDC-1 was investigated in patients with heart failure. A prospective study by Liu et al. included 96 patients with non-ischemic dilated heart failure. In this study, high SDC-1 levels were shown as an independent predictor of major adverse events[11]. Schellings et al. investigated the relationship between SDC-1 and hypertension in their experimental study. They demonstrated that SDC-1 expression increased significantly in experimental mice with angiotensin II-induced hypertension[12].

In the study conducted by Wernly et al., the prognostic importance of plasma SDC-1 levels in patients with acute reperfused ST-segment elevation myocardial infarction was investigated. A total of 206 patients were followed up for 6 months. SDC-1 levels were evaluated from blood samples taken after coronary intervention. No significant correlation was found in correlation analyses between various biochemical prognostic markers and SDC-1. However, a significant correlation was found between high SDC-1 levels and mortality[13].

Two studies were identified in the literature investigating the relationship between AKI and SDC-1 in cardiac surgery. Patients under the age of 18 years, who underwent cardiac surgery, were included in the prospective study conducted by Cavalcante et al. In this study, SDC-1 levels were measured in the early postoperative period. At the end of the study, high SDC-1 levels were found to be an independent predictor of the presence of severe AKI[5]. Different from this study, we included adult patients who underwent isolated CABG and evaluated their SDC-1 levels preoperatively. Xu et al.[14] used SDC-1 to predict the risk of AKI after cardiac surgery in adult patients. This study was carried out on patients who had undergone various cardiac surgeries (valvular, coronary, etc.), and blood samples of the patients were collected at the time of AKI diagnosis (in the first 48 h after the operation) for the SDC-1 measurements. In the multivariate analysis performed in the study, high plasma SDC-1 levels were shown as an independent predictor of AKI progression[14]. Unlike this study, we included only isolated CABG patients. In addition, SDC-1 levels were evaluated only preoperatively. In our study, preoperative high SDC-1 levels were shown as an independent predictor of the development of AKI after isolated CABG operations.

Our study is a prospective, single-center study and has some limitations. First, the number of patients was limited. SDC-1 value was evaluated only preoperatively; measurements could not be made during CPB or in the postoperative period. In addition, other AKI-related inflammatory parameters such as cystatin C, neutrophil gelatinase-associated lipocalin, and kidney injury molecule could not be evaluated. Therefore, new studies are needed.

CONCLUSION

Although CABG operations are performed with high success rates today, some morbid and mortal complications may occur in the postoperative period. Renal failure is one of the most common complications of this operation. For the first time in the literature, this study revealed that elevated preoperative SDC-1 is associated with AKI after isolated CABG accompanied by CPB and has prognostic utility independent of other recognized risk factors.

ETHICS APPROVAL

The study was approved by Bursa Yuksek Ihtisas Training and Research Hospital Clinical Research Ethics Committee (Protocol number: 2011-KAEK-25 2022/02-17).

AUTHORS' CONTRIBUTIONS

DA: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SAS:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ME:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **FA:** Conceptualization, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RFK:** Validation, Visualization, Writing – original draft, Writing – review & editing. YU: Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SY:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AFO:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of antidiabetics and exercise therapy on suppressors of cytokine signaling-1, suppressors of cytokine signaling-3, and insulin receptor substrate-1 molecules in diabetes and obesity

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SUMMARY

OBJECTIVE: Pathological destruction of insulin signaling molecules such as insulin receptor substrate, especially due to the increase in suppressors of cytokine signaling molecules, has been demonstrated in experimental diabetes. The contribution of suppressors of cytokine signaling proteins to the development of insulin resistance and the effects of antidiabetic drugs and exercise on suppressors of cytokine signaling proteins are not clearly known.

METHODS: A total of 48 Wistar albino adult male rats were divided into six groups: control group, obese group with diabetes, obese diabetic rats treated with metformin, obese diabetic rats treated with pioglitazone, obese diabetic rats treated with exenatide, and obese diabetic rats with applied exercise program. Immunohistochemical staining was performed in both the liver and adipose tissue.

RESULTS: There was a statistically significant decrease in suppressors of cytokine signaling-1, a decrease in suppressors of cytokine signaling-3, an increase in insulin receptor substrate-1, and a decrease in immunohistochemical staining in the obese group treated with metformin and exenatide compared to the obese group without treatment in the liver tissue ($p<0.05$). A statistically significant decrease in immunohistochemical staining of suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 was found in the obese group receiving exercise therapy compared to the obese group without treatment in visceral adipose tissue ($p<0.05$). Likewise, no significant immunohistochemistry staining was seen in diabetic obese groups.

CONCLUSION: Metformin or exenatide treatment could prevent the degradation of insulin receptor substrate-1 protein by reducing the effect of suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 proteins, especially in the liver tissue. In addition, exercise can play a role as a complementary therapy by reducing suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 proteins in visceral adipose tissue.

KEYWORDS: Obesity. Insulin resistance. Antidiabetic drugs. Exercise.

INTRODUCTION

Insulin resistance is an important mechanism in the development of type 2 diabetes and obesity. The effect of insulin in muscle, liver, and adipose tissue is closely related to the activity of insulin receptor substrate (IRS) proteins. Insulin resistance is characterized by a decrease in insulin signal, mainly in the IRS[1]. IRS-1 proteins are major factors in the insulin signaling pathway; it has been determined that the inhibition or degradation of these proteins leads to the decrease in the insulin signal[2]. Pathological destruction of insulin signaling molecules such as IRS, especially due to the increase in suppressors of cytokine signaling molecules (SOCS), has been demonstrated in experimental diabetes[3,4]. The SOCS are molecules that take part in the negative feedback loop to weaken the cytokine effect. Eight members of the SOCS family have been identified, sharing similar structural and functional properties [SOCS-1–7 and cytokine-inducible SH2-containing protein (CIS)][5]. Expression of SOCS family proteins can alter many different signaling pathways in different tissues. SOCS-1 and SOCS-3, in particular, are thought to play a role in the development of insulin resistance and diabetes. Decreased expression of SOCS-1 and SOCS-3 proteins, especially SOCS-3, improves insulin resistance in the liver and increases insulin sensitivity in diabetic mice[6,7]. However, there are uncertainties as to whether SOCS proteins are the cause or the result of insulin resistance.

The first molecular link between obesity and pro-inflammatory cytokines was revealed by Hotamisligil et al.[8] Since then, the relationship of some inflammatory processes with obesity

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has been a serious area of research. Obesity is now easily defined as a chronic inflammatory process[9]. At the molecular level, it has been shown that SOCS-1 and SOCS-3 bind to IRS-1 and IRS-2, disrupting the effect of insulin and leading to the development of insulin resistance in obesity[10,11]. With the inhibition of SOCS proteins, IRS could be increased and insulin resistance induced by obesity could be prevented.

Effect of antidiabetic drugs on IRS-1 degradation in the liver, vi\\sceral adipose tissue, and muscle tissue in patients with diabetes is not clear. We aimed to investigate the effect of certain antidiabetic drugs on IRS-1 protein via SOCS-1 and SOCS-3 proteins in diabetes and obesity.

METHODS

Animal models and study groups

A total of 48 Wistar albino adult male rats weighing between 180 and 220 g were used. They were divided into six groups (control, obesity + diabetes, obesity + diabetes + metformin, obesity + diabetes + pioglitazone, obesity + diabetes + exenatide, and obesity + diabetes + exercise), with eight rats in each group. The study was carried out in an internationally certified animal experimentation laboratory at Gaziantep University.

The control group was fed with standard chow for 8 weeks. The subjects in the groups other than the control group were given 1000 g: 228.0 g of casein, 2.0 g of DL-methionine, 170.0 g of maltodextrin, 175.0 g of sucrose, 25.0 g of soybean oil, 335.5 g of coconut oil, 40.0 g of mineral mix, 10.5 g of sodium bicarbonate, 4.0 g of potassium citrate (with H_2O), 10.0 g of vitamin mixture, 2.0 g of choline, and 0.1 g of FD&C Red Dye #4 containing high-fat (60% fat) special feed for 4 weeks. It was accepted that nonalcoholic fatty liver disease occurred at the end of this period in groups other than the control group[12,13].

Animals fed this diet were weighed daily, and rats with a 20% weight gain were considered obese. After that, 40 mg/kg of streptozocin (STZ) was administered to obese groups to create diabetes with obesity. Different drug treatments (metformin, pioglitazone, and exenatide) or exercise therapies were administered to these obese groups with STZ-induced diabetes for 4 weeks. The daily blood glucose levels of all animals were measured and recorded from the blood sample taken from the tail vein. The treatment lasted for 4 weeks, after which the study was terminated. During the study, the temperature of the environment where the rats were kept was maintained constant at 20–24°C and the room was provided with 12 h of light and 12 h of darkness (light between 07.00 and 19.00).

- Group 1: Control group (C): It was fed with standard feed.
- Group 2: Diabetic obese group with metformin treatment (Ob+D+M): Metformin (0.33 mg/mL/day) treatment was applied for 4 weeks (105).
- Group 3: Diabetic obese group with pioglitazone treatment (Ob+D+P): Pioglitazone (4 mg/kg/day) treatment was applied for 4 weeks (105).
- Group 4: Diabetic obese group with exenatide treatment (Ob+D+Exn): Exenatide (0.03 mg/kg/day) treatment was applied for 4 weeks (105).
- Group 5: Diabetic obese group with exercise program (Ob+D+Exc): 4 weeks of swimming exercise (1 h/day) was applied (106).
- Group 6: Diabetic obese group without any treatment (Ob+D): It was waited for 4 weeks without any application.

mRNA isolation and complementary DNA synthesis

Using the Qiagen RNeasy Plus Universal Mini Kit (50) (Hilden, Germany; cat. no. 73404) mRNA isolation kit from the liver, visceral fat, and muscle tissues obtained from the study groups, mRNAs were obtained in accordance with the kit protocol. Concentration determinations were made with the nanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and then the concentrations of all samples were fixed to the same range as 150 ng/µL by performing the necessary dilutions, and the stock RNAs were stored at -80°C. Complementary DNAs (cDNAs) were obtained from isolated mRNAs by reverse polymerase chain reaction (PCR) method using the Fluidigm Reverse Transcription Kit (San Francisco, CA, USA; cat. no. 100-6298), nanoDrop ND-1000 spectrophotometer (Thermo Scientific), and stock cDNAs were stored at -80°C.

Quantitative real-time (RT) PCR

BioMark qRT-PCR system (Fluidigm), a high-capacity qRT-PCR method, was used to determine the gene expression levels of SOCS-1, SOCS-3, and IRS-1. Pre-amplification process was performed using Pre-amplification Master Mix and targeted *SOCS-1*, *SOCS-3*, and *IRS-1* as well as *GAPDH* as housekeeping gene, using TaqMan™ (Thermo Scientific) gene expression primer assays. PCR mix with pre-amplified cDNA samples was loaded into Fluidigm Flex Six™ (cat. no. 100-6308) sample portion, 20X Assays diluted 1:1 with 2X Assay Loading Reagent, Flex Six ™ (Fluidigm; cat. no. 100-6308)

Gene Expression loaded into the assay portion of the IFC array. BioMark IFC Controller (Fluidigm; HX-10273) assay and sample mixes were loaded into FlexSix™ chambers using "Fluidigm's Integrated Fluidic Circuit Technology."

IMMUNOHISTOCHEMICAL ANALYSIS

For immunohistochemical study, 4-µm-thick sections from paraffin blocks obtained from formalin-fixed liver, visceral adipose tissue, and muscle tissues were taken on polylysine-coated slides. The slides were first incubated at 37°C for 15 min. Afterward, automatic staining with SOCS-1, SOCS-3, and IRS-1 polyclonal antibodies]SOCS-1 (Bios, bs-0113R, 1:50), SOCS-3 (Bios, bs-0580R, 1:50), and IRS-1 (Bios, bs-0319R, 1:50)] (Ventana® Bench Mark Ultra, SN:316054) immunohistochemical staining was performed. All the stained sections were evaluated under the Olympus BX46 light microscope for the extent and intensity of staining at 40×, 100×, and 200× magnifications.

Statistical analysis

The IBM Statistics SPSS version 20.0 was used for statistical analysis. While analyzing the data, it was determined whether or not the data of the groups showed normal distribution, and as a result, one-way ANOVA or Kruskal-Wallis test was applied to compare the groups. Data were summarized as mean±standard deviation. Gene expression analyses were evaluated with the QIAGEN Globe. In the analysis, the *GAPDH* was used as a housekeeping gene. In this analysis, the program calculates the analysis of raw data results (Ct values) with the $2^{-\Delta\Delta Ct}$ method, and after these values are calculated, it uses the basic Student's t-test method to make comparisons between the groups (calculation of p-values).

RESULTS

Body weight and HbA1c alteration in obesity and diabetes models

At the end of the first 11 weeks, obesity was induced by applying a high-fat diet to 48 animals (according to the Lee index).

There was a statistically significant difference between the control group and the obese group. This difference demonstrated the presence of obesity (control group 297.40±53.23 vs. obese group 354.10±75.91; p<0.01). At the end of the 16th week, when different interventions were applied, the mean weight changes of obese and diabetic obese animals were examined. The mean intracardiac plasma glucose value was 395.8±70.7 in the control group and 547.0±133.4 in the diabetic group. The HbA1c values of the obese and diabetic obese groups were 6.5±0.22% and 9.9±0.53%, respectively (p<0.01).

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obesity with diabetes models

There was a statistically significant decrease in SOCS-3 gene expression in the control group compared to the obese control group (p<0.05). Compared to the obese control group, the difference in SOCS-1 gene expression in the obese exenatide group was statistically significant, while the expression of the SOCS-1 gene was found to be decreased in the obese exenatide group (p<0.05). There was no statistical significance in the analyses of the obesity + metformin, obese + pioglitazone, and obese + exercise groups compared to the obese control group (p>0.05).

SOCS-1 gene expression was found to be decreased in the ob+STZ+P group compared to the control group (p<0.05) (Table 1).

Table 1. Gene expression analysis of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obesity with diabetes models.

	Control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	1.212	0.372	1.036	0.514	0.751	0.285	0.570	0.028[B]	0.6461	0.246
SOCS-3	0.393	0.016[A]	1.061	0.758	0.587	0.102	1.780	0.187	0.753	0.317
IRS-1	0.912	0.850	1.237	0.333	0.693	0.157	0.611	0.066	0.775	0.387
	Control		Ob+STZ+Metformine		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	1.187	0.279	0.673	0.059	0.542	0.019[C]	0.576	0.109	0.627	0.413
SOCS-3	0.498	0.053	1.703	0.304	0.816	0.467	1.773	0.136	0.814	0.496
IRS-1	1.094	0.541	0.908	0.952	0.805	0.463	1.011	0.647	0.977	0.959

SOCS: suppressors of cytokine signaling; IRS: insulin receptor substrate; Ob: obese; STZ: streptozocin. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene for normalization. [A]Statistically significant difference with obese control group, p<0.05. [B,C]Statistically significant difference with control, p<0.05.

There were statistically significant SOCS-1, SOCS-3, and IRS-1 immunohistochemical staining changes in the obese groups treated with metformin and exenatide compared to the obese group ($p<0.05$). There was a statistically significant immunohistochemical staining decrease in only SOCS-1 and SOCS-3 in the diabetic obese group treated with metformin compared to the obese group ($p<0.05$), and also there was a statistically significant decrease in only SOCS-1 in the diabetic obese group treated with exenatide compared to the obese group ($p<0.05$) (Table 2).

Immunohistochemistry analysis bar graph and histological staining of SOCS-1, SOCS-3, and IRS-1 in obese and diabetic obese groups in liver tissue are shown in Figures 1 and 2, respectively.

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in visceral adipose tissue in experimental obesity and obesity with diabetes models

Compared to the obese control group, *SOCS-1* and *SOCS-3* genes were found to be statistically significant in the control group, while the expressions of these genes were found to be increased in the control group ($p<0.05$). Compared to the obese control group, the *SOCS-1* gene was found to be statistically significant in the obese + exenatide group, while the expression of the SOCS-1 gene was increased in the obese + exenatide group ($p<0.05$). Compared to the obese control group, the *SOCS-1* gene was found to be statistically significant in the obese +

Table 2. Immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obese diabetes models.

	Obese control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	Score	p-value	Score	p-value	Score	p-value	Score	p-value	Score	p-value
SOCS-1	2.80		1.22	0.001[A]	1.75		1.20	0.001[A]	1.38	0.003[A]
SOCS-3	2.75		1.40	0.001[B]	1.75		1.33	0.001[B]	1.87	
IRS-1	1.40		2.75	0.001[C]	2.38		2.75	0.001[C]	2.25	
	Ob+Streptozotocin		Ob+STZ+Metformine		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	Score	p-value	Score	p-value	Score	p-value	Score	p-value	Score	p-value
SOCS-1	2.75		1.50	0.004[D]	1.70		1.30	0.001[D]	1.75	
SOCS-3	2.73		1.50	0.003[E]	1.45	0.001[E]	1.70	0.020	1.63	0.016[E]
IRS-1	1.25		2.25		2.50		2.70	0.005[F]	2.50	0.021[F]

SOCS: suppressors of cytokine signaling; IRS: insulin receptor substrate; Ob: obese; STZ: streptozocin. [A,B,C]Statistically significant difference with obese control, $p<0.05$. [D,E,F]Statistically significant difference with obese control with diabetes, $p<0.05$.

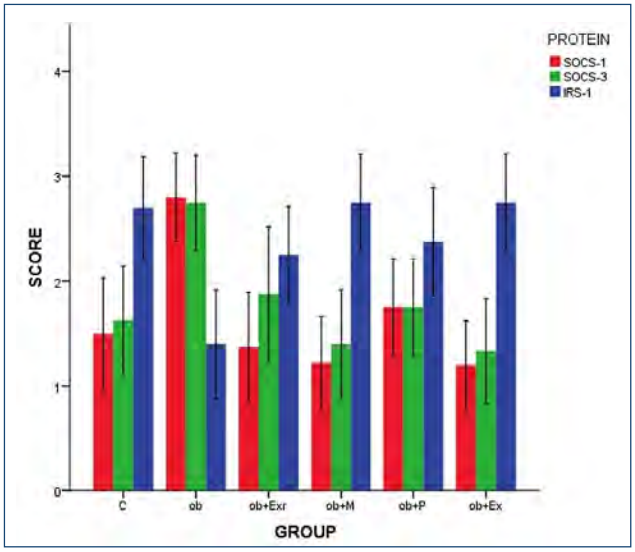

Figure 1. Immunohistochemistry analysis bar graph of SOCS-1, SOCS-3, and IRS-1 in obese group in liver tissue. C: control; Ob: obese control; Ob+Exr: obese + exercise; Ob+M: obese + metformin; Ob+P: obese + pioglitazone; Ob+Ex: obese + exenatide; Ob+STZ: obese + streptozocin; Ob+STZ+Exr: obese + streptozocin + exercise; Ob+STZ+P: obese + streptozocin + pioglitazone; Ob+STZ+Ex: obese + streptozocin + exenatide.

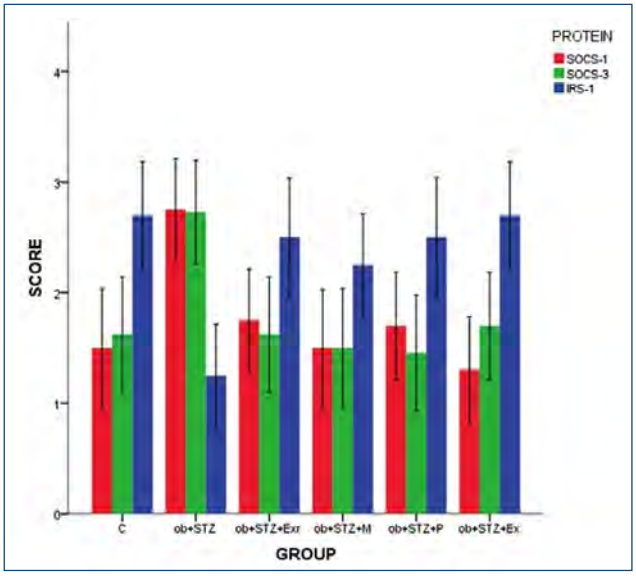

Figure 2. Immunohistochemistry analysis bar graph of SOCS-1, SOCS-3 and IRS-1 in diabetic obese groups. C: control; Ob: obese control; Ob+Exr: obese + exercise; Ob+M: obese + metformin; Ob+P: obese + pioglitazone; Ob+Ex: obese + exenatide; Ob+STZ: obese + streptozocin; Ob+STZ+Exr: obese + streptozocin + exercise; Ob+STZ+P: obese + streptozocin + pioglitazone; Ob+STZ+Ex: obese + streptozocin + exenatide.

exercise group, while the expression of the SOCS-1 gene was increased in the obese + exercise group (p<0.05). Compared to the diabetic control group, the *IRS-1* gene was found to be statistically significant in the obese + STZ + metformin group, and the expression of the IRS-1 gene was found to be decreased in the obese + STZ + metformin group (p<0.05) (Table 3).

A statistically significant decrease in immunohistochemical staining of SOC-1, SOCS-3, and IRS-1 was found in the obese group receiving exercise therapy compared to the obese group (p<0.05).

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in muscle tissue in experimental obesity and obesity with diabetes models

Compared to the control group in muscle tissue samples, no difference was found in SOCS-1, SOCS-3, and IRS-1 gene expression levels in neither obese nor diabetic obese groups. Likewise, no significant immunohistochemistry staining was seen on these proteins.

DISCUSSION

In this study, we evaluated the effect of several antidiabetic drugs and exercise on the alteration of SOCS-1, SOCS-3, and IRS-1 in the liver, adipose, and muscle tissues. SOCS proteins, mainly SOCS-1 and SOCS-3, have been associated with insulin resistance, obesity, and the development of diabetes[2]. However, there is still no clear conclusion that these SOCS proteins are the cause or consequence of insulin resistance. SOCS proteins should not be evaluated only on the basis of insulin effect. SOCS proteins play different roles in the functioning of many systems, such as immune, hematopoietic, and hormone receptor signaling pathways[12]. In our study, it was observed that some drugs and exercise used in the treatment of type 2 diabetes could affect the gene expressions of SOCS-1, SOCS-3, and IRS-1, as well as the degree of immunohistochemical staining of these proteins in different tissues.

Cytokines and growth factors regulated by SOCS-1 and SOCS-3 are important in both physiologic and neoplastic growth of hepatocytes[13]. Studies showed that SOCS-1 and SOCS-3 may be a therapeutic target in hepatic insulin resistance[14,15]. There are publications reporting that overexpression of SOCS-3 is associated with insulin resistance, particularly in hepatic tissue. In addition, some mutations in the *SOCS-1* gene have been associated with obesity and insulin resistance and obesity in the literature[2]. However, there is still a lack of sufficient and quality data to establish the effect of antidiabetic drugs and exercise on SOCS proteins and IRS-1. In our study, the decrease in immunohistochemical staining of SOCS-1 and SOCS-3 in the liver was accompanied by a significant increase in IRS-1 in groups who were obese and were treated with metformin or exenatide. In the liver tissue of diabetic obese group, the decrease in the staining of SOCS-1 and SOCS-3 proteins was accompanied by a significant increase in IRS-1 staining only in the exenatide-treated group.

In addition, three different IRS molecules were detected in visceral adipose tissue (IRS-1-2-3). It is thought that the chemical structures of IRS molecules and the signal pathways they cause are similar[16]. In our study, there was a significant decrease in SOCS-1 and SOCS-3 staining only in the obese adipose tissue group receiving exercise therapy (p=0.001). Interestingly, these decreases were accompanied by significant decrease in the staining of IRS-1. Other treatment modalities caused almost no change in IRS-1 staining. In the diabetic obese groups, a decrease was observed in SOCS-3 staining in the exenatide and exercise groups, while the decrease in only exenatide treatment was significant. Change in IRS-1 level did not observe in the groups receiving these treatments. In different studies, it has been shown that changes in IRS molecules in adipose tissue may vary according to the causes of insulin resistance. Different alterations

Table 3. Gene expression analysis of SOCS-1, SOCS-3, and IRS-1 in visceral adipose tissue in experimental obesity and obesity with diabetes models.

	Control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	20.452	0.049[A]	5.305	1.870	1.870	0.671	9.101	0.029[C]	4.265	0.031[D]
SOCS-3	6.567	0.008[B]	0.776	1.736	1.736	0.215	1.249	0.211	1.302	0.305
IRS-1	0.927	0.670	0.888	0.458	0.458	0.695	0.746	0.108	0.685	0.153
	Control		Ob+STZ+Metformin		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	2.584	0.208	0.490	0.754	1.141	0.337	1.089	0.614	1.763	0.277
SOCS-3	9.315	0.051	5.853	0.063	7.053	0.122	1.235	0.573	2.031	0.302
IRS-1	1.097	0.653	0.494	0.025[E]	1.242	0.342	1.066	0.804	1.227	0.500

Ob : obese; STZ: streptozocin. [A,B,C,D]Statistically significant difference with obese control, p<0.05. [E]Statistically significant difference with obese control with diabetes, p<0.05. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene for normalization.

in IRS molecules have been observed in different insulin resistance models. Modulation and signaling pathway of IRS and SOCS in the case of insulin resistance in adipose tissue is still not clearly defined. SOCS proteins can inhibit insulin signaling by five different mechanisms. The mechanism underlying SOCS-3-mediated insulin resistance involves the following:

1. Competition for binding to the activated insulin receptor
2. IRS protein degradation
3. Inhibition of the tyrosine kinase activity of the insulin receptor;
4. Negative feedback regulation of the Janus kinase 2 (JAK2)/STAT3 signal transduction pathway
5. Regulation of leptin signal transduction[17]. In addition, the presence and functionality of the IRS-1 molecule is necessary for the effectiveness of insulin in tissues. The changes in these molecules should be examined separately according to each different insulin resistance scenario.

One study showed that SOCS-1, SOCS-3, and IRS-1 proteins showed different alterations according to the relevant tissue. In addition, SOCS-1 binds to the IRS-2 recognition site in the IR kinase domain (IR) and primarily inhibits IRS-2-mediated insulin signaling, whereas SOCS-3 binds to IR and inhibits both IRS-1 and IRS-2[2]. SOCS-1 has been reported to increase the ubiquitin-mediated degradation of some proteins as part of the ubiquitin-ligase complex[18]. In another study, it was also shown that SOCS-1 and SOCS-3 bind IRS proteins in cultured cells in the liver and direct them to ubiquitin-mediated protein degradation[19]. However, there are also studies in which no interaction could be detected between SOCS and IRS. Chronic insulin therapy or long-term exposure to hyperinsulinemia has been shown to reduce IRS-2 mRNA in hepatocyte cell cultures, whereas IRS-1 is reduced mainly through protein degradation[2]. Pro-inflammatory cytokines, such as TNF-α, have been shown to inhibit insulin signaling via IR and IRS proteins[20]. It has been suggested that SOCS protein-mediated inhibition of IRS phosphorylation is also involved in TNF-α-mediated inhibition of insulin signaling[21].

The findings we obtained at different levels in different tissues differed according to the drug treatments and exercise status we used. It is not surprising that different interventions yield different results in various tissues. Because the mechanism of action of each drug is different, it is known that the main site of action of metformin is the liver. The reason is the dominance of the presence of organic cation transporter-1 in the liver. Therefore, the uptake of metformin in the liver is higher than in other organs[22].

Studies have shown that metformin increases AMP-activated protein kinase (AMPK) activation and decreases lipopolysaccharide-induced inflammatory responses[23]. In another study, it was shown that activation of AMPK significantly suppressed the acute-phase response and decreased SOCS-3 gene expression[24]. These findings are consistent with the data in our study. Metformin treatment significantly reduces SOCS-1–3 in liver tissue in the obese and diabetic obese groups. In our study, the fact that metformin treatment did not cause an increase in IRS-1 in the diabetic obese group, but also preserved the level of IRS-1, may suggest that metformin is more effective in the liver tissue by creating an anti-inflammatory effect.

The antioxidant effects of GLP-1 analogs are known. GLP-1 analogs are known to reduce free radicals and inflammation-induced oxidative stress. There are also studies in the literature showing that it reduces lipotoxicity and glucotoxicity[25]. Increasing the level of inflammation has a negative effect on IRS-1. In addition, the contribution of SOCS proteins to the inflammatory process is known. In our study, the fact that the most effective treatment was exenatide in both the obese group and the diabetic obese group can be attributed to the antioxidant effects of GLP-1 analogs.

The difference in the normal expression levels of these molecules in different tissues in the normal physiological process and the different mechanisms of action of the drugs used may have revealed these results. Comprehensive studies, including the mechanisms of action of drugs, may yield clearer results.

In light of the current literature, the decrease in SOCS-1 and SOCS-3 and the increase in the IRS-1 molecule are extremely important in terms of preventing diabetes-related complications due to better glycemic control in obese and especially obese patients with diabetes. In our study, exenatide was found to be the most effective drug in liver tissue in both obese and diabetic obese patients. Metformin treatment had similar effects to exenatide in the obese group. Although it caused a decrease in SOCS-1–3 in the diabetic obese group and an increase in IRS-1, this increase was not as significant as the increase caused by exenatide. When the effects on visceral adipose tissue were evaluated, it was determined that the most effective treatment was exercise. There was a decrease in both SOCS-1 and SOCS-3 in the obese group. These findings can be accepted as molecular evidence of the expected benefit of adding exercise to drug therapy in diabetes and obesity.

CONCLUSION

Metformin or exenatide treatment could prevent the degradation of IRS-1 protein by reducing the effect of SOCS-1 and SOCS-3 proteins, especially in liver tissue. Drugs that alter the SOCS effect and/or IRS-1 protein may be new agents for the treatment of obesity, insulin resistance, and type 2 diabetes. It also suggests that the use of exercise therapy as a complement to medical treatments may be beneficial.

AUTHORS' CONTRIBUTIONS

EA, ZB: Conceptualization, Methodology, Software, Writing – original draft, and Writing – review & editing. **ZAS:** Conceptualization, Writing – original draft, and Writing – review & editing. **SOB, CD:** Conceptualization, Formal Analysis, Methodology, Software, Writing – original draft, and Writing – review & editing. **MK, IY:** Formal Analysis, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Foley catheter plus misoprostol versus misoprostol alone for labor induction

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SUMMARY

OBJECTIVE: This study aimed to analyze the effects of Foley catheter combined with misoprostol in the labor induction process.

METHODS: This is a nonblinded, block randomized, controlled trial that compared the association between transcervical Foley catheter/vaginal misoprostol 25 μg combination and vaginal misoprostol 25 μg alone in normal-risk and healthy pregnant women undergoing labor induction in the south of Brazil.

RESULTS: A total of 230 patients with indications for labor induction were evaluated and classified into the "combined" group (Foley catheter plus misoprostol), consisting of 107 patients, and the "misoprostol" group (misoprostol only), consisting of 123 patients. The "combined" group was observed to have a shorter labor induction time (p=0.008). In addition, there was a lower need for misoprostol use for overall cervical ripening (p<0.001) and a lower relative risk of needing a second, third, or fourth misoprostol tablet in the "combined" group (risk ratio [RR] 0.80, 95% confidence interval [CI] 0.71–0.91; RR 0.41; 95%CI 0.31–0.56; and RR 0.29, 95%CI 0.17–0.52, respectively) (p<0.001). No statistically significant difference was found in induction failure rate, cesarean section rate, or perinatal outcomes.

CONCLUSION: A combination of methods leads to shorter labor induction, lower need for misoprostol doses, and lower risk of cesarean section, with no increase in the rate of perinatal complications. REBEC number is RBR-7xcjz3z.

KEYWORDS: Labor. Induced labor. Misoprostol.

INTRODUCTION

Labor induction is the stimulation of uterine contractions in a pregnant woman before labor begins to achieve vaginal delivery, reducing the cesarean section rates[1,2]. It is indicated when the birth is beneficial for the mother and/or the fetus. The most frequent causes are as follows: late-term pregnancies, premature membrane rupture, gestational diabetes, intrauterine growth restriction, and elective reasons[1,3].

Several factors can interfere with the response to induction, but the most important is cervical ripening[4]. If induction is indicated and the state of the cervix is unfavorable, agents for cervical ripening must be used[5]. In this sense, the Bishop index aims at assessing cervical ripening, taking into account the following characteristics of the cervix: dilation, fading, consistency, position, and fetal presentation height. Many studies have shown that values ≤6 present a lower probability of vaginal delivery. The use of artificial methods to prepare the cervix increases the chances of a successful vaginal delivery[6].

Due to the long time and experience of their use, prostaglandins misoprostol (E1) and dinoprostone (E2) are considered the main pharmacological agents. Misoprostol is a low-cost synthetic analog of prostaglandin E1 and can be kept at room temperature, advantages that make it the preferred method in Brazil[7]. The transcervical Foley catheter is a mechanical method that has long been used for cervical ripening. Its insertion is performed through a specular examination, with the catheter passing through the cervix or under direct vision, and subsequently inflating the cuff with 30–60 mL of distilled water. A few studies have shown that the mean response time to the catheter is 12 h, but it can safely remain for up to 24 h[2,8].

A synergistic effect has been shown when pharmacological and mechanical methods are used in association, in addition to the safety and benefits for both the mother and neonate[1,3,9,10]. However, no evidence was found regarding the use of this technique in the Brazilian population. Also, considering the overcrowding of maternity hospitals and the increasing number of patients who need labor induction, the benefits of this association could affect patients undergoing the fastest and effective methods as well as the health system by reducing costs of hospitalization and procedures. In this sense, this

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study was designed to compare the association between the transcervical Foley catheter/vaginal misoprostol 25 µg combination and vaginal misoprostol 25 µg alone and to evaluate the effectiveness of both the methods associated and their safety, in normal-risk pregnant women undergoing labor induction at two public teaching-maternity hospitals in the south of Brazil.

METHODS

This is a multicenter, open-label, two-arm randomized clinical trial executed between August and December 2021 and was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee. All participants signed the informed consent form, and the data collected were anonymized.

The investigation included healthy pregnant women with normal-risk pregnancies, aged between 18 and 40 years, admitted for late-term labor induction, and with a Bishop index ≤6 at admission, assisted at two public teaching-maternity hospitals in the south of Brazil. Both hospitals have similar induction protocols.

The selection of the participants was carried out by convenience. The patients who agreed to participate in the study were drawn by block randomization. For sample size calculation, the OpenEpi software (version 3.01) was used for a hypothesis test to compare means and detect a possible difference in labor induction time.

Patients with contraindications to the use of misoprostol (e.g., previous cesarean section or placenta previa) or use of a Foley catheter (e.g., membrane rupture, vaginal infection, or chorioamnionitis) and with cervical dilation ≥3 cm were excluded from the study, as well as those with nonreassuring preinduction cardiotocographic evaluation and the patients who underwent cesarean sections due to induction failure before completing the hospital protocol. Patients with difficulties in positioning the transcervical Foley catheter were also excluded from the "combined" group.

The patients were classified as the "combined" and "misoprostol" groups. An 18F transcervical Foley catheter was inserted in the patients from the "combined" group, inflated with a volume of 60 mL at admission, and removed after 24 h if it was not spontaneously expelled. In addition, they received misoprostol 25 µg vaginally every 4 h until satisfactory cervical ripening was achieved (Bishop index >6) or a maximum of six doses. The patients in the "misoprostol" group received misoprostol alone, according to the protocol described above. Oxytocin was administered to all patients who did not achieve at least three regular contractions in a maximum of 10 min within 4 h of administering the last misoprostol dose. Amniotomy was performed during labor at the discretion of the assisting professional or earlier if the induction protocol was near completion.

The primary outcomes of this study included the frequency of induction failure, defined as failure to achieve labor after six doses of misoprostol 25 µg; administration of oxytocin with a maximum infusion rate of 32 mUI/min in an infusion pump; and rupture of the amniotic membranes.

