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Bioethical and legal issues of termination of pregnancy resulting from rape

Fábio Roberto Cabar[1*], Juliana Bertoldi Franco[2], Maria Luiza Gorga[3]

From the obstetrical point of view, abortion is the interruption of pregnancy, spontaneous or provoked, with up to 22 weeks of gestation and/or with the fetus weighing up to 500 g[1].

From a Brazilian legal point of view, abortion is the interruption of pregnancy with the destruction of the product of conception, regardless of gestational age or fetal weight and whether the pregnancy is spontaneous or the result of assisted reproduction techniques, characterizing a crime. In these cases, the law criminalizes the voluntary termination of pregnancy with the plea of defending the life of the human being in formation, but there are three legal exceptions that expressly allow the performance of an abortion: pregnancy resulting from rape, a situation in which the termination of pregnancy is the only way to safeguard the mother's life (high-risk pregnancy), or pregnancy of an anencephalic fetus (this last hypothesis is allowed based on Supreme Court decision on ADPF 54, from 2012).

Recently, there has been intense discussion about the legal, bioethical, and health aspects related to the termination of pregnancy of a girl who was 11 years old at the time of the facts, making necessary to analyze some legal, obstetric, and bioethical aspects on the subject.

According to Brazilian law, it is the crime of rape of a vulnerable person to "have sexual intercourse or perform another lewd act with a person under the age of 14"[2] (Article 217-A). Complementing this understanding, the Superior Court of Justice determined in 2015 (AgRg REsp 1453155/SC) that this situation characterizes the crime, regardless of whether there is the victim's consent, previous sexual experience, or the existence of a romantic relationship with the one who committed the act. Therefore, in the specific case, as it is a girl under the age of 14, there is no doubt that there was rape of a vulnerable person.

As already discussed, abortion is allowed when the pregnancy is the result of rape and if there is a consent on the part of the pregnant woman. Furthermore, the Brazilian Penal Code does not impose a temporal limitation for its performance. This is exactly what happened in the case: as it was a pregnancy resulting from rape, it was the girl's right to terminate the pregnancy, being a decision and a right of the young pregnant woman, and judicial authorization is not required for the abortion.

From a healthcare viewpoint, pregnancy in girls under the age of 14 represents a real risk to the health of the adolescent and the fetus, which also provides medical support to the pregnant woman's decision. From specifically the obstetric position, the literature reports a higher prevalence of anemia, arterial hypertension, birth dystocia, postpartum hemorrhage, and a higher incidence of maternal death[3]. In addition, there is an association with the development of social, emotional, and economic problems, represented by school dropout, need to provide for their own support, emotional pressure exerted by the family and society in general[4].

Finally, considering the Bioethical principles, the interruption of pregnancy in these circumstances meets the principle of Beneficence, as it protected the girl from the complications of a pregnancy at an early age, Autonomy, as far as it respects the pregnant's decision of interrupting the pregnancy, and Justice, as far as the laws of the country were respected and applied.

The principle of Beneficence refers to the physician's obligation to maximize benefit and minimize harm. The medical professional must have the conviction and technical knowledge needed to ensure that the medical act is beneficial to the patient. Thus, the principle of Beneficence prohibits inflicting deliberate harm, so that the physician's action must always cause the least harm or no harm to the patient's health, a precept historically enshrined in the Hippocratic aphorism *primum non nocere* (first do no harm), whose purpose is to reduce the adverse or undesirable effects of diagnostic and

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therapeutic actions[5], always with the objective to treat, cure, and protect a patient's life.

Likewise, the principle of Autonomy advocates that individuals must be empowered to decide about their health pathway and should be treated with respect for their decision-making capacity. People have the right to decide on matters related to their body and their lives, and the patient's authorization is required to carry out any medical acts[6]. The Committee on Ethical Issues in Human Reproduction and Women's Health of the International Federation of Gynecology and Obstetrics stated that "the principle of autonomy emphasizes the important role that women must play in decision-making regarding their health care. Physicians should observe female vulnerability, expressly requesting their choice and respecting their opinions[7]." In the case of children, the principle of autonomy must be exercised by the family or legal guardian, which occurred in the case under discussion, as the child's mother was supporting her.

Based on all the discussions and myriad of opinions expressed about this case, it is essential to highlight the importance of knowing and applying the legal, technical, and bioethical aspects for the performance of best practices in medical activities, especially in vulnerable patients and those situations surrounded by possible controversies, so that one can act according to the rules and principles that must guide every medical professional and not based on societal pressures and fears.

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AUTHORS' CONTRIBUTIONS

FRC: Conceptualization, Supervision, Writing – review & editing. **JBF:** Writing – original draft. **MLG:** Investigation, Writing – review & editing.

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Use of cannabidiol in the treatment of epilepsy: Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex

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SUMMARY

OBJECTIVE: The objective of this systematic review with meta-analysis was to evaluate the efficacy, safety, and short- and long-term tolerability of cannabidiol (CBD), as an adjunct treatment, in children and adults with Dravet syndrome (SD), Lennox-Gataut syndrome (LGS), or tuberous sclerosis complex (TSC), with inadequate control of seizures.

METHODS: This systematic review was conducted through a search for scientific evidence in the Mediline/PubMed, Central Cochrane, and ClinicalTrials.gov databases until April 2022. Selected randomized clinical trials (RCTs) that presented the outcomes: reduction in the frequency of seizures and total seizures (all types), number of patients with a response greater than or equal to 50%, change in caregiver global impression of change (CGIC) (improvement ≥1 category on the initial scale), adverse events (AEs), and tolerability to treatment. This review followed Preferred Reporting Items for Systematic reviews and Meta-Analyses.

RESULTS: Notably, six RCTs were included, with a total of 1,034 patients with SD, LGS, and TSC, of which 3 were open-label extension RCTs. The meta-analysis of the studies showed that the use of CBD as compared with placebo, in patients with convulsive seizures refractory to the use of medications, reduces the frequency of seizures by 33%; increases the number of patients with a reduction ≥50% in the frequency of seizures by 20%; increases the number of patients with absence of seizures by 3%; improves the clinical impression evaluated by the caregiver or patient (S/CGIC) in 21%; increases total AEs by 12%; increases serious AE by 16%; increases the risk of treatment abandonment by 12%; and increases the number of patients with transaminase elevation (≥3 times the referral) by 15%.

CONCLUSIONS: This systematic review, with meta-analysis, supports the use of CBD in the treatment of patients with seizures, originated in DS, LGS, and TSC, who are resistant to the common medications, presenting satisfactory benefits in reducing seizures and tolerable toxicity.

KEYWORDS: Dravet syndrome. Lennox Gastaut syndrome. Tuberous sclerosis complex. Cannabidiol. Seizures. Seizures refractory.

INTRODUCTION

Epilepsy is one of the most common neurological disorders[1]. About one-third of all patients with epilepsy have drug-resistant seizures. The International League Against Epilepsy defines drug-resistant epilepsy as the "failure of ≥2 appropriate and tolerated antiepileptic drugs (either as monotherapy or in combination) to achieve the sustained freedom of seizures"[2]. Inadequate seizure control significantly affects the quality of life and cognitive function of these patients. Drug-resistant epileptic syndromes are associated with significant comorbidity and high rates of cognitive impairment, as well as psychiatric and physical disability. Currently, cannabidiol (CBD) is being used for three epileptic syndromes: Lennox-Gastaut syndrome (LGS), Dravet syndrome (DS), and tuberous sclerosis complex (CST). Both LGS and DS are early-onset encephalopathic epileptics with poor prognosis and associated with comorbidities.

LGS is a severe epileptic encephalopathy of varying presentation and is associated with high rates of seizure-related injury and cognitive impairment[3-5]. LGS has an incidence of approximately 1:4,000 births; estimates of uncertain prevalence, possibly around 15/100,000. LGS is believed to account for 1–4% of all infant epileptics[3-5].

DS is rare, intractable, occurs in early childhood and is characterized by prolonged and recurrent partial crises at onset, with progression to generalized polymorphic seizures resulting in developmental delay, cognitive impairment, and increased mortality. SD has an incidence of approximately 1:20,000 births; estimates of uncertain prevalence, possibly around 3/100,000. SD is believed to account for approximately 7% of all severe epileptics initiated before 3 years of age[6-8].

CBD was also evaluated under conditions with mainly focal seizures, such as TSC. TSC is a genetic disease that can

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present in any part of the body. The most common manifestations include benign tumors in the skin, brain, kidneys, lung, and heart that cause organic dysfunction[9]. The reported incidence ranges from 1 per 5,800 to 10,000 live births[9] and the prevalence of 1/20,000 people in the UK[9].

Cannabis has been used to treat epilepsy since antiquity, and interest in cannabis-based therapies has increased in the past decade. CBD, which is one of the main constituents of the *Cannabis sativa* plant, has anticonvulsant properties and does not produce euphoric or intrusive side effects[10]. The lack of regulation and standardization in the medicinal cannabis industry, however, raises concerns about the composition and consistency of the products that are dispensed[11]. Pharmaceutical grade oral CBD solution is the first product made directly from the cannabis plant, rather than created synthetically, to be authorized by regulatory agencies and the first of a new class of anticonvulsant drugs.

OBJECTIVE

The aim of this study was to assess the efficacy, safety, and short- and long-term tolerability of CBD, as an adjuvant treatment in children and adults with inadequately controlled DS, LGS, or TSC.

METHODS

This systematic review will be carried out in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)[12].

A clinical doubt arises: what is the impact of CBD use on outcomes reducing the frequency of seizures and total seizures (all types), number of patients with a response equal to or greater than 50%, impression of clinical improvement by the patient or caregiver, adverse events (AEs), and tolerability to treatment?

The eligibility criteria of the studies are as follows:

1. Patients with DS, LGS, and TSC;
2. Treatment with CBD plus usual therapy compared to placebo plus usual therapy;
3. Outcomes – reduction in the frequency of seizures and total seizures (all types), number of patients with a response greater than or equal to 50%, change in caregiver global impression of change (CGIC) (improvement of ≥1 category in the initial scale), AEs, and tolerability to treat;
4. Excluding outcomes – intermediaries;
5. Phase III RCT or observational cohort studies;
6. No period or language limit;
7. Text complete available for access; and
8. Follow-up: minimum of 16 weeks.

The search for evidence will be carried out in the Virtual Scientific Information Base Medline using the search strategy — (Cannabis OR Tetrahydrocannabinol OR Cannabinoids OR Cannabinol OR Cannabidiol) AND (Epilepsy OR infantile spasms OR Epilepsies, Myoclonic OR Tuberous Sclerosis OR Lennox Gastaut Syndrome OR Dravet Syndrome OR Sturge-Weber Syndrome OR Drug Resistant Epilepsy) AND Random*; CENTRAL/Cochrane with the search strategy — (Cannabis OR Tetrahydrocannabinol OR Cannabinoids OR Cannabinol OR Cannabidiol) AND (Epilepsy OR infantile spasms OR Epilepsies, Myoclonic OR Tuberous Sclerosis OR Lennox Gastaut Syndrome OR Dravet Syndrome OR Sturge-Weber Syndrome OR Drug Resistant Epilepsy) and ClinicalTrials.gov with the search — (Cannabinol OR Cannabidiol) AND (Tuberous Sclerosis OR Lennox Gastaut Syndrome OR Dravet Syndrome OR Sturge-Weber Syndrome). The search in these databases will be carried out until April 2022.