The secondary outcomes were as follows: the interval from induction initiation to labor in hours, the interval from labor initiation to birth in hours, maximum tablets of misoprostol used, maximum oxytocin infusion rate, frequency of adverse maternal outcomes, adverse perinatal outcomes, and the interval from induction initiation to discharge in hours.

The information obtained was analyzed using the IBM SPSS Statistics version 24.0 software (2016). The non-normal distribution of variables was determined using the Kolmogorov-Smirnov test and analyzed using Fisher's exact test and Mann-Whitney U test. In all the statistical inference processes, p-value ≤0.05 was considered significant.

RESULTS

Between August and December 2021, 250 pregnant women participated in the study. Of the 20 subjects excluded, 11 had a Bishop index of >6 or cervical dilation of >3 cm, 8 refused to participate, and 1 had a latex allergy. After randomization, six pregnant women could not progress the Foley catheter through the cervix, five withdrew from participating, and seven underwent cesarean sections before the induction protocol was completed in the "combined" group. There were only two losses in the "misoprostol" group due to cesarean section before the induction protocol was achieved.

Thus, 230 pregnant women at usual risk who required labor induction due to late-term pregnancy were evaluated. A total of 123 were allocated to the "misoprostol" group, representing 53.5% of the sample, and 107 to the "combined" group, representing 46.5% of the cases studied.

The study population consisted of pregnant women with a mean age of 27 years, 0.76 previous vaginal deliveries, 40.92 weeks of gestational age at admission, and a Bishop index of 2.2. There was no statistical difference between both groups, characterizing the homogeneity of the samples (Table 1).

When analyzing the mode of delivery, it was observed that 17% of the inductions progressed to cesarean sections. Regarding evolution to vaginal delivery, the corresponding rate in the "combined" group was 82.2% versus 83.7% in the "misoprostol" group, with no statistical difference between the groups (p=0.869). There was no difference in the induction failure rate between the groups (Table 2). In most of the cases, the indications for cesarean section were related to nonreassuring fetal status (60.0%), arrest of labor progression (24%), and

induction failure (10%), with no relevant statistical differences between the groups.

When evaluating the number of misoprostol doses used, the "combined" group required one fewer misoprostol tablet (median of two misoprostol tablets vs. the need for three tablets in the control group, p<0.001). There was no significant difference in the maximum oxytocin dose used in the two groups (Table 3).

Table 1. Demographic and obstetric characteristics of normal-risk pregnant women undergoing labor induction with misoprostol/Foley catheter combination or misoprostol alone at two public teaching-maternity hospitals in the south of Brazil, 2021 (n=230).

	Intervention				p-value
	Combined (n=107)		Misoprostol alone (n=123)		
	Mean	SD	Mean	SD	
Maternal age	27.75	6.82	26.9	6.11	0.120
Parity*	1.75	1.111	2	1	0.538
Gestation (weeks)	41	0.22	40.89	0.32	0.155
Bishop index	2.14	1.25	2.32	1.66	0.242
Birthweight (g)	3419.02	393.05	3413.54	425.73	0.619

SD: standard deviation. *Nulliparity was found in 65% on the "misoprostol" group and 68% on the "combined" group (p=0.610).

Table 2. Maternal, labor, and neonatal outcomes of normal-risk pregnant women undergoing labor induction with misoprostol/Foley catheter combination or misoprostol alone at two public teaching-maternity hospitals in the south of Brazil, 2021 (n=230).

	Intervention				RR (95%CI)	p-value
	Combined (n=107)		Misoprostol alone (n=123)			
	n	%	n	%		
Induction failure						
No	104	97.2	120	97.6	1.0 (Reference)	
Yes	3	2.8	3	2.4	1.08 (0.48–2.43)	0.869
Misoprostol (doses)						
1	107	100	123	100	1.0 (Reference)	
2	79	73.8	113	91.9	0.80 (0.71–0.91)	<0.001
3	33	30.8	92	74.8	0.41 (0.31–0.56)	<0.001
4	12	11.2	47	38.2	0.29 (0.17–0.52)	<0.001
>4	2	1.9	10	8.1	0.23 (0.52–1.03)	0.054
Mode of delivery						
Vaginal	88	82.2	103	83.7	1.0 (Reference)	
Cesarean section	19	17.8	20	16.3	1.09 (0.62–1.93)	0.762
Complications						
None	90	84.1	101	82.1	1.0 (Reference)	
Yes	17	15.9	22	17.9	0.92 (0.63–1.36)	0.821
PPH	5	4.8	7*	5.7	–	–
Uterine hyperstimulation	0	0	1	0.8	–	–
Abruptio placentae	0	0	1	0.8	–	–
Apgar score <7	12	11.2	14*	11.4	–	–
NICU admission	0	0	4	3.2	–	–

PPH: postpartum hemorrhage; NICU: neonatal intensive care unit; RR: relative risk; CI: confidence interval. *On one occasion, there was a concomitant PPH and Apgar score <7.

Table 3. Number of misoprostol tablets administered, labor induction and active labor time, length of stay, and oxytocin dose used in normal-risk pregnant women undergoing labor induction with misoprostol/Foley catheter combination or misoprostol alone at two public teaching-maternity hospitals in the south of Brazil, 2021 (n=230).

	Intervention				p-value
	Combined (n=107)		Misoprostol alone (n=123)		
	Median	Range	Median	Range	
Misoprostol (doses)	2	6	3	7	<0.001
Maximum oxytocin dose (mUI/min)	5	32	4	32	0.456
Induction time (h)	10	33	12	22	0.008
Labor time (h)	5	8	16	35	0.051
Length of stay (h)	56	42	60	132	<0.001

Regarding the need for additional misoprostol doses, the patients in the "combined" group were less likely to receive a second (73.8% vs. 91.9%, p<0.001), third (30.8% vs. 74.8%, p<0.001), or fourth misoprostol tablet (11.2% vs. 38.2%, p<0.001) when compared to the "misoprostol" group. This reduced the need for a second, third, or fourth misoprostol dose by 20% (relative risk [RR] 0.80; 95% confidence interval [CI] 0.71–0.91], p<0.001), 59% (RR 0.41; 95%CI 0.31–0.56; p<0.001), and 71% (RR 0.29; 95%CI 0.17–0.52; p<0.001), respectively. There were no differences in the need to administer the fifth or sixth misoprostol dose (Table 2).

When comparing the induction, labor, and hospitalization times, the pregnant women in the "combined" group had a 2-h reduction in the induction time (median of 10 h in the "combined" group vs. 12 h in the "misoprostol" group, p=0.008) and a 4-h reduction in the hospitalization time (median of 56 h in the "combined" group vs. 60 h in the "misoprostol" group, p<0.001). There was no significant difference in labor time (Table 3).

In most cases, no complication was observed during the hospitalization (83.0%). However, we noticed one patient with uterine hyperstimulation, abruptio placentae, 28 cases of Apgar score below 7, 4 cases where the newborns were admitted to the NICU, and 12 cases of postpartum hemorrhage. On one occasion, there was concomitant postpartum hemorrhage, and an Apgar score below 7. No relevant statistical differences were identified between the two groups (Table 2).

DISCUSSION

To the best of our knowledge, this is the first randomized study in the Brazilian population evaluating the Foley catheter/vaginal misoprostol combination or vaginal misoprostol alone for cervical ripening and labor induction.

This study did not identify any significant difference between the groups when evaluating the vaginal delivery and cesarean section rates, as was the case in the research by Osoti et al.[9] and Hill et al.[10] who observed that the cesarean section rates presented no statistical significance. However, Levine et al. evaluated 492 pregnant women paired in 4 groups with 123 participants each (i.e., misoprostol only, Foley catheter only, misoprostol plus Foley catheter, and oxytocin plus Foley catheter) and observed that the women who received the combined methods were twice as likely to have vaginal deliveries when compared to those who received misoprostol alone[7]. The induction failure rates found in this study also had no statistically significant difference, similar to the studies by Osoti et al.[9] and Kehl et al.[11].

We observed a significant difference when comparing the number of misoprostol tablets used in induction, in the same way that the propensity to need more misoprostol doses was reduced in the "combined" group up to the fourth dose. A similar result was observed by Osoti et al. with the "combined" group, presenting a reduced need to receive the second misoprostol tablet at 25% induction and the third tablet at 68%[9].

When assessing the time from induction to active labor, there was a significant difference of 2 h less in labor induction in the "combined" group. Aduloju et al. found a similar result with the transcervical catheter group, with a mean of 22.84 h (SD 4.69), misoprostol: 18.74 h (SD 4.43), and combined: 17.79 h (SD 2.85), with p=0.001[12].

We did not find any statistically significant difference between the two groups regarding labor time. In contrast, in a meta-analysis involving only the use of a balloon catheter associated with misoprostol versus misoprostol alone, Ornat et al. observed 15 randomized clinical trials that showed a statistically significant reduction in the time from induction initiation to delivery[8]. However, this difference may be related to the lack of distinction between the induction and labor times, which in this study were evaluated separately. Aduloju et al. also found a similar result, with a significant reduction in the

induction, labor, and delivery times[12]. Hill et al. also found a significantly shorter time to vaginal delivery (mean: 14.6±6.9 vs. 20.8±13.8 h, p<0.0001)[10]. Levine et al. also identified that the association of methods reduces labor time with statistical significance (p<0.001)[7].

Interestingly, most patients evaluated in this study did not present any complications during hospitalization. The most prevalent were nonreassuring fetal status and postpartum hemorrhage, with no significant difference between the groups. Osoti et al. found similar results, with most of the inductions progressing uneventfully, with the two main complications being postpartum and uterine hyperstimulation, without statistical significance between the groups[9].

We did not find any significant difference between the study groups when assessing fetal vitality. Osoti et al. found a similar result regarding the NICU admission rates in both the control (18.9%) and the combined (16.7%, p=0.697) groups[9]. In contrast, Ornat et al. found a lower NICU admission rate in the "combined" group (p=0.03)[8].

Despite this strong evidence, our research had some limitations mainly related to the nonblinding of the assistants due to the nature of the study, which may have influenced patient management. The higher occurrence of losses due to early interruption of the induction protocol can reflect this situation.

CONCLUSION

Combining the vaginal misoprostol and transcervical Foley catheter methods did not significantly reduce the induction failure rates. However, it did reduce the labor induction and hospitalization times, in addition to reducing the number of misoprostol tablets used for induction and the need for more than one misoprostol dose, without interfering with the risk of adverse maternal and perinatal outcomes, proving to be an interesting method to be added to the protocols of the services involved in the study.

Additional studies are suggested to evaluate other variables involved in the induction process, such as costs of the procedures and maternal satisfaction.

AUTHORS' CONTRIBUTIONS

JAE, BAA: Data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing – original draft, and writing – review & editing. **LKV:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, and writing – review & editing.

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ORIGINAL ARTICLE

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Clinical and electrophysiological efficacy of extracorporeal shock-wave therapy in carpal tunnel syndrome: a placebo-controlled, double-blind clinical trial

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the efficacy of radial extracorporeal shock wave therapy on pain, functionality, and electrophysiological measurements in carpal tunnel syndrome.

METHODS: Between June 2021 and January 2022, a total of 66 wrists in 45 participants with mild-to-moderate carpal tunnel syndrome were included in this double-blind, prospective, randomized, placebo-controlled study. Patients were randomized into two groups, namely, the radial extracorporeal shock wave therapy (group 1, n=33) and the sham radial extracorporeal shock wave therapy (group 2, n=33). Night splints and tendon nerve gliding exercises were given to all participants. The participants were evaluated at baseline and the first month after treatment. Participants were evaluated using a visual analog scale, the Boston Carpal Tunnel Questionnaire, Leeds Neuropathic Symptom and Symptom Assessment, and electrophysiological examinations.

RESULTS: A total of 37 participants (a total of 55 wrists, radial extracorporeal shock wave therapy n=27, and sham radial extracorporeal shock wave therapy n=28) completed the study. After the intervention, there was a significant decrease in visual analog scale values ($p<0.001$) and a significant increase in Boston Carpal Tunnel Questionnaire scores ($p<0.001$) and Leeds Neuropathic Symptom and Symptom Assessment scores ($p<0.001$). In electrophysiological measurements, there was a significant decrease in median nerve sensory ($p=0.002$) and motor ($p=0.003$) distal latency, and a significant increase in median nerve sensory conduction velocity ($p=0.026$) was found in the radial extracorporeal shock wave therapy group.

CONCLUSION: This study shows that radial extracorporeal shock wave therapy has positive effects on pain, functionality, and electrophysiological measurements for mild-to-moderate carpal tunnel syndrome 1 month after application.

KEYWORDS: Carpal tunnel syndrome. Extracorporeal shockwave therapy. Median neuropathy.

INTRODUCTION

Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy resulting from compression of the median nerve at the wrist level[1]. Diabetes, rheumatoid arthritis, amyloidosis, hypothyroidism, obesity, acromegaly, previous wrist fracture, menopause, and pregnancy are known risk factors for CTS[2]. Studies have shown that the use of vibrating tools, compelling movements of the wrist, and computer use affect the development of CTS[3]. Clinical features of CTS include night pain, numbness, and tingling sensation in the median nerve dermatome[4]. It has been reported that carpal tunnel pressure increases 8–10 times compared with normal during wrist flexion and extension and causes ischemic compression in the median nerve[1], and nighttime symptoms are also explained by the increase in pressure in the canal with the wrist flexed at night[5].

Extracorporeal shock wave therapy (ESWT) can be classified as focused (fESWT) and radial (rESWT) according to the targeted area, as well as low, medium, and high energy according to the energy level[6]. ESWT, which has become widespread in the treatment of different musculoskeletal diseases, has increasing evidence in the treatment of CTS[7]. The mechanism of action of ESWT in the treatment of neuropathy is generally explained by the stimulation of neurogenesis and angiogenesis through different molecules and by the anti-inflammatory effect[8]. Although positive effects were found on functional measurements, pain, and electrophysiological parameters in the meta-analysis examining the results of ESWT application in the treatment of CTS, there are uncertainties about the optimal treatment protocol[7].

In this study, we aimed to investigate the effect of rESWT treatment on symptom severity, functional outcomes, and

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electrophysiological parameters in patients diagnosed with mild-to-moderate CTS.

METHODS

The study protocol was approved by Istanbul Kanuni Sultan Suleyman Training and Research Hospital Training and Research Hospital Ethics Committee in accordance with the Declaration of Helsinki (Date: May 27, 2021, Number: 2021.05.162). All participants were informed about the study before inclusion in the study, and their written consent was obtained.

This single-center, prospective, double-blind, randomized, placebo-controlled study was conducted at Istanbul Kanuni Sultan Suleyman Training and Research Hospital Training and Research Hospital, Physical Medicine and Rehabilitation outpatient clinic, between June 2021 and January 2022. Standard electrophysiological tests were performed on 65 participants with a preliminary diagnosis of CTS. A total of 66 wrists from 45 participants with mild-to-moderate CTS who met the inclusion criteria were included in the study. The inclusion criteria were as follows:

1. diagnosis of mild-to-moderate CTS,
2. age 40–60 years,
3. no treatment for CTS in the last 6 months,
4. positive Phalen and Tinel tests, and
5. agreement to withhold other therapeutic interventions for CTS during the study and follow-up period.

Notably, 20 of the initially evaluated participants with mild-to-moderate CTS were excluded from the study according to the exclusion criteria. The exclusion criteria were as follows: brachial plexopathy, polyneuropathy, other upper extremity entrapment neuropathies and cervical radiculopathy, history of wrist fracture, history of cervical spinal and wrist surgery, steroid injection for CTS in the last 6 months, bleeding disorder, and pregnancy.

All participants were evaluated before treatment and 1 month after treatment. Participants were randomized into two groups (rESWT and sham rESWT groups) using a computer program. If a participant had bilateral CTS, both wrists were assigned to the same treatment group (i.e., rESWT or sham rESWT). The study flowchart is shown in Figure 1. The ESWT intervention was performed by an investigator blinded to outcome measures and randomization.

Treatment methods

Shock wave therapy

While the participant was in the sitting position, the forearm was placed on the table with the finger and palm facing up. The median nerve was found using musculoskeletal ultrasonography Digital Sonoace 5500 machine (Medison America Inc., Cypress, CA, USA), and the ESWT probe was placed perpendicular to the median nerve. rESWT was performed using a Vibrolith ESWT device (Elmed Medical Systems, Orlando, FL, USA). The proximal carpal tunnel located at the level of the pisiform bone in the transverse ultrasonography (USG) image was also included in the treatment area. rESWT was performed on the participants once per week for three consecutive weeks, for a total of three sessions (2,000 shots, with 1.6 bar and frequency of 6 Hz rESWT). ESWT was performed similarly to the participants in the sham rESWT group (group 2) but without skin contact. The participants were evaluated in terms of adverse effects and safety after treatment. In three participants in the rESWT group, no additional complications were observed except for paresthesia symptoms in the median nerve trace, which regressed in a short time without the need for additional intervention after the treatment session.

Wrist splint

All the participants included in the study were advised to use a wrist splint of the appropriate size as much as possible, every night and day during the study period. Splint use of the participants was monitored through weekly phone calls.

Exercise

An exercise program based on nerve and tendon gliding exercises developed by Totten and Hunter[9] was taught and given in written format to all participants in the study. To monitor exercise compliance, weekly phone calls were made to the participants, and they were asked to maintain a diary with the exercise details. Participants were asked to repeat each exercise 10 times with three times per day.

Outcome measures

The participants were evaluated two times, at the beginning and the first month after the treatment. Participants were evaluated using a visual analog scale (VAS), the Boston Carpal Tunnel Questionnaire (BCTQ), Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), and nerve conduction studies.

The VAS was used to measure the hand pain experienced by the participants, with a scale ranging from 0 (no pain) to 10 (extremely severe pain)[10].

The BCTQ is the most commonly used questionnaire in clinical trials to assess the symptom severity and functional status of patients with CTS. There are 11 questions in the symptom severity subscale and eight questions in the functional status subscale[11].

The LANSS pain scale is used to evaluate neuropathic pain. In the first part of the test, there are five descriptive questions about neuropathic pain. In the second part of the test, the painful and painless area is compared in a physical examination[12].

Nerve conduction study (NCS) was performed using a Neuropack S1 MEB-9400© (Nihon Kohden, Japan) device by a neurologist. The compound muscle action potential (CMAP) and sensory nerve action potential (SNAP) of

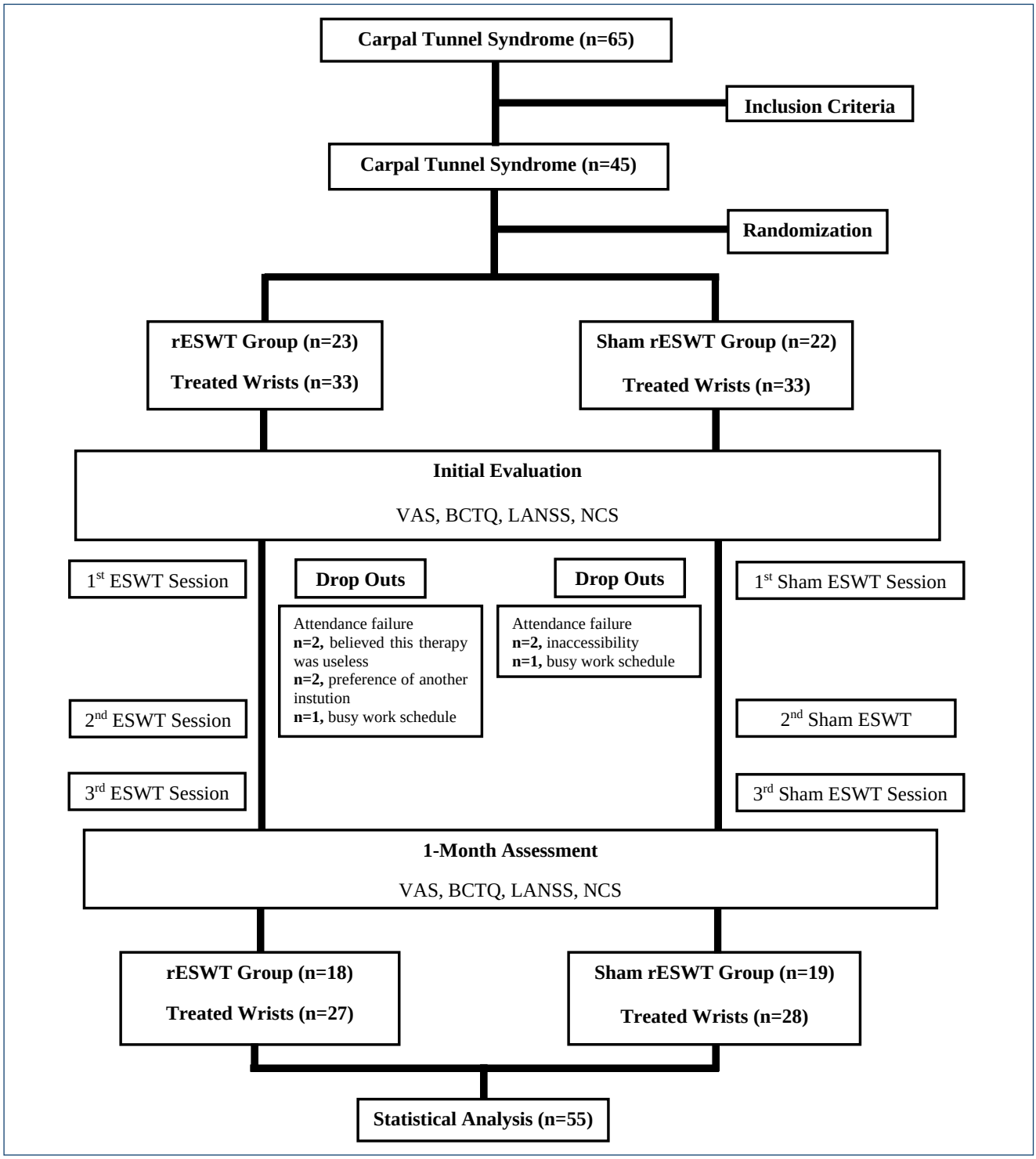

Figure 1. Patient flow and study profile. rESWT: radial extracorporeal shock wave therapy group; sham rESWT: sham radial extracorporeal shock wave therapy group; VAS: visual analog scale; BCTQ: Boston Carpal Tunnel Questionnaire; LANSS: Leeds Assessment of Neuropathic Symptoms and Signs; NCS: nerve conduction study.

the median and ulnar nerves were measured in the upper extremities. The onset latency and the baseline-to-peak amplitude of the CMAPs were measured. Onset latency, peak-to-peak amplitude, and conduction velocity were recorded for each measurement. The results were processed according to published reference values accepted by our EMG laboratory[13].

Data analysis

The sample size of the study was calculated using the G*Power version 3.1.9 program (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) based on the change in pain intensity. According to the sample size calculation, to achieve $\alpha<0.05$ and $\beta=95\%$ according to the VAS scores with an effect size of 0.98, it was calculated that a minimum of 24 participants would be required for each group as described by Xu et al.[14].

The IBM SPSS for Windows version 21.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. For intra-group analysis, the paired-sample t-test or Wilcoxon signed-ranks test was used, and for inter-group analysis, the independent samples t-test or Mann-Whitney U test was used according to the distribution of the variables.

RESULTS

Of 45 participants, 37 completed the study, and a total of 55 wrist results were analyzed (n=27 in the rESWT group and n=28 in the sham-rESWT group). The participants' reasons for leaving the study are given in Figure 1.

The demographic and clinical characteristics of the participants are shown in Table 1.

According to the intra-group analysis results, there were statistically significant changes in terms of VAS (p<0.001), BCTQ (p<0.001), LANSS (p<0.001), median nerve sensory distal latency (p=0.002), median nerve sensory conduction velocity (p=0.007), and median nerve motor distal latency (p=0.004) in the rESWT group. No statistically significant changes were found in any parameters in the sham-rESWT group.

A statistically significant decrease was found in the VAS (p<0.001), BCTQ (p<0.001), and LANSS scores (p<0.001) in the rESWT group in the inter-group analysis. In addition, in the rESWT group, there was a significant increase in median nerve sensory conduction velocity (p=0.026), and a significant decrease in the electrophysiological values of median nerve motor (p=0.003) and sensory distal latency (p=0.002). Detailed information is shown in Table 2.

DISCUSSION

This study aimed to investigate the effect of rESWT performed in addition to splint and exercise in the treatment of CTS. In this prospective, randomized, placebo-controlled, and double-blind study, the results showed that rESWT reduced pain and improved functional and electrophysiological findings in mild-to-moderate CTS.

In the literature, low-energy ESWT applied to nervous tissue is stated to be a safe and effective treatment[15], and high-energy ESWT has no negative effect on peripheral nerves. ESWT is used for peripheral neuropathies such as interdigital neuroma and distal symmetric polyneuropathy, as well as CTS[16,17].

Table 1. Demographic and clinical characteristics of the participants.

Variables	ESWT (n=27)	Sham ESWT (n=28)	p
Age (year)	43.8 (8.3)	46.9 (9.3)	0.415
BMI (kg/m^2)	29.8 (5.1)	30.9 (4.9)	0.715
Laterality			
Right wrist	17 (63)	13 (46.4)	0.221
Left wrist	10 (37)	15 (53.6)	
Duration of symptoms, month	16.5 (16.6)	17.3 (20.0)	0.376
Duration of symptoms, range			
3–6 months	11 (40.7)	12 (42.9)	0.529
6–12 months	4 (14.8)	7 (25.0)	
>12 months	12 (44.5)	9 (32.1)	
VAS	6.4 (2.1)	6.9 (1.6)	0.207
Boston SSS	2.7 (0.5)	2.8 (0.4)	0.979
Boston FCS	3.0 (0.8)	2.7 (0.7)	0.431
Boston-total score	5.7 (1.2)	5.5 (1.0)	0.744
LANSS	12.1 (4.7)	9.8 (5.0)	0.745
mSNDL (ms)	3.3 (0.8)	3.3 (0.7)	0.645
mSNA (mV)	12.6 (6.9)	11.3 (6.8)	0.852
mSNCV (m/s)	35.8 (6.9)	36.3 (6.5)	0.518
mMNDL (ms)	4.2 (0.8)	3.9 (0.6)	0.080
mMNA (mV)	7.8 (1.9)	8.5 (2.3)	0.512
mMNCV (m/s)	58.7 (4.7)	54.9 (5.4)	0.901

ESWT: extracorporeal shock wave therapy group; sham ESWT: sham extracorporeal shock wave therapy group; BMI: body mass index; VAS: visual analog scale; SSS: Symptom Severity Scale; FCS: Function Severity Scale; LANSS: Leeds Assessment of Neuropathic Symptoms and Signs; mSNDL: median sensory nerve (2. Finger-wrist) distal latency; mSNA: median sensory nerve (2. Finger-wrist) amplitude; mSNCV: median sensory nerve (2. Finger-wrist) conduction velocity; mMNDL: median verve motor distal latency; mMNA: median motor nerve amplitude; mMNCV: median motor nerve conduction velocity. p<0.05 is considered significant for the homogeneity of variables.

Table 2. Intra- and inter-group analysis of the outcome measures.

	ESWT (n=27)	p[a]	Sham ESWT (n=28)	p[b]	p[b]
VAS					
PreT	6.4±2.1	<0.001	6.9±1.6	0.05	<0.001
1st month	3.5±2.1		6.6±1.8		
Boston SSS					
PreT	2.77±0.50	<0.001	2.85±0.45	0.058	<0.001
1st month	1.89±0.61		2.68±0.59		
Boston FCS					
PreT	3.00±0.84	<0.001	2.70±0.71	0.155	<0.001
1st month	1.98±0.61		2.57±0.61		
Boston-total score					
PreT	5.77±1.20	<0.001	5.42±0.97	0.076	<0.001
1st month	3.87±1.16		5.24±1.03		
LANSS					
PreT	5.39±1.85	<0.001	5.33±1.54	0.302	<0.001
1st month	3.62±1.44		4.76±1.65		
mSNDL (ms)					
PreT	3.3±0.8	0.002	3.3±0.7	0.245	0.002
1st month	3.0±0.7		3.4±0.8		
mSNA (mV)					
PreT	12.6 ±6.9	0.641	11.3 ±6.8	0.061	0.189
1st month	13.4±8.9		9.6±5.6		
mSNCV (m/s)					
PreT	35.8±6.9	0.007	36.3±6.5	0.961	0.026
1st month	39.7±9.4		36.4±5.9		
mMNDL (ms)					
PreT	4.2±0.8	0.004	3.9 ±0.6	0.308	0.003
1st month	3.9±0.7		3.9±0.7		
mMNA (mV)					
PreT	7.8±1.9	0.332	8.5±2.3	0.553	0.251
1st month	7.4±1.3		8.7±2.1		
mMNCV (m/s)					
PreT	58.7±4.7	0.400	54.9±5.4	0.516	0.873
1st month	58.4±5.3		54.6±5.9		

ESWT: extracorporeal shock wave therapy group; sham ESWT: sham extracorporeal shock wave therapy group; VAS: visual analog scale; SSS: Symptom Severity Scale; FCS: Function Severity Scale; LANSS: Leeds Assessment of Neuropathic Symptoms and Signs; mSNDL: median sensory nerve (2. Finger-wrist) distal latency; mSNA: median sensory nerve (2. Finger-wrist) amplitude; mSNCV: median sensory nerve (2. Finger-wrist) conduction velocity; mMNDL: median verve motor distal latency; mMNA: median motor nerve amplitude; mMNCV: median motor nerve conduction velocity; PreT: pre-treatment; [a]p: p-value for intra-group analysis; [b]p: p-value for inter-group analysis; p<0.05 is considered significant.

Studies on the effectiveness of ESWT in the treatment of CTS were conducted in the following years, compared with oral nutraceutical capsule treatments[18], USG intervention[19], and sham rESWT[8], and it has been seen that ESWT is an effective and safe treatment method in the treatment of CTS. In a study investigating the optimal number of ESWT sessions to be performed in the treatment of CTS, better functional results were observed in three sessions of ESWT compared with one

session and a placebo group[20]. Similar to these results, in the present study, three sessions of ESWT were found to be effective on clinical and electrophysiological results compared with sham applications.

The mechanism of action of ESWT in an entrapment neuropathy such as CTS is not fully understood. Studies have reported that the biological effects of ESWT include tissue regeneration, wound healing, angiogenesis, bone remodeling, and anti-inflammation[21]. The anti-inflammatory effect of ESWT is generally like the mechanism of action indicated in other musculoskeletal diseases where ESWT is widely used[22]. Different studies have shown that ESWT provides anti-inflammatory effects by increasing nitric oxide (NO) levels through enzymatic and non-enzymatic NO production[23]. Reduction of inflammation in the carpal tunnel as a result of its anti-inflammatory effect can reduce perineural pressure and improve symptoms[7].

There are also studies on the effect of ESWT on peripheral nerve regeneration. Studies have shown increased Schwann cell proliferation and axonal regeneration in nerve tissue after treatment with ESWT[24]. The improvement in electrophysiological parameters detected in the present study can be explained by this mechanism.

In the evaluations made in terms of ESWT safety, no serious adverse effects of the treatment were reported. In general, mild complications such as pain and redness that resolve spontaneously have been reported[7]. In our study, ESWT treatment was found to be safe and well-tolerated, except for mild symptoms that resolved without the need for local and additional intervention.

The strengths of this study are as follows. This is a double-blind, randomized, sham-controlled, and prospective study. In addition, determining the treatment area with USG and using rESWT before ESWT is performed on patients are also strong points. The main limitation of this study is the short follow-up period after treatment. Studies investigating the long-term effectiveness of rESWT are needed.

CONCLUSION

This study shows that the rESWT application has positive effects on pain, functionality, and electrophysiological measurements in patients with mild-to-moderate CTS.

ETHICAL STATUS

The study protocol was approved by the Clinical Research Ethical Board of Kanuni Sultan Suleyman Training and Research Hospital (Approval no: KAEK/2021.05.162) in conformity with the Declaration of Helsinki. The study was registered at https://clinicaltrials.gov (ID number: NCT04896398).

AUTHORS' CONTRIBUTIONS

AKM: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **MDK**: Data curation, Formal Analysis, Writing – review & editing. **HS**: Data curation, Writing – review & editing.

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ORIGINAL ARTICLE

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Effectiveness of a course on family health in the knowledge of doctors of the *Mais Médicos* program

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the effectiveness of the distance education course in family health in the knowledge of physicians from the *Mais Médicos* program.

METHOD: This is a quantitative, quasi-experimental study, without a pretest and posttest control group, carried out from August 2019 to September 2021. In all modules, physicians responded to a pretest and posttest to verify their knowledge of the subject.

RESULTS: There was a statistically significant difference in all modules with higher average scores in the posttests; the modules with the greatest emphasis are child health care: growth and development; approach to cancer in primary health care; and family health strategy and territorialization.

CONCLUSION: The effectiveness of the distance education course was verified, as evidenced by the significant improvement of knowledge in all the modules studied.

KEYWORDS: Community health planning. Primary health care. Education, distance. Education, continuing. Medicine.

INTRODUCTION

Primary Health Care (PHC) emerged as a strategy to achieve the principles of the Unified Health System (*SUS – Sistema Único de Saúde*), such as integrality, equity, and universality, which comprises a set of individual or collective actions, with the purpose of promoting and protecting health, preventing diseases and conditions, and meeting the needs of the individual, the family, and the community[1,2].

Faced with this demand, PHC professionals face constant challenges regarding their performance in different areas of knowledge[3]. Many professionals feel the need for continuous education that adds knowledge to professional practice due to the mechanisms provided by routine and constant changes in the health area. This drives them to a constant update for qualified care, making the need for Permanent Education in Health (PEH) evident[4,5].

The National Policy on Permanent Education in Health (*PNEPS – Política Nacional de Educação Permanente em Saúde*) encourages pedagogical proposals based on the problematization and meaningful learning of professionals[6,7]. From this, the Permanent Education Program in Family Health (*PEPSUS – Programa de Educação Permanente do Sistema Único de Saúde*) emerged as an interdisciplinary strategy of support and strengthening for PHC, linked to provision policies such as the *Mais Médicos* program (*PMM – Programa Mais Médicos*) of the Ministry of Health (MoH).

PEPSUS is carried out through distance education (DE) tools from the SUS Virtual Learning Environment (*AVASUS – Ambiente Virtual de Apredizagem do SUS*)[8,9]. This teaching modality, through virtual tools, allows professionals new learning opportunities, a reduction of distances, and flexibility of time to reconcile studies and work activities[10].

Considering the importance of this type of education and PEH for PHC professionals, as well as the scarcity of studies that evaluate the results of these courses, this study aims to analyze the effectiveness of a DE course in family health on the knowledge of physicians from the *Mais Médicos* program.

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METHOD

This is a quantitative, quasi-experimental research, without a pretest and posttest control group. The study was carried out from August 2019 to September 2021 using the *AVASUS* online platform.

The theme of the program is the family health strategy (FHS), and its objective is to contribute to the consolidation and improvement of PHC as a guide for the health care model. The course lasts 48 weeks, has a workload of 360 h, and allows for autonomous construction using the problematization method[11].

The specialization was organized into modules; the mandatory ones were Reception of Spontaneous and Scheduled Demand; Reproductive, Prenatal, and Puerperium Planning; Attention to Child Health and Attention to Mental Health; Health of the Elderly; Approach to Cancer; and Control of Chronic Non-Communicable Diseases in PHC. The optional modules were chosen based on the student's interest and choice among 33 topics. This study verified knowledge related to mandatory modules[11].

Before classes, the professionals signed a free and informed consent term (ICF) and answered an instrument with 10 multiple-choice questions related to the topic addressed in each module. After each class, the participants were again subjected to the instrument to identify the knowledge acquired in the studied module. Students had only one chance to respond in the pretest and posttest and had access to their successes/mistakes in the posttest.

This study used a sample of 620 specialist students enrolled in the specialization course in family health. Physicians who did not complete the activities and who dropped out of the study were excluded from the research.

The paired *t*-test was used, and the confidence coefficient adopted was equal to 5% ($p<0.05$). The Cohen effect size estimate was calculated, and the cutoff points related to Cohen's[12] effects were classified as follows: negligible ($d<0.20$), small ($d^3 0.20$ and <0.50), medium ($d^3 0.50$ and <0.80), and large ($d^3 0.80$).

The project was approved in 2019 by the Research Ethics Committee of the Universidade Federal de Pernambuco (UFPE), Opinion Report No. 3,745,515, in line with Resolution No. 466/2012[13].

RESULTS

The specialist students working in Family Health Units in the states of Amazonas (93), Amapá (72), Bahia (2), Ceará (1), Minas Gerais (1), Pará (4), Paraíba (1), Pernambuco (1), Paraná (160), Rio Grande do Norte (135), Roraima (54), Sergipe (81), and São Paulo (15) (458 Brazilians trained in Brazil and 162 individual exchange students, that is, professionals regardless of nationality, trained abroad). At the end of the course, the total dropout rate was approximately 28%, totaling 441 specialist students who completed it. The pandemic period may have influenced the dropout rate of the course subjects, which was taken into account in the analysis.

It was observed that the difference was statistically significant in all modules. Table 1 presents a description of these results in the pretest and posttest.

The effect size of the DE course from Cohen's *d* was considered large and therefore clinically important in all modules of the course.

DISCUSSION

The DE modality encourages the participation and engagement of professionals in seeking to deepen their knowledge, providing greater autonomy, skill development, and an exchange of experiences with other participants in the learning process. It is noteworthy that DE does not require the replacement of other traditional forms of teaching but intends to be integrated as a complementary methodology, especially in the continuing education of health professionals[14].

The main advantage of courses in the DE modality, such as Massive Open Online Courses (MOOCs), is the flexibility of access to materials, the integration of participants, and the possibility of interaction through forums. However, the participants' persistence and completion of the course are challenges[15].

Results of a study that evaluated the ease of engagement and persistence of students in MOOCs observed that academic self-efficacy, teaching presence, and perceived usefulness had significant effects on learning engagement ($p<0.05$). Also, the presence of teaching, perceived ease of use, and learning engagement had significant effects on learning persistence ($p<0.05$)[16].

The findings are consistent with the training of physicians for PHC, with challenges arising from the course's own curricular matrix, which emphasize specialized areas on the diagnosis and treatment of diseases that focused less on public policies, mental health, and management in the SUS. A study carried out with managers of 125 courses showed that the skills of risky prenatal care, follow-up of psychiatric patients, and psychiatric urgency and emergency care were considered the least developed in medical training courses. Course coordinators also point out as problems in this scenario the fragmentation of teaching and practice, emphasis on specializations, shortage of faculty and practical scenarios, and interprofessional development[17].

Table 1. Differences between the pretest and posttest of *Programa de Educação Permanente do Sistema Único de Saúde* modules.

Modules	n	Mean	Standard deviation	Standard error	CI*		*p*-value	Cohen's *d*	r
Public health policies and health reform									
Pretest	557	5.27	1.81	0.08	5.12	5.42	<0.001	2.41	0.77
Posttest	557	8.90	1.12	0.05	8.80	8.99			
Family health strategy and territorialization									
Pretest	556	6.53	1.89	0.08	6.37	6.68	<0.001	1.71	0.65
Posttest	556	9.13	1.03	0.04	9.05	9.22			
Observation of the health unit									
Pretest	552	5.68	1.96	0.08	5.51	5.84	<0.001	2.18	0.74
Posttest	552	9.11	1.06	0.04	9.02	9.19			
Advice on spontaneous and scheduled demand									
Pretest	524	7.11	1.62	0.07	6.97	7.24	<0.001	1.28	0.54
Posttest	524	8.87	1.07	0.05	8.78	8.96			
Reproductive planning, prenatal, and postpartum									
Pretest	518	5.85	1.74	0.08	5.70	6.00	<0.001	1.96	0.70
Posttest	518	8.67	1.05	0.05	8.58	8.76			
Child health care									
Pretest	464	7.87	1.45	0.07	7.74	8.00	<0.001	1.27	0.54
Posttest	464	9.39	0.86	0.04	9.31	9.47			
Approach to cancer in PHC									
Pretest	458	7.02	1.73	0.08	6.86	7.18	<0.001	1.65	0.64
Posttest	458	9.37	1.03	0.05	9.27	9.46			
Control of noncommunicable chronic diseases in PHC									
Pretest	457	6.80	1.74	0.08	6.64	6.96	<0.001	1.50	0.60
Posttest	457	8.92	0.98	0.05	8.83	9.01			
Mental health care in PHC									
Pretest	448	6.21	1.77	0.08	6.05	6.38	<0.001	1.84	0.68
Posttest	448	8.83	0.95	0.05	8.74	8.92			
Elderly health care in PHC									
Post	448	5.85	2.18	0.10	5.65	6.05	<0.001	1.18	0.51
Posttest	448	7.97	1.30	0.06	7.85	8.09			

*CI: confidence interval.

Medical training, especially in the PHC context, has challenges because there is an emphasis on hospital disciplines focused on specialized areas since graduation[18]. This is also observed in the international scenario, where there were problems in specialized training to work in PHC, as doctors had deficient and outdated knowledge, lack of security, and problems in dealing with other professionals and patients[19].

These aspects are the main factors that limit the strengthening of PHC, given the scarcity of investments, low salaries, and deficient education, which reinforce the discouragement of professionals to work in PHC. From this, the need for changes to intensify the workforce of physicians in PHC is identified, with an emphasis on qualification for leadership, motivation, resolution, and protagonism[20].

In this sense, there is a need to reformulate curricula and teaching strategies for adequate training and qualification within the scope of PHC. Studies show that competency-based education, with the development of skills for the practical scenario,

active participation, teamwork, and implementation of technological resources are some of the trends for improvement in the training of physicians[21].

It is noteworthy that the course of this study took place during the pandemic, when educational programs canceled face-to-face activities and migrated to online resources, strengthening DE initiatives. The use of these technologies proved to be a resource for promoting the learning of skills in training[22].

Online teaching has the potential for self-directed learning and competency-based teaching when done properly. For this, it requires good practices in the implementation of this tool, such as the alignment of the curriculum and objectives; synchronous and asynchronous interaction; encouragement of active learning; and feedback focused on individuality. Furthermore, it is essential to train teachers to achieve the necessary teaching skills[23].

In this context, continuing education provides an opportunity to deepen the deficient knowledge linked to the experience of professional practice, and, especially in DE, there is a flexible schedule for the participation of active professionals and the incorporation of online educational technologies[24].

Online technologies provide interactive environments anchored in pedagogical assumptions that allow the construction of the learning process from the relationship between students, educators, and resources[25].

The positive impact of DE is also evidenced in health care, within the scope of PHC, with a decrease in hospitalizations for conditions sensitive to primary care and chronic conditions after the completion of the DE course by health professionals. This demonstrates the need for constant programs of courses and training for professionals in order to improve knowledge and performance in professional practice[25].

However, there is a need to implement strategies in DE that combine the most appropriate resources, activities, and methodologies for the actors involved in the learning process, as this combination is fundamental to avoid the dropout of students from the courses and the lack of engagement[24].

This study highlights the importance of DE teaching in the continuing education of medical professionals working in PHC, as well as suggests that this teaching modality can be beneficial in other contexts and for other health professionals if anchored to the selection of appropriate methodologies and technological resources.

The limitations are related to the instruments not covering the entirety of the sociodemographic characterization and information that would allow further analysis for the effectiveness of the course. Therefore, more studies are needed to verify the benefits of these resources in other contexts and areas of medical training and what factors are related to the results.

CONCLUSION

It was possible to observe the effectiveness of the DE course in family health due to the significant improvement in the general knowledge of the participants in all the tested mandatory modules of the course, pointing out that the DE modality is a valid strategy for the development of continuing education for physicians in the context of PHC. However, it should be noted that such results are not generalizable to all courses in this educational format.

The offer of courses in the DE modality for the practice of permanent education in health is configured as an innovative and very useful tool for a professional qualification in *SUS* services.

AUTHOR'S CONTRIBUTIONS

LRC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **JAS:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. **IKFC:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **IPS:** Validation, Visualization, Writing – original draft, Writing – review & editing. **LSF:** Validation, Visualization, Writing – original draft, Writing – review & editing. **RMN:** Validation, Visualization, Writing – original draft, Writing – review & editing. **SKCM:** Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Fetal thymus in growth-restricted fetuses due to placental insufficiency

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SUMMARY

OBJECTIVE: The aim of this study was to assess fetal thymus size by ultrasound in growth-restricted fetuses due to placental insufficiency and compare to high-risk and low-risk pregnancy fetuses with normal placental function.

METHODS: This is a nested case-control study of pregnant women followed up at a university hospital (July 2012 to July 2013). In all, 30 pregnant women presenting small fetuses for gestational age (estimated fetal weight <p10) due to placental insufficiency (umbilical artery Doppler >p95) were compared to 30 high-risk and 30 low-risk pregnancies presenting normal Doppler indices. The thymus transverse diameter and perimeter were converted into zeta score according to the normal values for gestational age. Head circumference and femur length were used to calculate ratios.

RESULTS: Fetal thymus were significantly lower in pregnancies with placental insufficiency when compared to high-risk and low-risk pregnancies presenting, respectively, transverse diameter zeta score (-0.69±0.83 vs. 0.49±1.13 vs. 0.83±0.85, p<0.001) and P zeta score (-0.73±0.68 vs. 0.45±0.96 vs. 0.26±0.89, p<0.001). There was also a significant difference (p<0.05) in the ratios among the groups: pregnancies with placental insufficiency (TD/HC=0.10, P/FL=1.32, and P/HC=0.26), high-risk pregnancies (TD/HC=0.11, P/FL=1.40, and P/HC=0.30), and control group (DT/HC=0.11, P/FL=1.45, and P/HC=0.31).

CONCLUSION: Fetal thymus size is reduced in growth-restricted fetuses due to placental insufficiency, suggesting fetal response as a consequence of the adverse environment.

KEYWORDS: Thymus gland. Fetal growth restriction. Placental insufficiency.

INTRODUCTION

The relationship between nutrition and immunity is a well-known subject in the literature. It is widely recognized that malnutrition state interferes with the proper functioning of immune system, thus increasing morbidity and mortality due to infectious diseases[1]. During fetal life, chronic malnutrition state can be detected in pregnancies with placental insufficiency and fetal growth restriction (FGR). Studies in *postmortem* babies have shown that FGR is associated with a reduced weight of thymus gland[2], an important organ of immune system, and this event is attributed to atrophy of the lymphoid tissue.

Thymus atrophy seems to be part of a fetal response to intrauterine adversities. In situations such as placental insufficiency, activation of fetal hypothalamic-pituitary-adrenal axis increases glucocorticoid levels, which stimulate morphological and functional changes in a wide range of tissues to ensure fetal survival including thymus involution[3]. This process is also reported in chorioamnionitis and fetal inflammatory response syndrome, and recently, it has been investigated in preeclampsia, maternal diabetes, and COVID-19 infection[4-8]. Small thymus is associated with an increased child mortality, and its postnatal evaluation can predict the likelihood of survival in preterm infants[9,10].

Prenatal evaluation of fetal thymus has been described in the literature[11-14], along with nomograms for measurements of transverse diameter (TD) and perimeter (P), both assessed by ultrasound. This organ can be identified at the level of three-vessel and trachea views as a hypoechogenic and homogeneous element located between the vessels and the sternum. Magnetic resonance imaging (MRI) was also taken, providing an accurate representation of the structure[15]. Some authors tested the hypothesis that intrauterine growth restriction (IUGR) is associated with reduced fetal thymus size and were successful in finding that the organ was significantly smaller in growth-restricted fetuses compared to the control group[16-18].

The aim of this study was to make a sonographic evaluation of fetal thymus size in growth-restricted fetuses due to placental insufficiency and compare to high-risk and low-risk pregnancy fetuses with normal placental function. It was hypothesized that growth-restricted fetuses present reduced thymus size, as a consequence of chronic starvation and intrauterine adversity.

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METHODS

This is a prospective comparative study comprising 90 singleton pregnancies. The research was conducted at the University Hospital (Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo) from July 2012 to July 2013. The protocol (No. 0790/11) was approved on November 11, 2011, by the local ethics committee (Comissão de Ética para Análise de Projetos de Pesquisa – CAPPesq), and all the participants signed informed consent form.

Inclusion criteria for the study group were as follows: high-risk pregnancies presenting small fetuses with gestational age ranging from 26 to 37 weeks, singleton pregnancies, absence of fetal malformations, intact membranes, not in labor, no signs of maternal or fetal infection, and no use of corticoids before ultrasound evaluation. High-risk pregnancies were defined as those with clinical or obstetric complications or maternal morbidity. FGR was characterized by small fetuses presenting estimated fetal weight p10 for gestational age[19] and increased placental resistance characterized by abnormal umbilical artery (UA) pulsatility index (PI)[20] above the 95th centile for gestational age. This population was compared to 30 high-risk pregnancies with normal UA Doppler and normal fetal growth, which presented the following inclusion criteria: high-risk singleton pregnancies presenting adequate gestational age fetuses, with gestational age ranging from 26 to 37 weeks, absence of fetal malformations, intact membranes, not in labor, no signs of maternal or fetal infection, and no use of corticoids before ultrasound evaluation. The normal control group included 30 low-risk pregnancies without maternal or fetal morbidities, with gestational age ranging from 26 to 37 weeks, absence of fetal malformations, intact membranes, not in labor, no signs of maternal or fetal infection, and no use of corticoids before ultrasound evaluation. Gestational age was determined based on a reliable last menstrual period and early ultrasonography. The exclusion criteria for all groups were postnatal diagnosis of anomaly of the newborn and postpartum diagnosis of any maternal or fetal pathology of infectious origin.

All ultrasound evaluations were performed using a transabdominal 3.5-MHz curved-array transducer (Envisor, Philips, The Netherlands, or Voluson Expert, General Electric Medical Systems, Austria) by two examiners. The thymus was measured after its identification at the three-vessel and trachea views of fetal thorax, according to the technique described by Gamez et al.[13]. The measurements were obtained three times by the same observer, in a 3- to 5-min interval between examinations, and the mean of the values obtained was used for the analysis. Thymus TD was standardized by measurement of the diameter perpendicular to the line connecting the center of the sternum and the spine, with the calipers placed at the interface between the thymus and the lungs. P was also measured at the level of three-vessel and trachea views, using the trace function drawing the organ's boundary. All thymus parameters were transformed into z-scores (SD values from the mean) according to normative references[13]. The reproducibility of measurements of the fetal thymus by ultrasound showed an intraobserver correlation for DT of 0.97 (95%CI, 0.93–0.99) and for P of 0.97 (95%CI, 0.93–0.99) and an interobserver correlation for DT of 0.84 (95%CI, 0.68–0.99) and for P of 0.79 (95%CI, 0.55–0.90).

Ultrasound evaluation was performed weekly in placental insufficiency group, and the last assessment right before birth or antenatal corticosteroid was utilized in the analysis. Patients from high-risk and low-risk groups underwent ultrasound evaluation only once, at a similar gestational age as the study group. High-risk pregnancy group was composed of patients presenting maternal or obstetrical diseases with normal UA Doppler indices, during the same period of the study. Pregnant women without maternal or obstetrical morbidities built the control group; they were evaluated at an outpatient clinic during prenatal care appointment.

Conventional fetal biometric measurements were consistently evaluated. Ratios between thymus TD and P with femur length (FL) and head circumference (HC) were established. All Doppler recordings were done in the absence of fetal body or breathing movements. The high-pass filter was set at the minimum, and the size of the sample volume was adapted to the vessel diameter. To adjust for gestational age, all Doppler parameters were transformed into z-scores (SD values from the mean) according to reference curves[20].

Statistical analysis

Data were analyzed using the MedCalc program, version 11.5.1.0 (MedCalc Software, Belgium). The Kruskal-Wallis test was applied to compare continuous nonparametric variables between the groups. Categorical data were compared using the chi-square or Fisher's exact test. To minimize a possible influence of fetal size on the dimension of the thymus, the ratio between thymus measurements and fetal anthropometric parameters was calculated. The level of significance was set at $p<0.05$ for all tests.

RESULTS

A total of 90 patients were included in the study: 30 in growth-restricted fetuses due to placental insufficiency group, 30 in high-risk group, and 30 in low-risk group. Maternal characteristics, perinatal data, and Doppler velocimetry results of all groups

are shown in Table 1. The proportion of nulliparas was similar between the groups (66.7, 43.3, and 50%, p=0.285). The same was observed for maternal age and other characteristics. In growth-restricted fetuses, UA-PI was significantly increased, and so was the corresponding z-score (4.6, -0.5, and -0.2, p<0.001). As expected, the MCA-PI z-score was significantly decreased in this group compared to the others (-2.6, 0.1, and -0.6, p<0.001).

Thymus measurements were successfully obtained in all cases. Table 2 shows that the mean fetal TD and P (z-scores) (-0.689 and -0.734, respectively) and the ratios TD/HC, P/FL, and P/HC were significantly lower in placental insufficiency group (0.096, 1.318, and 0.261, respectively), compared to high-risk and low-risk groups. The TD/FL failed to reveal significant differences among the groups. Figure 1 displays fetal thymus TD and P in placental insufficiency group, high-risk group, and low-risk group plotted on the reference ranges published by Gamez et al.[13].

DISCUSSION

The purpose of this study was to investigate if there is an association between placental insufficiency and reduced fetal thymus

Table 1. Maternal and neonatal characteristics groups with fetal growth restriction, and high-risk and low-risk pregnancies.

Characteristics	Pregnancy with FGR and abnormal Doppler (n=30)	High-risk pregnancy and normal Doppler (n=30)	Low-risk pregnancy and normal Doppler (n=30)	p-value
Maternal age, years	27.4 (7.1)	29.9 (7.4)	28.5 (6.0)	0.138
Parity 0	20 (66.7%)	13 (43.3%)	15 (50.0%)	0.285
Maternal disease				
Hypertension	7 (23.3%)	12 (40.0%)	0 (0%)	<0.001
Diabetes	3 (10.0%)	9 (30.0%)	0 (0%)	0.002
Heart disease	4 (13.3%)	4 (13.3%)	0 (0%)	0.111
Renal disease	3 (10.0%)	1 (3.3%)	0 (0%)	0.198
Lupus	1 (3.3%)	4 (13.3%)	0 (0%)	0.064
Doppler				
UA-PI (z-score)	4.6 (2.7; 28.6)	-0.5 (-2.1; 0.8)	-0.2 (-1.8; 1.2)	<0.001
GA at examination, weeks	33.7 (27.7; 36.9)	34.9 (28.7; 37.1)	31.9 (26.4; 37.0)	0.008
GA at delivery, weeks	34 (28–37)	37 (32–40)	40 (37–41)	<0.001
Birth weight, g	1375 (770–2480)	2890 (1970–4040)	3296 (2465–3900)	<0.001
Newborn gender				
Female	10 (33.3%)	15 (50.0%)	19 (63.3%)	0.067
Male	20 (66.7%)	15 (50.0%)	11 (36.7%)	

Data are expressed as n (%), mean (SD), or median (range). FGR: fetal growth restriction; UA: umbilical artery; MCA: middle cerebral artery; PI: pulsatility index; GA: gestational age.

Table 2. Transverse diameter of the fetal thymus evaluated by ultrasonography in groups with fetal growth restriction, and high-risk and low-risk pregnancies.

Thymus measurements	Pregnancy with FGR and abnormal Doppler (n=30)	High-risk pregnancy and normal Doppler (n=30)	Low-risk pregnancy and normal Doppler (n=30)	p-value
TD (z-score)	-0.689 (0.832)	0.487 (1.125)	0.830 (0.853)	<0.001*
TD/FL ratio	0.485 (0.073)	0.501 (0.073)	0.509 (0.054)	0.392
TD/HC ratio	0.096 (0.011)	0.107 (0.017)	0.109 (0.011)	0.001*
P (z-score)	-0.734 (0.680)	0.447 (0.958)	0.258 (0.885)	<0.001*
P/FL ratio	1.318 (0.198)	1.404 (0.197)	1.448 (0.184)	0.035*
P/HC ratio	0.261 (0.031)	0.299 (0.044)	0.310 (0.035)	<0.001*

Data are expressed as mean (SD). FGR: fetal growth restriction; TD: transverse diameter; P: perimeter; FL: femur length; HC: head circumference. *FGR vs. HR: p<0.05; FGR vs. LR: p<0.05; HR vs. LR: p<0.05.

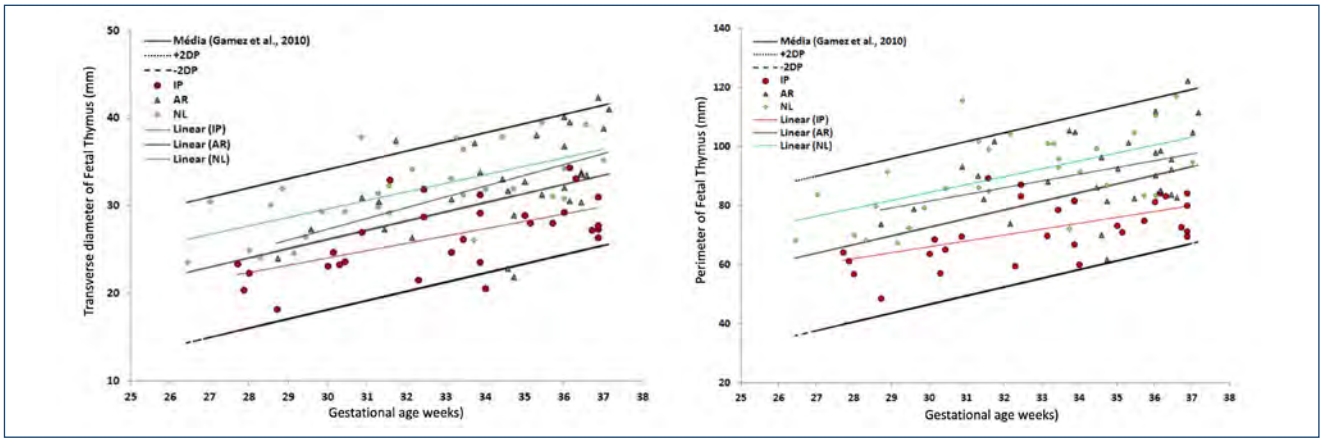

Figure 1. Transverse diameter and perimeter of fetal thymus plotted on reference ranges of Gamez et al. (media, +2DP, -2DP) in patients of placental insufficiency group (●), high-risk group (▲), and low-risk group (◆). The red, green, and blue lines represent the tendencies of placental insufficiency, high-risk, and low-risk groups, respectively.

size, suggesting the process of atrophy under hostile conditions. Our results confirmed that fetal thymus was smaller in growth-restricted fetuses due to placental insufficiency, compared to other groups without this condition. Other studies in the literature also presented similar findings[16-18]. Both high-risk and FGR groups were composed of patients with similar diseases, so they could not be considered confounding factors. The gestational age of inclusion of the cases presented a wide variation, and this may have caused the inclusion of early and late cases of FGR.

To correct the influence of fetal size, we analyzed the ratios of thymus dimensions/biometric parameters, similar to Cromi et al.[3]. We were successful in finding lower values in the ratios TD/HC, P/HC, and P/FL in FGR group, demonstrating that the structure was disproportionately reduced in this group. The only parameter that failed to show significant difference was ratio TD/FL. Cromi et al.[3] studied only thymus P, using reference range of Zalel et al.[11]. However, since the organ presents an irregular shape, in our study, TD was also measured, as a complement evaluation. We used the study of Gamez et al.[13] as a reference because it included the largest number of patients (678 fetuses), compared to other published references for normal ultrasound measurements of fetal thymus, and they assessed both TD and P.

Similarly, Ekin et al.[18] also studied only one parameter of fetal thymus measurement. They established a reference range of TD based on their control group and verified that the proportion of fetuses with this measurement below 5% for gestational age was higher in IUGR with abnormal Doppler group compared to IUGR with normal Doppler group. It apparently suggests that the more critical the situation of placental insufficiency is, the worse the thymus atrophy becomes[21].

In such cases of fetal chronic starvation, thymus involution seems to be mediated by activation of hypothalamic-pituitary-adrenal axis and glucocorticoid release[3]. The increased production of these steroids induces thymocyte depletion, and probably this is the mechanism responsible for thymus shrinkage[22]. Experimental studies in mice have confirmed that exposure to high concentrations of glucocorticoid leads to reduction in the total number of thymocytes, and this is due to an increased rate in cell death. On the contrary, adrenal insufficiency in humans and adrenalectomy of animals result in thymic hypertrophy[22]. The mechanism by which glucocorticoids cause thymocyte apoptosis is not totally known, but it may involve caspases, in a cell type-specific process. In 2020, Jones et al.[23] concluded that antenatal corticosteroid exposure was associated with a significant reduction in thymic size by ultrasound evaluation. Therefore, in our study, we excluded cases that received corticosteroid before assessment.

Fetal thymus can be evaluated in antenatal period either by ultrasound or by MRI[17,24]. The comparison of these two imaging modalities demonstrated good reproducibility, but the high cost of MRI made it not feasible for our study. Although 3D ultrasound assessment method seems to be promising[25], we chose not to use it, because thymus has a complex 3D shape, which could adversely affect the reproducibility of the method. In addition, the learning curve for volume measurement of the structure by 3D ultrasound is much longer and more difficult than standard 2D. Some authors preferred to use a second trimester thymus-thorax ratio, defined as the quotient of anteroposterior thymus diameter and anteroposterior thoracic diameter. However, they failed to predict preterm birth, as there is no gold standard for thymus measurements[5].

Small thymus is associated with increased neonatal adverse outcomes in very low birth weight infants, such as bronchopulmonary dysplasia, respiratory distress syndrome, patent ductus arteriosus, retinopathy of prematurity, periventricular leukomalacia, and sepsis[26], as thymic involution presumably occurs because of depletion of thymocytes. It is known that IUGR infants present low T-lymphocyte count, and this is apparently responsible for an increased susceptibility to infection. A meta-analysis that explored the association between small fetal thymus on ultrasound and adverse obstetrical outcome reported that small thymus increased the risk of neonatal sepsis and morbidity[27]. Small thymus size was also found in fetuses of diabetic mothers when compared to healthy controls[28].

This study had some limitations. There is no gold standard for validating ultrasound methods for measuring the fetal thymus. The small sample size does not allow to cause-and-effect association, and this restricted the performance of multivariate analyses and the construction of predictive curves for the parameters studied. We also did not perform a detailed assessment of factors related to the immunity or immunocompetence of neonates. The study of thymic function in prenatal life may help establish implications for later immune competence. As a limitation, we also observed a significant difference between the groups regarding hypertension. Although UA Doppler is normal in the high-risk group, placental insufficiency can be seen with altered uterine artery Doppler, but this has not been investigated in this population. So, further longitudinal studies must be conducted linking such prenatal assessment to a long-term follow-up of the infants.

CONCLUSION

Fetal thymus size is reduced in growth-restricted fetuses due to placental insufficiency, suggesting fetal response to adverse environment. Currently, we do not recommend the practical use of fetal thymus evaluation to predict perinatal outcome or to determine the timing of delivery in FGR. However, perhaps we should focus more on the fetal thymus in routine of morphological and obstetrical ultrasound. Besides, this field of study has recently started to be applied in association with other diseases during pregnancy, hence there is more to investigate. In the future, studies may combine prenatal assessment with the analysis of immune status markers and long-term follow-up of the infants and, then, establish a clear application of them in clinical practice.

AUTHORS' CONTRIBUTIONS

RMYN: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Writing original draft, Writing – review & editing. **MAT:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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The relationship between premature ventricular complexes and index of cardiac-electrophysiological balance

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SUMMARY

OBJECTIVE: Premature ventricular complexes are common in healthy individuals' ambulatory monitoring. The index of cardiac-electrophysiological balance may predict malignant ventricular arrhythmias. This study investigated the relation between Premature ventricular complex burden and index of cardiac-electrophysiological balance in 24-h Holter monitoring.

METHODS: A total of 257 patients who were admitted to a cardiology outpatient clinic without structural heart disease and underwent 24-h Holter monitoring were included in the study. Demographic features, laboratory parameters, and electrocardiographic and echocardiographic values of all patients were obtained from the hospital database. Patients were categorized into the following four groups according to their premature ventricular complex burden: ≤5% premature ventricular complexes as group 1, >6 and ≤10% premature ventricular complexes as group 2, >11 and ≤20% premature ventricular complexes as group 3, and >20% premature ventricular complexes as group 4. QRS, QT, and T peak to end interval were measured by resting electrocardiography. QT interval was corrected using Bazett's formula. T peak to end interval/QT, T peak to end interval/corrected QT interval, index of cardiac-electrophysiological balance, and corrected index of cardio-electrophysiological balance ratios were calculated.

RESULTS: There was no significant difference between groups regarding cardiovascular risk factors. In group 4, beta-blocker usage was significantly higher, and the serum magnesium levels were significantly lower than in other groups. There was no difference in QT duration or index of cardiac-electrophysiological balance values; however, corrected index of cardio-electrophysiological balance was significantly lower in the highest premature ventricular complex group (5.1, 5.1, 4.8, 4.7, p=0.005). In multivariate backward logistic regression analyses, it was found that lower corrected index of cardio-electrophysiological balance, lower serum magnesium levels, lower serum creatinine levels, larger left atrium size, and higher T peak to end interval were associated with higher premature ventricular complexes.

CONCLUSION: Corrected index of cardio-electrophysiological balance is a novel and noninvasive marker that can predict premature ventricular complex burden in patients with structurally normal hearts.

KEYWORDS: Premature ventricular complexes. Index of cardiac-electrophysiological balance. Electrocardiography.

INTRODUCTION

Premature ventricular complexes (PVCs) are a complex clinical entity in structurally normal hearts. Till date, many studies demonstrated different numbers of PVCs in healthy people[1-3]. Previously, the Framingham Heart Study showed that 12% of patients without coronary artery disease experienced PVCs or complex ventricular arrhythmias in 1 h of monitoring[4]. In another study, >200 PVCs per 24 h was found in 3.3% of 1273 healthy volunteers[1]. Detection of PVC burden depends mostly on the follow-up duration. Burden of PVCs of more than 10% in 24 h highly increases the risk of tachycardiomyopathy and heart failure[5].

The index of cardiac-electrophysiological balance (iCEB), which is the ratio of QT/QRS duration on the surface electrocardiography (ECG), was found to be related to Torsades de Pointes (TdP) or non-TdP-like ventricular arrhythmias in an animal model[6]. A change in cardiac-electrophysiological balance affects the heart's systolic and diastolic potentials and changes ECG parameters. Not only QT duration but also QRS duration could have an impact on ventricular arrhythmias. Therefore, iCEB could be used as a new biomarker for predicting arrhythmic potential in normal individuals.

This study aimed to determine the relationship between iCEB and PVC burden in patients with structurally normal hearts.

METHODS

This retrospective study analyzed 257 patients admitted to a cardiology outpatient clinic without structural heart disease and underwent 24-h Holter monitoring. Patients with any known genetic or structural heart disease were excluded. Patients with coronary intervention and/or coronary surgery history, valvular surgery history, ejection fraction (EF) <50%, atrial fibrillation, intracardiac

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implantable devices, thyroid abnormalities, liver or kidney diseases, active infection, presence of malignancy, psychiatric diseases, and taking anti-arrhythmic drugs were excluded from the study. The study was conducted as per the Declaration of Helsinki, and the study protocol was approved by the hospital's ethics committee.

Patients' demographic features, cardiovascular risk factors, and laboratory parameters were collected from the hospital database. Patients' medications at the time of Holter monitoring were also recorded. All patients underwent 24-h rhythm Holter monitoring. Analyses of PVCs and corrections of the automated computer system results were made by a cardiologist. The total number of PVCs was determined by the automated system. Patients were categorized into the following four groups according to their PVC burden: ≤5% PVCs as group 1, >6% and ≤10% PVCs as group 2, >11% and ≤20% PVCs as group 3, and >20% PVCs as group 4.

The iCEB was determined by resting ECG. All the measurements were performed using the MUSE software (GE HealthCare). QRS duration, QT interval, and heart rates were determined. The measurements were performed on lead II and lead V5, and then the longest QT interval was selected for analysis. QT interval was corrected using the Bazett's formula (QTc=QT/(RR1/2)). iCEB and corrected iCEB (iCEBc) were calculated by dividing QT to QRS duration for iCEB and QTc to QRS duration for iCEBc.

Statistical analysis was performed using the SPSS 24.0 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the distribution pattern of parameters. Continuous parameters with normal distribution were presented as the mean ± standard deviation, whereas parameters with non-normal distribution were presented as median and categorical variables were presented with numbers and percentage values. An analysis of variance test or Kruskal-Wallis tests were used to compare continuous variables according to iCEB groups. A χ^2 test was used to compare categorical variables. Multivariable logistic regression analysis was used to determine the independent parameters related to >20% PVCs. Possible confounding factors for which the unadjusted p<0.10 in univariate regression analysis (age, sex, heart rate, beta blocker usage, creatinine, potassium, calcium, magnesium, corrected iCEB, T peak to end interval, EF, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and left atrial [LA] size) were identified as potential risk markers and included in multivariable logistic regression model. The effects of different variables on in-hospital mortality were assessed by Cox regression analysis. A p<0.05 was considered statistically significant.

RESULTS

Demographic, clinical, and laboratory characteristics of the study population are shown in Table 1. There was no significant difference between groups regarding cardiovascular risk factors, including diabetes, hypertension, hyperlipidemia, or smoking status. In the highest PVC group, beta-blocker usage was significantly higher (54.3%, p<0.001), and the serum magnesium levels were significantly lower (1.9 mg/dL, p=0.011) than in other groups. In echocardiographic parameters, EF was similar between groups; however, LA size was increased from group 1 to group 4.

Electrocardiographic parameters of the patients are presented in Table 2. The duration of QRS was significantly different between groups, and group 4 had the highest QRS duration (84.8±11.6, 85.2±13.5, 91.2±13, 94.1±15 ms, p<0.001). There was no difference in QT duration or iCEB values; however, iCEBc was significantly lower in the highest PVC group (5.1, 5.1, 4.8, 4.7, p=0.005) (Figure 1). Tp-e interval was increased from group 1 to group 4, but there was no difference in the Tp-e/QT and Tp-e/QTc ratios between groups.

When we made a multivariate backward logistic regression analyses with a model including age, sex, heart rate, beta blocker usage, creatinine, potassium, calcium, magnesium, iCEBc, T peak to end interval, EF, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and LA size, it was found that lower iCEBc (hazard ratio [HR] 0.552; 95% confidence interval [CI] 0.530–0.925, p=0.024), lower serum magnesium levels (HR 0.043; 95%CI 0.003–0.535, p=0.014), lower serum creatinine levels (HR 0.057; 95%CI 0.005–0.705, p=0.026), larger LA size (HR 1.143; 95%CI 1.053–1.242, p=0.001), and higher Tp-e interval (HR 1.022; 95%CI 1.005–1.040, p=0.014) were associated with higher PVCs (Table 3).

DISCUSSION

This study demonstrated that lower iCEBc, lower serum creatinine levels, lower magnesium levels, larger LA size, and higher Tp-e interval were associated with higher PVCs in patients with structurally normal hearts.