The following data were extracted from the studies: author name and year of publication, population studied, methods of intervention and comparison, absolute number of events reductions in the frequency of seizures and total seizures (all types), number of patients with response equal to or greater than 50%, impression of clinical improvement by the patient or caregiver (CGIC), AEs, in addition to follow-up time. The results of the median percentage change (minimum – maximum) in relation to baseline in the monthly frequency of seizures were also extracted.

The risk of bias scans for RCTs will be assessed using the rob 2 tool items[13], plus other key elements, and expressed as low, moderate, serious or critical risk of bias, and no information. For cohort studies, the tool currently recommended by the Cochrane Collaboration will be used to assess the risk of bias in estimates of effectiveness and safety in nonrandomized Risk of Bias In Non-Randomized Studies — of Interventions (ROBINS-I) intervention studies[13]. ROBINS-I evaluates seven domains of bias, classified by moment of occurrence. The bias risk assessment will be conducted by two independent reviewers (AS and IF), and in case of disagreements, a third reviewer (WB) can deliberate on the evaluation. The quality of evidence will be extrapolated from the risk of bias obtained from the study(s) (if there is no meta-analysis) using the TERMINOLOGY GRADE[14] in very low, low, and high, and through the software GRADE pro[15] (if there is meta-analysis) in very low, low, moderate, and high.

The results for categorical outcomes will be expressed by the difference in risk (DR) between CBD therapy and placebo treatment. If the DR between groups is significant (95% confidence), this will be expressed with the 95% confidence interval (95%CI) and a number needed to treat (NNT) or to produce a Harm (NNH). In continuous measures, the results are expressed as mean difference or median difference with 95%CIs. Data from observational studies are reported as the percentage of participants who experienced a result.

If there is more than one study included with common outcomes, this will be aggregated through meta-analysis, using the RevMan 5.4 software[16], with the overall risk difference with 95%CIs being the final measure used to support the synthesis of evidence, which will answer the clinical doubt of this assessment. The estimated size of the combined effects was performed by a fixed or random effect model after the evaluation of heterogeneity results. Heterogeneity was also calculated using the value I^2. The results will be evaluated by study design (RCTs and observational cohorts) and presented individually.

Included studies

In the search for evidence, 145 articles were retrieved, and 15 studies evaluated the use of CBD plus usual therapy as compared with placebo in the treatment of patients with DS, LGS, and TCS or were observational cohort studies "open-label extension" (OLE). The 15 studies were assessed because they met the eligibility criteria for analysis of the full text. Of these 15 studies, 6[17-22] ECRs and 3[23-25] OLE studies were included to support this evaluation, whose characteristics are described in Tables 1 and 2, respectively. The excluded list and the reasons are available in the references and are shown in Figure 1[26].

The six RCTs enrolled 1,034 patients with DS, LGS, and TSC, with 485 patients undergoing treatment with CBD (all dosages) compared to 325 placebo patients. This population was followed to measure the outcomes of reduction in the frequency of seizures and total seizures (all types), number of patients with response greater than or equal to 50%, change in CGIC, AEs, and tolerability to treatment. The follow-up was 14–16 weeks after the start of treatment (Table 3).

These patients who had previously participated in the RCTs were allowed to continue in an OLE study for each pivotal study (Table 1), evaluating the efficacy, safety, and tolerability of CBD in the long term (median on days ranging from 267 to 1,090; n=880).

Risk of bias in included studies

For this update of the review, a combination of two out of three review authors (from AS, IF, and WB) independently re-assessed the risk of bias in each included trial according to predefined criteria stated in the Methods section (Table 3 and Figure 2)[27].

Regarding the risk of bias of the six RCTs included[13-17,27], none of them were blinded by the evaluator and one did not perform a sample calculation, and the overall risk of the studies may be considered nonsevere (Table 3).

The assessment of the risk of bias in the observational cohort OLE studies was made with the use of the ROBINS-I tool. The three studies included[23-25] presented a risk of critical bias to the loss domain (bias due to missing data), while all other domains presented a low risk of bias. Therefore, the overall risk of bias can be considered moderate (Figure 2).

Results of randomized clinical trials

Five studies18-22, assessing 726 participants, allowed the evaluation of the outcome "absolute reduction in seizures" treated with CBD as compared to placebo, with a follow-up time of 12–16 weeks. This analysis demonstrated increase in the number of patients who obtained absolute reduction in the frequency of seizures [risk difference (RD)=0.31 (95%CI 0.18–0.44; I2=77%)], NNT=3. Moderate evidence quality (Analysis 1.1; Figure 3 and Table 2).

Meta-analysis of five studies18-22, assessing 726 participants, found there was an increased in the "number of patients with ≥50% reduction in seizures" for treatment with CBD as compared to placebo, and the follow-up time was 12–16 weeks [RD=0.20 (95%CI 0.13–0.26; I2=0%)], NNT=5. High evidence quality (Analysis 1.2; Figure 4 and Table 2).

Five studies18-22, assessing 726 participants, have been submitted for a meta-analysis and demonstrated a less difference in the outcome "number of patients with absence of seizures" comparing treatment CBD as to placebo, with a follow-up time of 12–16 weeks [RD=0.03 (95%CI 0.01–0.03; I2=44%)]. Moderate evidence quality (Analysis 1.3; Figure 5 and Table 2).

The CGIC (7-point Subject/Caregiver Global Impression of Change, S/CGIC), evaluated through a questionnaire with seven items [improvement (mild, moderate, or intense), worsening (mild, moderate, or intense), and without change] was applied to caregivers and patients. Five studies18-22, assessing 726 participants, with a follow-up time of 12–16 weeks, demonstrated improved in S/CGIC. In the patients who received CBD as compared to placebo [RD=0.21 (95%CI 0.14–0.28; I2=0%)], NNT=5. High evidence quality (Analysis 1.4; Figure 6 and Table 2).

AEs, six studies17-22, assessing 733 participants, evaluated the "frequency of total adverse events" (any), with a follow-up time of 4–16 weeks, comparing the use of CBD to placebo. This

Table 1. Characteristics of clinical studies evaluating the use of cannabidiol in patients with Dravet syndrome, Lennox-Gastaut syndrome, and tuberous sclerosis complex.

Study	Drawing	Population	Intervention	Comparison	Denouement	Follow-up time
Devinsky et al.[17]	RCT	Pivotal phase 3 study; patients (n=120) diagnosed with Dravet syndrome; 2–18 years; with uncontrolled epileptic seizures, using more than one anticonvulsant drug, for more than 4 weeks. Multicenter (USA, UK, and Poland)	Staggered dose 5.10–20 mg/kg/day divided into two times a day. Maintenance doses for 12 weeks: 20 mg/kg/day	Placebo	Primary: reduction in the median monthly frequency of convulsions. Secondary: change in the overall impression of caregivers and patients (S/CGIC); reduction in percentage of the number of seizures (25, 50, 75, and 100%); adverse events (number, type and severity)	14 weeks
Miller et al.[19]	RCT	Pivotal phase 3 study; patients (n=199) diagnosed with Dravet Syndrome; 2–18 years; more than one anticonvulsant drug for more than 4 weeks. Multicenter (USA, Spain, Poland, the Netherlands, Australia, and Israel)	Staggered dose 5.10–20 mg/kg/day, divided into two times a day. Maintenance doses for 12 weeks: 10 or 20 mg/kg/day	Placebo	Primary: reduction in the frequency of the number of convulsions. Secondary: change in the overall impression of caregivers and patients (S/CGIC); reduction in percentage of the number of seizures (25, 50, 75, and 100%); adverse events (number, type, and severity)	14 weeks
Devinsky et al.[18]	RCT	Patients (n=34); age between 4 and 10 years; with DS. Evaluation of pharmacokinetics and safety of cannabidiol	Staggered dose 5.10 or 20 mg/kg/day divided into two times a day; treatment maintained for 3 weeks	Placebo	Dose titration and adverse events	4 weeks
Devinsky et al.[20]	RCT	Pivotal phase 3 study; patients (n=225) with LGS; age 2–55 years; diagnosed by electroencephalographic alterations; anticonvulsants drugs for more than 4 weeks, without seizure control	Staggered dose 5.10–20 mg/kg/day divided into two times a day. Maintenance doses for 12 weeks: 10 or 20 mg/kg/day	Placebo	Primary: median reduction in the number of drop seizures and total monthly seizures. Secondary: change in overall impression by caregivers and patients (S/CGIC); reduction in percentage of the number of seizures (25, 50, 75, and 100%); adverse events (number, type, and severity)	14 weeks
Thiele et al.[21]	RCT	Pivotal phase 3 study; patients (n=171) with LGS; age 2–55 years; clinically diagnosed by electroencephalogram (including documented history of slow electroencephalograms [<3.0 Hz]), associated with more than one type of generalized seizure, including falls, for at least 6 previous months; on use of anticonvulsant drugs for more than 4 weeks	Staggered dose 5.10–20 mg/kg/day divided into two times a day. Maintenance doses for 12 weeks: 20 mg/kg/day	Placebo	Primary: median reduction in the number of drop seizures and total monthly seizures. Secondary: change in the overall impression of caregivers ant patients (S/CGIC); reduction in percentage of the number of seizures (25, 50, 75, and 100%); adverse events (number, type and severity)	14 weeks
Thiele et al.[22]	RCT	Pivotal phase 3 study; patients (n=255) with diagnostic tuberous sclerosis complex; age between 1 and 65 years; using more than one anticonvulsant drug, for more than 4 weeks. (Poland, Australia, Spain, the Netherlands, UK, and USA)	Staggered dose, with an increase of 5 mg, up to 25 or 50 mg/kg/day, divided into two doses per day. Maintenance doses for 12 weeks: 25 or 50 mg/kg/day	Placebo	Primary: reduction in the number of seizures. Secondary: proportion of patients with a 50% reduction in the number of seizures, change in the overall impression of patients and caregivers (S/CGIC) and adverse events	14 weeks

DS: Dravet syndrome; LGS: Lennox-Gastaut syndrome; RCT: randomized controlled trial.

Table 2. Quality of evidence (GRADE).