It is well known that an increase or decrease in the QT interval leads to TdP, non-TdP-related ventricular tachycardia (VT), or ventricular fibrillation (VF). However, PVCs and VT/VF can be seen in patients with even normal QT intervals. This may raise the question of whether other factors may be related to arrhythmias in these patients. We speculated that the QRS interval, which shows ventricular depolarization and is similar to conduction velocity, and the QT interval, which is similar to the ventricular refractory period, can be used to determine arrhythmia risk. Therefore, the ratio of QT/QRS, which is called iCEB, may reflect the cardiac-electrophysiological balance and related to cardiac arrhythmias.

Alteration of the cardiac muscle membrane ion current, intracellular calcium metabolism, sympathetic and parasympathetic system balance, and external factors like some drugs are responsible for PVCs in normal hearts[5-7]. In patients with long QT syndrome, it

was found that iCEB was increased compared to genotype-negative family members, while it was decreased in the Brugada syndrome group[8]. This study showed that not only QT interval but also QRS duration is important in arrhythmia risk in patients with genetic diseases. A change in the iCEB value in any direction causes changes in ventricular depolarization and repolarization duration and may

Table 1. Demographic, clinical, and laboratory characteristics of the study population.

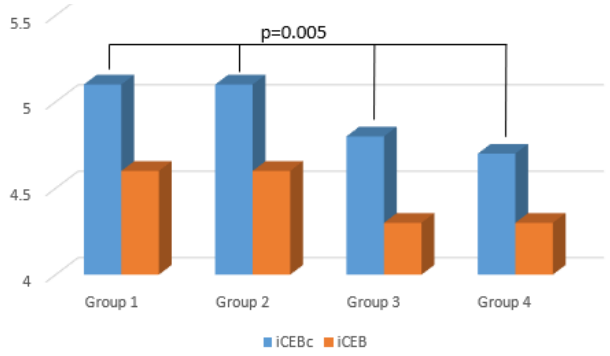
Characteristics	Group 1 (≤5% PVCs) (n=135)	Group 2 (>6% and ≤10% PVCs) (n=40)	Group 3 (>11% and ≤20% PVCs) (n=47)	Group 4 (>20% PVCs) (n=35)	p
Age (years, mean±SD)	51.3±15.9	54.9±19	56.2±15.2	56.2±15.2	0.096
Male, n (%)	61 (45.2)	24 (60)	30 (63.8)	23 (65.7)	**0.036**
SBP (mm Hg, mean±SD)	124±14.4	125.3±15.7	123±14.1	124.3±15.9	0.914
DBP (mm Hg, mean±SD)	72.6±10.4	73±8.9	70.2±9.4	71.1±10.3	0.119
Current smoker, n (%)	3 (2.2)	3 (7.5)	2 (4.3)	3 (8.6)	0.156
Hypertension, n (%)	44 (32.6)	17 (42.5)	15 (31.9)	13 (37.1)	0.661
Diabetes mellitus, n (%)	27 (20)	9 (22.5)	11 (23.4)	8 (22.9)	0.952
Hyperlipidemia, n (%)	13 (9.6)	3 (7.5)	7 (14.9)	6 (17.1)	0.419
Medication, n (%)					
ASA	38 (28.1)	11 (27.5)	20 (42.6)	16 (45.7)	0.092
ACEI/ARB	38 (28.1)	35 (87.5)	32 (68.1)	8 (23.5)	0.965
Beta blocker	71 (52.6)	49 (52.7)	38 (39.6)	19 (54.3)	**<0.001**
Calcium channel blocker	22 (16.3)	3 (7.5)	4 (8.5)	6 (17.1)	0.324
Diuretic	12 (8.9)	7 (17.5)	4 (8.5)	2 (5.7)	0.367
Hemoglobin (g/L, mean±SD)	13.8±1.5	13.9±1.5	13.7±1.6	14±1.4	0.691
WBC (10^3/μL, mean±SD)	7.9±2	7.8±1.8	7.7±1.8	8.3±3	0.553
Platelets (10^3/μL, mean±SD)	251±60.5	241±53.9	248.7±61	238.5±98.7	0.697
Creatinine (mg/dL, mean±SD)	0.8±0.2	0.87±0.2	0.87±0.18	0.8±0.17	**0.027**
GFR (mL/min, mean±SD)	95.8±17.6	90.9±20.5	91±16.2	95±15.6	0.316
Sodium (mmol/L, mean±SD)	140±2.8	140.2±2.9	139.7±2.8	140.4±2.8	0.656
Potassium (mmol/L, mean±SD)	4.5±0.4	4.6±0.3	4.6±0.4	4.4±0.3	0.051
Calcium (mg/dL, mean±SD)	9.2±0.4	9.3±0.4	9.2±0.5	9.1±0.5	0.426
Magnesium (mg/dL, mean±SD)	2±0.17	2.1±0.2	2±0.2	1.9±0.1	**0.011**
AST (U/L, mean±SD)	17.3±6.4	17.6±7.3	17.2±6.2	17.5±6.4	0.996
ALT (U/L, mean±SD)	17.7±9.6	16.1±8.5	16.1±6.3	16.4±11.4	0.653
Total cholesterol (mg/dL, mean±SD)	176.3±41.8	172±32.1	168.5±39.5	164.4±30.2	0.346
HDL-C (mg/dL, mean±SD)	47.5±15.3	47.2±14.3	48.4±12.6	44.5±10.4	0.648
LDL-C (mg/dL, mean±SD)	97±27.6	97.2±24	92.6±30	97.8±24.3	0.769
Triglyceride (mg/dL, mean±SD)	140.1±67	131.1±60.6	135±66.6	115.3±36.7	0.216
Echocardiographic features					
EF (%, mean±SD)	58.6±4.2	58±5	57.1±5.1	57.4±4.1	0.174
LVEDD (cm, mean±SD	4.8±0.5	4.8±0.6	5.1±0.6	5±0.4	**0.003**
LVESD (cm, mean±SD)	3.1±0.5	3.2±0.5	3.5±0.5	3.4±0.5	**<0.001**
LA size (cm, mean±SD)	3.5±0.5	3.6±0.6	3.7±0.5	3.9±0.6	**0.006**

ACEI: angiotensin converting enzyme inhibitor; ALT: alanine transaminase; ARB: angiotensin receptor blocker; ASA: acetyl salicylic acid; AST: aspartate transferase; DBP: diastolic blood pressure; EF: ejection fraction; GFR: glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LA: left atrium; LDL-C: low-density lipoprotein cholesterol; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; PVCs: premature ventricular complexes; SBP: systolic blood pressure; SD: standard deviation; WBC: white blood cell. Bold values indicate statistical significance at the p<0.05 level.

Table 2. Electrocardiographic parameters of the groups.

Variables (mean±SD)	Group 1 (≤5% PVCs) (n=135)	Group 2 (>6% and ≤10% PVCs) (n=40)	Group 3 (>11% and ≤20% PVCs) (n=47)	Group 4 (>20% PVCs) (n=35)	p
Heart rate (bpm)	77.2±13.6	75.4±12.9	75.5±14	72.9±13.9	0.390
QRS interval (ms)	84.8±11.6	85.2±13.5	91.2±13	94.1±15	**<0.001**
QT interval (ms)	382.8±39.2	382.7±44.8	386±43	397±48.8	0.343
QTc interval (ms)	429.5±34.8	422.3±40.6	429±39	432.5±42.6	0.666
Tp-e interval (ms)	88.3±22	85.1±26.6	89.9±22.7	100±24.7	**0.032**
Tp-e/QT ratio	0.23±0.06	0.23±0.08	0.24±0.07	0.25±0.07	0.278
Tp-e/QTc ratio	0.2±0.06	0.2±0.07	0.21±0.05	0.23±0.07	0.113
iCEB (QT/QRS ratio)	4.6±.0.6	4.6±0.8	4.3±0.8	4.3±0.8	0.072
iCEBc (QTc/QRS ratio)	5.1±0.7	5.1±0.9	4.8±0.7	4.7±0.9	**0.005**

Bpm: beat per minute; iCEB: index of cardio-electrophysiological balance; iCEBc: corrected index of cardio-electrophysiological balance; ms: millisecond; PVCs: premature ventricular complexes; QTc: corrected QT interval; Tp-e: T peak to end interval. Bold values indicate statistical significance at the p<0.05 level.



iCEB: index of cardio-electrophysiological balance; iCEBc: corrected index of cardio-electrophysiological balance.

Figure 1. iCEB and iCEBc values of the groups.

Table 3. Multivariable Cox-regression analysis of risk factors for premature ventricular complexes burden.

Variables***	Hazard ratio, 95%CI	p
iCEBc	0.552 (0.530–0.925)	**0.024**
Tp-e	1.022 (1.005–1.040)	**0.014**
Creatinine	0.057 (0.005–0.705)	**0.026**
Magnesium	0.043 (0.003–0.535)	**0.014**
LA size	1.143 (1.053–1.242)	**0.001**

CI: confidence interval; iCEBc: corrected index of cardio-electrophysiological balance; LA: left atrium; Tp-e: T peak to end interval. *Model included age, sex, heart rate, beta-blocker usage, creatinine, potassium, calcium, magnesium, corrected index of cardio-electrophysiological balance, T peak to end interval, ejection fraction, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and left atrial size. **Selection of covariates for multivariable models is explained in the Methods section. Unless otherwise indicated, hazard is interpreted as the presence (vs. absence) of each categorical variable or an increase of 1 unit of each continuous variable. Bold values indicate statistical significance at the p<0.05 level.

cause PVCs. Therefore, other than well-known ECG parameters like QT interval and Tp-e duration[9], iCEB may add more valuable information about the risk of arrhythmias.

Ventricular repolarization markers such as Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios have been evaluated in many clinical situations[10-14]. The Tp-e interval may represent the transmural distribution of repolarization, and an increased Tp-e interval is associated with malignant ventricular arrhythmias. Furthermore, the Tp-e/QT ratio is found higher in diseases with increased arrhythmogenicity, such as long QT syndrome, Brugada syndrome, short QT syndrome, or organic heart disease[15]. Zhao et al. found that the Tp-e interval and Tp-e/QT ratio were increased in polymorphic VT/VF patients with idiopathic PVCs[16]. In another study, Tp-e interval and Tp-e/QTc ratio have been associated with a high PVC number[17]. Similar to this study, in our study, the Tp-e interval was found to be higher in the highest PVC group compared to the other groups.

In the Atherosclerosis Risk in Communities (ARIC) study, a cross-sectional analysis of the 15,792 individuals, according to 2-min ECG, revealed that increasing age, male sex, lower educational attainment, and lower serum magnesium or potassium levels are directly related to PVC prevalence[18]. Similar to the ARIC study, we found lower serum magnesium and creatinine levels and a higher proportion of males in patients with higher PVCs.

In our study, QRS duration increased from the lower PVC group to the higher PVC group. Although QTc duration was similar between the groups, the ratio of QTc/QRS was lower in the highest iCEBc group. In one study, QRS duration was related to the development of PVC-induced left ventricular dysfunction[19]. In that study, patients who developed cardiomyopathy had significantly longer PVC QRS duration (159 vs. 142 ms; p<0.001) and a longer sinus QRS duration (97 vs. 89 ms; p=0.04).

Limitations

Our study has a few limitations. First, this is a single-center study involving less number of patients. Second, the patients were not followed up, and the PVC burden was determined at a single time. Third, we cannot totally exclude the presence of cardiac disease in the study group because cardiac magnetic resonance imaging was not performed.

CONCLUSION

Corrected index of cardio-electrophysiological balance is a novel, noninvasive marker that may predict PVCs in patients with structurally normal hearts. Beyond the other ECG parameters, iCEBc can be used as a definite marker of electrophysiological balance and arrhythmia risk.

AUTHORS' CONTRIBUTIONS

MKA: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **YD:** Data curation, Investigation, Software, Validation, Writing – original draft. **SY:** Formal Analysis, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of patients of vaccine side effects after the COVID-19 vaccine

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SUMMARY

OBJECTIVE: Postvaccine side effects were evaluated in patients presenting to our emergency department with complaints of vaccine side effects after taking COVID-19 vaccine, and new unknown side effects ranging from mild complaints to life-threatening risks, and frequency of all side effects were investigated. This study aimed to establish a scientific resource to identify the potential side effects of the vaccine.

METHODS: Patients' demographic information, clinical characteristics, epicrisis reports, COVID-19 disease and vaccination histories, vital values, and blood values were examined. The SPSS 20.0 package program was used for statistical evaluation. $p<0.05$ was considered statistically significant.

RESULTS: Notably, 13.1% of patients presenting to the emergency department started to have complaints after taking Sinovac vaccine, whereas 86.9% of them had complaints after taking BioNTech vaccine. Also, 36.9% of patients stated that they had COVID-19. All patients had a Glasgow coma scale score of 15 during admission. No patient was hospitalized, ventilator was not needed, and all patients were discharged. While the most common presenting complaint to the emergency department after vaccination was fatigue in 29.7%, the most common diagnoses after examination in the emergency department were myalgia in 32.1% and upper respiratory tract infection in 28.6%.

CONCLUSION: Results and conclusions of our study will guide healthcare workers and patients on the side effects of COVID-19 vaccine.

KEYWORDS: COVID-19. Coronavirus. Vaccines.

INTRODUCTION

The WHO declared COVID-19 as a pandemic on March 11, 2020[1]. Since COVID-19 affects people of all ages and has a mortal course with severe clinical pictures even in healthy people, scientists and states aimed to find protective methods to prevent the transmission of the disease. The spread of the disease is prevented in two practices. The first practice consists of general control measures such as mask, interpersonal social distancing of 1.5 m, hand washing, and staying away from non-ventilated areas[1,2]. The second practice consists of developing vaccine suitable for the disease, and thus preventing the transmission of the disease by vaccinating the general population.

The World Health Organization has authorized the use of vaccines for which pharmaceutical companies applied for approval to use and the necessary studies were completed. The effectiveness of vaccines in reducing the mortality and preventing severe diseases in vaccinated people was found to be 99%[3-5]. Nowadays, the vaccines used to combat COVID-19 have been designed to teach the body's immune system to recognize the virus that causes COVID-19 and to destroy the coronavirus in case of facing COVID-19[3,5,6].

In Turkey, two vaccines are mainly used: BioNTech vaccine, which is an mRNA vaccine with a reported efficacy of 95% and efficacy against new variants, and Sinovac vaccine, which is an inactivated vaccine with an efficacy of 50–70% and may require the administration of the third dose due to a decrease in antibody levels 6 months after vaccination administered as two doses[5,7]. Moderna vaccine: Moderna vaccine was produced similar to the BioNTech vaccine; however, its structure surrounding the mRNA is different[8]. Johnson & Johnson vaccine: Adenovirus type 26 is used as a vector in this vaccine[9].

In this study, post-vaccine side effects were evaluated in patients presenting to our emergency department with the complaints of vaccine side effects after the COVID-19 vaccine, and new unknown side effects ranging from mild complaints to life-threatening risks, and the frequency of all side effects

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were investigated. Furthermore, with this study, it is aimed to establish a scientific resource to know the potential side effects in case of vaccine side effects in the vaccinated person.

METHODS

After taking the medical history of 84 patients presenting to the Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Department of Emergency, between October 1, 2021, and December 31, 2021, due to vaccine-related side effects, the patients were examined, their findings were identified, and then the patient files were examined through the automation system and additional information about the patients was obtained. The study was conducted retrospectively, and the data of the patients presenting with the complaint of vaccine adverse effects between the specified dates were obtained from the hospital automation system and the patient examination file. Vaccination information was received from the Ministry of Health's "vaccination" program. The Kolmogorov-Smirnov test was used to analyze the normal distribution of the data. The SPSS 20.0 package program was used for statistical evaluation and necessary tests were performed. The categorical variables were expressed using numbers and percentages. Measurement-based continuous variables were analyzed for normal distribution and presented as median (minimum–maximum) and mean ± standard deviation. In the evaluation of difference between the groups, the level of change was evaluated by the Mann-Whitney U test, and the relationship between the variables was evaluated by Spearman correlation analysis. $p<0.05$ was considered statistically significant. Ethical approval was obtained from the Ministry of Health of the Republic of Turkey and the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with the resolution number 01 in session 2022/14 on April 26, 2022.

RESULTS

The median age of patients presenting to the emergency department with complaints after taking the COVID-19 vaccine was 36.0, ranging between 18 and 79 years, and 51.2% were female. While body temperatures measured during admission to emergency department were found to be between 35.5 and 39.0°C with median 36.7°C, pulse values were between 53 and 161/min with median 90.0/min, respiratory rates were 92.5±1.9/min, oxygen saturation values were between 92 and 100% with median 97.0%, and systolic blood pressure values were between 95 and 240 mmHg with median 130.0 mmHg. Notably, 31 (36.9%) patients stated that they had COVID-19 (Table 1).

It was determined that 13.1% of the patients presenting to the emergency department due to various complaints after

Table 1. Patient's vital signs, hemogram values, and vaccination-complaint-admission times.

Variable	Variable value	
	Mean ± standard deviation	Median (minimum–maximum value)
Age	37.7±15.5	36.0 (18–79)
Body temperature (°C)	36.8±0.7	36.7 (35.5–39.0)
Pulse/min	92.5±18.9	90.0 (53–161)
Respiratory rate/min	18.0±1.9	18.0 (12–23)
Oxygen saturation (%)	97.1±1.7	97.0 (92–100)
Systolic blood pressure (mmHg)	133.0±21.0	130.0 (95–240)
Hemoglobin (g/dL)		
Female	12.6±1.1	12.7 (10.9–15.3)
Male	13.7±1.1	15.1 (11.2–17.1)
Leukocyte (10^3/mm^3)	8.2±2.8	7.8 (3.6–17.0)
Lymphocyte (10^3/mm^3)	2.0±1.9	1.64 (0.4–14.1)
D-dimer (µg/L)	0.44±0.30	0.36 (0.18–1.57)
Time between vaccination and complaint	1.1±1.2	1.0 (0–4)
Time between vaccination and admission to the emergency department	2.0±1.6	1.0 (0–8)
Time between complaint and admission to the emergency department	0.7±1.4	0.0 (0–8)

vaccination started to have complaints after taking the Sinovac vaccine, while 86.9% of them had complaints after taking the BioNTech vaccine. While 5 patients stated that their complaints occurred after the fourth dose of vaccine, 23 patients indicated that their complaints occurred after the third dose of vaccine, and 40 patients and 16 patients indicated that their complaints occurred after the second dose of vaccine and after the first dose of vaccine, respectively (Table 2).

There was a positive correlation between the age of the patients and the time between vaccination and the onset of complaints. The time between vaccination and the onset of complaints increased as the age of the patients increased (r=0.274, p=0.02). While a weak positive correlation was found between the patients' D-dimer values and age (r=0.319, p=0.003), there was a weak negative correlation with hemoglobin values (r=-0.346, p=0.001). The time of vaccination-complaint and complaint-admission increased as the time between vaccination and admission to the emergency department increased, and the time of complaint-admission decreased as the time of vaccination-complaint increased.

Table 2. Vaccination information, symptoms, diagnosis, and treatment information of the patients.

	n	%
Gender		
Female	43	51.2
Male	41	48.8
Additional disease		
Yes	10	11.9
No	74	88.1
Positive for COVID-19 in the past?		
Yes	31	36.9
No	53	63.1
Hemoglobin value		
Normal and high	70	83.3
Low	14	16.7
Last vaccine		
Sinovac	11	13.1
BioNTech	73	86.9
After which dose of vaccine		
1	16	19.0
2	40	47.6
3	23	27.4
4	5	6.0
Admission complaint		
Fatigue	25	29.7
Joint pain	14	16.6
Fever	13	15.5
Headache	12	14.2
Chest pain	8	9.5
Sore throat	8	9.5
Nausea or vomiting	8	9.5
Shivering	6	7.1
Myalgia	6	7.1
Cough	6	7.1
Stomach ache	6	7.1
Chill	4	4.8
Allergic rash and itching	4	4.8
Dizziness or fainting	4	4.8
Back pain	4	4.8
Dyspnea	4	4.8
Diarrhea	3	3.6
Palpitation	3	3.6
Bone pain	3	3.6
Numbness and pain in the extremities	3	3.6
Axillary swelling	3	3.6
Diagnosis in the emergency department		
Upper respiratory tract infection (URTI)	42	50.1
Myalgia	27	32.1
Acute gastroenteritis	6	7.2
Urticaria	5	6
Angina (chest pain)	5	6
Headache	3	3.6
Arrhythmia	2	2.4
Syncope	2	2.4
Deep vein thrombosis (DVT)	1	1.2
Anaphylaxis	1	1.2
Treatment in the emergency department		
No medication	41	48.8
Diclofenac	19	22.6
Paracetamol	18	21.4
Pheniramine Hydrogen Maleate – Methylprednisolone –Dexketoprofen	2	2.4
Metoclopramide	2	2.4
Ondansetron Hcl	1	1.2
Adrenaline + methylprednisolone	1	1.2

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DISCUSSION

The side effects that occur within the first 48 h after vaccine administration are early side effects. Swelling and pain at the vaccine injection site, fatigue, fever, allergic reactions, and headache are the most common early side effects. It has been reported that severe allergic reactions are mostly found in Pfizer-BioNTech and Moderna vaccines[10]. These vaccines are contraindicated in people who are allergic to any vaccine component. Moreover, in the study of Yoo et al., it was reported that four patients had facial paralysis, two patients had transverse myelitis, and one patient had myocardial infarction[11]. Idiopathic neuralgic amyotrophy that may also occur after COVID-19 was found in one patient after taking Pfizer-BioNTech vaccine[12]. Thrombocytopenia and cerebral venous sinus thrombosis were reported in six cases after Johnson & Johnson's Janssen vaccine[11]. In the evaluations, it was reported that there was a risk of vaccine-induced thrombocytopenia and thrombosis in women under 50 years of age[13]. Most of the vaccine side effects are mild and short term[14]. In our study, similar to other studies, the complaints of the patients presenting to the emergency department due to vaccine side effects were mild, the Glasgow Coma Scale score of all patients was found to be 15, none of the patients were hospitalized, and ventilator was not needed for any of the patients.

In the study of Park et al., fever, myalgia, headache, shivering, injection site redness/pain, urticaria, and itching were the most commonly reported symptoms by patients presenting to the emergency department after taking the COVID-19 vaccine. Anaphylaxis was not found in any patient[15]. When the side effects of our patients after taking the COVID-19 vaccine were examined, chills, shivering, allergic rash or itching, fever, joint/bone pain, fatigue, myalgia, headache, cough, dizziness, syncope, back pain, sore throat, nausea or vomiting, diarrhea, palpitations, chest pain, dyspnea, abdominal pain, numbness, and pain in the extremities were found. In the study of Park et al., intravenous hydration was administered to 68.2% of the patients as parenteral therapy; however, antipyretic and antiemetics were required in 11.5% of the patients. While the mean number of days from vaccination to the emergency department visit was 1.83, 79% of patients came to the emergency department within 2 days. No hospitalization was required for the patients; however, outpatient follow-up was required for 10.2% of the patients, and four patients came to the emergency department at least twice[15].

According to Fertel et al., the mean age of the patients was 57.5 years. They also reported that 70.3% of the patients had a blood test, intravenous (IV) drugs were given to 61.6% of the patients, 45.8% of the patients had a chest X-ray, 20.3% of the patients had a thorax computed tomography, 81% of the patients were discharged, and 17.9% of the patients were hospitalized. The first three main complaints during admission were dyspnea, chest pain, and allergic reaction[16].

All patients included in this study received outpatient treatment, and all patients were discharged. Hemogram and D-dimer examinations of the patients were performed, and blood test results are presented in the "Results" section. The time between patients' vaccination and the onset of complaints was found to be 0–4 days. The time between patients' admission to the emergency department after the onset of post-vaccine side effects was 0–8 days, and it was observed that the date of onset of complaints and the date of admission to the emergency department were the same.

Although data from the studies examining COVID-19 vaccine reactions cover a short time frame, they show that COVID-19 vaccine reactions may also occur with high-risk complaints to the emergency department. The clinician will initiate treatment with an accurate diagnosis by evaluating the side effects of the vaccine and considering it as a trigger for potential severe complications. Most common high-risk side effect complaints and diagnoses associated with the vaccine administration in the past were Guillain-Barré syndrome, febrile convulsions, seizures, anaphylaxis, and meningitis/encephalitis[17].

In Turkey, two types of vaccines are mainly used as COVID-19 vaccine: "Sinovac" and "BioNTech." It was determined that 13.1% of the patients presenting to the emergency department due to various post-vaccine complaints occurred after taking the Sinovac vaccine, while 86.9% of the complaints occurred after taking the BioNTech vaccine. It was found that while the complaints occurred after the fourth dose of vaccine in 5.95% of the patients included in our study, the complaints occurred after the third dose of vaccine in 27.3% of them, the complaints occurred after the second dose of vaccine in 47.6% of them, and the complaints occurred after the first dose of vaccine in 19.04% of them.

There was a positive correlation between the age of the patients and the time between vaccination and the onset of complaints. The time between vaccination and the onset of complaints increased as the age of the patients increased. While a positive correlation was found between the patients' D-dimer values and age, there was a negative correlation with hemoglobin values. The time of vaccination-complaint and complaint-admission increased as the time between vaccination and admission to the emergency department increased. The time of complaint-admission decreased as the time of vaccination-complaint increased (Table 3).

Table 3. COVID-19 histories, vaccine, and additional disease information of patients.

	n	Median (minimum–maximum)	Test p
Gender			
Female	36	1.0 (0–4)	z=-0.748
Male	36	1.0 (0–4)	0.454
Positive for COVID-19 in the past?			
Yes	25	0.0 (0–4)	z=-1.578
No	47	1.0 (0–4)	0.114
Hemoglobin value			
Below normal	12	0.0 (0–3)	z=-1.716
Normal and above	60	1.0 (0–4)	0.086
Last vaccine			
Sinovac	9	1.0 (0–3)	z=-0.321
BioNTech	63	1.0 (0–4)	0.748
Additional disease			
Yes	8	1.0 (0–4)	z=-0.028
No	64	1.0 (0–4)	0.978

In this study, we aimed to ensure that the COVID-19 vaccine side effects are not overlooked, to evaluate the complaints in the best way, and to perform the correct diagnosis and treatment. We hope that our study on vaccine side effects will guide healthcare workers and patients on the side effects of COVID-19 vaccine in the future.

DECLARATION OF INTEREST STATEMENT

Our study was carried out in accordance with the Declaration of Helsinki, and we declare that it complies with the ethical standards of the Republic of Turkey. Ethical approval was obtained from the Ministry of Health of the Republic of Turkey and the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with the resolution number 1 in session 2022/14 on April 26, 2022.

AUTHORS' CONTRIBUTIONS

MSG: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **AİK:** Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing – original draft, Writing –review & editing. **HH:** Data curation, Formal Analysis, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **ÖFK:** Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing – review & editing. **YS:** Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing – review& editing. **NMB:** Data curation, Formal Analysis, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **YEÇ:** Formal Analysis, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of enhancer of zeste homolog 2 and mucin 1 expressions on treatment response in breast cancer

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SUMMARY

OBJECTIVE: Breast cancer is the most common malignancy in women. In the treatment of these patients, pathological complete response is defined as the absence of invasive cancer in breast or lymph node tissue after the completion of neoadjuvant chemotherapy. In this study, we aimed to investigate the relationship of enhancer of zeste homolog 2 and mucin 1 expressions with pathological complete response in patients with breast cancer receiving neoadjuvant chemotherapy.

METHODS: A total of 151 patients were included in the study. Enhancer of zeste homolog 2 and mucin 1 expressions were evaluated in the biopsy materials pre-neoadjuvant chemotherapy and post-neoadjuvant chemotherapy surgical material, and their relationship with pathological complete response was investigated.

RESULTS: The pathological complete response rates were significantly higher among the hormone receptor-negative patients, those with a high Ki-67 score, and patients with HER2-positive. Higher pathological complete response rates were obtained from patients with enhancer of zeste homolog 2 expression positivity pre-neoadjuvant chemotherapy. In addition, after neoadjuvant chemotherapy, enhancer of zeste homolog 2 expression was found to be completely negative in materials with pathological complete response; that is, in breast tissues considered to be tumor-free. While there was no significant relationship between mucin 1 expression and pathological complete response pre-neoadjuvant chemotherapy, mucin 1 expression was determined to significantly differ between the tissues with and without pathological complete response among the surgical materials examined.

CONCLUSION: In our study investigating the relationship between enhancer of zeste homolog 2 and mucin 1 expression and pathological complete response in patients who received neoadjuvant chemotherapy, we found that enhancer of zeste homolog 2 expression could be used as a predictive marker for pathological complete response. However, mucin 1 expression was not associated with pathological complete response.

KEYWORDS: Breast neoplasms. Enhancer of zeste homolog 2 protein. MUC1 protein, human. Neoadjuvant chemotherapy.

INTRODUCTION

Breast cancer is the most common malignancy in women and the second most common cause of cancer-related mortality[1,2]. As a systemic control regimen, chemotherapy has dramatically increased the rate of disease-free and overall survival. Chemotherapy can be administered before or after surgery. When chemotherapy is applied before surgery, it is called neoadjuvant chemotherapy (NAC)[3,4]. Pathological complete response (pCR) is defined as the absence of invasive cancer in breast or lymph node tissue after the completion of NAC[5]. Patients with this response to chemotherapy have a significantly lower risk of tumor recurrence than those with residual carcinoma[6]. Enhancer of zeste homolog 2 (EZH2) is a polycomb group protein involved in stem cell regeneration and carcinogenesis. In breast cancer, increased EZH2 expression is associated with tumor aggressiveness. EZH2 expression in the normal breast epithelium is accepted as an independent risk factor for the development of breast cancer, and, therefore, it has been suggested that this expression can be used in the risk classification of benign breast biopsies[7]. Mucin 1 (MUC1) is a transmembrane protein normally expressed at low levels on the apical surfaces of epithelial cells, including the pancreas, breast, lung, and gastrointestinal tract. It has been shown to be associated with metastasis and invasion in many cancer types. It has also been reported that the overexpression of MUC1 is associated with a poor prognosis in breast cancer[8]. However, there are insufficient data on the relationship of EZH2 and MUC1 expressions with pCR in patients with breast cancer receiving NAC. Therefore, in this study, we aimed to investigate the relationship of EZH2 and MUC1 expressions with pCR in patients with breast cancer receiving NAC.

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METHODS

In this study, 175 patients who received NAC and had fully accessible data were evaluated. Eligibility criteria were 18–70 years of age, stage 2 or 3 breast cancer, and noninflammatory invasive ductal carcinoma subtype. Patients who did not have laboratory test results, pre-NAC biopsy results, post-NAC surgical material pathology reports, or preparations were excluded from the study. After NAC, all the patients underwent breast-conserving surgery or modified radical mastectomy. A total of 151 patients were determined to meet the inclusion criteria.

The biopsy and surgical materials of the cases included in the study group were fixed in 10% formaldehyde. From the prepared paraffin blocks, 5-micron sections were obtained. EZH2 expression was determined with the EZH2 Mouse Monoclonal Antibody (415M-15, Cell Marque) and INI-1 (MRQ-27) Mouse Monoclonal Antibody (272M-15, Cell Marque) using the ultraView Universal DAB Detection Kit (Ventana, 760-500) on the Ventana BenchMark XT automated immunohistochemistry stainer. The results were evaluated using a light microscope (Nikon Eclipse E200) by a pathologist. Nuclear staining >1% of tumor cells was considered positive (Figure 1A).

The ab15481 antibody (Abcam, USA) was used for MUC1 expression. MUC1 was scored as 0 (no staining), 1+ (weak staining), 2+ (moderate staining), and 3+ (strong staining). While a score of 0 was evaluated as negative, scores 1+, 2+, and 3+ were considered to indicate positivity (Figure 1B). EZH2 and MUC1 expressions were evaluated in the biopsy materials pre-NAC and post-NAC surgical materials, and their relationship with pCR was investigated.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local ethics committee of the university (approval number: date: June 10, 2016, meeting no: 54, decision no: 27).

The Statistical Package for the Social Sciences (SPSS) v. 23.0 software package was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and continuous measurements as mean and standard deviation values (median and minimum–maximum where appropriate). The Shapiro-Wilk test was used to determine whether the parameters in the study showed a normal distribution. The chi-square and Fisher's exact tests were used to compare categorical data. The Student's t-test was used for normally distributed parameters and the Mann-Whitney U test for non-normally distributed parameters. The statistical significance level was 0.05 in all tests.

RESULTS

While pCR was achieved in 57 of the 151 patients included in the study, this response was not observed in the remaining 94 patients. There was no statistically significant difference between the patients with and without pCR in terms of age, grade, and menopausal status. However, a statistically significant difference was detected between the patients with and without pCR in relation to estrogen receptor (ER) and progesterone receptor (PR) status and percentages, and Ki-67 level. HER2 status also significantly differed between these two groups. Finally, there was no statistically significant difference in the rates of patients with T and N stages between the pCR and non-pCR groups (Table 1).

Enhancer of zeste homolog 2 expression significantly differed between the groups with and without pCR based on the examination of biopsy and surgical materials. While MUC1 expression did not statistically significantly differ between these two groups in the examination of biopsy materials, there was a statistical significance difference MUC1 expression among the surgical materials (Table 2).

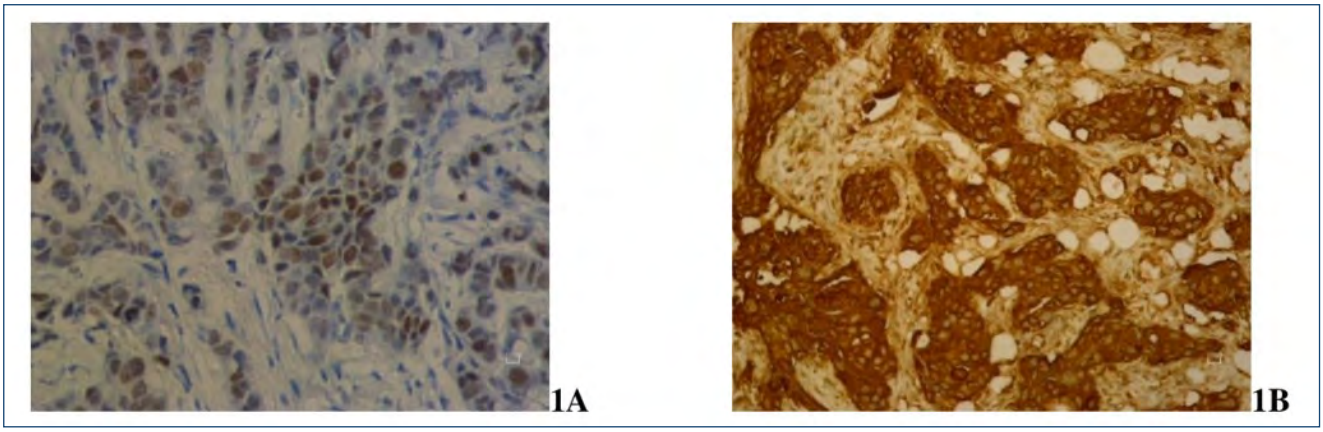

Figure 1. **(A)** Enhancer of zeste homolog 2 nuclear staining (400´). **(B)** Mucin 1 membranous and cytoplasmic staining (400´).

Table 1. Association between clinicopathological factors and pathological complete response (n=151).