Cannabidiol compared to placebo for seizures					
Patient or population: Lennox-Gastaut syndrome, Dravet syndrome, and tuberous sclerosis complex Context: Efficacy, safety, and tolerability Intervention: Cannabidiol Comparison: Placebo					
Outcomes	**Number of participants (studies) follow-up**	**Certainty of the evidence (GRADE)**	**Relative effect (95%CI)**	**Potential absolute effects**	
				Risk with placebo	**Risk difference with cannabidiol**
Absolute reduction in seizures follow-up: range 12–16 weeks	726 (5 ECRs)	⊕⊕⊕◯ Moderate[a]	not priceless	188 per 1,000	188 less per 1,000 (188 less for 188 less)
Number of patients with a reduction equal to or greater than 50% in seizures follow-up: range 12–16 weeks	726 (5 ECRs)	⊕⊕⊕⊕ High	RR 1.88 (1.50 to 2.35)	224 per 1,000	197 more per 1,000 (112 more to 303 more)
Number of patients without seizures follow-up: range 12–16 weeks	726 (5 ECRs)	⊕⊕⊕◯ Moderate[b]	RR 4.29 (1.24 to 14.87)	6 per 1,000	18 more per 1,000 (1 more to 77 more)
Improvement of clinical impression evaluated by patient or caregiver (S/CGIC) follow-up: range from 12–16 weeks	726 (5 ECRs)	⊕⊕⊕⊕ High	RR 1.54 (1.32 to 1.80)	385 per 1,000	208 more per 1,000 (123 more to 308 more)
Total adverse events follow-up: range 4–16 weeks	733 (5 ECRs)	⊕'very Low[b,c]	RR 1.15 (1.00 to 1.32)	801 per 1,000	120 more per 1,000 (0 less for 256 more)
Severe adverse events follow-up: range 12–16 weeks	727 (5 ECRs)	⊕⊕⊕◯ Moderate[d]	RR 3.25 (1.56 to 6.74)	72 per 1,000	162 more per 1,000 (40 more to 413 more)
Risk of treatment abandonment follow-up: range 4–16 weeks	741 (6 ECRs)	⊕⊕⊕⊕ High	RR 8.70 (3.80 to 19.89)	14 per 1,000	105 more per 1,000 (38 more to 257 more)
Number of patients with transaminase elevation equal to or greater three times the follow-up reference: range 4–16 weeks	721 (6 ECRs)	⊕⊕' Low	RR 11.20 (4.03 to 31.16)	5 per 1,000	55 more per 1,000 (16 more to 164 more)
The risk in the intervention group (and its 95%CI) is based on the risk assumed from the comparator group and the relative effect of the intervention (and its 95%CI). RR: risk ratio.					
GRADE Working Group grades of evidence high certainty: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low certainty: We have very less confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.					

Explanations
a. Heterogeneity equal to 77%.
b. Wide confidence interval.
c. Heterogeneity equal to 83%.
d. Heterogeneity equal to 72%.
e. Heterogeneity equal to 85%.

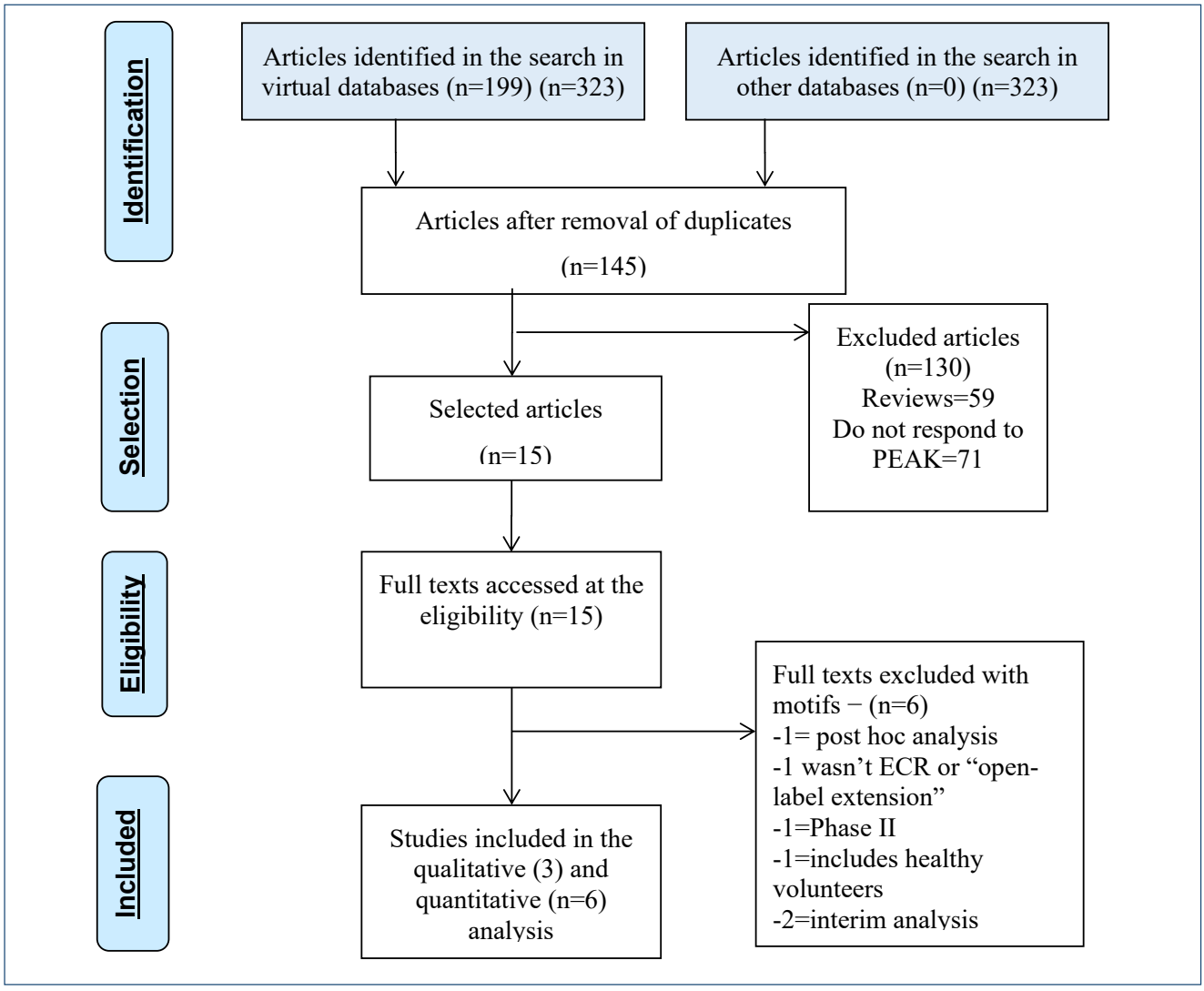

Figure 1. Evidence retrieval and selection diagram[26].

Table 3. Risk of biases from randomized clinical trials studies included.

Study	Random	Blind folded allocation	Double-blind	Blinding of the evaluator	Losses <20%	Characteristic prognostic	Outcome	Simple size calculation	Early interruption
Devinsky et al.[17]									
Devinsky et al.[18]									
Miller et al.[19]									
Devinsky et al.[20]									
Thiele et al.[21]									
Thiele et al.[22]									

Red: presence; green: absence; yellow: risk of unclear bias.

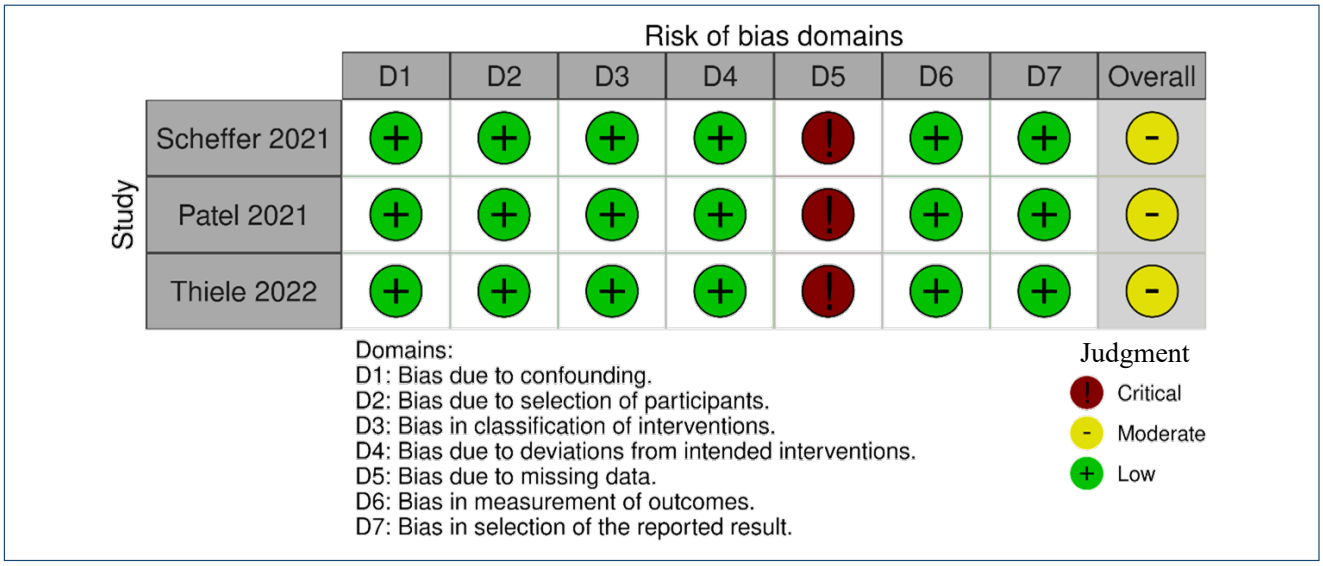

Figure 2. Risk-of-bias plot – result of the risk assessment of bias of the observational cohort studies ("open-label extension") included[27].

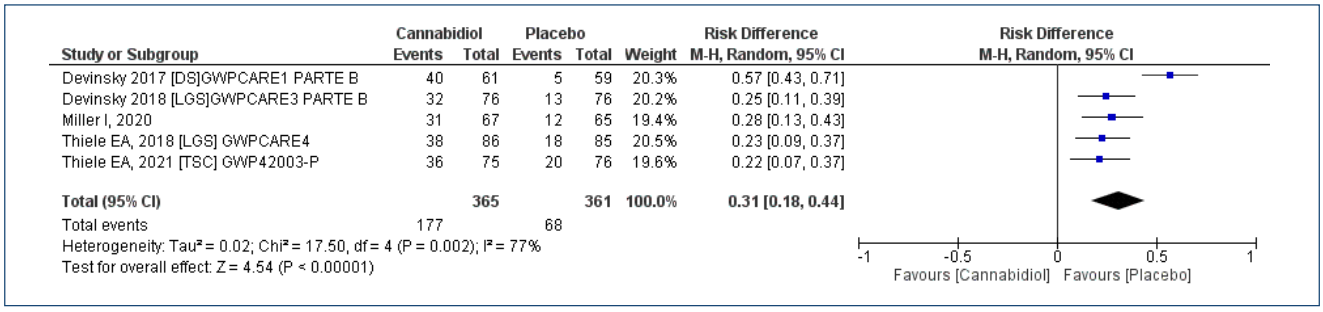

Figure 3. Meta-analysis of the results of absolute reduction in seizures with cannabidiol[17,18,21,22].

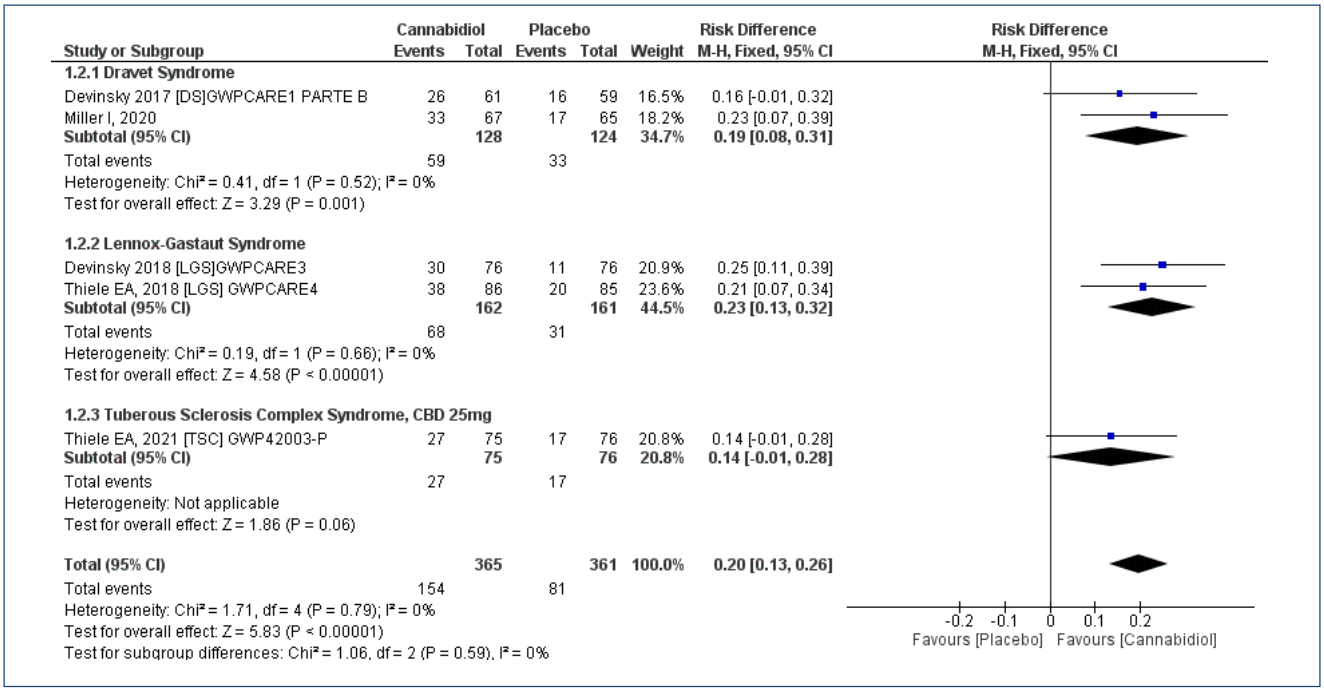

Figure 4. Meta-analysis of the results of reduction equal to or greater than 50% in seizures[17-19,21,22].

analysis demonstrated an increase in the risk of AEs with the use of CBD in the treatment of DS, LGS, and TSC [RD=0.21 (95%CI 0.14–0.28; I2=83%)], NNT=8. Very low evidence quality (Analysis 1.5; Figure 7 and Table 2).

The frequency of "severe adverse events" was evaluated in five studies18-22, assessing 727 participants, and the follow-up time was 12–16 weeks. This analysis demonstrated an increased risk of serious AEs with the use of CBD when compared to

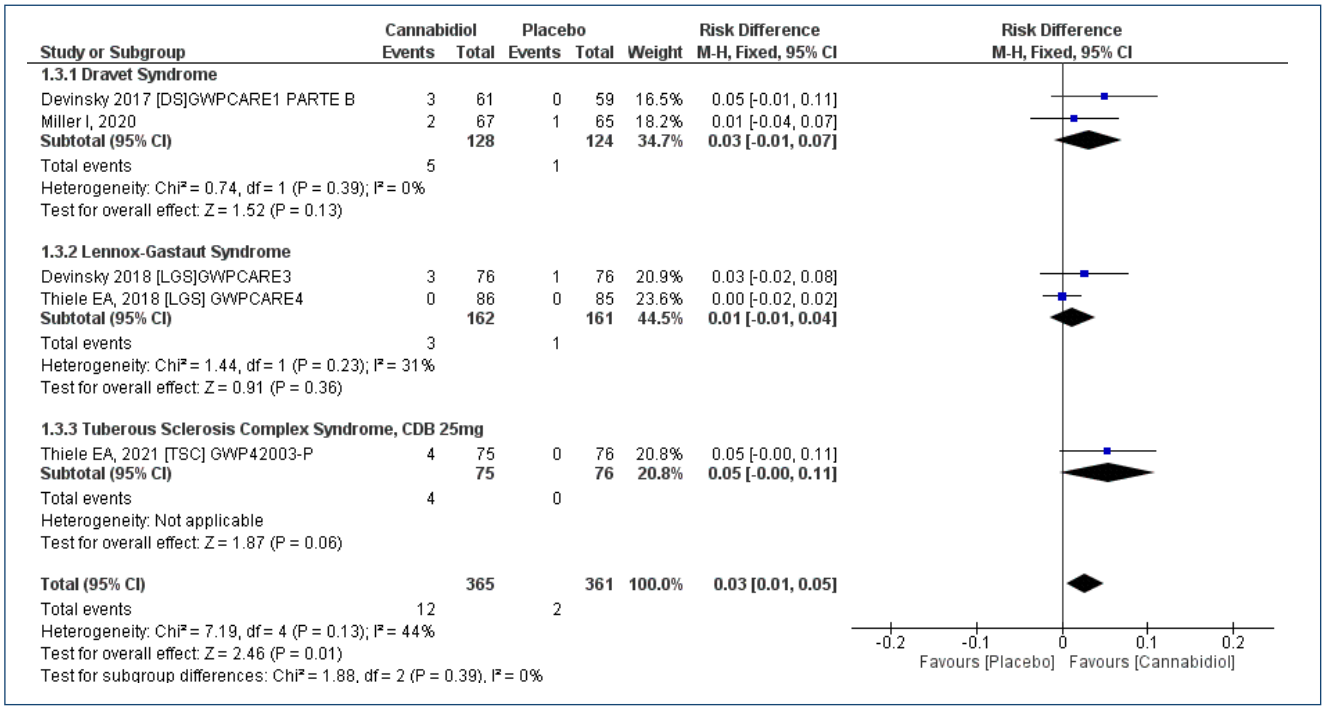

Figure 5. Meta-analysis of the results of patients with absence of seizures and use of cannabidiol[17-19,21,22].

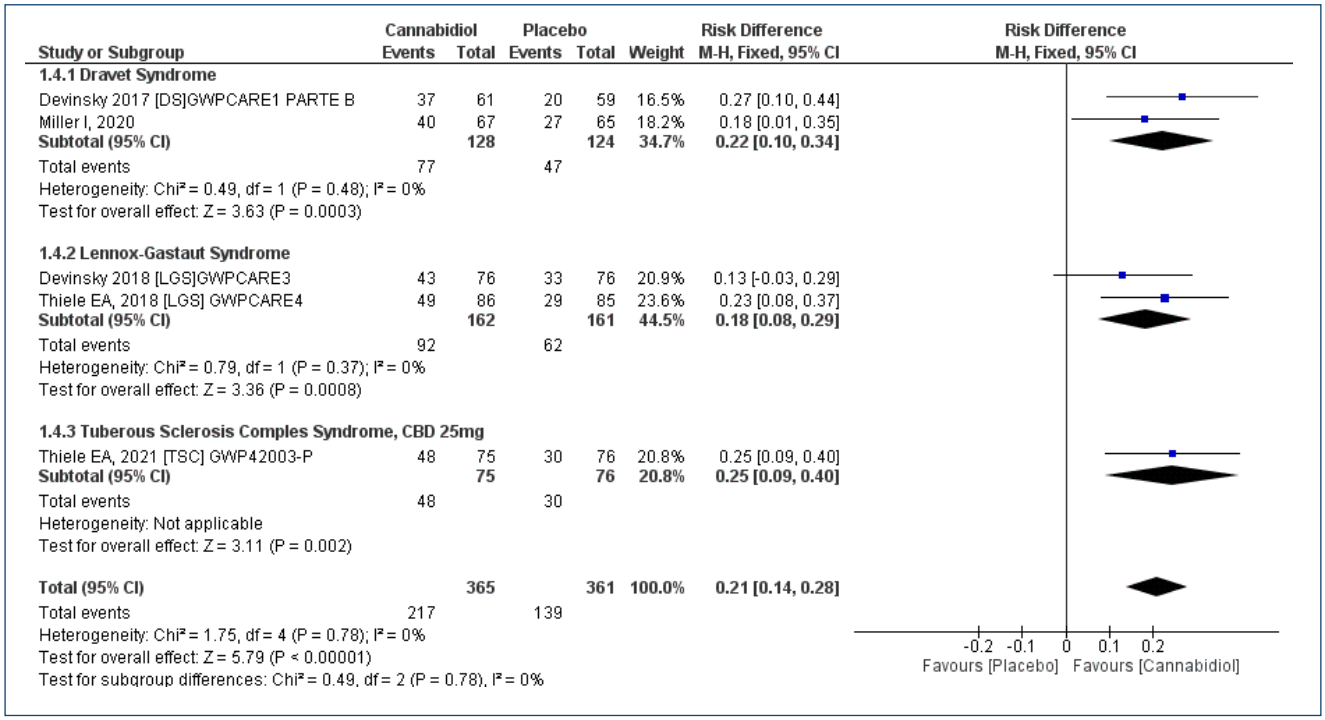

Figure 6. Meta-analysis of the results of caregiver global impression of change[17-19,21,22].

placebo [RD=0.16 (95%CI 0.07–0.26; I2=72%)], NNT=6. Moderate evidence quality (Analysis 1.6; Figure 8 and Table 2).

The "risk of treatment abandonment" was evaluated in six studies17-22, assessing 741 participants, and the follow-up time was 4–16 weeks. CBD increased the risk of treatment abandonment in the patients who received CBD as compared to placebo [RD=0.12 (95%CI 0.06–0.17; I2=50%)], NNH=8. High evidence quality (Analysis 1.7; Figure 9 and Table 2).