	Patients with pCR (n=57)	Patients without pCR (n=94)	p-value
Age (mean – years)	52.8±11.7	50.9±10.5	0.292[a]
	53 (28–76)	51 (28–70)	
Grade			
II	27 (49.1)	49 (53.3)	0.624[c]
III	28 (50.9)	43 (46.7)	
Menopausal status			
Premenopausal	22 (38.6)	42 (44.7)	0.463[c]
Postmenopausal	35 (61.4)	52 (55.3)	
ER status			
Positive	27 (47.4)	83 (88.3)	**<0.001**[*c]
Negative	30 (52.6)	11 (11.7)	
ER level (% mean)	30.7±39.3	65.2±37.5	**<0.001**[**b]
	0 (0–90)	90 (0–100)	
PR status			
Positive	17 (29.8)	71 (75.5)	**<0.001**[**c]
Negative	40 (70.2)	23 (24.5)	
PR level (% mean)	16.1±30.1 0 (0–90)	36.5±36.0 20 (0–95)	**<0.001**[**b]
Ki-67 level (mean)	52.1±25.7	28.7±18.5	**<0.001**[**b]
	60 (10–90)	22.5 (3–80)	
HER2 status			
0–I	12	36	**0.001**[c]
II	12 (3 FISH +)	32 (2 FISH +)	
III	33	26	
T stage			
T1	8 (14.0)	7 (7.4)	0.120[c]
T2	30 (52.6)	38 (40.4)	
T3	3 (5.3)	11 (11.7)	
T4	16 (28.1)	38 (40.4)	
N stage			
0	5 (8.8)	4 (4.3)	0.421[c]
1	7 (12.3)	19 (20.2)	
2	33 (57.9)	49 (52.1)	
3	12 (21.1)	22 (23.4)	

*p<0.05, **p<0.001. [a]Student's t-test. [b]Mann-Whitney U test. [c]Chi-square and Fisher's exact tests. pCR: pathological complete response; ER: estrogen receptor; PR: progesterone receptor; FISH +: FISH positive. Fisher's exact test was used if the expected minimum was <5 according to the chi-square test. Statistically significant p-values are shown in bold (p<0.05).

DISCUSSION

In breast cancer, which is the second most common cause of cancer-related mortality, it has been shown that patients with pCR have a longer disease-free and overall survival than those with residual cancer. Recent studies have identified pCR as the primary goal in predicting disease-free and overall survival times in NAC[9,10].

While it is very important to predict which patients will achieve pCR with NAC, this treatment is not completely

Table 2. Association between enhancer of zeste homolog 2 and mucin 1 expressions and pathological complete response (n=151).

	Patients with pCR (n=57)	Patients without pCR (n=94)	p-value
EZH2 – biopsy			
Positive	46 (88.5)	51 (62.2	**0.001****[a]
Negative	6 (11.5)	31 (37.8)	
MUC1 – biopsy			
Positive	49 (94.2)	78 (95.1)	0.821[a]
Negative	3 (5.8)	4 (4.9)	
EZH2 – surgical			
Positive	–	10 (11.8)	**0.013***[a]
Negative	49 (100.0)	75 (88.2)	
MUC1 – surgical			
Positive	43 (87.8)	88 (100.0)	**0.002****[a]
Negative	6 (12.2)	–	

*p<0.05, **p<0.001. [a]Chi-square and Fisher's exact tests. pCR: pathological complete response; EZH2: enhancer of zeste homolog 2; MUC1: mucin 1. Fisher's exact test was used if the expected minimum was <5 according to the chi-square test. Statistically significant p-values are shown in bold (p<0.05).

risk-free. Although the prediction of patients that will achieve longer disease-free and overall survival after NAC facilitates patient management, NAC may increase the ipsilateral tumor recurrence rate compared with adjuvant therapy. In addition, the existence of healthcare access barriers and socioeconomic inequalities are the main reasons for late-stage diagnosis in developing countries and delaying surgery may result in decreased overall survival[11,12].

Previous studies have explored many factors to predict pCR after NAC. Compared with luminal A tumors, HER2 overexpression and triple-negative subtypes are reported to be more sensitive to NAC[13]. High Ki-67 expression and lack of ER and PR expressions are associated with higher pCR[14,15]. A meta-analysis of 36 studies evaluating the pCR rate in patients with breast cancer with different Ki-67 labeling indices who received NAC showed that those with a high Ki-67 index had a significantly higher pCR rate[16]. Gomes da Cunha et al. examined the relationship between the Residual Cancer Burden (RCB) index and overall and disease-free survival in women undergoing NAC. It was found statistically significant that the RCB 0 subgroup had a better prognosis (pCR) than RCB 1, 2 and 3[17]. In a study evaluating pCR status according to HER2 status, 51 of 413 samples were HER2-positive and 287 were HER2-negative, while HER2 results of 75 patients could not be reached. In 94 (14.3%) of these patients, pCR was obtained from breast tissue and lymph nodes. pCR was found to be three times more common in HER2-positive patients (23.5%) than in HER2-negative patients (7%)[18]. In our study, consistent with the literature, higher rates of pCR were obtained from the patients with hormone receptor negativity, high Ki-67 score, and HER2 expression positivity.

The functions of EZH2 in cell proliferation, apoptosis, and aging have been previously described[19]. EZH2 dysregulation is highly tumorigenic and has been observed in various cancers where EZH2 acts as an oncogene or a tumor suppressor[20]. In a meta-analysis evaluating 11 studies (2,330 patients in total), 1,052 EZH2-positive and 1,278 EZH2-negative patients were examined. It was determined that EZH2 overexpression was significantly associated with ER and PR negativity, HER2 positivity, invasive ductal cancer, race, high histological grade, and triple-negative status, resulting in a poor overall survival rate. The authors concluded that EZH2 could be used as a prognostic marker in breast cancer[21].

In a study investigating the effect of MUC1 expression on treatment response and survival in patients with breast cancer receiving NAC, it was stated that MUC1, which could be detected at mRNA and protein levels, was frequently expressed in breast cancer. High MUC1 protein and mRNA expressions were associated with a lower probability of pCR and longer patient survival. Thus, MUC1 expression was suggested to be an independent predictor of treatment response and survival after NAC[22].

Predicting patients who will achieve pCR after NAC is very important for patient management, which increases the need for predictive markers of NAC response. In our study investigating the relationship of EZH2 and MUC1 expression with pCR in patients receiving NAC, we found that EZH2 expression could be used as a predictive marker

of pCR. Higher pCR rates were obtained from the patients with EZH2 expression positivity pre-NAC, while EZH2 expression was completely negative in materials with pCR after NAC. MUC1 expression was not associated with pCR, but there was a statistically significant difference in MUC1 expression between the tissues with and without pCR based on the examination of the surgical materials. Due to the small number of patients, we were not able to explore the relationship of pCR separately with each breast cancer subgroup and not examining more parameters, which can be considered a limitation of our study. The strengths of our study, i.e., the promising results of EZH2 and the insignificance of MUC1, may lead to further studies.

CONCLUSION

Enhancer of zeste homolog 2 is a good predictive marker of pCR in patients receiving NAC. Further studies are needed to validate the use of EZH2 expression in the prediction of pCR in patients receiving NAC.

ETHICAL APPROVAL

The study was approved by the local ethics committee of the university (approval number: 10.06.2016-54).

AUTHORS' CONTRIBUTIONS

AEY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **SP:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **MB:** Conceptualization, Data curation, Formal Analysis, Methodology. **AO:** Data curation, Formal Analysis, Investigation, Resources, Software. **ÖY:** Conceptualization, Resources, Software, Visualization. **SZ:** Conceptualization, Formal Analysis, Supervision, Validation, Visualization. **ME:** Formal Analysis, Supervision, Validation, Visualization. **İK:** Data curation, Methodology, Resources, Software, Writing – review & editing. **MMK:** Data curation, Investigation, Resources, Software.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20221129

A novel labeling modality of intra-abdominal lesions with Magseed magnetic marker and extirpation by Sentimag probe navigation

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SUMMARY

OBJECTIVE: This study aimed to evaluate our experience with the use of Magseed, the magnetic metallic marker, as a localization technique followed by Sentimag probe detection in patients with solitary intra-abdominal local metastases with subsequent resection of the lesions.

METHODS: Five patients underwent resection after the lesion was marked with the Magseed magnetic marker. Prior to the surgery, a computed tomography scan of the chest and abdomen and/or positron emission tomography was performed to rule out the dissemination of the disease. The indication for surgery was evaluated in a meeting of a multidisciplinary team, and the placement of the magnetic marker under computed tomography control had been performed the day before the planned procedure.

RESULTS: The present preliminary outcomes have revealed that Magseed might be a promising technique that is feasible and safe, particularly when the postsurgical anatomic conditions in the abdominal cavity are altered and the lesions are not visible or palpable. Surgical extirpation of lesions occurred without complications in each case. In all the cases, the resection was complete and curative, and one wound infection in all (20%), without any major complications, had occurred. The mean hospital stay was 6.6 days.

CONCLUSION: Magseed utilization, as a localization technique, followed by Sentimag probe detection in intra-abdominal tumors has not been reported before. Improving the visualization and, consequently, the precise marking of the lesion with subsequent radical removal can prevent insufficient or excessive removal of healthy tissue, leading to a faster diagnosis and better overall clinical outcomes.

KEYWORDS: Magnetic. Magseed. Sentimag. Thyroid gland. Thyroidology.

INTRODUCTION

Perioperative localization and surgical removal of intra-abdominal local recurrences can be technically difficult because recurrent lesions may be small and localized in relatively inaccessible areas or surrounded by necrotic, adipose, or other tissue. The lesions are usually not easily visible or palpable. Accurate localization is important because resection of recurrences can improve survival; conversely, inaccurate targeting of the lesion can lead to either insufficient excision with an increased risk of recurrence or excessive removal of healthy tissue.

Localization and surgical treatment of nonpalpable lesions of the breast, lung nodes, liver, and other parts of the body are often performed by preoperative marking with wire, the harpoon technique, a contrast agent such as indocyanine green (ICG), liquid markers, a radioactive iodine seed, or, as in our case, a magnetic seed, Magseed. Magseed (Endomagnetics Ltd., Cambridge, UK) is a 1 mm × 5 mm magnetic metallic marker that is preloaded in a sterile 18-G needle. The seed is detectable using the Sentimag probe and can be detected from any direction, regardless of seed orientation. The Sentimag probe produces an alternating magnetic field that transiently magnetizes the iron oxide particles within the Magseed. The probe shows a numerical count and produces an audio tone related to the strength of the magnetic field and, therefore, to the distance of the seed in the tumor from the detector probe. The seed is cylindrical with no barbs, has no moving parts, and cannot be

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damaged when implanted. Magseed is usually and frequently used in patients with nonpalpable breast tumors for tumor localization before surgery[1].

METHODS

This study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin. We retrospectively analyzed data from five patients who had undergone surgery for intra-abdominal local recurrences of different cancers from October 2021 to May 2022. The age of the patients (three males and two females) was between 60 and 74 years, and all had previously undergone an oncological surgery procedure, presenting recurrences during the follow-up. The primary tumors were recognized as colorectal adenocarcinomas (3/5), endometrial carcinoma (1/5), and neuroendocrine tumors (1/5). A thoracoabdominal computed tomography (CT) scan and/or positron emission tomography (PET) scan was performed in all the cases in order to exclude disseminated disease or the presence of multiple nodules, which was correlated with specific tumor markers. The indication for surgery was evaluated at a meeting of a multidisciplinary team. Placement of a CT-guided magnetic seed, Magseed, was undertaken after the patient's written consent was obtained, and they were admitted 1 day before surgery when the radiological procedure was performed under local anesthesia. The lesions were localized by CT scan, and the puncture route was anterior (2/5), lateral transperitoneal (2/5), or dorsal (1/5). For the procedure, the patient was positioned depending on the lesion location and seed placement. The site of entry was then cleansed, prepped, and draped in a sterile manner, and the magnetic seed was placed less than 1 cm from the lesion. Correct placement and settlement of the Magseed was checked by CT scan (Figures 1 and 2). The patients were transferred back to the surgical ward, and the position of the marker was directly checked using the Sentimag magnetic probe. The same control was also used directly before the surgical procedure (Figure 3). Depending on the location of the lesions, a conventional surgical approach through a median laparotomy (one patient), a lateral (one patient), or a transverse approach (three patients) was performed. To this end, the lesions were easily located using the Sentimag probe and then resected.

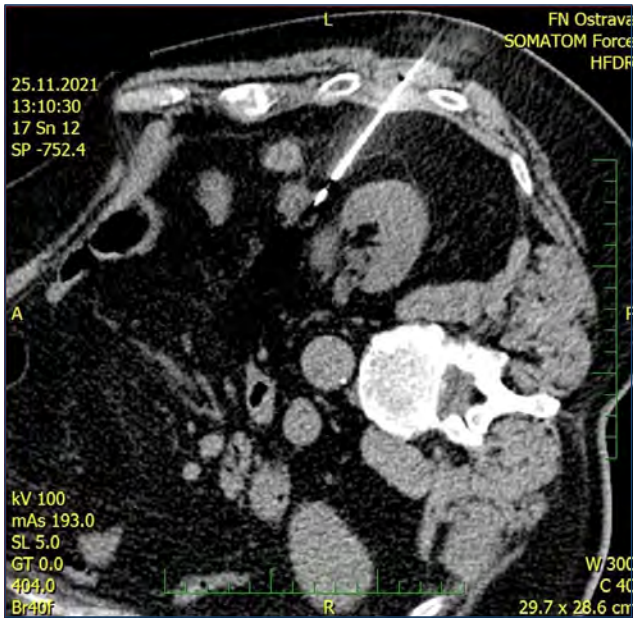

Figure 1. Placement of the Magseed via a CT scan navigation.

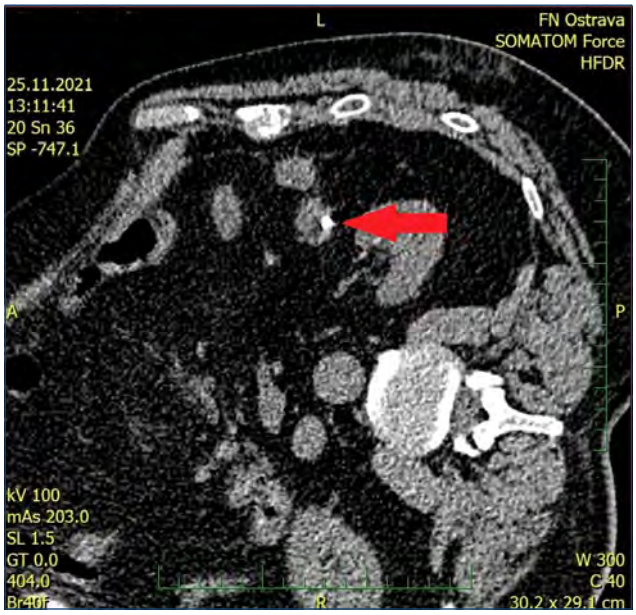

Figure 2. Settlement of the Magseed, utilizing a CT scan.

RESULTS

The Magseed placement was successful in all five patients. No patients reported pain during the radiological procedure. No placement-related complications had been noted, and no intra-abdominal organ injuries occurred. In all cases, the tumor was located near the seed, and the seed was extirpated with the tumor lesion. A safety margin of healthy tissue of at least 1 cm was excised together with the lesion. A colon resection was necessary for one patient due to neoplastic infiltration, and this case also involved resection and primary suture with partial resection of the spleen. One patient necessitated a resection of tail of the pancreas. Surgical excision of the lesions

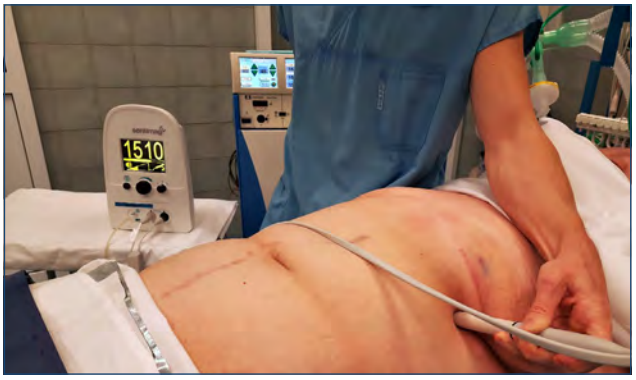

Figure 3. Diagnostic settlement of the Magseed, directly checked using a magnetic Sentimag probe prior to the surgical procedure.

was performed without perioperative complications in any case. One (20%) patient had a wound infection with secondary healing. The mean hospital stay was 6.6 days. Resection was complete and curative in all cases. In addition, four (80%) cases exhibited the metastatic lesions:

I. Two of them were metastatic adenocarcinomas.
II. One was an endometrioid carcinoma.
III. One was a neuroendocrine metastatic cell, all possessing negative margins of more than 1 mm. In one patient, there was a histopathological finding of pseudocyst with eosinophils, with a positive preoperative PET/CT scan finding that was suspicious for metastatic disease from the primary colon tumor.

DISCUSSION

The development of interventional radiology (IR) in the past decade has often paved the way for new therapies that are later performed routinely and not only by interventional radiologists. For instance, based on long-term results, minimally invasive ablative techniques, originally proposed as alternative surgical options, are now recommended treatments in many indications, such as in the liver and kidneys[2].

IR focuses on anatomical localization using ultrasound (US), CT, and magnetic resonance imaging (MRI) to improve the accuracy and specificity of diagnosis. It is also desirable to avoid excessive medical treatment and waste of clinical resources[3]. Percutaneous approaches can be performed under the guidance of US, x-rays, CT, MRI, or even PET/CT[4].

The main anatomic parts of *Homo sapiens* in which image-guided localization techniques are useful are the breast, lung, liver, thyroid, parathyroid, kidney, and other soft tissues and organs such as lymph nodes. Localization studies remain significant in neck-endocrine surgery and thyroidology to date and provide vital clues for both surgeon-performed US examinations and all thyroidologists[5-9]. In breast lesions, the main indications are preoperative localization of nonpalpable lesions that are only visible on imaging; this imaging guides surgeons for a safe intervention aiming at an R0 resection and a good cosmetic result. Another indication is the permanent marking of a tumor in candidates for neoadjuvant therapy when, after complete tumor regression, the surgeon is able to identify and remove the "tumoral bed." Frequently used methods are wire localization, radioactive-seed localization, magnetic seed localization, carbon marking, US-visible hydrogel-based markers, and radiotracer injection[10].

In lung lesions, it is convenient to mark those that are small or deeply located. In addition, during video-assisted thoracoscopy, only the tactile sensation of the thoracoscopic instruments can be applied, and this is sometimes not informative enough. There are also multiple techniques used in the thoracic cavity to localize tumors, such as percutaneous hook-wire placement, which is mainly used for lesions ≤10 mm in size and located >5 mm from the pleural surface; dyes such as ICG, indigo carmine, and methylene blue; an ethiodized oil like Lipiodol; or a radioisotope. A radiopaque metallic marker has been used before stereotactic body radiotherapy (SBRT) of lung tumors[10].

In liver lesions, an ethiodized oil (Lipiodol) is used to mark hepatocellular carcinoma before the thermal ablation procedure, and implanted fiducial markers as surrogates for liver tumors are frequently used before SBRT of liver tumors. This procedure is usually performed under US and/or CT guidance, depending on the imaging modality that better exhibit the lesion, but it can also be performed during laparoscopy or laparotomy in case of an inoperable tumor. For better results, two or more fiducials should be placed <2 cm from the tumor[11]. In addition, for soft-tissue lesions, frequently used markers include liquids, metallic coils, and hook and curved-end wires. Liquid markers are not used, because they have a tendency to distribute nonspecifically into the adjacent tissue[12].

Lymph nodes are usually marked before the initiation of neoadjuvant chemotherapy when it is necessary to mark the node for later surgical removal. The insertion of different markers is typically performed under US guidance. Markers are standard hook and curved-end wires, low-activity radioactive seeds using the ^{125}Iodine, or a Magseed magnetic seed[13].

The technique for locating intra-abdominal local recurrences with this CT-guided placement technique is similar to CT-guided fine-needle aspiration (FNA) of intra-abdominal masses. The duration of the procedure and patient tolerance are also similar. The incidence of possible secondary complications of seed placement can be extrapolated from the incidence of

FNA, which is about 1.3%[14]. Complications like hemorrhage and bile peritonitis in liver FNA or biopsy can be recognized within 4 h after FNA application. Post-liver biopsy bleeding can occur in 0.32–0.35% of patients, with morbidity related to hemorrhage at 0.24% and mortality from severe bleeding at around 0.11%[15]. To the best of our knowledge, in English-language literature, the mortality from FNA has been reported as 0.006%, occurring mainly in the liver and pancreatic biopsies[16]. Pulmonary complications post-liver biopsy are considered to be rare, which may occur as the needle biopsy passes through the costophrenic angle above the reflection between the parietal and visceral pleura. The patients can develop pneumothorax and hemothorax[17]. The incidence of pneumothorax is in the range of 0.08–0.28%, and the symptoms are usually mild, with pulmonary collapse not exceeding 10%[18]. Hemothorax post-liver biopsy is also scarce, accounting for 0.18–0.49%, and can be managed conservatively without thoracotomy[17]. In our series, we had not encountered any complications during radiological placement of the seed or during the surgical extirpation. We have not recognized any complications from the Magseed placement or during the surgery. It is usually used to preoperatively localize the nonpalpable breast lesions or as a marker of lymph nodes before the neoadjuvant chemotherapy in patients with breast carcinoma[19]. To the best of our knowledge, this series of the Magseed placements in intra-abdominal lesions and the lesions' extirpation by Sentimag probe navigation is the first to be reported in English-language literature, to date.

CONCLUSION

Effective treatment requires the optimal placement of targeted treatment devices without affecting other organs. The precise marking of the lesion can improve preoperative planning by providing the surgeon with valuable information regarding the optimal incision and lead to a less invasive and more biologically sparing excision. The use of Magseed, a magnetic metallic marker, as a localization technique followed by Sentimag probe detection in patients with solitary intra-abdominal local metastases with subsequent resection of lesions can prevent insufficient or excessive removal of healthy tissue and thus speed up treatment and improve overall clinical results.

AUTHORS' CONTRIBUTIONS

DT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, and Writing – original draft. **IS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, and Writing – review & editing. **OK, PG:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, and Writing – original draft. **TJ:** Investigation, Project administration, Resources, Software, Validation, Visualization, and Writing – review & editing. **JP:** Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, and Writing – review & editing. **LT:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Validation, Visualization, and Writing – original draft. **PI:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, and Writing – review & editing. **AP**: Investigation, Project administration, Validation, Visualization, and Writing – review & editing. **DS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, and Writing – review & editing.

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ORIGINAL ARTICLE

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Cryopreservation as a strategy for prevention of ovarian hyperstimulation syndrome in a public assisted reproduction service in São Paulo – Brazil

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SUMMARY

OBJECTIVE: This study aimed to evaluate the prevalence of ovarian hyperstimulation syndrome (OHSS) and associated risk factors in patients undergoing fertilization cycles at risk of OHSS (≥15 antral follicles or ≥15 oocytes aspirated) and submitted to cryopreservation of all embryos in the Human Reproduction Service of the Pérola Byington Hospital (Referral Center for Women's Health) in São Paulo, SP, Brazil.

METHODS: This cross-sectional, institutional, descriptive study of secondary data from patients' charts enrolled in the Assisted Reproduction Service of the Pérola Byington Hospital at risk of OHSS after controlled ovarian stimulation and submitted to cryopreservation of all embryos was conducted between January 2015 and September 2017.

RESULTS: OHSS occurred in 47.5% of cycles, all with mild severity, and there were no moderate or severe cases of OHSS.

CONCLUSION: The cryopreservation of all embryos is associated with a reduction in moderate and severe forms of OHSS. Risk factors for OHSS should be evaluated prior to initiation of treatment, with less intense stimulation protocols accordingly.

KEYWORDS: Ovarian hyperstimulation syndrome. Cryopreservation. Fertilization in vitro.

INTRODUCTION

One in six couples experience fertility problems and, for 20% of this group, the only way to achieve a pregnancy is by using assisted reproduction technology (ART)[1,2]. These techniques, such as in vitro fertilization (IVF), seek to attain pregnancy by replacing or facilitating the defective stage in the reproduction process[3]. The ART, however, can lead to side effects, such as ovarian hyperstimulation syndrome (OHSS)[4].

The syndrome can occur iatrogenically due to the high hormone dose administered to the patient during the oocyte stimulation phase. Human chorionic gonadotropin (hCG) is one of the hormones used in the stimulation process. The greater number of oocytes produced increases the chance of fertilization and success of the technique. However, this boosting of hormone level to increase success is associated with a 2–3% incidence of moderate and severe forms of OHSS in ARTs[5]. By comparison, OHSS incidence in a Referral Hospital for Assisted Reproduction in São Paulo, Brazil, was 1.9%[6].

The syndrome affects 6020 patients annually in the United States and Europe, with an estimated mortality of 1 in 450,000–500,000[7]. Risk factors for OHSS include younger age, history of polycystic ovary syndrome (PCOS), and personal history of high response during a previous IVF cycle and on evaluation of biomarkers, such as anti-Müllerian hormone (AMH) level and follicles using ultrasound[8].

The pathophysiology of OHSS is complex and not yet fully understood. However, the syndrome involves increased vascular permeability of the mesothelial layer of ovaries and leakage of protein-rich fluids into the interstitial or "third" space. Clinical symptoms reflect the degree of third spacing and hemoconcentration resulting from the depletion in intravascular volume[9].

Pro-inflammatory vasoactive mediators, such as vascular endothelial growth factor (VEGF), are believed to be involved in this pathogenesis[10]. When stimulated under supraphysiological conditions, ovaries oversecrete VEGF to above the normal levels, promoting excessive vascular permeability with leakage to the third space, leading to reduced perfusion of organs[11]. Some studies have shown stronger association of VEGF with hCG and higher VEGF in peritoneal fluid of patients who used

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hCG when compared to the gonadotropin-releasing hormone (GnRH) agonist[12].

The signs and symptoms vary with the severity of the syndrome. Mild symptoms occur in roughly 30% of patients, such as slight discomfort and distended abdomen due to increased volume of one or both ovaries[5,13]. More severe forms can present with ascites of varying severity; pleural effusion, oliguria secondary to renal failure, thromboembolism, and death can occur as a result of hemoconcentration and reduced perfusion of other organs such as the kidneys, heart, and brain[14,15].

The two main types of OHSS are early and late onset. In early OHSS, symptom onset takes place 7 days after the application of hCG (administered for final oocyte maturation), whereas late OHSS manifests 10 days after hCG application and is triggered by endogenous hCG produced by trophoblasts following pregnancy. The late form of OHSS, compared with the early form, has a higher probability of becoming severe (72.2% vs. 42%)[15].

One method of preventing OHSS is by performing cryopreservation of embryos. The technique entails freezing embryos and implanting them during a later cycle, when ovarian response has normalized after previous hyperstimulation for follicle production. In addition, the use of an antagonist protocol and final follicle maturation with a GnRH agonist trigger, followed by the freeze-all strategy, constitutes an effective option for the prevention of OHSS with high live birth rate[16,17].

Taken together, this evidence suggests that cryopreservation represents one of the best prevention approaches for patients at high risk of OHSS, given that fresh embryo transfer triggers a further hCG surge following implantation. Therefore, the objective of the present study was to assess cryopreservation as a strategy for the prevention of OHSS and identify the risk factors associated with the syndrome.

METHODS

This retrospective, cross-sectional, institutional, descriptive study of secondary data from patients' charts enrolled in the Assisted Reproduction Service of the Pérola Byington Hospital at risk of OHSS after controlled ovarian stimulation and submitted to cryopreservation of all embryos was conducted between January 2015 and September 2017.

Those women who had ≥15 antral follicle count (AFC) by ultrasound or ≥15 oocytes aspirated after controlled ovarian stimulation and submitted to cryopreservation of all embryos were included in the study.

Patients undergoing cryopreservation of embryos for preservation of fertility, genetic factors, egg donation/recipients, and/or those with surplus embryos were excluded. In addition, patients' charts with incomplete data were excluded.

The variables evaluated were as follows: age, body mass index (BMI) (calculated as weight/height^2, kg/m^2), categorized according to the World Health Organization (WHO) criteria; infertility factors; the levels of AMH, follicle-stimulating hormone (FSH), and luteinizing hormone (LH); AFC by ultrasound; number of aspirated oocytes and number of mature oocytes; fertilization rate; fertilization technique employed, such as intracytoplasmic sperm injection (ICSI) or IVF; embryo transfer; occurrence of OHSS and related complications using the classification of Golan et al.[18], for mild, moderate, and severe forms; type of OHSS treatment; occurrence of biochemical pregnancy (detection of hCG in maternal plasma 14 days after embryo transfer); and clinical pregnancy (detection of gestational sac on ultrasound from 7 weeks' gestation).

The protocols for controlled ovarian stimulation for IVF/ICSI employed in the human reproduction service studied were assessed. The protocol used was dictated by AFC as follows:

Protocol A (AFC≤15): 300 IU FSH/hMG (human menopausal gonadotropin) per day; GnRH antagonist (Cetrorelix or Ganirelix) 0.25 mg; trigger with hCG (Choriomon 5000 IU) or GnRH agonist (Gonapeptyl 0.2 mg/day) when at risk of OHSS.

Protocol B (AFC=11–15): long block with GnRH agonist (1.875 mg Triptorelin); stimulation with 300 IU FSH/hMG per day; trigger with hCG (Choriomon 5000 IU).

Protocol C (AFC≥15): stimulation with 150 IU FSH/hMG per day; GnRH antagonist (Cetrorelix or Ganirelix) 0.25 mg; trigger with GnRH agonist (Gonapeptyl 0.2 mg/day).

Sample size calculation and statistical analysis

The sample size was calculated using a confidence interval, adopting an initial prevalence of 3% based on the outcome of OHSS incidence in the study population by Papanikolaou et al.[19]. A 95% level of confidence and 4.5% error margin yielded n=55.

Data collection was carried out based on a review of medical charts using a data collection instrument containing the variables of interest for the study. A database was created using the statistical software SPSS (Statistical Package for Social Sciences) for Windows version 17.0, where data were tabulated and analyzed. The variables were categorized based on the criteria used by the institution's protocol to predict ovarian hyper-response (risk of OHSS). The prevalence of OHSS and respective confidence interval were calculated for a 95% confidence level. Associations between categorical variables were explored with bivariate analysis using chi-square or Fisher's exact test, considering a p-value <0.05 as significant.

RESULTS

A total of 64 fertilization cycles of patients at risk of developing OHSS undergoing cryopreservation of all embryos were assessed (Figure 1). The mean age of patients was 32 years (range 23–40), and 56.1% were nulliparous. The patients' characteristics are summarized in Table 1.

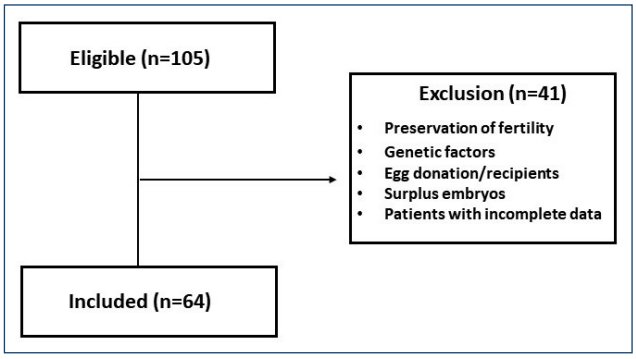

Figure 1. Patient distribution flowchart as determined by inclusion/exclusion criteria.

Table 1. Characteristics of patients undergoing cryopreservation of all embryos.

	Mean	SD
Age	32.0	3.8
BMI	26.6	4.9
AFC	16.9	6.1
Number of aspirated oocytes	19.0	8.6
Number of mature oocytes	15.6	7.9
AMH	5.1	4.1

SD: standard deviation; BMI: body mass index; AFC: antral follicle count; AMH: anti-Müllerian hormone.

With regard to BMI, 45.7% of patients were of healthy weight, 34.8% were of overweight, 15.2% belong to Class 1 obesity, and 4.3% belong to Class 3 obesity.

Of the cases evaluated, 43.8% had tubule factor, 28.1% PCOS, 17.2% male factor, 15.6% endometriosis, and 7.8% had unexplained infertility. Of these cases, six had two or more infertility factors.

The level of AMH averaged 5.1 ng/mL, and in 48.4% of cases, it was 3.5 ng/mL. Mean FSH level was 5.9 mIU/mL and LH level was 6.4 mIU/mL.

Protocol C was the most used (50.0%), followed by B (42.2%) and A (7.8%). All patients underwent cryopreservation of embryos to prevent OHSS.

Mean number of antral follicles was 16.9, and 64.1% of cases had ≥15 follicles. Mean number of aspirated follicles was 19, and mean fertilization rate was 72.8%.

ICSI was the most commonly used fertilization technique (78.1% of cases). Embryo transfer was performed in 87.5% of the cycles. Biochemical pregnancy occurred in 50% of these cycles and clinical pregnancy in 39.2%. There were a total of 22 pregnancies, 4 of which were twin pregnancies.

OHSS occurred in 47.5% (95%CI 22.7–68.2) of cycles, all with mild symptoms. There were no cases of moderate or severe OHSS.

The results for risk factors associated with OHSS are given in Table 2.

DISCUSSION

This study showed that cryopreservation of all embryos prevented moderate and severe forms of OHSS in patients at risk for OHSS, in that there were no moderate or severe cases of the syndrome. This evidence supports embryo freezing as a key strategy for reducing

Table 2. Factors associated with ovarian hyperstimulation syndrome in cycles of patients undergoing cryopreservation of all embryos.

		Mild OHSS		p*
		Yes	No	
Number of aspirated oocytes	≤15	8 (42.1)	11 (57.9)	0.567
	>15	21 (50.0)	21 (50.0)	
Trigger	Gonapeptyl	13 (39.4)	20 (60.6)	0.212
	Choriomon	15 (55.6)	12 (44.4)	
AMH	≤3.4	5 (31.3)	11 (68.8)	0.213
	>3.4	8 (53.3)	7 (46.7)	
BMI	<25	10 (45.5)	12 (54.5)	0.654
	≥25	13(52.0)	12 (48.0)	
PCOS	No	21 (46.7)	24 (53.3)	0.819
	Yes	8 (50.0)	8 (50.0)	
Age	>35	5 (55.6)	4 (44.4)	0.724
	≤35	24 (46.2)	28 (53.8)	

OHSS: ovarian hyperstimulation syndrome; AMH: anti-Müllerian hormone; BMI: body mass index; PCOS: polycystic ovarian syndrome. *Significance level <0.05.

the risk in susceptible patients, given that fresh embryo transfer triggers a further hCG surge following implantation.

These findings are consistent with previous results reported in the literature. A randomized clinical trial describing the use of freeze-all compared elective cryopreservation of all embryos with a new fresh embryo transfer in patients at risk of OHSS. Ferraretti et al. found a reduced risk of moderate/severe OHSS, where 6% of patients in the fresh embryo transfer group developed severe OHSS versus zero cases in the freeze-all group. In addition, there was no significant difference in live birth rates between the two groups[20].

Several principal clinical parameters have been established, such as the number of follicles on the day of the trigger, to help attenuate the risk of moderate and severe OHSS[21]. Thus, the use of freeze-all strategy after a GnRH agonist trigger is the gold standard strategy for patients at risk of OHSS. With regard to clinical aspects, it is important to note that embryo freezing is a well-established technique with similar pregnancy rates as fresh embryo transfer[4,22].

In the present study, mild OHSS occurred in 47.5% of the patients assessed. Other studies report mild syndrome in around 30% of IVF cycles[5]. One of the determinants of this rate is the fact that total prevention of OHSS is not possible until the pathogenesis of the syndrome has been fully elucidated. Thus, although it can prevent late OHSS (moderate and severe forms), cryopreservation is not totally effective for the prevention of the syndrome because the strategy cannot prevent early-onset OHSS (mild form)[9].