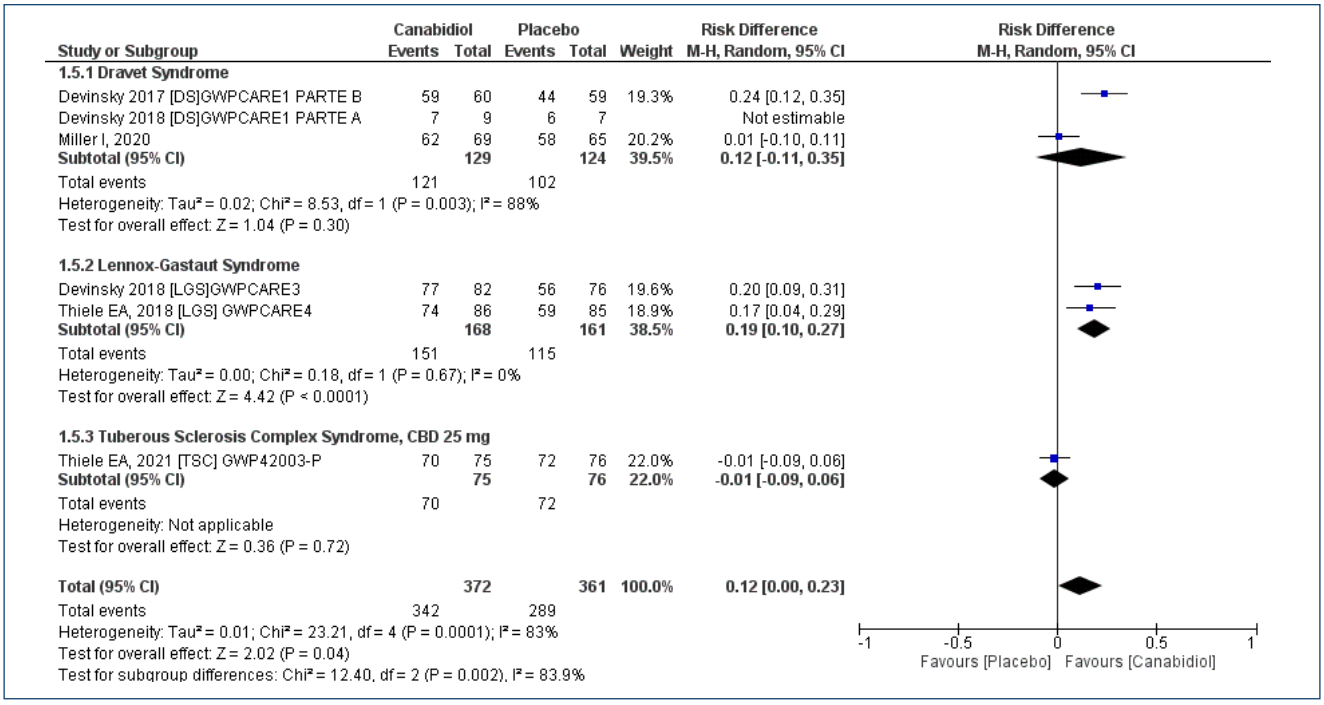

Figure 7. Meta-analysis of the results of total adverse events[17-19,21,22].

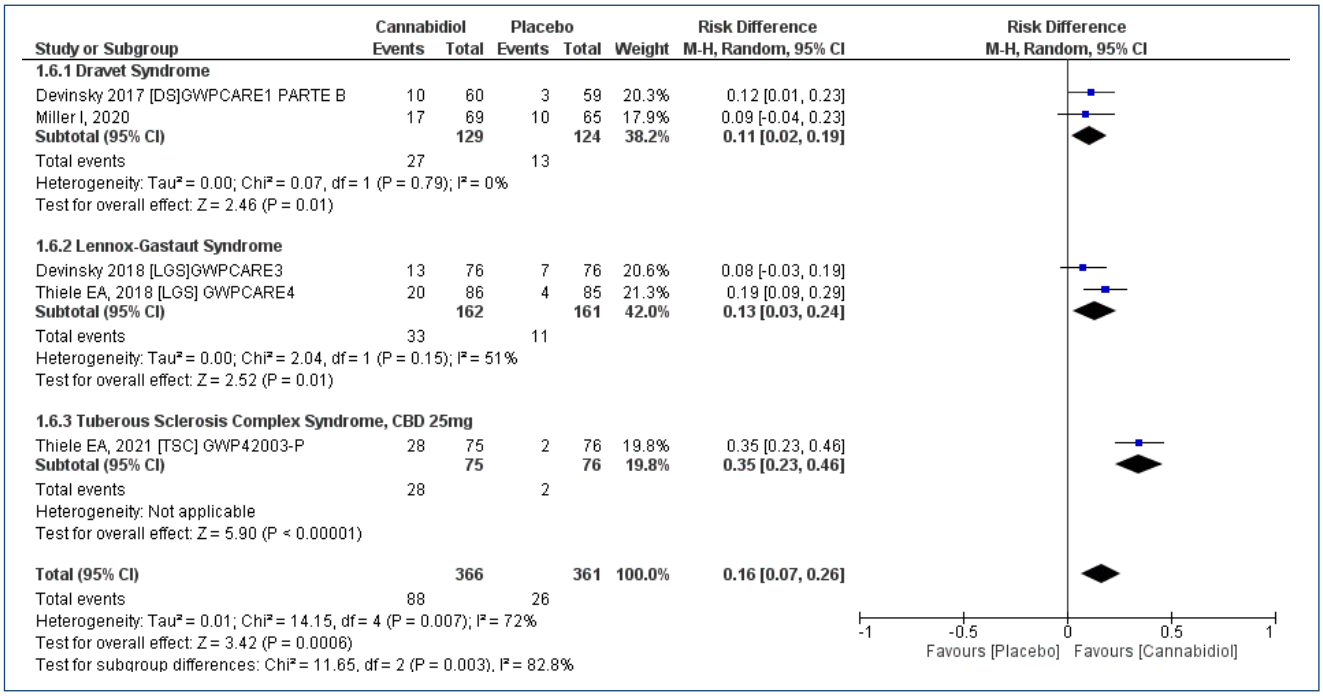

Figure 8. Meta-analysis of the results of severe adverse events with cannabidiol[17-19,21,22].

Meta-analysis of studies17-22, assessing 721 participants, with a follow-up time of 4–16 weeks, evaluated the number of patients with "transaminase elevation (≥3 times the reference)" comparing the use of CBD to placebo. This analysis demonstrated an increased risk of transaminase elevation ≥3 times the reference value in patients who received CBD, as compared to placebo [RD=0.15 (95%CI 0.05–0.24; I2=85%)], NNH=6. Low evidence quality (Analysis 1.8; Figure 10 and Table 2).

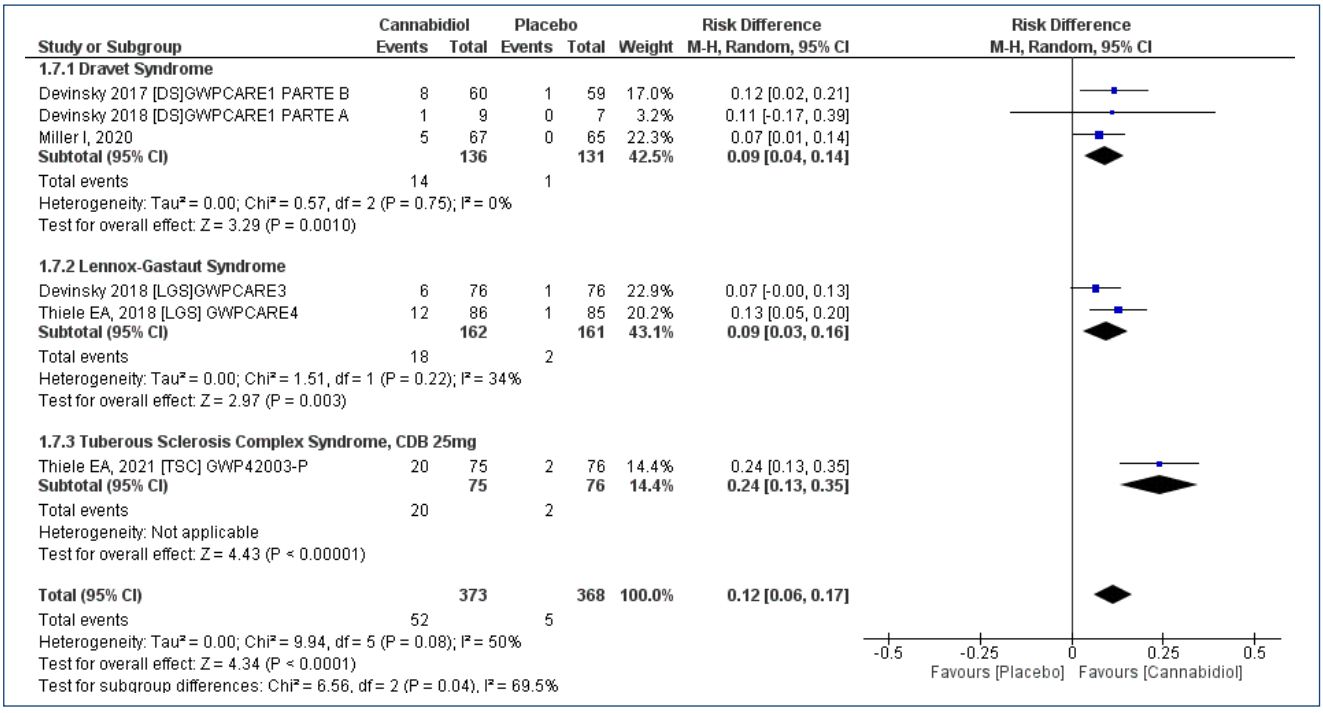

Figure 9. Meta-analysis of the results of the risk of abandonment to cannabidiol treatment[17-19,21,22].

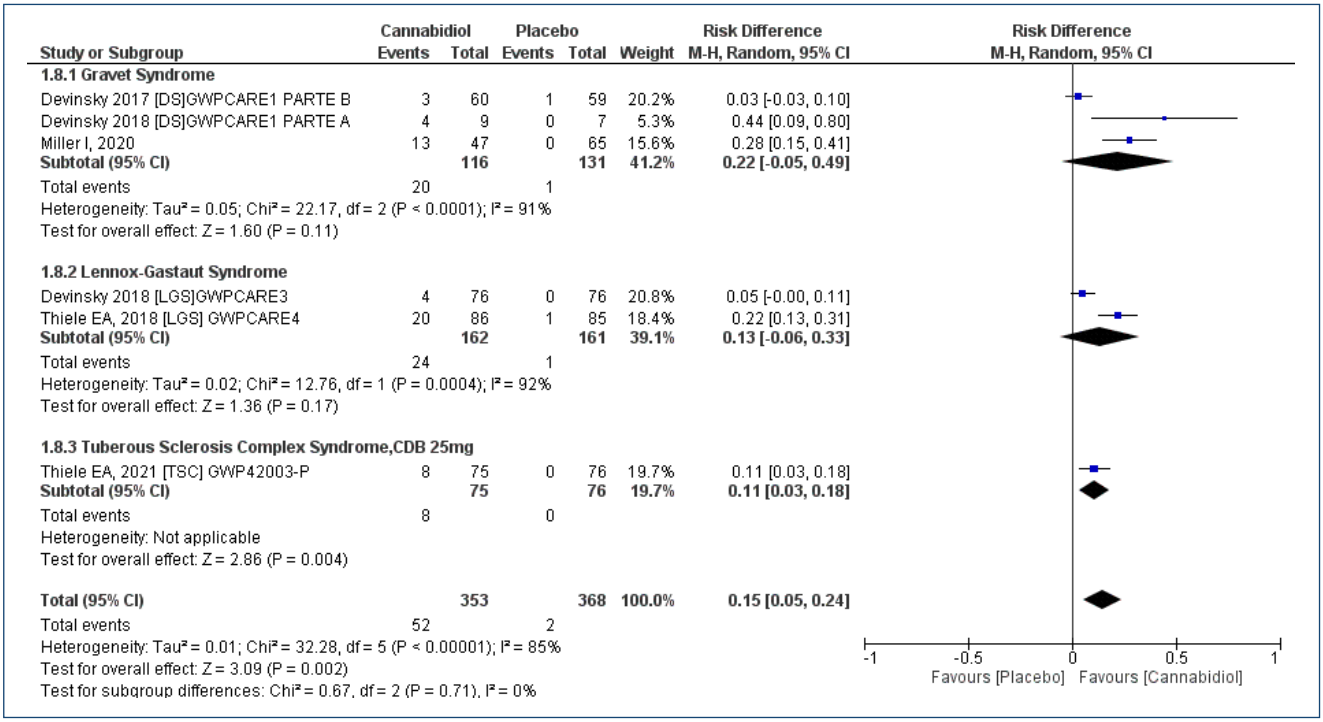

Figure 10. Meta-analysis of the results of the elevation of transaminases ≥3 times the reference[17-19,21,22].

RESULTS OF THE "OPEN-LABEL EXTENSION"

Safety and tolerability

Three OLE studies[23-25] allow the evaluation of treatment-emergent adverse events (TEAEs) in the use of CBD, in different types of primary seizures, and in the long term (median treatment time between 267 and 1,090 days). AEs for LGS, DS, and CTS groups are summarized by pathology in Table 4.

The majority (95.8%) of all patients had at least one TEAE during follow-up; there was no significant difference between disease groups (97% in DS, 96.4% in LGS, and 92% in TSC).

The incidence of severe AEs was much lower in the TSC group (29 [15%]) compared to that in DS (132 [42%]) and LGS (155 [42.3%]) groups; a similar result occurred with the elevation of transaminases (>70% had associated valproic acid). However, we should consider that the follow-up time for CST group [median of 267 (range 18–910) days] was shorter compared to that in DS [444 (18–1,535)] and LGS [1,090 (3–1,421)] groups.

The most commonly reported TEAEs were pyrexia and others related to the gastrointestinal tract, including diarrhea, vomiting, and reduced appetite, but also neurological issues including drowsiness.

Overall, the reported TEAEs, including the observed frequencies and severity, are comparable with previous observations of pivotal assays.

The percentage of patients who permanently discontinued treatment with CBD was 9.4% (n=83). The most common reasons were seizures and increased liver enzymes. Both are events known to cause the discontinuation of CBD treatment.

Summary of evidence

Randomized clinical trials

The use of CBD in patients with DS, LGS, and TSC as compared to placebo, follow-up time of 12–16 weeks:

- Shows an absolute reduction in the frequency of seizures of 33%; three patients for one benefit (NNT=3) are needed. Moderate evidence quality.
- Increases the number of patients with a 50% reduction in the frequency of seizures by 20%; NNT=5. High evidence quality.
- Increases the number of patients with absence of seizures by 3%; NNT=33. Moderate evidence quality.
- Improves the change in S/CGIC by 21%; NNT=5. High evidence quality.
- Increases all AEs by 12%, and it is necessary to treat eight patients to obtain damage (NNH=8). Very low evidence quality.
- Increases serious AEs by 16%; NNH=6. Quality of evidence was moderate.
- Increases the risk of treatment abandonment by 12%; NNH=8. High evidence quality.
- Increases the number of patients with transaminase elevation (≥3 times the reference) by 15%; NNH=6. Low evidence quality.

Observational studies' cohort "open-label extension"

In treatment with CBD of different types of primary seizure, in the long term (follow-up median 1–3 years):

- 95.8% of all patients have at least one TEAE with CBD;
- The rate of severe TEAEs can be up to 36%;

Table 4. Summary of adverse events emerging from cannabidiol treatment for grouped (open-label extension) Lennox-Gataut syndrome, Dravet syndrome, and tuberous sclerosis complex, with median follow-up time of 267–1,090 days.

Emerging adverse events of treatment during OLE				
Type of adverse event	**Dravet syndrome (n=315) n (%)**	**Lennox-Gastaut syndrome (n=366) n (%)**	**Tuberous sclerosis complex (n=199) n (%)**	**Total (n=880) n (%)**
All TEAEs	306 (97)	353 (96.4)	184 (92)	843 (95.8)
Graves TEAEs	132 (42)	155 (42.3)	29 (15)	316 (36)
Abandonment due to adverse events	28 (9)	43 (11.7)	12 (6)	83 (9.4%)
Elevated hepatic transaminases* (ALT or AST) >3 × higher	69 (22%); 58 of which (84%) had concomitant use of valproic acid.	55 (15%); 40 of which (73%) with concomitant use of valproic acid.	17 (9%); 12 of which (71 %) with concomitant use of valproic acid	141 (16)

OLE: open-label extension; TEAE: treatment-emergent adverse event. *Elevations of liver enzymes include only those reported as adverse events.

- Transaminase levels (ALT, AST), ≥3 times the reference, may occur in 16% of patients;
- The most commonly reported TEAEs are pyrexia, diarrhea, vomiting, reduced appetite, and drowsiness;
- The percentage of patients who can permanently discontinue treatment with CBD is 9.4%. The most common reasons are seizures and increased liver enzymes.

These results have a very low evidence quality.

CONCLUSIONS

This systematic review, with meta-analysis, supports the use of CBD in the treatment of patients with seizures, originating in DS, LGS, and TSC, who are resistant to the common medications, satisfactory benefits in reducing seizures, and tolerable toxicity.

AUTHORS' CONTRIBUTIONS

AS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **IF:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **WMB:** 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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https://doi.org/10.1590/1806-9282.20220381

Comment on "Role of monocyte to high-density lipoprotein cholesterol ratio in predicting left atrial enlargement in hypertensive patients"

Like Qiu[1], Lian-Ping He[1], Chang-Wei Liu[2*]

Dear editor,

We are pleased to read the article published by Celik and Karaaslan[1] entitled "Role of monocyte to high-density lipoprotein cholesterol ratio in predicting left atrial enlargement in hypertensive patients". The results of this study revealed that the increased monocyte-to-high-density lipoprotein cholesterol ratio level was associated with hypertension and increased left atrial volume indexes. This study may provide new information on the prevention measure to hypertensive patients with left atrial enlargement. However, according to our opinion, some concerns should be raised.

The main concern is that baseline was inconsistent across two groups (Table 1). The monocyte-to-high-density lipoprotein cholesterol ratio may be increased with age. We found that the mean age in hypertension group was higher than that in the control group (53.1 years vs. 44.8 years). Thus, the age may be a confound factor that has an influence on the relationship between monocyte-to-high-density lipoprotein cholesterol ratio and hypertension. The correlation analysis of this study revealed significant associations between age and monocyte-to-high-density lipoprotein cholesterol ratio. Prior studies in population-based multicenter Danish Cardiovascular Screening trial of elderly men have also found an association between age and left atrium size[2]. The female predominance tended to be higher in the hypertension group than that in the control group (68.7% vs. 60.4%), although there was no significant difference in sex distribution between the two groups. In addition, since this is a prospective study, the relationship of monocyte-to-high-density lipoprotein cholesterol ratio and hypertension cannot be determined.

Another concern is that the inclusion and exclusion criteria for the control group are not provided in "Methods" section. Baseline characteristics of hypertension group and control group should be well balanced. Furthermore, we found a mismatch in gender, body mass index (BMI), and age between the two groups (Table 1).

Table 1. Demographic and clinical features of the study group.

	Overall (n=216)	Hypertension group (n=115)	Control group (n=101)	p-value
Age (year)*	49.2±9.2	53.1±7.7	44.8±8.7	<0.001†
Sex‡				
Female	140 (64.8)	79 (68.7)	61 (60.4)	0.258§
Male	76 (35.2)	36 (31.3)	40 (39.6)	
Diabetes mellitus‡	13 (6.0)	9 (7.8)	4 (4.0)	0.365§
Smoking‡				
Nonsmoker	136 (63.0)	80 (69.6)	56 (55.4)	0.048§
Ex-smoker	35 (16.2)	18 (15.7)	17 (16.8)	
Active smoker	45 (20.8)	17 (14.8)	28 (27.7)	
Alcohol consumption‡	1 (0.5)	0 (0.0)	1 (1.0)	0.468§
BMI (kg/m^2)//	29.4 (16.5–46.2)	31.2 (19.4–46.2)	28.3 (16.5–45.5)	<0.001¶
BSA (m^2)*	1.9±0.2	1.9±0.2	1.8±0.2	0.030†
LAVI (mL)*	37.8±12.1	43.3±12.4	31.4±7.9	<0.001†

BMI: body mass index; BSA: body surface area; LAVI: left atrial volume index; *Mean±standard deviation; †Independent samples t-test; ‡n (%); §Pearson's χ^2 and Fisher's exact test; //Median (range); ¶Mann-Whitney U test.

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CONCLUSION

We found that the mean age in the hypertension group was higher than that in the control group. The female predominance tended to be higher in the hypertension group than in the control group (68.7% vs. 60.4%), although there was no significant difference in sex distribution between the two groups. As age and gender are associated with the left atrium size, they affect the association between monocyte-to-high-density lipoprotein cholesterol ratio and left atrial enlargement in hypertensive patients.

AUTHORS' CONTRIBUTIONS

LQ: Validation, Visualization, Writing – original draft, Writing – review & editing. **LPH:** Methodology, Project administration, Resources, Software, Supervision. **CWL:** Methodology, Project administration, Resources, Software, Supervision.

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https://doi.org/10.1590/1806-9282.20220556

Comment on "Relationship between different body composition and bone mineral density in Qinhuangdao city"

André Pontes-Silva[1,2,3]*

Dear editor,

I read with great appreciation the article entitled "Relationship between different body composition and bone mineral density in Qinhuangdao city" by Zhang et al.[1] In this study, the authors concluded that body composition was related to bone mineral density and bone mineral salt content, and the correlation between different body composition indexes, bone mineral density, and bone mineral salt content was different. The article has a very elegant rationale and I am sure that it will generate further research; however, there are some methodological weaknesses that may compromise the results.