Another factor that may influence the outcome of this study for mild cases of OHSS is the protocol employed for the cycle. The choice of protocol was dictated by AFC and risk factors, where women with AFC>15 underwent the protocol with antagonist and GnRH agonist trigger (Protocol C). However, 42.2% of cycles were performed using Protocol B (use of agonist and hCG trigger) because AFC count was between 11 and 15. Nevertheless, this number of follicles subsequently rose following ovarian stimulation to over 15 follicles aspirated, a factor that may have influenced the development of OHSS.

In addition, another possibility for preventing OHSS and its severity reduction is the use of dopamine agonist. But the protocols are not well defined, and more studies are needed to evaluate the potential of dopaminergic agonist in the prevention of OHSS. A recent study showed that bromocriptine did not prevent the moderate or severe cases of early-onset OHSS in high-risk patients subjected to IVF[23].

Generally, there is a tendency for fewer moderate and severe cases of OHSS. This reduction is due to the greater screening of risk factors, with ovarian marker studies that predict supraphysiological response and allow the use of individualized ovarian stimulation protocols. In addition, wider use of GnRH antagonists for the prevention of premature release of LH and expansion of freeze-all procedures are factors contributing to a reduction in complications related to OHSS[24].

The specific risk factors (markers of ovarian reserve) are AMH level and AFC[6]. Randomized prospective studies have shown that a basal AMH level to OHSS and allow the use ofresponse with high sensitivity (90.5%) and specificity (81.3%). Women with levels exceeding 5 ng/mL are at 3 times greater risk of developing OHSS[25]. Another study suggests that an AFC>16 has 89% sensitivity and 92% specificity of predicting high ovarian response[26].

In the present study, risk factors were evaluated and no statistically significant association with OHSS was found, possibly explained by the small sample size, since this was not a primary objective of the study.

The findings of this research are strengthened by the selection of an appropriate sample size and the use of broad eligibility criteria. The prevalence of OHSS in patients undergoing fertilization cycles at risk of OHSS and submitted to cryopreservation of all embryos found in the Service of Human Reproduction of the Pérola Byington Hospital proved to be within the range suggested by the literature. The characterization of patients with OHSS and ARTs employed did not differ from that described in other studies.

This study has some limitations, such as the final pregnancy rate, where some patients had not undergone embryo transfer and were still being treated at the assisted reproduction service during the study period. Other patients also went on to receive a further stimulation cycle. The retrospective design of the study represents another limitation. Further studies involving larger casuistics will allow investigation of other aspects related to this outcome, improving the success of ARTs and preventing complications such as severe forms of OHSS.

CONCLUSION

Cryopreservation of all embryos is associated with a reduction in moderate and severe forms of OHSS. Risk factors for OHSS should be evaluated before commencing treatment, with less intense stimulation protocols adopted accordingly. Thus, efforts should be made in OHSS prevention, given that once the syndrome has developed, there is no reliable form of treatment, particularly in severe cases.

AUTHORS' CONTRIBUTIONS

LCGMM, MC, AD, SMRRL: Conceptualization, data curation, formal analysis, writing – original draft, writing – review & editing. **ARR:** Data curation, formal analysis.

ETHICAL COMMITTEE

The study was approved by the Plataforma Brasil by the Research Ethics Committee under CAAE: 46741721.6.0000.0069 and Permit no. 4.735.050, for report dated May 26, 2021. The study complied with the National Board of Health resolution CNS 196/96 on research involving humans.

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ORIGINAL ARTICLE

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Ultra-processed foods and the nutritional quality of the diet of Brazilian pregnant women

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the consumption of ultra-processed foods by Brazilian pregnant women and its association with the nutritional quality of the diet.

METHODS: This is a prospective and cross-sectional study with food consumption data of Brazilian pregnant women from the 2017 to 2018 Family Budgets Survey (*Pesquisa de Orçamentos Familiares)*. Food consumption was measured using two 24-h food recalls, and the foods were categorized according to the NOVA classification. The averages of absolute and relative energy consumption for each of the NOVA groups and subgroups were estimated. The sociodemographic characteristics described the diet's caloric contribution of ultra-processed and non-ultra-processed food fractions. Linear regression models were used to describe the association between quintiles of the caloric contribution of ultra-processed foods and the average content of nutrients in the diet.

RESULTS: Consumption of ultra-processed foods represented 20.9% of the total calories in the diet of Brazilian pregnant women. There was a higher energy contribution of ultra-processed foods in the diet of pregnant women living in urban areas (22%), with higher per capita income (23.7%), and in the south region of the country (26.9%). In addition, the data showed an association between higher consumption of ultra-processed foods with reduced intake of protein, carbohydrate, fiber, potassium, iron, zinc, and folate and increased intake of total fat, saturated fat, trans fat, and free sugar.

CONCLUSION: Results show that higher consumption of ultra-processed foods is associated with a reduction in the nutritional quality of the diet of Brazilian pregnant women.

KEYWORDS: Food-processing industry. Nutritional quality. Pregnant women.

INTRODUCTION

In recent years, the diet pattern of the Brazilian population has changed, with a decrease in the consumption of vegetables, cereals, and tubers and an increase in the consumption of foods rich in fats and sugars such as ultra-processed foods (UPFs)[1,2]. The NOVA classification categorizes foods based on the extent and purpose of industrial processing. UPFs are formulations of food substances modified by chemical processes and assembled into ready-to-eat foods and beverages, using numerous cosmetic additives such as colorings, flavors, emulsifiers, sweeteners, and thickeners[3].

Several studies have shown that a diet based on UPFs increases the risk of developing overweight, obesity, and chronic noncommunicable diseases (NCDs)[4-6] and contributes to inadequate intake of micronutrients due to the low nutritional quality of these foods, which have a high energy density; high content of free sugar, sodium, and saturated and trans fats; and low content of fiber, vitamins, and minerals[7-9].

The increase in the consumption of UPFs is especially worrying in the population of pregnant women, a group vulnerable to nutritional inadequacies[10], as micronutrient deficiency in the gestational period is considered a global public health problem[10,11]. According to the World Health Organization (WHO) (2017)[11], inadequate nutrient intake during pregnancy can increase the prevalence of NCDs, which, in turn, increases the risk of adverse outcomes during pregnancy[12]. Thus, the nutritional quality of the diet is critical to ensuring a healthy pregnancy for both the mother and the fetus[10-12].

Although the importance of an adequate diet in the gestational period is well established in the literature[10-12] and the negative impact of the consumption of UPFs on the nutritional quality of the diet for the general population[4-9], this relationship

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has been less investigated in the population of pregnant women. This fact motivated us to evaluate the consumption of UPFs by Brazilian pregnant women and the association with the nutritional quality of the diet.

METHODS

The data analyzed come from the individual food consumption module of the Brazilian Family Budgets Survey (POF – *Pesquisa de Orçamentos Familiares*), carried out by the Brazilian Institute of Geography and Statistics (IBGE) between 2017 and 2018[13].

The POF uses a complex two-stage cluster sampling plan. In the first stage, geographic and socioeconomic stratification are selected by systematic sampling, with probability proportional to the number of households in each sector. In the second stage, families are selected by simple random sampling.

Standardized questionnaires were applied to obtain data such as age, sex, pregnancy, ethnicity or race, educational level, household income, geographic region, and urban or rural area. The POF sample, referring to 2017–2018, was 57,920 households. The individual food consumption module was applied to a probabilistic subsample of 20,112 households. All residents over 10 years of age residing in these households were selected, totaling a subsample of 46,164 individuals.

Women between 10 and 50 years old who answered "yes" to the question "Are you pregnant?" regarding the POF questionnaire 7 (Personal Food Consumption Block)[13] were included in the study, totaling a sample of 379 pregnant women. In addition, data from the individual food consumption module were obtained from two 24-h food recalls, applied on nonconsecutive days. The recalls contained all meals and beverages (except water) consumed within 24 h, including the preparation, homemade measure, daily servings, meal times, and place of consumption.

We used data from the Brazilian Food Composition Table (TBCA)[14] to estimate the nutrients for each food and drink reported in the survey. Additionally, the "Model of Nutritional Profile" method of the Pan American Health Organization (PAHO)[15] was used to evaluate free sugar. Analyses were performed using the 2-day 24-h recall, when available. The Multiple Source Method® (MSM) program (version 1.0.1)[16] was used to adjust the estimate of habitual food and nutrient intake.

The consumption items (n=1,593) reported in the survey were categorized according to the NOVA food classification, which divides foods into four groups[3]:

- *Natural or minimally processed foods* (group 1) are obtained directly from animals or plants with no modifications/alterations after separation from nature such as cleaning, milling, freezing, pasteurization, fermentation, and other processes that do not include the addition of substances to the original foods.
- *Processed culinary ingredients* (group 2) are condiments extracted directly from foods of the first group or nature such as sugar, salt, oils, and fats.
- *Processed foods* (group 3) are produced by the industry with the addition of salt or sugar (or another substance commonly used in cooking) to natural or minimally processed food.
- *Ultra-processed foods* (group 4) are formulations of food substances modified by chemical processes and assembled into ready-to-eat foods and beverages, using numerous cosmetic additives such as dyes, flavors, emulsifiers, sweeteners, and thickeners.

The nutritional quality of the diet was evaluated by dietary indicators, for which WHO established consumption goals for the prevention of NCDs[16,17]. The parameters used were protein, carbohydrate, free sugar, fiber, total fat, saturated fat, trans fat, sodium, and potassium. In addition to the micronutrients that WHO points out, there is a need for greater nutritional surveillance during the gestational period: iron, folate, zinc, calcium, and vitamin A[18,19].

The total and relative energy consumption averages of the NOVA groups and subgroups were estimated to describe food consumption. The diet was divided into two fractions, one composed only of UPFs and the other of non-UPFs. In addition, the caloric contribution of each fraction was described for the total sample of pregnant women and by sociodemographic characteristics, using a 95% confidence interval to analyze the statistical difference.

The sample of pregnant women was first stratified according to the quintiles of energy contribution of UPFs to the total caloric value of the diet to evaluate the association between the consumption of UPFs and the nutritional quality of the diet. The first quintile had the lowest caloric contribution of UPFs, and the last quintile had the highest. Then, the average intake of energy and nutrients by consumption quintiles of UPFs was estimated. Linear regression was used to identify the association's direction and statistical significance. Models were adjusted for confounding variables such as age group, per capita income, educational level, urban or rural residence, and country macro-regions, considering $p<0.05$ as the level of statistical significance. All analyses were performed using the Stata/MP software, version 14.0, considering the complex design of the 2017–2018 POF sample and its weighing factors.

The Research Ethics Committee approved this project of the Federal University of São Paulo (protocol 5682270619).

RESULTS

A total of 379 (11.9%) pregnant women were adolescents (10–18 years old), and 88.1% were adult women (19–50 years old). Most lived in urban areas (76.3%), had a per capita income of up to R$ 1,667.8 (78.9%), and were from the north (21.9%) and northeast (37.2%) regions. There was a more significant energy contribution from UPFs in the diet of pregnant women living in urban areas (22%), with higher per capita income (23.7%), and in the south of the country (26.9%) (Table 1).

The average daily energy consumption was 1791.2 kcal, of which 52.6% came from natural or minimally processed foods, 15.7% from processed culinary ingredients, 10.8% from processed foods, and 20.9% from UPFs. In the group of natural or minimally processed foods, the three items that had the most significant contribution to total energy consumption were rice (9.6%), beef (7.0%), and beans (5.6%). Among products of animal origin, after meat, poultry had the highest energy contribution (5.3%). Fruits and vegetables contributed 3.8 and 1.7% to the total daily energy, respectively.

In the group of processed culinary ingredients, vegetable oil and sugar presented the highest energy contribution of 8.0 and 5.7%, respectively. Processed breads alone accounted for more than 70% of the calories consumed in the processed food group. As for UPFs, ready-to-eat or semi-ready meals were the items with the highest consumption in terms of total energy intake (3.2%), followed by margarine (2.8%) and salted biscuits and chip-type salty snacks (2.6%) (Table 2).

Table 3 shows that the energy contribution in the last quintile was 165% (925 kcal) more significant than the energy contribution in the first quintile. The relative content of free sugar, total fat, saturated fat, and trans fat increases significantly with the increase in the contribution of UPFs to the diet. In contrast, the opposite occurs for protein content, carbohydrate, fiber, potassium, iron, folate, and zinc. For sodium, calcium, and vitamin A, no significant association was found between the quintiles of consumption of UPFs and the content of these micronutrients in the diet.

Table 1. Mean percentage of total energy intake from two fractions of the diet according to sociodemographic characteristics – pregnant women aged 10–50 years (Brazil, Family Budget Survey, 2017–2018) (N=379).

	N	%	Non-ultra-processed food diet fraction[a]	Ultra-processed food diet fraction
			% (95%CI)	% (95%CI)
Age group (years)				
10–18	45	11.9	77.2 (71.76–82.64)	22.8 (17.36–28.24)
19–50	334	88.1	79.4 (77.66–81.07)	20.6 (18.93–22.34)
Educational level (years)				
≤5	35	9.2	79.0 (72.14–85.87)	21.0 (14.13–27.86)
6–11	150	40.6	79.9 (77.95–81.83)	20.1 (18.17–22.05)
12–16	194	51.2	78.6 (75.974–81.16)	21.4 (18.84–24.03)
Area				
Urban	289	76.3	78.0 (76.11–79.85)	22.0 (20.14–23.88)
Rural	90	23.8	84.7 (82.48–86.84)	15.3 (13.16–17.52)
Per capita income				
Up to R$ 440.6	106	28.0	83.8 (81.36–86.30)	16.2 (13.70–18.64)
R$ 442.3–913.8	116	30.6	79.0 (76.04–82.00)	21.0 (18.00–23.96)
R$ 915.2–1,667.8	77	20.3	77.4 (75.07–79.71)	22.6 (20.29–24.93)
> R$ 1,667.8	80	21.1	76.3 (71.73–80.84)	23.7 (19.16–28.27)
Region				
North	83	21.9	84.8 (81.91–87.67)	15.2 (12.33 -18.09)
Northeast	141	37.2	82.2 (80.12–84.28)	17.8 (15.72–19.88)
Southeast	72	19.0	77.3 (73.75–80.88)	22.7 (19.12–26.25)
South	43	11.4	73.1 (68.85–77.44)	26.9 (22.56–31.15)
Midwest	40	10.6	76.7 (73.14–80.27)	23.3 (19.73–26.86)

[a]Dietary fraction composed of natural or minimally processed foods, processed culinary ingredients, and processed foods.

Table 2. Mean absolute and relative daily energy intake according to NOVA food groups and subgroups – pregnant women aged 10–50 years (Brazil, Family Budget Survey, 2017–2018) (N=379).

NOVA Food Groups	kcal/day	% Total energy intake
Natural or minimally processed foods	**924.0**	**52.6**
Rice	167.9	9.6
Beef	120.2	7.0
Beans	95.8	5.6
Poultry	91.3	5.3
Fruits	67.1	3.8
Pasta	58.8	3.2
Milk	56.0	3.1
Beans	40.0	2.3
Vegetables and legumes	29.0	1.7
Pork	33.5	1.9
Cassava flour	28.8	1.5
Natural fruits juices	27.3	1.5
Fish	18.9	1.2
Corn, oats, wheat, and other cereals	19.0	1.1
Eggs	20.9	1.2
Wheat flour	12.2	0.7
Coffee and tea	9.9	0.6
Other[a]	27.4	1.3
Processed culinary ingredients	**280.4**	**15.7**
Plant oils	140.1	8.0
Sugar	103.5	5.7
Butter	17.2	1.0
Animal fats	5.9	0.3
Other[b]	13.6	0.7
Processed foods	**194.2**	**10.8**
Processed breads	147.6	8.4
Cheese	25.9	1.4
Salted, smoked, or canned meat or fish	9.4	0.5
Other[c]	11.3	0.5
Ultra-processed foods	**392.6**	**20.9**
Ready-to-eat or semi-ready-to-eat meals[d]	77.5	3.8
Margarine	49.8	2.8
Salted biscuits and chip-type salty snacks	46.6	2.6
Cold cuts	34.3	1.9
Cookies, cakes, and sweet pies	41.7	2.2
Bread, hamburger, hot dog, and similar	33.7	2.0
Chocolates, ice cream, candies, or other industrialized desserts	33.7	1.6
Milk-based drinks	22.2	1.1
Carbonated soft drinks	20.3	1.1
Fruit drinks industrialized	14.4	0.8
Other[e]	18.4	1.0
Total	**1,791.2**	**100.0**

[a]Natural yogurt, lentils, chickpeas and other legumes, nuts and seeds, other flours, seafood, and other meats. [b]Coconut milk, starch, vinegar, and salt. [c]Legumes/vegetables/fish preserves, fruit jam, tomato sauce, beer, and wine. [d]Pizzas, sandwiches, fast food, snacks, frozen pasta dishes and noodles, soup, and other "instant" foods. [e]Breakfast cereals, ultra-processed cheeses, industrialized sauces, cream, spirits, other nonalcoholic beverages, and caloric supplements.

Table 3. Mean dietary nutritional indicators according to quintiles of consumption of ultra-processed foods – pregnant women aged 10–50 years (Brazil, Family Budget Survey, 2017–2018) (N=379).

Nutritional indicators	Quintile of consumption of ultra-processed foods (% total energy)					β	p-value*
	Q1	Q2	Q3	Q4	Q5		
Total energy (kcal/day)	1,412.5	1,654.4	1,655.3	1,910.2	2,337.7	209.95	0.000
Percentage of total energy from							
Protein	15.3	14.9	14.4	13.7	14.1	-0.38	0.003
Carbohydrates	60.4	59.1	58.7	58.6	57.2	-0.67	0.028
Free sugars	11.0	15.1	16.5	16.2	19.2	1.73	0.000
Total fats	24.3	26.0	26.9	27.7	28.7	1.04	0.000
Saturated fat	7.3	8.3	8.3	8.7	8.8	0.34	0.000
Trans fat	0.5	0.6	0.6	0.7	0.9	0.10	0.000
Nutrients density							
Fiber (g/1,000 kcal)	14.6	12.1	11.5	11.4	10.1	-0.94	0.002
Sodium (g/1,000 kcal)	1.4	1.4	1.4	1.4	1.3	-27.29	0.093
Potassium (mg/1,000 kcal)	1363.6	1164.3	1160.6	1088.7	993.8	-80.55	0.000
Iron (mg/1,000 kcal)	6.0	5.9	5.6	5.7	5.2	-0.18	0.025
Folate (μg/1,000 kcal)	254.2	221.7	215.9	219.3	190.6	-12.73	0.002
Zinc (mg/1,000 kcal)	5.7	6.1	5.3	4.4	4.3	-0.48	0.000
Calcium (mg/1,000 kcal)	244.2	218.4	224.9	241.5	219.5	-2.36	0.601
Vitamin A (μg/1,000 kcal)	143.0	253.1	232.7	267.4	178.2	7.99	0.476

*p<0.05 for linear trend in the variation of the nutritional indicator according to quintiles of consumption of ultra-processed foods (with adjustment for confounding variables).

DISCUSSION

In this study, we showed that the higher consumption of UPFs contributes to a reduction in the intake of protein, carbohydrates, fiber, potassium, iron, zinc, and folate and an increase in the intake of total fat, saturated fat, trans fat, and free sugar. Thus, in proportion to the increased consumption of UPFs, it reduced the nutritional quality of the diet. These findings corroborate with other studies in Brazil, which also showed that the higher consumption of UPFs favors a decrease in the intake of essential micronutrients for the human body (iron, zinc, and folate) and those that prevent NCDs (fiber and potassium)[7-9,20].

Different types of UPFs such as ready-to-eat and semi-ready-to-eat meals, sweet and savory cookies, cold cuts, and cold cuts, which are rich in free sugar, trans fat, and sodium, had a significant quota in the diet of pregnant women. This is worrying since the intake of these nutrients above the recommended level is associated with overweight, obesity, and NCD[5,6]. Studies have reported that women with a high pre-pregnancy body mass index, who present excessive gestational weight gain, and a higher risk of obstetric complications, may contribute to the intergenerational expansion of the obesity and NCD epidemic[12,21].

The percentage of caloric diet in the NOVA groups among pregnant women was similar to that of Brazilian nonpregnant women[13], showing that during the period of pregnancy, there was no reduction in the consumption of UPFs. The consumption of UPFs also presented sociodemographic characteristics like the patterns observed in the general population[1], being higher in the urban region, in the higher income bracket, and in the south part of the country. One factor that explains the greater consumption of UPFs is the greater availability and ease of acquisition of UPFs[22]. This scenario confirms the importance of public policies to strengthen nutritional assistance in prenatal care and reduce exposure to UPFs.

The NOVA classification is fundamental for policies to promote healthy eating, encouraging reflection on food composition and its impacts on health such as its use in the "Food Guide for the Brazilian Population"[23]. In 2020, the Ministry of Health launched the "Protocol for the Use of the Food Guide for the Brazilian Population in the Dietary Guidelines for Pregnant Women"[24] as a support material for clinical practice in primary health care, whose central recommendation is to encourage the consumption of natural or minimally processed foods to the detriment of ultra-processed.

This study has limitations such as the lack of information on the women's gestational age and those from the 24-h recall, the underreporting of food consumption, the differences between real and standardized cooking recipes, and the differences between the real nutritional composition and that of the TBCA[14].

Among the strengths of this study are the use of two 24-h food recalls, which provided detailed information on the foods consumed, and the use of the NOVA classification to assess the nutritional quality of the diet since studies with the population are still scarce for pregnant women.

CONCLUSION

The negative impact of the consumption of UPFs on the nutritional quality of the diet of Brazilian pregnant women was evidenced. In this way, encouraging the reduction of consumption of UPFs has the potential to improve the nutritional profile of the diet of pregnant women.

AUTHORS' CONTRIBUTIONS

GCA: Conceptualization, Visualization, Writing – original draft. **KRM:** Data curation, Validation, Visualization. **MLCL:** Formal Analysis, Visualization. **MUN:** Investigation, Methodology, Visualization. **ES:** Project administration, Supervision, Visualization. **EAJ:** Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Impact of the coronavirus disease 2019 pandemic on the management of acute peptic ulcer perforation: to be reconsidered(?)

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SUMMARY

OBJECTIVE: Peptic ulcer perforation presents the most serious complication of ulcer disease with mortality that varies significantly depending on the age and conditions. The coronavirus disease 2019 pandemic was effective worldwide in 2020 and continues to date. The aim of this study was to investigate the initial clinical parameters and short-term outcomes of patients with acute peptic ulcer perforation before and during the coronavirus disease 2019 pandemic.

METHODS: A retrospective cohort study was conducted in the Department of Surgery, University Hospital Ostrava, Czech Republic. The patients undergoing surgical modality of a simple suture of peptic ulcer perforation with/without omentoplasty in the post-coronavirus disease 2019 (January 1, 2020 to December 31, 2021) and the pre-coronavirus disease 2019 (January 1, 2018 to December 31, 2019) had been incorporated in this study.

RESULTS: This study included a total of 46 cases (26 in the pre-coronavirus disease 2019, 20 in the post-coronavirus disease 2019). The age, body mass index, Boey score, duration of symptoms, surgery time, and length of hospital stay were comparable in both study subgroups. During the coronavirus disease 2019 pandemic, patients were admitted with a statistically significantly lower degree of perioperative risk according to the American Society of Anesthesiologists classification (p=0.013). Notably, 30-day postoperative morbidity was significantly higher in pre-coronavirus disease 2019 (73.1 vs. 55.0%, p=0.038). The mortality rate in the laparoscopic group was 13.6%, in the laparotomy group 41.4%, and the mortality rate was higher in pre-coronavirus disease 2019 than in post-coronavirus disease 2019 (34.6 vs. 20.0%, p=0.166).

CONCLUSION: In fact, the coronavirus disease 2019 pandemic had not significantly influenced therapeutic management and short-term outcomes of patients undergoing acute surgical repair of peptic ulcer perforation.

KEYWORDS: Peptic ulcer perforation. COVID-19. Pathology. Mortality. Morbidity.

INTRODUCTION

Peptic ulcer (PU) disease is the most common disease of the upper gastrointestinal tract with a worldwide incidence of about 90 cases per 100,000 inhabitants per year. The most serious and life-threatening complication of PU disease is perforation, the worldwide average incidence of which is approximately 9 cases per 100,000 inhabitants[1]. The mortality of PU perforation is generally around 10–40%[2,3] but varies significantly depending on the patient's age and condition prior to and at the moment of admission to the hospital. For the patient with PU perforation, some specific classification systems can be used to determine the degree of risk of a patient with PU perforation. Currently, the simple and accurate Boey score is used mainly for this purpose[4]. The standard treatment option for patients with PU perforation is a surgical suture of perforation, which can be performed by laparotomy or using minimally invasive surgical techniques.

The coronavirus disease 2019 (COVID-19) pandemic affected the whole world in 2020 and continues to date. In total, more than half a billion people have been proven to be infected. Although the number of reported deaths from COVID-19 reached 5.94 million worldwide at the end of

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2021, it is estimated that up to 18.2 million people worldwide have died from the disease to this date (95%CI 17.1–19, 6)[5]. The Czech Republic, similar to Brazil, had one of the highest incidences of disease and subsequent mortality per million inhabitants in the world[6].

In addition to wearing masks, the basic mechanism of the fight against COVID-19 was the isolation of the population. Due to the isolation of the population and the overload of the health system, patients with PU disease temporarily lost the possibility of these regular checks and examinations that could have resulted in a deterioration of their health status, stress, and an augmentation in the risk of subsequent complications, including acute PU perforation. Recently, some studies have suggested a change in the spectrum of patients and treatment modality for acute conditions during the pandemic period. These studies reported an attenuated number of patients with acute problems, but an increase in their more severe conditions[7-9].

The aim of this study was to investigate the initial clinical parameters and the short-term outcomes of patients undergoing acute surgical repair of PU perforation pre- and post-COVID-19. We ask ourselves whether the COVID-19 pandemic affected the availability of acute care for patients with PU perforation and, therefore, worsened their condition before admission and subsequently increased the risk of postoperative complications and mortality.

METHODS

A retrospective cohort study with a total of 46 cases had been conducted in the Department of Surgery, University Hospital Ostrava, Ostrava, Czech Republic. To this end, the cases undergoing surgical repair, the simple suture of PU perforation, with or without omentoplasty in the post-COVID-19 (January 1, 2020 to December 31, 2021) and the pre-COVID-19 (January 1, 2018 to December 31, 2019) had been incorporated in this study. All the cases had undergone simple acute surgical closure of PU perforation with or without omentoplasty by conventional or laparoscopic approach. In addition, the cases with different or other associated procedures and with incomplete data in the hospital document database had been excluded from the present study design.

Demographic and clinical data from all the studied cases, such as the age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) classification, duration of symptoms of PU perforation, surgical modality, surgery time, length of hospital stay, and 30-day postoperative morbidity were extracted from hospital medical records. The preoperative clinical condition of all the cases had been assessed according to the Boey score (Table 1). The duration of perforation was determined as the time interval between the onset of severe acute abdominal pain and arrival time at the hospital. Systolic blood pressure <100 mmHg was considered a preoperative shock and the health conditions of systemic heart, lung, liver, or kidney disease, cancer, and diabetes were considered concomitant severe medical illnesses[10]. The postoperative complications were classified according to the Clavien-Dindo classification[11].

The obtained data were analyzed using descriptive statistics (mean, standard deviation, median, range, relative frequency). The Student's t-test or the Mann-Whitney U test was used for quantitative variables while the χ^2 test was used for the categorical variables. A level of significance of α=0.05 and p<0.05 were considered statistically significant.

RESULTS

The study included a total of 46 cases after the surgical suturation of the PU perforation. In the pre-COVID-19 (which was considered the reference period), 26 cases had been included while 20 were in the post-COVID-19. The basic demographic and clinical characteristics of all the studied patients are presented in Table 2. No statistically significant differences between the pre- and post-COVID-19 in age (p=0.164), BMI (p=0.288), or surgical modality (p=0.249) were recognized.

Regarding the Boey score and ASA classification, patients in post-COVID-19 had better general health status at the moment of hospital admission. Herein, 50.0% of the cases in post-COVID-19 had a Boey score of 0 and no one had a Boey score of 3. In contrast, 15.4% of the cases in the pre-COVID-19 had a Boey score of 3, and solely 26.9% had a Boey score of 0. Notably, the differences in Boey scores between the study subgroups were not statistically significant (p=0.207). Similarly, 35.0% of the cases of post-COVID-19 were preoperatively classified as ASA I–II and only 15.0% as ASA IV–V. In contrast, only 19.2% of patients in pre-COVID-19 were classified as ASA I–II and 50.0% as ASA IV–V. The differences in ASA classification between the study subgroups were statistically significant (p=0.013). The average duration of symptoms of perforation was 24.1±21.8 h in 43.5% of the cases with perforation lasting longer than 24 h and the differences

Table 1. The Boey score.

Boey score	Findings
1 point	Duration of perforation >24 h
1 point	Preoperative shock
1 point	Concomitant severe medical illness

in the duration of perforation between study subgroups were not significant (p=0.365, p=0.188).

The intra- and postoperative outcomes are presented in Table 3. The average operation time was 53.0±17.7 min, and the difference between the subgroups was not statistically significant (p=0.291). The mean postoperative hospital stay was 13.0±8.6 days (13.6±8.9 days, post-COVID-19 and 12.6±8.6 days, pre-COVID-19 with a range of 3–35 days), which did not reveal any significance (p=0.720). In addition, the 30-day postoperative morbidity rate was 65.2%, whereas 55.0% in post-COVID-19 and 73.1% in pre-COVID-19 had possessed postoperative complications with a statistical significance in the postoperative morbidity rate (p=0.038). The severity of postoperative complications between the subgroups was not significant considering the Clavien-Dindo classification (p<0.0112). Besides, 30-day postoperative mortality was 28.3%, which was higher in patients in pre-COVID-19 compared to post-COVID-19, without significance (34.6 vs. 20.0%, p=0.166).

Table 2. The demographics and clinical data of the studied cases.

	Post-COVID-19 n=20	Pre-COVID-19 n=26	p	Total n=46
Age, years, mean±SD	58.8±17.7	65.8±14.7	0.164	62.7±16.3
Gender, n (%)			1.000	
Female	7 (35.0%)	10 (38.5%)		17 (37.0%)
Male	13 (65.0%)	16 (61.5%)		29 (63.0%)
BMI (kg/m^2), mean±SD	22.6±4.4	24.2±5.2	0.288	23.5±4.9
ASA, n (%)			0.013	
I–II	7 (35.0%)	5 (19.2%)		12 (26.1%)
III	10 (50.0%)	8 (30.8%)		18 (34.8%)
IV–V	3 (15.0%)	13 (50.0%)		16 (39.1%)
Boey score, n (%)			0.207	
0	10 (50.0%)	7 (26.9%)		17 (37.0%)
1	4 (20.0%)	6 (23.1%)		10 (21.7%)
2	6 (30.0%)	9 (34.6%)		15 (32.6%)
3	0 (0.0%)	4 (15.4%)		4 (8.7%)
Duration of symptoms, h, mean±SD	20.7±23.1	26.7±20.9	0.365	24.1±21.8
Duration of perforation >24 h, n (%)	6 (30.0%)	14 (53.8%)	0.188	20 (43.5)
Surgery approach, n (%)			0.249	
Laparoscopy	12 (60.0%)	10 (38.5%)		22 (47.8%)
Laparotomy	8 (40.0%)	16 (61.5%)		24 (52.2%)

Table 3. The intraoperative and postoperative outcomes of study patients.

	Post-COVID-19 n=20	Pre-COVID-19 n=26	p	Total n=46
Surgery time (min, mean±SD)	49.6±17.1	55.4±18.0	0.291	53.0±17.7
Length of hospital stay (day, mean±SD)	13.6±8.9	12.6±8.6	0.720	13.0±8.6
30-Day postoperative morbidity, n (%)	11 (55.0%)	19 (73.1%)	0.038	30 (65.2%)
Clavien-Dindo classification, n (%)			0.112	
Grade 0	9 (45.0%)	7 (26.9%)		16 (39.1%)
Grades I–II	4 (20.0%)	5 (19.2%)		9 (19.6%)
Grades III–IV	3 (15.0%)	5 (19.2%)		8 (17.4%)
Grade V (postoperative mortality)	4 (20.0%)	9 (34.6%)	0.166	13 (28.3%)

DISCUSSION

Patients with COVID-19 overwhelmed hospitals, which had to attenuate care for the remaining patients. This might have worsened the care of chronic patients and augmented the risk of hidden problems turning into acute and life-threatening conditions. A good example of the effect of delayed care on chronic patients is reported by Mun et al.'s[12] study in 2021. The authors expressed a set of 1,453 cases with chronic pain due to the postponement or cancellation of all regular checkups and the restriction of access to prescription opioids during the COVID-19 period, where approximately 25–30% of individuals reported exacerbation of their chronic pain. Similar conclusions were also described by Pagé et al.[13], Chatkoff et al.[14], and Lang-Illievich et al.[15].

The deterioration in care for patients without COVID-19 can be found in all medicines. Some authors[16,17] described an increase in the numerical value of seizures and a worsening of sleep quality in cases with epilepsy during the COVID-19 period. Rabbone et al.[18] reported that the COVID-19 pandemic could have altered diabetes presentation, whereas Brown et al.[19] showed disrupted medical care, exercise, and social activities that led to worsening of motor and nonmotor symptoms in about half of the cases.

The neglect of care for patients without COVID-19 was also reflected in surgery. Serban et al.[20] investigated the effects of the COVID-19 pandemic on the clinical presentation and therapeutic management of acute surgical abdomen. They found that the number of patients with therapeutically neglected or undiagnosed colorectal cancer who developed urgent complications such as tumor obstruction or perforation increased during the pandemic period[21,22]. The change in the frequency of the most frequent acute surgical diagnoses was investigated by Cano-Valderrama et al.[23], who reported that a significant reduction was observed in the number of acute surgery procedures performed and the delay in the arrival of patients in the hospital during the pandemics[24-25]. Higher morbidity was observed in patients undergoing acute surgery during the pandemic period, although mortality did not change. Herein, our study noted a slight decrease in the incidence of acute PU perforation during the pandemic period but did not observe a delay in the arrival of patients to the hospital or a worsening of their clinical condition at the time of admission.

To the best of our knowledge, no study has reported the effects of health restrictions during the COVID-19 pandemic on the treatment of acute PU perforation in the English-language literature. Preliminary outcomes of this study exhibited that during the COVID-19 pandemic there was no expected increase in PUP cases; in contrast, a slight decrease might be due to the patients' fear of visiting the hospital during the pandemic. Herein, an important question is whether the change in the demographic curve of the population is behind the decline. As is known, mainly older and already ill people died from COVID-19; hence, the population most at risk of complications from ulcer disease was significantly attenuated by the pandemic. Therefore, fewer patients at risk for PU disease complications may have led to a lower incidence of PU perforation in the era of COVID-19.

The alterations in the demographic curve can also explain some of the outcomes of the present study. In post-COVID-19, younger patients and patients with a lower average BMI (than those pre-COVID-19) came to the hospital. However, this change was not statistically significant, and, therefore, we cannot take it as the result of an alteration in the distribution of the studied population. In contrast, post-COVID-19, patients came to the hospital with a statistically significantly lower degree of risk according to the ASA classification, indicating the selection of a relatively healthier population compared with pre-COVID-19. The higher number of cases with a low degree of risk according to the Boey score in post-COVID-19 can be explained similarly.

The better clinical condition of the patients post-COVID-19 was also reflected in their postoperative outcomes. We recorded a statistically significantly lower overall patient 30-day postoperative morbidity in post-COVID-19, and the cases in post-COVID-19 had significantly more often an uncomplicated postoperative course or a lower grade complication according to the Clavien-Dindo classification. Contrary to the expectation that the mortality of patients with PU perforation would increase during the pandemic period, we recorded a substantial decrease. Mortality in patients in post-COVID-19 was lower, which might be explained by the better clinical condition and the younger age of patients at admission to the hospital.