The authors used the body mass index (BMI) to classify participants as normal, overweight, and/or obese. Although BMI is an internationally used index, it is not appropriate to use it to classify healthy individuals (like those in this study), as it is a confounding variable. For example, an individual with stature (height) of 190 cm and body mass (weight) of 108 kg has a BMI of 29.9 kg/m^2; this means the individual is classified as obese (do you agree?). However, if the individual has an above-average muscular component (such as an athlete or someone who does resistance training), then he is not obese, but an outlier—do study participants exercise? If yes, what kind of exercise? Why was not it described?

BMI can be useful for a study with this structure; however, it is necessary to collect other variables (to help in the interpretation of the findings); for example, waist-to-stature ratio (the waist circumference divided by the height in centimeters)[2,3]. This ratio, together with BMI, will help verify if the "obese" are really obese (or just outliers). The problem is that the authors did not present the variables capable of correcting this error: what was the waist perimeter? Mean stature of the participants? Body mass?

In addition, the authors describe that the DEXA was calibrated; however, considering that it is error-sensitive equipment, it is necessary to test the calibration, evaluating the same participants by doubly indirect methods (e.g., skinfolds, bone diameters, and body perimeters). How do you know that DEXA has been calibrated correctly?

I hope that the authors understand my concern about these very fragile findings. Good luck to everyone!

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Comment on "Do biomarkers have predictive value in the treatment modality of the patients diagnosed with bowel obstruction?"

Shuang Cao[1], Ya Wang[1*]

Dear Editor,

We read with great interest a retrospective study entitled "Do biomarkers have predictive value in the treatment modality of the patients diagnosed with bowel obstruction?" by Sahin et al, who investigated the ability of biomarkers to predict surgical treatment and mortality in patients diagnosed with intestinal obstruction[1]. In this study enrolling 179 patients, the authors found a significant predictive value of procalcitonin concentration in predicting treatment strategy and mortality in patients with intestinal obstruction. However, in our opinion, some concerns need further clarification.

First, in Table 1, the standard deviation (SD) of procalcitonin in the conservative management group was 11.1, which was significantly higher than its average value of 2.2. Similarly, the SD of procalcitonin in the surgical management group was also considerably higher than its mean value (15.8 vs. 7.5). Statistically, continuous variables whose SD is significantly greater than its mean are unlikely to be normally distributed data, but skewness distributed data. Therefore, normality test is highly recommended before data analysis so that appropriate statistical methods can be used to obtain more accurate and reliable conclusions.

Second, in Table 1, patients in the surgical management group were older and had more comorbidities than those in the conservative management group. For instance, patients in the surgical management group had more hypertension, coronary artery disease (CAD), renal insufficiency, and congestive heart failure compared with patients in conservative management group. In this case, the higher mortality rate in the surgical management group might be caused by the underlying diseases rather than the elevated procalcitonin concentration.

In addition, previous studies have shown that a variety of diseases can lead to an abnormal elevation in procalcitonin concentration[2,3,4]. A previous study[2] exhibited that procalcitonin was associated with coronary artery severity and proved to be an independent prognostic factor in CAD patients. Another study indicated that procalcitonin concentration can be used as an important serological marker for early systemic bacterial infection in patients with renal insufficiency[3]. Furthermore, an elevation in procalcitonin concentration is also common in patients with heart failure[4]. Thus, it is challenging to conclude whether the increase in procalcitonin was caused indirectly by the underlying diseases or directly by intestinal obstruction. If the elevated procalcitonin is due to the underlying diseases, its role in predicting treatment strategies in intestinal obstruction patients is limited.

AUTHORS' CONTRIBUTIONS

SC: Conceptualization, Investigation, Project administration, Resources, Writing – original draft. **YW:** Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Comment on "Survival outcome of pulmonary metastasectomy among the patients with colorectal cancers"

Jian Huang[1], Jia Zuo[2], AJun Gu[1*]

Dear Editor,

We read with interest the article entitled "Survival outcomes of lung metastases resection in colorectal cancer patients" by Yildiz et al.[1], who summarized prognostic factors after lung metastases resection in colorectal cancer (CRC) patients. We agree with the gist of this review and would like to share some thoughts after careful review and further analysis of this article.

First, we consider this topic to be of practical significance. CRC ranks third in the global ranking of cancer deaths, with more than 1.85 million cases and 850,000 deaths annually. Among newly diagnosed rectal cancer patients, 20% had metastatic disease at presentation, and another 25% developed metastases after localized disease[2]. Therefore, the more accurate diagnostic criteria for CRC are urgently needed in clinical practice. The research findings of Yildiz et al.[1] provide a new biological marker for the prognosis of clinical colon cancer.

We note that the authors included 607 patients with mCRC at the Adana Dr Turgut Noyan Research and Treatment Centre at Baskent University School of Medicine when selecting the sample, and after rigorous screening, only 33 patients met the study criteria. However, the authors did not give detailed sample information, such as the patient's daily diet, drinking water[3], and liver condition. As the daily diet and drinking water will have a greater impact on the serum albumin concentration, errors may occur while determining the albumin concentration. Liver disease[4] can cause a series of complications and body's inflammatory response, resulting in fluctuations in the albumin concentration. Therefore, we recommend the authors to supplement the corresponding information of the samples.

In general, this review provides a valuable clinical reference for the treatment of CRC and helps clinicians understand new prognostic biomarkers for CRC. It clarifies the clinical significance of the combination of lymphocyte count and albumin concentration. However, we believe that it is important to include patient's details, such as daily diet, water intake, and liver condition, and we recommend that these should be displayed in the study in order to enhance the convincing and scientific results.

AUTHORS' CONTRIBUTION

JH: Writing – original draft. **JZ:** Writing – review & editing. **AJG:** Conceptualization, Writing – review & editing.

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Comment on "Comparison of C-reactive protein and C-reactive protein-to-albumin ratio in predicting mortality among geriatric coronavirus disease 2019 patients"

Xiaojing Wang[1], Junna Li[1*]

Dear Editor,

We read with great interest a retrospective cohort study entitled "Comparison of C-reactive protein and C-reactive protein-to-albumin ratio in predicting mortality among geriatric coronavirus disease 2019 patients" by Rohat et al.1, who investigated the value of C-reactive protein and C-reactive protein-to-albumin ratio in predicting mortality in geriatric coronavirus disease 2019 (COVID-19) patients. In this study involving 404 participants, the authors found that both serological indicators were important in predicting mortality in elderly COVID-19 patients. However, in our opinion, we have some concerns which need clarification.

First, a detailed elucidation of the cause responsible for the mortality is extremely necessary. Notably, the population of this study1 was patients over the age of 65 years. In the vast majority of cases, mortality was caused by the underlying diseases (such as chronic obstructive pulmonary disease, coronary heart disease, or malignant tumor)2,3, but not COVID-19. The existence of COVID-19 is likely to be a coincidence and not a major factor responsible for the mortality. Therefore, it is highly recommended to provide detailed information on patient's mortality.

Second, in Table 11, there were significant differences in baseline characteristics between survivor and nonsurvivor patients. Remarkably, the nonsurvivor patients were older (78 years vs. 75 years) and had a higher incidence of congestive heart failure (57.8 vs. 42.2%) and chronic renal failure (65.6 vs. 34.4%) compared with the survivor patients. Advanced age4, congestive heart failure5, and chronic renal failure6 are all important factors leading to a significant increase in mortality. In particular, congestive heart failure and chronic renal failure are also common reasons in patients present to the emergency department. In this case, the abnormal C-reactive protein and C-reactive protein-to-albumin ratios are most likely due to congestive heart failure and chronic renal failure, but not COVID-19. Therefore, it is reasonable to assume that there is no definite correlation between the abnormal C-reactive protein-to-albumin ratio and COVID-19. From our perspective, one of the best solutions is to adjust potential confounding factors when ROC curve was calculated, in order to truly obtain the value of C-reactive protein and C-reactive protein-to-albumin ratio in predicting mortality in elderly COVID-19 patients.

AUTHORS' CONTRIBUTIONS

XW: Conceptualization, Writing – original draft. **JL:** Conceptualization, Writing – original draft.

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ORIGINAL ARTICLE

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Evaluation of cytokine levels as possible predicting elements in patients with chronic lymphocytic leukemia

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SUMMARY

OBJECTIVE: This study aimed to assess the patterns of serum cytokines in chronic lymphocytic leukemia patients at baseline and post-chemotherapy and investigate their association with response to treatment and chronic lymphocytic leukemia prognosis.

METHODS: Blood samples were taken from 32 subjects at their first medical visit after being diagnosed with chronic lymphocytic leukemia and 1 year after chemotherapy. Then, levels of cytokines and blood parameters in peripheral blood were measured. Correlation analysis was used to assess the indexes before and after chemotherapy as well as at different disease stages.

RESULTS: Most of the patients (45.80%) had stages I and III before initiation of treatment and after treatment, respectively. There were significant differences between levels of interleukin (IL)-6 (p=0.006) and IL-10 (p=0.009) before and after treatment. Notably, the difference in IL-10 levels before and after treatment was significantly higher in the advanced stages compared to that in the non-advanced stages (p=0.007). IL-6 and IL-10 were also higher in the expired patients compared to the survived cases.

CONCLUSIONS: Cytokines such as IL-6 and IL-10 may be considered predicting factors for chronic lymphocytic leukemia prognosis.

KEYWORDS: Chemotherapy. Cytokines. Lymphomas. Prognosis.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is considered the most prevalent adult leukemia[1]. CLL is accompanied by dysregulation of the cytokine profile and immune response[1]. Evidence from *in vitro* experiments indicated gene upregulation of inflammatory cytokines in the presence of CLL cells[2]. As recently demonstrated, chronic inflammation contributes to cancer progression and even plays a role in cancer predisposition[1]. An inflammatory microenvironment of CLL cells was suggested to be involved in the prolonged survival of the malignant cells[2]. Thus, inflammatory conditions have been suggested to be important in the pathogenesis of CLL[1,2]. The molecular interactions of adaptive immune cells (B and T cells) and innate immune cells (macrophages, dendritic, and nurse-like cells) with CLL cells contribute to the formation of a tumor-supportive microenvironment[1].

Immunologic abnormalities in CLL, such as the release of inflammatory (such as tumor necrosis factor [TNF] and interleukin [IL]-6, IL-8, IL-17) and anti-inflammatory cytokines (such as IL-10, IL-13, IL-4, and transforming growth factor β), have been linked to type 1 and type 2 diabetes, as well as complications such as diabetic ketoacidosis and nephropathy[1,3]. T-cell abnormalities, monocyte activation, and inflammatory markers were assumed to play a role in the loss of insulin secretory function by islet cells in this context[3,4]. Interestingly, recent studies have proposed an association between insulin resistance and microvascular complications with levels of specific cytokines such as omentin and neuregulin-4[5-7].