Limitations

The main limitations of the study are the size of the data set, in which a trend is clearly visible but without statistical significance, and the retrospective study design.

CONCLUSION

The therapeutic management of patients with PU perforation has not been significantly influenced by the COVID-19 pandemic. In contrast, we have recorded a better clinical condition of patients before admission to the hospital and lower postoperative morbidity/mortality after the surgical repair of

PU perforation. It might be explained by the alteration in the demographic distribution of the study population as a result of the COVID-19 pandemic. Finally, we postulate that it is critical for physicians and health providers to stay informed of the growing spectrum and clinical presentation of PU issues in this pandemic to ensure appropriate clinical care and the relevant treatment modalities to minimize both disease-induced injury and disease transmission.

AUTHORS' CONTRIBUTIONS

LT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **IS:** Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – review & editing. **PI:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **MM:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **DT:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **AP:** Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Visualization. **LM:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **DS**: Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Autonomic heart rate modulation in patients with coronavirus disease 2019 in mechanical ventilation

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SUMMARY

BACKGROUND: Patients with coronavirus disease 2019 on automatic mechanical ventilation have greater heart rate modulation with greater parasympathetic modulation.

OBJECTIVE: To analyze the autonomic modulation of heart rate in critically ill patients with coronavirus disease 2019 on invasive mechanical ventilation.

METHODS: A cross-section study was carried out with 36 individuals divided into two groups. The control group included patients of both genders, in orotracheal intubation with invasive mechanical ventilation under controlled assisted mode, hospitalized in the intensive care unit for another 24 h. In the non-COVID group, patients diagnosed with coronavirus disease 2019 in the same condition mentioned in the control group.

RESULTS: There was a significant increase in heart rate variability (standard deviation of all normal RR intervals recorded at an interval of time; p=0.001; triangular interpolation histogram of RR intervals; p=0.048; and SD2; p=0.014) in the coronavirus disease group compared to the non-COVID group. Successively, the parameters that demonstrate parasympathetic modulation are shown to be higher in the group of patients with coronavirus disease 2019 (root mean square of the square of differences between adjacent normal RR intervals in an interval of time; p<0.001; pNN50; p<0.001; SD1; p=0.002; and high frequency; p=0.022).

CONCLUSIONS: There was a greater autonomic modulation of heart rate with a greater parasympathetic modulation in patients with coronavirus disease 2019 on mechanical ventilation.

KEYWORDS: Autonomic nervous system. Coronavirus. Artificial respiration.

INTRODUCTION

In December 2019, an outbreak of pneumonia caused by a new coronavirus, called coronavirus disease 2019 (COVID-19), occurred in Wuhan, Hubei Province, China. After the initial outbreak, it spread rapidly around the world in the following months, leading to more than millions of cases and hundreds of thousands of deaths. Although most patients seem to have a favorable prognosis, elderly people and those with chronic diseases may have an unfavorable prognosis, with a greater need for interventions such as endotracheal intubation and invasive mechanical ventilation. Recent research has shown that, similarly to SARS-CoV, this virus can invade various tissues by binding to the angiotensin-converting enzyme 2 receptor, expressed mainly in lung alveolar epithelial cells, small intestine enterocytes, vascular endothelial cells, and airway epithelial cells and renal cells[1,2].

Sympathetic hyperactivity and/or decreased parasympathetic activity are related to an increased risk of various cardiac outcomes[3,4]. Heart rate variability (HRV) is a technique used to noninvasively estimate the characteristics of the autonomic nervous system and analyze the modulation of sympathetic and parasympathetic inputs[1,5]. The use of mechanical ventilation can influence heart rate (HR) due to significant changes in alveolar and intrathoracic pressure and subsequent changes in cardiac output and mean arterial pressure (MAP). Ventilation can profoundly alter the functioning of the cardiovascular system through complex and opposing processes that reflect the interaction between myocardial and ventricular reserve, pump function, circulating blood volume, blood flow, distribution, autonomic tone, endocrinological responses, pulmonary volume, intrathoracic pressure, and the surrounding pressures for the rest of the circulation[6].

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Some of the critical patients with COVID-19 require invasive mechanical ventilation, and the effects of positive pressure on the autonomic nervous system have been less explored; hence, it is of paramount importance to obtain a more detailed understanding of the association between invasive mechanical ventilation and autonomic modulation of HR in these patients. Therefore, the aim of this study was to analyze the autonomic modulation of HR in critically ill patients with COVID-19 on invasive mechanical ventilation.

METHODS

The research project was approved by the Research Ethics Committee of the Universidade Municipal de São Caetano (#2.912.528) and was applied only after approval by the Committee and signature of the consent term by the legal guardian of each research subject. This research follows STROBE checklist.

A cross-sectional study was carried out from July 2020 to March 2021, in which 36 patients admitted to the intensive care unit (ICU) in Brazil were evaluated.

The subjects were divided into two groups. The control group (CNG) included patients of both genders, and for the COVID group (CG), all patients of both sexes under invasive mechanical ventilation in controlled assisted mode admitted to the ICU in orotracheal intubation and who had authorization from the guardians to participate in the study were considered eligible. Patients with artificial cardiac pacemakers were excluded.

Data on prognosis and mortality risk were quantified by the Simplified Acute Physiology Score (SAPS III).

Data collection instruments

A data collection form filled at the bedside and from the patient's medical record was used through the online platform. The measurements taken included cardiorespiratory and hemodynamic data, such as peripheral oxygen saturation (SpO_2), HR, systolic blood pressure (SBP) and diastolic blood pressure (DBP), MAP, and ventilatory data. All patients were ventilated in the Newport E500® device. SpO_2 data were collected by viewing the DX 2010 multiparameter monitor from Dixtal®, and SBP, DBP, and MAP data were obtained by a single measurement provided by the same monitor. HR was recorded using a Polar® V800 HR monitor.

The recording strap was placed on the chest of the volunteers, and, on their wrist, the individuals were kept in the supine position in Fowler's position (semi-sitting, at 45°), with ventilatory parameters at the discretion of the physician on duty, and physiotherapist of the sector.

The Kubios HRV® software was used to obtain consecutive RR intervals and analyze HRV. Only sets with more than 95% sinus beats were included in the study; for this, a digital filtering was performed, followed by manual filtering, to eliminate premature ectopic beats and artifacts. The analysis of HRV was performed using linear methods, analyzed in the domains of time and frequency and through geometric índices[7].

Statistical analysis

Excel and SPSS (Statistical Package for Social Research) version 17.0 programs for statistics were used. Each variable was checked for normal distribution using the Shapiro-Wilk test. The comparison of the clinical profile and physiological parameters between positive and negative individuals for SARS-CoV-2 was performed using the independent Student's t-test and expressed as mean and standard deviation. The comparison between variables of HRV between groups was performed using the Mann-Whitney U test and expressed as median and percentiles. Descriptive statistics were used with mean values, and standard deviations of the differences were statistically significant when the probability of type I error was less than 5% ($p<0.05$). The sample was selected from a pilot test using the online software available at www.lee.dante.br, considering the root mean square of the square of differences between adjacent normal RR intervals in an interval of time (RMSSD) index as a variable. The sample size determined was a minimum of 13 individuals per group.

RESULTS

Thirty-six patients with a mean age of 70 years were analyzed. There were no statistical differences in the measurements of cardiorespiratory, hemodynamic data, and SAPS III severity score (Table 1).

Table 1. Comparison of the clinical profile and physiological parameters between positive and negative individuals for SARS-CoV-2 (COVID-19).

	COVID group	Non-COVID group	p-value*
	Mean±SD	Mean±SD	
Age, years	70±5	70±6	0.38
Predicted weight, kg	63±9	59±13	0.06
SBP, mmHg	121±17	127±25	0.25
DBP, mmHg	66±9	64±12	0.16
MAP, mmHg	84±9	80±13	0.26
SpO_2, %	96±1	96±2	0.07
SAPS III	66±14	70±18	0.51
Sex, male	61.1% (11)	72.2% (13)	0.48
Race, white	66.6% (12)	55.5% (10)	0.09
Use of vasoactive drugs	50.0% (9)	61.1% (11)	0.31

*Student's t-test. Data presented as mean±standard deviation (SD) and frequency {n (%)}. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate; SpO_2: oxygen pulse saturation; SAPS III: Simplified Acute Physiology Score III.

The standard deviation of all normal RR intervals recorded at an interval of time (SDNN) (p=0.001), pNN50 (p<0.001), RMSSD (p<0.001; Figure 1), and triangular interpolation histogram of RR intervals (TINN) (p=0.048) indices were higher in the CG compared to the non-COVID group (NCG).

In the frequency-domain indices, lower values were recorded in the CG in the following parameters: LFms^2 (p=0.002), LFnu (p=0.001), and the LF/HF ratio (p<0.001) and higher in the HFms^2 indices (p=0.022; Figure 2) and HFnu (p<0.001). The SD1 (p=0.002), SD2 (p=0.014), and SD1/SD2 (p<0.001) indices were higher in the CG.

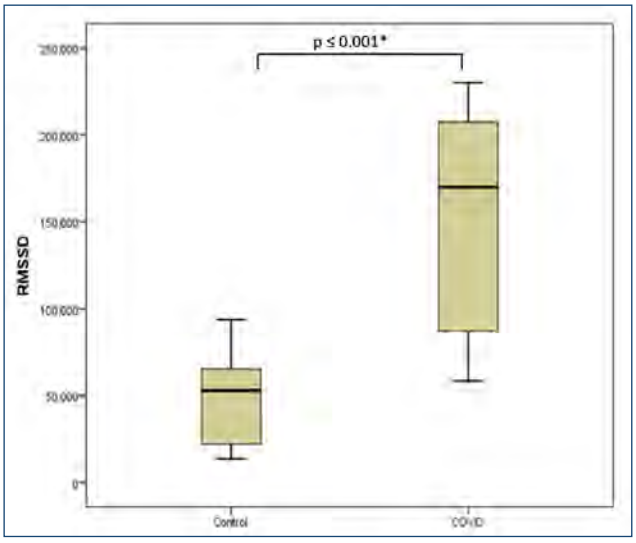

Figure 1. Comparison of RMSSD analysis indices in the time domains between individuals with SARS-CoV-2 and the control group. *Mann-Whitney test; RMSSD: square root of the mean square of the differences between successive normal RR intervals.

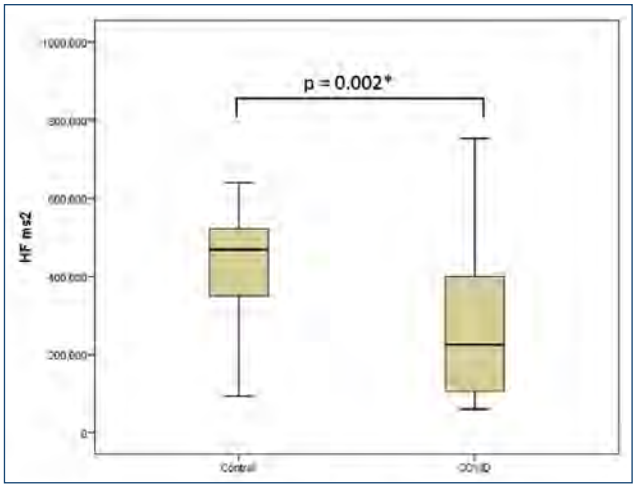

Figure 2. SARS-CoV-2. Comparison of HF between individuals with SARS-CoV-2 and the control group. *Mann-Whitney test; HF: high frequency; ms: milliseconds.

DISCUSSION

The study indicates the differences in HRV parameters in the COVID-19 group, which supports the hypothesis that infection influences HRV in patients undergoing invasive mechanical ventilation. The SDNN (p=0.001), TINN (p=0.048), and SD2 (p=0.014) were higher in the CG compared with the NCG. These parameters show a higher overall HRV in patients with COVID-19. Successively, the parameters that demonstrate parasympathetic modulation are shown to be higher in the group of patients with COVID-19 (RMSSD; p<0.001, pNN50; p<0.001, SD1; p=0.002; and HF; p=0.022).

Patients with COVID-19 are variably susceptible to the "pro-inflammatory cytokine storm" that simultaneously increases sympathetic noradrenergic system flows (SNS) and sympathetic adrenergic system[8]. Hyperactivation of the SNS, an important component of autonomic dysregulation in the setting of infections and hyperinflammatory release, together with attenuation of vagal activity caused by the vagus nerve injury produced by the virus, can lead to a serious autonomic imbalance in COVID-19 infection[9]. After an inflammatory response, afferent signals travel through the vagus nerve primary nerve of the parasympathetic nervous system to the nucleus of the solitary tract. A subsequent efferent signal via the vagus inhibits the synthesis of proinflammatory cytokines through the neurotransmitter acetylcholine. In short, a basic set of physiological structures involving the vagus nerve is responsible for a rapid reflex action in response to inflammation, known as the cholinergic anti-inflammatory pathway[8,9].

Few reports of autonomic dysfunction among patients with COVID-19 have been described. However, the relationship between HRV and inflammatory states has been widely studied. A meta-analysis demonstrated an inverse relationship between HRV and inflammation; this study examined the association of HRV with inflammation markers such as interleukins, C-reactive protein, interferon-gamma, and factor alpha tumor necrosis. This study showed a significant positive association with HR, and negative associations with SDNN, pNN50, RMSSD, and HF[9,10].

Haensel et al.[11] showed that SDNN was negatively correlated with markers of inflammation compared with HF and RMSSD. In summary, the results were particularly robust for SDNN and inflammatory markers compared with primarily SNP influence[12]. In contrast to the findings in our study, there was a significant increase in the values of SDNN, pNN50, and RMSSD in patients with COVID-19. In our study, we also evidenced an increase in sympathetic activity and a decrease in vagal tone, and a decrease in the LF/HF ratio, considered a marker of sympathovagal balance. This

is in spite of a previous study demonstrating that the RRtri geometric index was significantly lower in older women, with lower global HRV indices[13]. Age and gender can significantly contribute to the association between HRV and inflammatory states.

Monitoring of vagal tone in patients with COVID-19 may be a predictive marker of the course of the disease, with the idea that people with very low vagal tone at the onset of symptoms may be at high risk of developing an overstimulated dysregulated pro-inflammatory response during infection, leading to sudden death or transfer from the ICU. In the analysis of HRV, the RMSSD and HF indices are considered primary indices of HRV, mediated by the vagus nerve[14]. In our study, these variables had a positive correlation in patients with COVID-19 considered severe.

Several comorbidities are commonly associated with patients in the ICU, including systemic arterial hypertension, heart failure, type II diabetes mellitus and chronic kidney disease, and sympathetic hyperactivity, which in many cases contributes to disease progression[14,15]. These findings suggest that HRV analysis may have diagnostic value in intensive care. HRV is not only affected by pathophysiological conditions but also by treatments and events commonly performed in the ICU, such as sedation, mechanical ventilation, and stressful stimulation[16-20].

The analysis of the HRV is an important tool for evaluating the functioning of the organism under normal and pathological conditions, providing the development of actions aimed at the prevention, treatment, and/or detection of pathological conditions.

The data on the effect of positive pressure on the autonomic nervous system analyzed in our study are relevant to gain an understanding of the association between mechanical ventilation and autonomic modulation of HR in patients with COVID-19[17].

LIMITATIONS

This study has potential limitations. It is noteworthy that HRV analysis is a noninvasive tool and easy to apply at the bedside, which are its main advantages; however, the interpretation of results must be done carefully in the context of its limitations. The RR time series were measured under uncontrolled conditions; hence, the results may have been affected to some extent by unknown external factors. In addition, we did not measure recordings in the study group before SARS-CoV-2 infection; hence, a direct comparison of the results of HRV analysis before and after the advent of COVID-19 was not possible and their comparison was made with a control group without COVID-19 infection.

CONCLUSION

Our study demonstrated a greater autonomic modulation of HR with a greater parasympathetic modulation in patients with COVID-19 on mechanical ventilation.

AUTHORS' CONTRIBUTIONS

PJ: Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **JZ-R:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **JAM:** Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **ICES:** Data curation, Formal Analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **RDR:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Health in prison: coronavirus disease 2019's challenges in the Brazilian criminal justice system

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INTRODUCTION

On December 31, 2019, China reported to the World Health Organization (WHO) an outbreak of pneumonia in Wuhan City, the capital of Hubei Province. The disease, coronavirus disease 2019 (COVID-19), quickly spread around the world, resulting in a pandemic, declared by WHO on March 11, 2020[1,2].

Faced with the worsening of the health situation, governments have adopted measures aimed at limiting the spread of the disease in order to avoid the collapse of health systems. The main measures include the reinforcement of hygiene actions and of the health system and social distancing/isolation. These provisions consider the transmission dynamics of SARS-CoV-2, which is occurred by aerosols, to reduce the intensity with which the disease spreads in the population[3,4].

Therefore, it is necessary to worry about possible places where the disease can become an even more serious problem, with incidence and lethality rates higher than the national average. This is the case of the persons deprived of liberty (PDL), and within this group, the prison population deserves to be highlighted.

PDL is any form of detention, imprisonment, institutionalization, or custody of a person, for reasons of humanitarian assistance, treatment, guardianship, or protection, or for offenses and infractions of the law, ordered by a judicial or administrative authority or any other authority, or under their de facto control, in a public or private institution in which they do not have freedom of movement[5].

Brazil has the third largest prison population in the world, leaving behind only the USA and China, which occupy the first and second positions, respectively. There are about 750,000 prisoners in prison units with over 150% overcrowding and living in conditions that violate the most basic human rights[5].

It should be noted that the entry of the new coronavirus into the criminal justice system will bring damages that will extend to the entire Brazilian society. That said, we reflect on the following five aspects: *i. characterization of the prison population in Brazil, ii. risk groups for COVID-19 in the prison population and the supply of health services, iii. confinement, the rapid spread of the virus, and unsanitary conditions as a determinant of risk, iv. the way the virus can enter the prison system, and v. difficulties in adopting preventive measures.*

Characterization of the prison population in Brazil

The Brazilian criminal justice system presents difficulties related to the quality of its operation, the treatment offered to prisoners, and its resocializing effectiveness. A reflection of this is the disorderly growth of the prison population, which, when associated with the current management problems, aggravates the situation even more[6].

In Brazil, unlike other countries, such as China and the USA, the prison population has been increasing systematically in recent years. Between 2001 and 2019, this population increased more than threefold, reaching about 755,000 individuals, of which about 748,000 are located in prison units and 7,300 are in police station jails[6,7].

The Brazilian prison system is composed of 1,435 confinement institutions, distributed throughout the 27 Federative Units. They are concentrated mainly in the Southeast region (n=493; 34.4%), followed by the Northeast region (n=314; 21.9%)[6]. Data from 2019 show that the Brazilian prison population is predominantly composed of males (95.1%), young people aged between 18 and 24 years (23.1%), browns (43.4%), and people with incomplete elementary education (43.4%). With regard to the sentence regime issued, serving

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the sentence in a closed regime accounted for almost half of the cases (48.46%)[7].

In 2019, these prison institutions held the capacity to accommodate 442,349 individuals; however, by means of the total number of incarcerated persons in the same period, there was also a surplus population of 312,925[7]. This leads to overcrowding, in which the inmate/vacant ratio is about 1.7, leading to unhealthy situations, marked mainly by poor hygiene and environmental conditions. These factors are a strong potential for the transmission of respiratory diseases, such as tuberculosis, which represents an obstacle for an already deficient system.

The excess population in confinement generates a high maintenance cost for public authorities. On average, 2,700 *Reais* are spent per month per prisoner, which becomes 2 billion *Reais* when considering the total contingent of PPL in Brazil in 2019[8]. This situation is inconsistent with the Brazilian economic state and may be worsened if there is an increased demand for investment with the likely arrival of COVID-19 in prisons.

Risk groups for coronavirus disease 2019 in the prison population and the supply of health services

The health of people in prison is a right established in international and national laws that define the responsibility of the state in preserving their health. For the analysis of the pandemic of COVID-19 in the prison system, it should be evidenced that the profile of this population is far from being a reflection of the population outside the prison walls. These epidemiological differences are of fundamental importance for the identification of the risk profile in the prison system. For COVID-19, the literature13 points out the main risk groups: *i. the elderly population (60 years or older); ii. people with chronic diseases* (heart disease, lung disease, diabetes, hypertension, cancer, among others)*; iii. pregnant women; and iv. individuals with infectious diseases and compromised immune system*.

Elderly prison population

In 2019, 1.3% of inmates over the age of 60 years are part of the incarcerated contingent in Brazil[7]. These people deserve special attention for their tendency to develop more aggressive forms of COVID-19, requiring medical care and often interventions at the hospital level.

People with chronic diseases

Chronic diseases are not contemplated in prison information surveys, but it is known that these are present in prison. Chronic diseases, such as diabetes mellitus (DM) and systemic arterial hypertension (SAH), were found in 3.2 and 11.6% of the inmates, respectively, and from the total of these prisoners, 26.1% used controlled medication[9].

However, in people with cardiovascular diseases (i.e., hypertension, stroke, heart failure, and dyslipidemias), it can reach rates of up to 10.5%. In the sequence, diabetics (7.3%), people with chronic respiratory disease (6.3%), hypertensive people (6%), and cancer patients (5.6%) have the highest mortality rates[10].

In the case of patients with heart disease, impaired circulation and impaired pulmonary function seem to favor the aggressiveness of the infection. *DM*, mainly type 2 (DM2), is a risk factor for the aggravation of several infections because it impairs the body's defenses against viruses and bacteria[11].

Pregnant women in the prison system

In the period from January 25, 2018 to March 5, 2018, the National Council of Justice inspected 24 women's correctional facilities, finding that at the time, there were 179 pregnant and 167 lactating women. The vast majority serve their sentences in precarious and unsanitary situations[12].

Individuals with infectious diseases and compromised immune system

In 2019, according to the National Prison Information Survey (InfoPen), among the Brazilian prison contingent (n=755,274), 8,523 individuals living with HIV (1.13%), 3,030 (0.40%) cases of hepatitis, and 9,113 (1.21%) cases of tuberculosis were recorded[7]. However, the literature points out that these numbers can be even higher, with up to 28.5% for tuberculosis, up to 33.8% for STIs, and 35.2% for HIV. In addition, inmates are classified as vulnerable to infectious diseases: syphilis, tuberculosis, scabies, mycosis, HIV, gonorrhea, genital herpes, HPV, meningitis, and hepatitis as highly prevalent in the incarcerated population[9].

These diseases weaken the host, are potential aggravating factors in new coronavirus infections, and reveal a compromised immune system in these individuals. Additionally, as these diseases, especially HIV, are highly prevalent, the number of severe forms of COVID-19 may increase significantly, overburdening the health care system[13].

As for drug use, about 58% of the inmates use licit drugs and 38% use illicit ones[9]. Smoking is highly noted among inmates and contributes to an increase in the chance of opportunistic infections, worsening of preexisting infections, and impairment of the respiratory system[14].

Only 69% of the country's prison units offer medical assistance, and only 37% of these units have health modules to attend to the prison population[5,15]. In some regions, such as the Northeast, which is second in the number of inmates, the

offer of health care is even lower, with only 57.3% of the units providing some type of service[7].

Confinement, rapid spread of the virus, and unsanitary conditions as a risk determinant

Besides the biological determinants, environmental risk factors, such as inadequate prison architecture, poor sanitation, overcrowding, and poor ventilation conditions, are present in daily prison life[7,15]. The characterization of environments as unhealthy is given by precarious environmental and personal hygiene, which remains the target of preventive intervention. Such measures, along with social isolation, are the main ways to avoid the transmission of the virus[16].

Part of the overcrowding stems from the delay in judging cases. At least 40% of the prison population is composed of people waiting to know whether they will be convicted or not. This makes the environment favorable for the spread of infectious and contagious diseases since the vacancy deficit is more than 350,000[7,8]. Thus, overcrowding forces the sharing of cells between healthy and sick individuals.

This situation has repercussions for the professionals who perform services inside prisons, who are in direct contact with the prison population; therefore, a failure in the health system[17] indicates an increased risk of infection for these workers, who may become potential facilitators of transmission, in a two-way street: from inside to outside and from outside to inside the prison system.

How the virus can enter the prison system?

The mechanism of virus transmission from person to person, by contact and aerosols, favors the massive transmission in closed environments that promote the union of droplets containing the virus in the ambient air, promoting the formation of aerosols[18,19]. This aspect becomes even more relevant when added to a majority of asymptomatic carriers[20] and the average incubation time that ranges from 2 to 11 days[20].

The sum of these factors sows a favorable environment and strengthens the silent entry of the infection in the prison population through its servers who carry out the transfer from home to work. In this context, only one contaminated employee can act as a vector of mass transmission, since it is impossible to maintain the minimum distance of 1.5 m from person to person[20], unworkable in an overcrowded system.

Besides the fixed prison and penitentiary employees, it is necessary to reiterate that around 1,033 units make use of outsourced services, referring to health, food, cleaning, laundry, security, administrative services, and educational, labor, social, and legal assistance[7]. This aspect leads to an environment of even greater contacts, whether due to employee turnover in these companies or their circulation in the environment outside the workplace.

Visits to detainees also present themselves as a possible mechanism, facilitating the entry of the virus into the prison environment. This is because, first, visits generate population clusters, and second, viruses of the *coronaviridae* family can remain alive on surfaces for long periods of time (metal for up to 5 days and glass surfaces for up to 4 days)[21]. The third mechanism concerns the release of inmates for medical care outside the units.

Controlling the movement of people is one of the most effective strategies in controlling the pandemic[21,22]. This way, the temporary exits of prisoners, foreseen in the Brazilian legal system, act as another facilitator of the virus dissemination by the transit of people in prison and hospital environments.

Difficulties in adopting preventive measures

The Penitentiary System, as part of the Brazilian population, must adapt to the changes in order to also fight against COVID-19. These measures basically involve changes in lifestyle habits that reduce the probability of infection and delay the peak of the epidemic, thus reducing the burden caused by the disease[23]. They are based on the reduction or prevention of contact through distancing, segregation, or social isolation and sanitization of hands and shared objects[24].

However, it should be noted that Brazilian inmates are facing a specific situation imposed by the lack of operational conditions in the prison system[7,8]. This situation presents several factors that are contrary to international recommendations, turning prisons into high-risk sites for the dissemination of COVID-19.

Social distancing is one of the measures that aim to reduce the speed of virus transmission[20,23]. The basic premise of this measure is to move away, therefore, to have space for it. When it comes to the Brazilian prison system, the impossibility of implementing this measure must be emphasized. The overcrowding resulting from these environments makes it impossible to apply social distancing as a prevention measure.

Added to the exchange of shifts and work schedules, these professionals, evidently, when they return to their families and their leisure activities, may come into contact and potentially expose themselves to the virus. Thus, they serve as a vector for transmission inside prisons. It is estimated that the system has more than 40,000 professionals that enter and leave the penitentiary units every day[25].

Due to the lack of space to hold all the inmates, the prison system is insufficient to take the basic measures in the situation of suspected and/or confirmed cases of COVID-19, when it is necessary to keep the individual in isolation for 14 days[20,23], a

difficult measure in an overcrowded system without the minimum necessary structure.

In overcrowded prisons, a single toilet can be shared with up to 70 inmates, and since it does not have a working flush, it receives water only once a day. Besides this, the supply of water for sanitation is deficient. A similar situation occurs for other items of support to personal hygiene.

FINAL NOTES

The pandemic of COVID-19 poses challenges to all governments and health systems to ensure the health protection of the population. In the Brazilian context, a country marked by health inequities[24], population groups are more vulnerable to the effects of the pandemic.

To ponder about the prison population is not limited to thinking about health care strategies for this group, but it involves all workers and other professionals who work directly or indirectly in the prison system. It is necessary for an articulated action of the health sector with the other areas of the social protection system, in order to plan and execute actions that favor the adhesion of protective measures and minimize the deleterious impacts of the pandemic. It is essential that these decisions are based on the best scientific evidence available.

AUTHORS' CONTRIBUTIONS

CDFS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **JPSP:** Conceptualization, Methodology, Project administration, Resources Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LFS:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources Software, Supervision, Validation, Visualization, Writing – original draft. **TCL:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources Software, Visualization, Writing – original draft, Writing – review & editing. **GBAS:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources Software, Supervision, Validation, Visualization, Writing – original draft. **LGS:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **MFM:** Conceptualization, Investigation, Methodology, Project administration, Resources Software, Supervision, Validation, Visualization, Writing – review & editing.

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REVIEW ARTICLE

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Clinical effect of different dialyzers used in patients with kidney disease: a meta-analysis of randomized clinical trials

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INTRODUCTION

As an effective treatment for end-stage renal disease, hemodialysis has been used in clinic for more than 80 years and it is an effective measure for treating acute and chronic renal failure[1]. The incidence and prevalence of chronic kidney disease and kidney failure are increasing worldwide. In the United States, the number of people receiving dialysis has risen by about 20,000 cases per year[2]. Dialyzer is a necessary instrument for each hemodialysis. Blood (inside the membrane) convects with dialysis fluid (outside the membrane) in the dialyzer to remove toxic substances such as creatinine and urea from the patient's body through a concentration gradient or pressure gradient. At present, there are various kinds of dialyzer membrane materials commonly used in clinical practice, including polyether sulfone (PES), polysulfone, cellulose acetate, polymethyl methacrylate, and polyacrylonitrile membranes[3]. It is generally believed that the dialysis membranes affect the quality of dialysis in patients undergoing maintenance hemodialysis, but recent evidence-based studies have failed to provide strong evidence[4,5]. This meta-analysis was undertaken to evaluate the efficacy and safety between PES dialyzers and dialyzers with a different membrane material by collecting clinical data from randomized clinical trials (RCTs).

METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[6] statement for conducting a high-quality meta-analysis.

Data sources and searches

The Cochrane Library, EMBASE, PubMed, and ClinicalTrials.gov databases were searched for RCTs. The search period was set from January 1990 to February 2021. The following keywords were used in search strategies and a sensitive filter for RCTs was also used: "hemodialysis," "hollow fiber dialyzer," "polyether sulfone," "kidney disease," and "dialysis." In addition, the references listed in the selected trials were also reviewed for additional trials and information.

Study selection

Studies from the literature independently searched were screened by two investigators (CCM and YML); a third investigator will be consulted when encountering disagreements. We included studies that met the following inclusion criteria: (1) RCTs conducted in humans; (2) patients with kidney disease underwent randomly one study week of three consecutive hemodialysis treatments; (3) full-text articles of controlled trials examining PES hollow fiber dialyzer versus other member dialyzers, including polysulfone dialyzer, cellulose acetate dialyzer, and polymethyl methacrylate dialyzer; and (4) the change of blood urea nitrogen, creatinine, β2-microglobulin, hemoglobin, albumin, phosphoric acid, or myoglobin was examined. If there were duplicate studies or reports of similar results from the same trial, the literature with the most comprehensive data will be included. Reviews, meta-analyses, editorials, observational studies, and studies without results or a control group will be excluded.

Data extraction and quality assessment

A standardized data extraction form was used to extract clinical data independently by two different authors, and a third investigator was consulted to resolve conflicting opinions. The following information was extracted from the included studies: authors' names, year of publication, baseline characteristics of the participants, total number of individuals per arm, mean age, primary disease or condition, and the device used per arm. The change in value before and after hemodialysis of the following endpoints was extracted: urea nitrogen, creatinine, β2-microglobulin, hemoglobin, albumin, phosphoric acid, and myoglobin. In addition, information regarding blinding, random sequence generation, allocation concealment, indications

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for incomplete outcome data, indications for selective reporting, and other biases were also collected to evaluate the quality of the included investigations.

Statistical analysis

Data analysis was based on the intention-to-treat principle. Dichotomous outcomes were reported by risk ratio and 95% confidence interval (CI). Differences in continuous outcomes were reported by standard mean differences (SMDs) including the 95%CI. Heterogeneity was assessed through the Cochran's Q test and I^2 statistic; a Cochran's $p<0.10$ and an $I^2>50$ were considered significant heterogeneity. Pooled analyses were conducted using a fixed effect model, whereas a random effect model was used if there was significant heterogeneity.

RESULTS

Search results

A total of 527 potentially relevant publications were identified according to the search strategy. Among which, 72 publications were reviewed through full-text reading and 6 studies that met the selection criteria were finally included, as shown in Supplementary Figure S1[7-12]. The baseline characteristics of the included studies were shown in Supplementary Table S1. We included 232 participants in our meta-analysis, including 116 treated with PES dialyzer and 116 with other dialyzers. The quality assessment of the included studies was detailed in Supplementary Table S2 and Supplementary Figures S2 and S3.

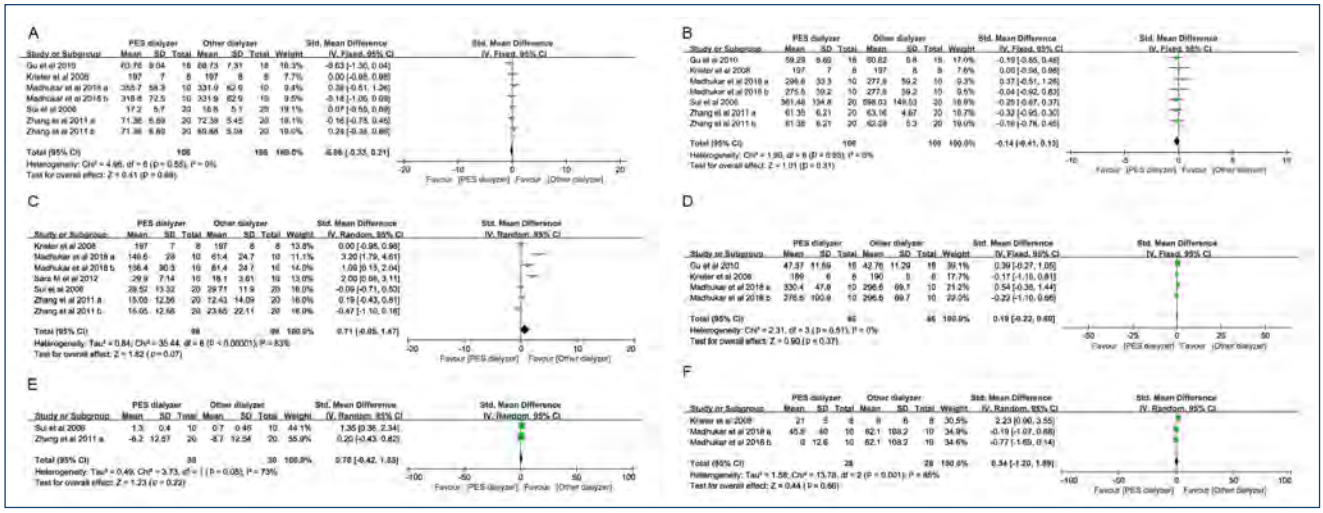

Figure 1. (A) Forest plot of urea nitrogen clearance. (B) Forest plot of creatinine clearance. (C) Forest plot of β2-microglobulin clearance. (D) Forest plot of phosphoric acid clearance. (E) Forest plot of hemoglobin removal rate. (F) Forest plot of myoglobin removal rate.

Table 1. Different dialyzers with various manufacturers based on the included membrane materials.

Member material	Manufacturer	Dialyzer	Country
Polyether sulfone	Nipro	PES series, ELISIO series	Japan
	Peony	PES series	China
	OCI	HD series	China
Polysulfone	Fresenius	HF series, F series, FX series, Hemoflow series, Optiflux series, Revaclear, and Revaclear Max	USA/Japan
	Toray industries	TS series	Japan
Cellulose acetate	NISSHO Corporation	FB series	Japan
	KAWASUMI	CTA series	Japan
	Nipro	SUREFLUX series, FB series	Japan
Polymethyl methacrylate	Toray industries	B series	Japan

Clinical results

The efficacy endpoints included the clearance of urea nitrogen, creatinine, β2-microglobulin, and phosphoric acid, while the safety endpoint was the change of hemoglobin and myoglobin.

Clearance of urea nitrogen

Five RCTs involving 212 patients reported the clearance of urea nitrogen, with 106 patients randomized to PES dialyzer group and 106 randomized to other dialyzer groups. No differences existed in the clearance of urea nitrogen (SMD -0.06; 95%CI -0.33 to 0.21; p=0.68; I^2=0%; Figure 1A).

Clearance of creatinine

Five RCTs involving 212 patients reported the clearance of creatinine, with 106 in each of the PES dialyzer and other dialyzer groups. No differences existed in the clearance of creatinine (SMD, -0.14; 95%CI -0.41 to 0.13; p=0.31; I^2=0%; Figure 1B).

Clearance of β2-microglobulin

Five RCTs involving 196 patients reported the clearance of β2-microglobulin, with 98 patients in PES dialyzer group and 98 in other dialyzer groups. No differences existed in the clearance of β2-microglobulin (SMD 0.71; 95%CI -0.05 to 1.47; p=0.07; I^2=83%; Figure 1C).

Clearance of phosphoric acid

Three RCTs involving 98 patients reported the clearance of phosphoric acid, with 46 patients in PES dialyzer group and other dialyzer groups. No differences existed in the clearance of phosphoric acid (SMD 0.19; 95%CI -0.22 to 0.60; p=0.37; I^2=0%; Figure 1D).

Change of hemoglobin

Two RCTs involving 60 patients (30 in PES dialyzer group and 30 in other dialyzer groups) reported the change of hemoglobin. No differences existed in the change of hemoglobin (SMD 0.34; 95%CI -1.20 to 1.89; p=0.66; I^2=85%; Figure 1E).

Change of myoglobin

Two RCTs involving 56 patients reported the change of myoglobin, with 28 patients in each group. No differences existed in the change of myoglobin (SMD 0.34; 95%CI -1.20 to 1.89; p=0.66; I^2=85%; Figure 1F).

Sensitivity and publication bias analysis

The meta-analysis results of the clearance of urea nitrogen were as follows: SMD -0.06; 95%CI -0.33 to 0.21; p=0.68; I^2=0%. For sensitivity analysis, the results were consistent after excluding each individual study, which demonstrated that the heterogeneity among the studies did not affect the combined results as shown in Supplementary Figure S4. The results of Egger's test showed no significant evidence of publication bias, as shown in Supplementary Figure S5.

DISCUSSION

This meta-analysis included 232 patients with kidney disease, who were randomized to PES dialyzer or other dialyzer groups during maintenance hemodialysis in six RCTs. Based on this meta-analysis, the small solute clearance (urea, creatinine, and phosphate) in PES dialyzer was comparable and not significantly different from other dialyzers. In addition, the clearances and removal rates of low-molecular-weight proteins (β2-microglobulin and myoglobin) were not significantly different.

The basic principle of dialysis treatment is that blood and dialysate exchange solutes through the dialysis membrane. Electrolytes and excess water in the blood enter the dialysate to be removed, and some bicarbonate and electrolytes in the dialysate enter the blood to remove toxins and water, maintaining acid-base balance and internal environmental stability. At present, several kinds of dialyzer membrane materials are commonly used in clinical practice, including PES, polysulfone, cellulose acetate, polymethyl methacrylate, and polyacrylonitrile membranes. Dialyzers were developed by various manufacturers based on the above membrane materials, as shown in Table 1. Studies have shown that PES has good heat resistance, corrosion resistance, and hydrophilicity. Furthermore, clinical trials have reported the efficacy and safety of using PES dialyzer in clinical practice[13-15]. However, there was no comprehensive analysis about PES dialyzer, compared with other dialyzers. As the first meta-analysis included RCTs to compare PES dialyzer with other dialyzers, quality assessment, sensitivity analysis, and publication bias analysis were addressed to obtain high-quality evidence. No clinically meaningful difference was found among the PES dialyzer and other dialyzers when small solute clearance and low-molecular-weight protein parameters were studied. These results had less difference from the previous published literature in that the clearance of β2-microglobulin was higher in PES dialyzer group than in other dialyzer groups[9,10]. Based on the sensitivity analysis, the results of this meta-analysis were stable, which was consistent in each heterogeneity analysis. No significant publication bias was revealed in the meta-analysis.

This meta-analysis included all the available RCTs that met the inclusion criteria. In addition, the quality of included clinical trials was all middle-to-excellent, and the results of our meta-analysis were reliable based on the sensitivity and publication bias analyses.

However, the study also had several limitations. First, the included clinical trials had a relatively small sample size, which caused to a restricted power with the results. Second, different PES dialyzers used in each clinical trial, including PES series (Nipro), ELISIO series (Nipro), and HD series (OCI), may cause the heterogeneity of the results. However, no strong heterogeneity was found among the trials. Third, the different calculation method for clearance used in different clinical trials and various substances used, especially the drugs, may have an influence on the results. Furthermore, polyacrylonitrile-derived filter was not included in this meta-analysis. Further RCTs with large sample sizes are needed to explore the efficacy and safety profile of PES dialyzer in clinical practice. In addition, detailed subgroup analysis can be conducted when enough clinical trials are published in the future.

CONCLUSION

No differences were demonstrated between PES dialyzer and other dialyzer groups with respect to the clearance of urea, creatinine, phosphate, and β2-microglobulin. In addition, the removal rates of microglobulin and myoglobin were not significantly different between PES dialyzer and other dialyzer groups.

AUTHORS' CONTRIBUTIONS

CM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **YL:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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REVIEW ARTICLE

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Craniofacial findings in syndromes associated with cafe-au-lait spots: a literature review

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INTRODUCTION

Cafe-au-lait spots (CALS), also called cafe-au-lait macules, are uniformly pigmented light to dark brown spots on the skin that may be present at birth or develop in childhood[1]. They usually appear as light brown in light-skinned people and medium to dark brown in dark-skinned people. The size of the spots can vary from 1–2 mm up to >20 cm^2. Morphologically, CALS are more frequently oval-shaped and have smooth edges, although other formats are described[3].

Histologically, an increase in melanin content has been demonstrated in both melanocytes and basal keratinocytes, and in some pathological conditions, an increase in the number of melanocytes, although proliferation of melanocytes is not seen[2]. CALS can occur anywhere in the body with the exception of the scalp, palms, and plantae, but they appear more frequently on the trunk and extremities, and less commonly on the face[4].

Several steps are involved in determining the color of the skin. Melanocytes arise from the neural crest. During embryonic development, melanoblasts migrate toward the dermis, and then through it to reach the overlying epidermis, where they undergo extensive proliferation and begin the production of melanin. In the next step, melanosomes are transferred from melanocytes to keratinocytes[5]. Furthermore, the configuration of the "ordered three-dimensional cellular arrangement" of the skin, called "epidermal melanin unit," also influences the determination of pigmentation[6].

Many genes encode protein components or regulators of signaling pathways involved in the development, migration, and function of melanocytes and, therefore, in the control of physiological and pathological pigmentation of the skin. A large group of syndromes associated with CALS result from germline mutations in these associated genes[7].

In addition to melanocytes, the neural crest gives rise to several other cell types. As a transient structure present during embryonic development, the neural crest is composed of highly multipotent progenitor cells, characterized by populations of already determined precursors and heterogeneous and multipotent cells[6,8], capable of giving rise to different phenotypes, depending on various growth factors and the microenvironment at the migration sites. Thus, the cephalic neural crest gives rise to most of the craniofacial skeleton (chondrocytes, osteocytes and odontoblasts) and other facial tissues such as nerve ganglia, muscles, connective tissue and pigment cells, while the trunk neural crest cells give rise to neurons and glial cells of the peripheral nervous system, in addition to secretory cells of the endocrine system and skin pigment cells[6,9]. This explains the wide phenotypic variation observed in syndromes associated with CALS.

It is important to highlight that isolated CALS may occur as a common finding (10–36% of healthy people) with no clinical significance when dissociated from other findings[4]. However, the presence of multiple CALS, large segmental CALS, other skin anomalies, facial dysmorphism, and other unusual findings on physical examination may suggest the presence of an associated genetic disorder and should be investigated[2].

The study aimed to provide a comprehensive understanding of the syndromes associated with CALS that exhibit craniofacial abnormalities as part of the clinical phenotype.

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METHODS

A review of the literature was conducted from January to July 2021. The identification of genetic diseases associated with CALS was carried out in the *Online Mendelian Inheritance in Man* (OMIM)[10]. The descriptors used for the search were as follows: "genetic diseases" or "hereditary diseases" and "cafe-au-lait spots" or "hyperpigmentation."

Once the related syndromes were identified, the presence (or not) of associated craniofacial abnormalities was determined from a survey carried out at OMIM, using the specific name of each syndrome and observing the signs/symptoms in the clinical synopsis. Subsequently, the evaluation of the clinical signs associated with each syndrome was extended to other databases, such as PubMed (www.pubmed.com) and Virtual Health Library (www.bvsalud.org).

Literature review, case report, and case series were included in the research. Since these are rare diseases, the date filter was not used.

RESULTS

A total of 60 syndromes associated with the presence of CALS are described[11]. Among them, craniofacial abnormalities can be part of the clinical phenotype in 45 syndromes.

The affected gene and the typical, general, craniofacial, and orodental alterations observed in each syndrome are described in Table 1. The identified syndromes were classified into groups according to the altered signaling pathway and/or the function of the mutated gene.

Among the 45 syndromes identified, 39 different genes were recognized, considering that different syndromes can be linked to the same gene and that some entities have not been related to any gene until nowadays.

DISCUSSION

Neurofibromatosis type 1 (NF1) is the disease with the highest incidence among all syndromes associated with CALS and one in which this association is well recognized and considered a diagnostic hallmark[2]. However, several other genetic syndromes are associated with café au lait spots, with a total of 60 syndromes described in the scientific literature[11].

Most of these diseases that present multiple CALS are part of the developmental diseases known as RASopathies. This group includes genetic syndromes caused by germline mutations in genes encoding components of the Ras/MAPK (mitogen-activated protein kinases) pathway. This regulatory pathway is an essential intracellular signaling cascade that controls many cell functions such as differentiation, survival, and proliferation – functions that are critical for normal development[7].

With regard to syndromes associated with CALS and craniofacial abnormalities, most of them are also included in the group of RASopathies. In this condition, we have NF1[7], Legius syndrome[7,12], Leopard syndrome 1[12], Leopard syndrome 2[12], Leopard syndrome 3, Costello syndrome[7,12], cardio-facio-cutaneous syndrome[7,12], Noonan syndrome, Noonan syndrome-like disorder with loose anagen hair 2[13], and Noonan syndrome 13.

Considering that dysregulation of the underlying Ras/MAPK pathway is common to all RASopathies, the diseases included in this classification exhibit numerous overlapping phenotypic characteristics, such as craniofacial dysmorphism, cardiovascular anomalies, abnormalities in tissues of ectodermal origin, neurocognitive impairment, and increased risk of cancer[12].

However, it is important to consider that each of the RASopathies exhibits a unique phenotype, as it is caused by mutations at different points in the metabolic pathway[12]. In this sense, and considering the importance of the Ras/MAPK pathway in craniofacial development, the characterization of craniofacial and orodental changes in each of the RASopathies can provide valuable information for the diagnosis of a specific syndrome[7].

Another group to be considered corresponds to phakomatoses. Defects at any stage of neural crest cell development such as migration, proliferation, cell-to-cell interaction, differentiation, or growth are associated with the pathophysiology of neurocutaneous syndrome or phakomatoses[14]. This group includes pathologies with different genetic mechanisms. The encompassing diseases that share CALS and craniofacial abnormalities are Watson syndrome, Peutz-Jeghers syndrome[15], tuberous sclerosis complex[15], Cowden syndrome[16], McCune-Albright syndrome[17], and Johnson neuroectodermal syndrome[18].

As a common feature in the group, all the diseases represent neurocristopathies and, therefore, include abnormalities in the tissues of ectodermal origin, especially the skin, eyes, and central nervous system[15]. Craniofacial alterations can also occur, mainly related to structures originating from the ectodermal embryonic leaflet[17].

Another important signaling pathway involved in the development and function of melanocytes is the KIT signaling pathway. Waardenburg syndrome type 2E, piebaldism, peripheral demyelinating neuropathy-central dysmyelination-Waardenburg syndrome-Hirschsprung disease, and familial progressive hyperpigmentation with or without hypopigmentation are genetic disorders of aberrant melanoblast differentiation and migration during embryogenesis[5]. The binding of the KIT ligand to its receptor KIT triggers

Table 1. Genetic syndromes associated with CALS and craniofacial abnormalities.

RASopathies	*Gene*	*Typical and general features*	*Craniofacial and orodental manifestation*	*Ref.*
Neurofibromatosis type I	*NF1*	*Typical:* multiple CALS; Lisch nodules; neurofibromas; freckling. Increased risk neoplasms. *General:* mild mental retardation, hydrocephalus; renal artery stenosis; skeletal anomalies.	Macrocephaly, hypoplasia mandibular; hypertelorism; dental irregularities, congenitally missing second molars.	OMIM (162200)[2,7]
Legius syndrome	*SPRED1*	*Typical:* multiple CALS (99%), variable dysmorphic features, lipomas, learning disabilities. Not associated with neurofibromas, optic gliomas, Lisch nodules, or tumor predisposition. *General:* freckling; pectus deformities; learning difficulties, attention deficit-hyperactivity.	Macrocephaly, triangular face, low-set ears; downslanting palpebral fissures, epicanthal folds, hypertelorism; low-posterior hairline; short neck; high arched palate; micrognathia; deeply philtrum.	OMIM (611431)[2,22,23]
Leopard syndrome 1	*PTPN11*	*Typical:* multiple lentigines, hypertelorism, pulmonic stenosis, abnormal genitalia, short stature, electrocardiographic abnormalities, deafness. *General:* CALS (70–80%); mental retardation; thoracic deformities, spina bifida.	Prognathism, triangular face, biparietal bossing; prominent and low-set ears; ptosis, epicanthus folds, strabismus; broad nose; short neck. Cleft palate, deep nasal-labial folds, thick lips, dental anomalies.	OMIM (151100)[7]
Leopard syndrome 2	*RAF1*	*Typical:* short stature, hypertrophic cardiomyopathy, craniofacial anomalies, CALS, lentigines. *General:* delayed puberty; cubitus valgus.	Dolichocephaly; prominent chin; short webbed neck; low-set ears; hypertelorism, downslanting palpebral fissures. Thick lips.	OMIM (611554)
Leopard syndrome 3	*BRAF*	*Typical:* pigmented lesions, short stature, hyperkeratosis, craniofacial anomalies. *General:* lentigines, CALS, multiple nevi spread on the whole body; cognitive deficits, seizures; heart and thoracic defects; delayed bone age.	Low-set ears, sensorineural deafness; short webbed neck; hypertelorism; depressed nasal bridge; curly hair.	OMIM (613707)
Costello syndrome	*HRAS*	*Typical:* coarse facies, short stature, distinctive hand posture, severe feeding difficulty. Predisposition to cancer. Mental retardation. *General:* deep creases, cutis laxa, acanthosis nigricans, papilloma, palmar nevi, isolated CALS (9-31%), multiple CALS (rare); cardiac defect; small lung; renal failure; nail abnormalities.	Macrocephaly, high forehead, bitemporal narrowing; hypertelorism, strabismus, epicanthal folds, ptosis; short nose; full cheeks; low-set ears; short neck. Arched palate, micrognathia, gingival hypertrophy, enamel defect, delayed tooth eruption; thick lips, large mouth, bifid uvula; macroglossia.	OMIM (218040)[7,24,25]
Cardio-facio-cutaneous syndrome syndrome 1	*BRAF*	*Typical:* coarse facies, heart defects, mental retardation. Ectodermal abnormalities, short stature. *General:* one or two CALS (9–31%); multiple CALS (rare), hyperkeratosis, ichthyosis, hemangioma; cortical atrophy, peripheral axonal neuropathy.	Relative macrocephaly, high forehead, bitemporal narrowing, hypertelorism, ptosis, strabismus, epicanthal folds; short nose, low-set ears; webbed neck. Sparse/curly hair. High arched palate, open bite.	OMIM (115150)[7,24,25]
Noonan Syndrome	*PPTN11*	*Typical:* short stature, facial dysmorphism, congenital heart defects. *General:* CALS (frequent), linfedema, skeletal defects, mental retardation, cryptorchidism, bleeding diathesis.	Broad forehead; hypertelorism, downslanting palpebral fissures; low set ears, hearing loss; webbed neck; deeply grooved philtrum, high-arched palate, dental malocclusion.	OMIM (163950)[7,25]
Noonan syndrome 13	*MAPK1*	*Typical:* global developmental delay, behavioral problems, craniofacial anomalies. *General:* lentigines, CALS; cubitus valgus, broad thorax; heart defects. Short stature.	High/broad forehead; long philtrum; low-set ears; ptosis, hypertelorism; wide nasal bridge; short neck; hypertrichosis. Marked upper lip vermilion, everted lower lip; dental anomalies.	OMIM (619087)
Noonan Syndrome-like disorder with loose anagen hair 2	*PPP1CB*	*Typical:* distinctive features of hair and skin, short stature, heart defects. *General:* hypopigmentation, freckling, CALS, loose skin; developmental delay, Chiari I (1 patient), Dandy-Walker malformation (1 patient); delayed bone age; pectus excavatum.	Macrocephaly, prominent forehead, low posterior hairline; large and low-set ears, preauricular pits; hypertelorism, ptosis, epicanthal folds; short and webbed neck. High-arched palate, dental malocclusion.	OMIM (617506)[25]

Continue...

Table 1. Continuation.

Neurocutaneous syndrome or phakomatoses	*Gene*	*Typical and general features*	*Craniofacial and orodental manifestation*	*Ref.*
Watson syndrome	*NF1*	*Typical:* pulmonary valvular stenosis, CALS, decreased intellectual ability, short stature. *General:* multiple CALS, neurofibromas, freckling.	Relative macrocephaly, Lisch nodules.	OMIM (193520)
Peutz-Jeghers syndrome	*STK11*	*Typical:* hyperpigmented spots, multiple gastrointestinal hamartomatous polyps, neoplasms. *General:* multiple CALS (unusual); polyps; digital clubbing; precocious puberty.	Hyperpigmented patches (lips and buccal mucosa). Vermilion zone of the lips. Nasal polyps.	OMIM (175200)[26]
Tuberous sclerosis 1	*TSC1*	*Typical:* hamartomas in multiple organ. *General:* white ash leaf-shaped macules, subcutaneous nodules, CALS (<50%), subungual fibromata; epilepsy, mental handicap, paraventricular calcifications; skeletal disorders.	Angiofibromas, fibrous plaques (forehead/ scalp), enamel pits, confluent gingival nodules (cobblestone appearance). Ophthalmic tumors.	OMIM (191100)[23,27]
Tuberous sclerosis 2	*TSC2*	*Typical:* hamartomas in multiple organs. *General:* Same features as tuberous sclerosis 1, with more severe disease.	Angiofibromas, fibrous plaques (forehead/ scalp), enamel pits, confluent gingival nodules (cobblestone appearance).	OMIM (613254)[23,27]
Cowden syndrome 1	*PTEN*	*Typical:* hamartomatous disorder characterized by macrocephaly, acral keratoses, facial trichilemmomas, papillomatous papules, risk for breast, thyroid and endometrial carcinoma. *General:* pigmentation of the glans penis, CALS (<50%), multiple skin tags; mental retardation; vascular anomalies; pectus excavatum; intestinal polyps, colonic diverticulosis.	Macrocephaly; hearing loss; hypoplastic mandible/ maxilla; cataract. Oral papillomas, scrotal tongue, high arched palate, microstomia, gingival hypertrophy, multiple gingival hyperplastic papules.	OMIM (158350)[23,28]
Johnson neuroectodermal syndrome	Not identified	*Typical:* deafness, anosmia, hypogonadotropic hypogonadism, alopecia. Growth retardation. *General:* hypohidrosis, truncal CALS (rare); mental retardation; short stature; heart defect.	Microcephaly (rare); sparse hair; microtia, conductive deafness; absent eyebrows/eyelashes; choanal stenosis. Facial nerve palsy, cleft palate (rare), retrognathia.	OMIM (147770)[29]
McCune-Albright syndrome	*GNAS*	*Typical:* polyostotic fibrous dysplasia, CALM>50% (large and segmental), precocious puberty. *General:* gastrointestinal polyps; pathologic fracture; hyperthyroidism, hyperparathyroidism, acromegaly, hyperprolactinemia, Cushing syndrome.	Craniofacial hyperostosis, facial asymmetry, deafness, blindness; pituitary adenoma.	OMIM (174800) Ref.[23,30]
DNA repair disorders	***Gene***	***Typical and general features***	***Craniofacial and orodental manifestation***	***Ref.***
Bloom syndrome	*WRN/ RECQL3*	*Typical:* pre and postnatal growth deficiency, short stature; facial telangiectatic, hypo/hyperpigmented skin, sun-sensitive; predisposition to malignancy. *General:* CALS (>50%), hypertrichosis, photosensitivity; infertility; digital defects; chronic lung disease; mild mental retardation; recurrent infections.	Dolichocephaly skull, microcephaly, narrow face, prominent ears/nose. Absent upper lateral incisors, highly arched palate.	OMIM (210900)[23]
Nijmegen breakage syndrome	*NBN*	*Typical:* microcephaly, cancer predisposition, short stature, immunodeficiency. *General:* CALS (<50%), vitiligo; mental retardation, hyperactivity, neurodegeneration; primary ovarian failure; radiation hypersensitivity. Premature death.	Typical facial appearance, prominent midface, microcephaly; upward slanting of palpebral fissures; dysplastic ears; choanal atresia, long nose. Periodontal diseases; cleft lip/palate.	OMIM (251260)[23]
Seckel syndrome 2	*RBBP8*	*Typical:* "bird-headed" facial appearance, mental retardation, short stature, microcephaly. *General:* CALS (some); ectopic kidneys; digital defects, slender extremities.	Microcephaly, proptosis; beaklike nose; narrow face, receding mandible; nystagmus; ear defect. Dental anomalies, cleft palate, high arched palate, hypoplastic enamel, macroglossia, gingival hyperplasia.	OMIM (606744)[31]
Nijmegen breakage syndrome-like disorder	RAD50	*Typical:* severe growth restriction; congenital microcephaly, impaired intellectual development. *General:* CALS, multiple nevi; short stature; spasticity, Chiari malformation; brachydactyly, clinodactyly; vascular anomalies, Wolff-Parkinson-White anomaly; widely spaced nipples.	Microcephaly, sloping forehead, micrognathia; hypertelorism; broad nasal bridge; hypoplastic nasal septum.	OMIM (613078)
Fanconi anemia (FANCA, FANCC, FANCI, FANCD2)	*FANCA FANCC FANCI FANCD2*	*Typical:* developmental abnormalities in major organ systems, early-onset bone marrow failure, high predisposition to cancer (leukemia). Small stature. *General:* malformations: skeleton (radial aplasia, thumb deformity, vertebrae defects), skin (CALS, others), cardiopulmonary, gastrointestinal, central nervous systems, urogenital.	Microcephaly; strabismus, microphthalmia. "Fanconi facies"; ear malformations; short neck.	OMIM (227650, 227645, 227646, 609053)

Continue...

Table 1. Continuation.

KIT signaling pathway	*Gene*	*Typical and general features*	*Craniofacial and orodental manifestation*	*Ref.*
Waardenburg syndrome type 2E	*SOX10*	*Typical:* auditory-pigmentary syndrome. Congenital hearing loss, neurologic abnormalities. *General:* hypopigmented patches, CALS (mild), premature graying; pectus excavatum.	Ocular albinism, white forelock/ eyelashes/eyebrows; nystagmus; anosmia. Delayed deciduous tooth eruption, large central incisors, irregularly placed dentition.	OMIM (611584)
Familial progressive hyper- and hypopigmentation	*KITLG*	*Typical:* larger hypopigmented ash-leaf macules, diffuse hyperpigmentation, CALS. *General:* lentigines, vitiligo, multiple CALS, hyperkeratosis.	Hyperpigmented patches.	OMIM (145250)
Genomic imprinting disorders	***Gene***	***Typical and general features***	***Craniofacial and orodental manifestation***	***Ref.***
Silver-Russell syndrome 1	*ICR1*	*Typical:* growth retardation, craniofacial features, body asymmetry, others malformations. *General:* CALS (<50%); developmental delay; cardiac defects; digital defects; neoplasms.	Relative macrocephaly, triangular face, prominent forehead; blue sclera; micrognathia, thin lips, downturned corners of mouth, retrognathia.	OMIM (180860)[23]
Mulchandani-Bhoj-Conlin syndrome	*GRCh38*	*Typical:* short stature, profound feeding difficulties. *General:* CALS (infrequent); hypotonia; horseshoe kidney; digital defects.	Microcephaly, triangular face, dolichocephaly, low-set ears, thick helices; epicanthal folds. Retrognathia, narrow palate.	OMIM (617352)
Miscellaneous	***Gene***	***Typical and general features***	***Craniofacial and orodental manifestation***	***Ref.***
Multiple endocrine neoplasia type I	*MEN1*	*Typical:* endocrine tumors. *General:* CALS (40%), hypopigmented macules, lipomas, collagenomas; prolactinoma; vasointestinal peptide tumor, gastrinoma; carcinoid tumors.	Multiple facial angiofibromas, collagenomas. Multiple gingival papules.	OMIM (131100)[23]
Multiple endocrine neoplasia type IIB	*RET*	*Typical:* hamartoneoplastic syndrome: thyroid carcinoma, pheochromocytoma, mucosal neuromas, thick corneal nerves. Failure to thrive. *General:* CALS (sometimes); ganglioneuroma, developmental delay; parathyroid hyperplasia; goiter; colonic diverticulum, megacolon; myopathy, skeletal abnormalities.	Characteristic facial appearance: swollen lips, flat nasal bridge; ptosis. High arched palate, prognathism, thick lips.	OMIM (162300)
Smith-Kingsmore syndrome	*MTOR*	*Typical:* macrocephaly, seizures, umbilical hernia, facial dysmorphic features. *General:* CALS (11.1%); intellectual disability, heterotopic gray matter, corpus callosum hypogenesis, polymicrogyria, hypotonia; small thorax; limb shortening; small toenails.	Midface hypoplasia, frontal bossing; hypertelorism, downslanting palpebral fissures; short nose; curly hair. Macrostomia, long philtrum, thin lip.	OMIM (616638)[32]
Rubinstein-Taybi syndrome 1	*CREBBP*	*Typical:* microcephaly, mental retardation, growth deficiency, broad thumbs/ halluces, dysmorphic facial features. *General:* single transverse palmar creases, CALS; agenesis corpus callosum, seizures; heart defect; sternal anomalies; digital defects; hirsutism.	Striking facial features, low anterior hairline, prominent forehead; strabismus, ptosis; hypoplastic maxilla, micro/retrognathia; low-set ears, hearing loss; beaked nose. Dental anomalies, high-arched palate, malocclusion, hypoplastic enamel, thick lip.	OMIM (180849)
OHDO syndrome, X-linked	*MED12*	*Typical:* blepharophimosis, ptosis; long filter; micrognathia, deafness. *General:* CALS; developmental delay; cryptorchidism; clinodactyly.	Facial coarsening, epicanthal folds; small ears; wide nasal bridge; blepharophimosis, ptosis; microstomia, dental anomalies.	OMIM (300895)
Chung-Jansen syndrome	*PHIP*	*Typical:* impaired global and intellectual development, dysmorphic features, obesity. *General:* CALS (40%); hands: tapering fingers, clinodactyly; feet: syndactyly; hypotonia.	High forehead; large ears; thick eyebrows, hypertelorism, synophrys, epicanthal folds, strabismus. Micrognathia, thin lips, high palate.	OMIM (617991)
Kabuki syndrome	*KMT2D*	*Typical:* mental retardation, postnatal dwarfism, peculiar facies, characteristic skeletal and dermatoglyphic changes. *General:* CALS, cutis aplasia; seizures, hypotonia; heart defect; anal defects; renal anomalies; vertebral/hip anomalies, digital defects.	Microcephaly; long palpebral fissure, eversion of eyelids, arched eyebrows, long eyelashes, hypertelorism; prominent earlobes, hearing loss; wide nose. High palate, cleft lip/palate, bifid tongue/uvula, micrognathia, diastema, dental anomalies.	OMIM (147920)

Continue...

Table 1. Continuation.

Miscellaneous	*Gene*	*Typical and general features*	*Craniofacial and orodental manifestation*	*Ref.*
Roberts syndrome	*ESCO2*	*Typical:* tetraphocomelia, mental retardation, cranial/cardiac/renal anomalies. *General:* hypopigmented patches, CALS; rudimentary gallbladder; talipes equine-valgus, rudimentary digits; encephalocele, hydrocephalus. Growth retardation. Short neck.	Microcephaly, craniosynostosis, midfacial hemangioma; exophthalmos, corneal clouding, blue sclera, hypertelorism; hypoplastic nasal alae; malformed ears; fissured lips, high arched palate, cleft lip/palate.	OMIM (268300)
Adams-Oliver syndrome 4	*EOGT*	*Typical:* aplasia cutis and terminal transverse limb defects. *General:* cutis marmorata, CALS (rare); dysplastic/aplastic toenails; temporal/ occipital infarct; heart defect; umbilical hernia; digital defects.	Cutis aplasia and bony defect (scalp).	OMIM (615297)
Johanson-Blizzard Syndrome	*UBR1*	*Typical:* short stature, mental retardation, dysmorphic features. *General:* CALS, scalp defects, tranverse palmar crease; hypotonia; heart defect; small nipples; liver failure; pancreatic insufficiency; imperforate anus; clinodactyly.	Microcephaly; hearing loss; hypertelorism, cutaneous-lacrimal fistulae; hypoplastic nasal wing; blonde and unruly hair. Oligodontia, cleft lip/palate.	OMIM (243800)
Carney complex	*PRKAR1A*	*Typical:* multiple neoplasia syndrome; pigmented lesions. *General:* lentigines, nevi, CALS (<50%); adrenal dysplasia, Cushing disease, acromegaly, thyroid hyperplasia; mammary fibroadenoma, pheochromocytoma, pituitary adenoma.	Conjunctival pigmentation, eyelid myxoma; hirsutism, red hair.	OMIM (160980)[4,23]
Russell-Silver syndrome, X-linked	Unknown	*Typical:* pigmentary anomaly, X-linked – severe in males, mild in females. *General:* CALS; achromatic areas of trunk and limbs; growth retardation.	Triangular facies.	OMIM (312780)
Chromosome 17q11.2 deletion syndrome	Not reported	*Typical:* variable facial dysmorphism, mental retardation, excessive number neurofibromas, increased risk for malignant peripheral nerve tumors. *General:* CALS (93%), freckling; attention-deficit hyperactivity disorder; tall stature; heart defects; pectus excavatum; bone cysts, large hands/feet.	Macrocephaly; coarse facies; Lisch nodules (93%), hypertelorism, optic glioma.	OMIM (613675)
Chromosome 15q26-qter deletion syndrome	*IGF1R*	*Typical:* deletion of chromosome 15q26-qter encompassing the insulin-like growth factor 1 receptor gene. Short stature is stablished hallmark. *General:* CALS; mental retardation; congenital cardiac anomalies; digital defects.	Microcephaly; low-set ears; blepharophimosis, strabismus; broad bridge nose.	OMIM (612626)
Microcephaly, growth restriction and increased sister chromatid exchange 2	*TOP3A*	*Typical:* growth restriction with short stature, microcephaly. *General:* CALS; mild developmental delayed; dilated cardiomyopathy.	Microcephaly; dysmorphic facial features progeroid-like.	OMIM (618097)
Autosomal recessive primary microcephaly	*CDK5RAP2*	*Typical:* microcephaly, developmental delay, variable dysmorphic facies. *General:* CALS; behavioral problems; atrophic cortical, absence of corpus callosum, seizures.	Microcephaly; conical-shaped and widely spaced teeth; hearing loss; prominent nose.	OMIM (604804)
Ring chromosome 14 syndrome	*RC14R MAX*	*Typical:* developmental delay, early-onset epilepsy, microcephaly, dysmorphic facial features. *General:* Pigmentary abnormalities, CALS; hypotonia, seizures, poor speech. Short stature.	Micro/dolichocephaly; low-set ears; downslanting palpebral fissures, epicanthal folds, hypertelorism; flat nasal bridge, anteverted nostrils.	OMIM (616606)[2]
Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	*CBL*	*Typical:* facial dysmorphism, cardiac disease, reduced growth, cognitive deficits, ectodermal/ musculoskeletal anomalies. Susceptibility to juvenile myelomonocytic leukemia. *General:* CALS, lymphedema, thin skin; delayed psychomotor development; language delay; cubitus valgus, joint laxity; pectus excavatum, widely spaced nipples.	Thin hair; frontal bossing, triangular face, long philtrum; large ears, low-set ears; hypertelorism, ptosis, downslanting palpebral fissures; depressed nasal bridge, thick lips.	OMIM (613563)
Microcephalic osteodysplastic primordial dwarfism type II	*PCNT*	*Typical:* severe short stature, microcephaly. Skeletal malformations. *General:* CALS, hypopigmentation; mental retardation, aneurysms; digital defects.	Retrognathia; small ears; prominent nasal root. Enamel hypoplasia, microdontia.	OMIM (210720)

CALS: cafe-au-lait spots; OMIM: Online Mendelian Inheritance in Man; Ref.: reference.

the Ras/MAPK signaling pathway, which regulates the differentiation, migration, and survival of melanocytes, as well as proliferation, melanogenesis, and melanosome transfer[7]. Among the diseases of this group, Waardenburg syndrome and familial progressive hyper- and hypopigmentation are those that present associated craniofacial alterations.

Bloom syndrome, Nijmegen breakage syndrome, Seckel syndrome 2, and Fanconi anemia are diseases that present CALS and craniofacial alterations classified as DNA repair disorders. Germline pathogenic mutations in genes encoding key proteins in DNA repair and telomeres biology result in a high risk of cancer associated with these syndromes[18,19].

In addition, we have the genomic imprinting disorders, associated with an epigenetic phenomenon that causes genes to be expressed or not, inherited from the mother or father. Silver-Russell syndrome 1[20] and Mulchandani-Bhoi-Conlin syndrome[21] are diseases of this group.

Besides these classifications, localized or generalized melanotic hyperpigmentation might be part of the clinical presentation of many other congenital systemic disorders that result from ubiquitous protein defects and/or basal cell processes. This suggests that melanocytes are a cell type with high sensitivity to such perturbations[6]. In these cases, CALS occurs as isolated lesions with low occurrence.

CONCLUSION

The observation of CALS in the assessment of a patient can be of great significance, especially the presence of multiple CALS, large and segmental CALS, other skin anomalies, facial dysmorphism and orodental changes, and other unusual findings on physical examination. These findings should suggest an associated genetic disorder.

Furthermore, it is important to highlight that the craniofacial structures and skin tissue share a similar embryological origin. Thus, the characterization of craniofacial abnormalities in the assessment of a patient with a genetic syndrome associated with CALS can be of great relevance for the diagnosis of the specific syndrome related to this condition.

AUTHORS' CONTRIBUTIONS

AAC: Conceptualization, Research, Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review and editing. **DRBM:** Formal analysis, Writing – review and editing. **LDAF:** Research, Data Curation, Formal Analysis, Writing – Original Draft, Writing – review and Editing. **RAM:** Data curation, Formal analysis, Writing – original draft, Writing – review and editing. **HMJ:** Conceptualization, Data curation, Formal analysis, Project administration, Writing – review and editing.

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