Aberrant secretion of multiple inflammatory and immunosuppressive cytokines, such as IL-10, TNF-α, and chemokine (C-X-C motif) ligand 9, has been linked to worsening CLL conditions[1]. Secreted inflammatory cytokines such as IL-6 and IL-8 are thought to affect the CLL microenvironment and thus facilitate CLL development[8]. T-helper cells (Th), monocytes, and other immune cells produce IL-10, an anti-inflammatory and immunoregulatory cytokine that has strong immunosuppressive effects by suppressing the synthesis of Th1 cytokines such as TNF-α. IL-6 is released by a variety of cell types such as monocytes, normal hematopoietic cells, and lymphocytes[1]. Serum cytokine

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patterns were suggested to reveal the underlying immune and inflammatory mechanisms involved in the survival and migration of CLL cells[9]. Hence, this study was designed to determine the role of cytokine status as a biomarker of treatment response and to investigate its association with CLL prognosis.

METHODS

Study design

This study was conducted in the Hematology and Oncology Department, Imam Reza Hospital, Mashhad, Iran, and enrolled 32 patients (14 females and 18 males) with a definite CLL diagnosis. Informed consent was obtained from the patients, and the study was approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1396.600). All patients received either chlorambucil-based or RCHOP treatment regimens. The patients were followed up every 3 months for 1 year. A medical interview and a complete physical examination were performed as part of the follow-up examinations to classify the disease stages.

Blood samples

Peripheral venous blood samples (4 mL) were taken from CLL patients at the initial medical visit following the diagnosis of CLL and again 1 year later.

Determination of serum levels of cytokines (IL-6 and IL-10) in CLL patients

The IL-6 and IL-10 serum levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA, and ENDOGEN Inc., Cambridge, MA, USA). For this purpose, the serum samples were dispensed (in triplicates) into the 96-well plate pre-coated with monoclonal anti-IL-6 and anti-IL-10 antibodies. To remove the unbound subjects, the plate was washed, followed by the addition of a conjugate solution containing polyclonal anti-IL-6 and IL-10 antibodies conjugated to horseradish peroxidase. Afterward, a substrate solution containing stabilized hydrogen peroxide and stabilized chromogen was added. Finally, the stop solution was added to terminate the reactions, and the absorbance was read using a microplate reader (BioTek, USA) at a wavelength of 450 nm. Finally, the cytokine levels were determined using the standard curves and presented as pg/mL.

Statistical analysis

The collected data were statistically analyzed using the SPSS software version 16 (17.0.1, SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov tests were performed to assess data normality. To compare the differences in median and mean of serum IL-6 and IL-10 before and after treatment, Wilcoxon signed-ranks and paired samples tests were applied. Furthermore, the independent samples test and Mann-Whitney U-test were used to compare the mean and median of cytokines among disease stages. The results were presented as mean±SD. A p-value less than 0.05 was considered significant.

RESULTS

This study was performed on 32 CLL patients including 58.3% males and 41.7% females, with a mean age of 68.25±1.2 years (Table 1). B-symptoms (including severe

Table 1. Demographic and clinical features of chronic lymphocytic leukemia patients.

Parameters	Amount
Age, years, n (mean±SD)	68.25±1.2
Sex	
Female, n (%)	14 (41.7)
Male, n (%)	18 (58.3)
Rai stage of disease (before treatment), n (%)	
0	2 (8.3)
I	11 (45.8)
II	3 (12.5)
III	4 (16.7)
IV	4 (16.7)
Rai stage of disease (after treatment), n (%)	
0	0 (0)
I	6 (25)
II	2 (8.3)
III	10 (41.7)
IV	6 (25)
B symptoms	
Yes, n (%)	18 (75)
NO, n (%)	6 (25)
Treatment regimen	
Chlorambucil, n (%)	15 (62.5)
RCHOP, n (%)	2 (8.3)
NO change n (%)	7 (29.2)
Changed in the middle of treatment, n (%)	7 (29.2)
Changed at the beginning of treatment, n (%)	10 (41.7)
Recurrence, n (%)	7 (29.2)

RCHOP: rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine (Oncovin), prednisolone.

fatigue, weight, fevers ≥38.0°C, and night sweats) were observed in 25% of the patients.

As presented in Table 1, most of the patients (45.80%) had stage I of the disease before initiation of treatment. However, most of the patients experienced stage III of the disease during the follow-up (45.80) and after treatment (41.70).

The median values for the IL-6 serum levels before the initiation of treatment and after treatment were 0.045 pg/mL (range of 0.01–166.20 pg/mL) and 1.90 pg/mL (range of 0.08–106 pg/mL), respectively. In the case of IL-10 serum levels, the median values before the initiation of treatment and after treatment were 1.25 pg/mL (range of 0.00–38.10 pg/mL) and 2.85 pg/mL (range of 0.03–43 pg/mL), respectively. Before the initiation of treatment, serum levels of IL-6 and IL-10 were significantly lower than those in post-chemotherapy (p=0.006 and p=0.009, respectively).

Table 2 shows IL-6 and IL-10 serum levels in CLL patients with advanced (III, IV) and early stages (0, I, II). Cytokine levels in patients with different disease stages were evaluated to see if they could be used to identify patients based on disease stage and severity. There was no significant difference in IL-6 and IL-10 serum levels between patients with advanced stages and those with early stages before treatment starts. After treatment, IL-6 and IL-10 serum levels were found to be higher in patients with advanced stages than in patients with early stages, although the differences were not statistically significant. The median value for the difference between IL-10 before and after treatment was significantly higher in the advanced stages compared to that in the nonadvanced stages (p=0.007). However, the median value for the difference between IL-6 before and after treatment did not show any significant difference in the advanced stages compared to that in the early stages (p=0.06).

As shown in Table 3, the median IL-6 level in non-survivors was significantly higher than that in survivors following the chemotherapy (2.95 [166.20–0.06] vs. 0.03 [6.20–0.01], p=0.006). Similarly, the mean IL-10 level in the dead patients was higher than in the alive patients (23.83±17.55 vs. 2.67±6.7, p=0.008) before treatment initiation.

DISCUSSION

In this study, we determined the serum levels of IL-6 and IL-10 to investigate their association with response to treatment and CLL prognosis. Our findings revealed an increasing pattern of IL-6 and IL-10 levels after chemotherapy. These results were in line with the previous studies[10,11]. Zhu et al. reported higher levels of IL-6 in the plasma of CLL patients who received chemotherapy compared to untreated controls in this regard[10]. Another recent study also showed that an increased production of autocrine IL-6 was associated with a worse clinical course of CLL[11].

Interestingly, our results showed that IL-10 levels were significantly higher in the end stages of the disease (Rai stage III or IV). Evidence from cellular experiments confirmed the secretion of IL-6 and IL-10 in the supernatant of cultured CLL[8,12,13]. There are some reports suggesting that IL-6 in the CLL microenvironment inhibits proliferation but prolongs survival through suppressing apoptosis of CLL

Table 2. Comparison of cytokine levels in the chronic lymphocytic leukemia stages.

Cytokines	Status	Early stages (0, I, II), median (min–max)	Advanced stages (III, IV), median (min–max)	p-value+
IL-6	Before treatment	0.035 (0.01–166.20)	0.06 (6.20–0.01)	0.90
	After treatment	3.1 (106.00–0.6)	1.10 (3.50–0.08)	0.05
	Difference of before and after treatment (ΔIL-6)	1.70 (-6.90–60.20)	0.73 (-2.03–2.70)	0.06
IL-10	Before treatment	1.55 (0.01–16.00)	1.15 (38.10–0.05)	0.64
	After treatment	1.35 (18.00–0.03)	3.65 (43.00–0.90)	0.06
	Difference of before and after treatment (ΔIL-10)	0.065 (-3.25–5.60)	2.62 (-36.10–2.00)	0.007

IL: interleukin. +Mann-Whitney U-test.

Table 3. The comparison of the levels of IL-6 and IL-10 between chronic lymphocytic leukemia non-survivors and survivors.

Cytokine, Value	Dead	Alive	p-value
IL-6, median (min–max)	2.95 (166.20–0.06)	0.03 (6.20–0.01)	0.006+
IL-10, mean (mean±SD)	23.83±17.55	3.67±2.32	0.008#

IL: interleukin. +Mann-Whitney U-test. #Independent samples test.

cells[1,11,14]. *In vitro* studies showed that secretion of specific inflammatory cytokines like IL-6 alters the biology of the CLL microenvironment, which correlates with progressive CLL[8]. el-Far et al. claimed prognostic values of IL-10 and IL-6 for non-Hodgkin's lymphoma[15]. These findings support the idea that inflammatory and regulatory cytokines and related immune molecules have been raised in the serum of CLL patients[9]. There is accumulating evidence suggesting that the secretion of certain autocrine cytokines, such as IL-6 and IL-10, may contribute to the severe course of CLL[16]. In this regard, Fayad et al. showed the correlation of IL-6 and IL-10 serum levels with the severity of the disease[16]. Furthermore, an increased percentage of monocytic myeloid-derived suppressor cells producing IL-10 in CLL patients was reported at Rai I/II and III/IV stages. IL-10, an immunoregulatory cytokine, inhibits the production of other cytokines and suppresses immune responses[1]. It seems that IL-10 secretion in CLL patients may be associated with the suppression of the immune response against CLL[13]. Recent evidence indicates poor prognosis and outcome of the disease, accompanied by increased levels of IL-6 and IL-10 in the advanced stages of CLL[17-19]. Hence, in this study, aberrant secretion of IL-6 and IL-10 seems to be involved in worsening the CLL conditions.

In this study, we found that the expired patients had much greater levels of IL-6 and IL-10 than the survivors. Consistently, IL-6 or IL-10 serum levels in CLL patients were shown to be associated with adverse disease features and 3-year survival[16].

Based on our data, the median IL-6 levels in survived and dead patients were 2.95 and 0.03 pg/mL, respectively. Similarly, another study proposed that survival chance was higher in patients with the IL-6 level less than 3 pg/mL[18]. Wang et al. also reported a higher mean value of IL-6 linked to shorter progression-free survival[11].

Regarding the IL-10 level, a higher value was observed in the expired patients compared to the survived patients. Consistent with our findings, according to Fayad et al., the 3-year survival rate of CLL patients with high levels of IL-10 was 45%, while it was 85% in those with low levels of IL-10. They proposed an IL-10 cutoff of greater than 10 pg/mL as a predictor of poor prognosis[16]. Our data support the previous findings[9,15]. In this context, Yan et al. indicated a higher level of IL-10 with reduced survival[9]. el-Far et al. indicated higher levels of IL-6 and IL-10 with poor survival[15]. Therefore, IL-6 and IL-10 may predict the outcome of patients with CLL.

CONCLUSIONS

Our findings revealed that serum levels of IL-6 and IL-10 increased in parallel with the CLL progression, with high levels of the particular cytokines in non-survivors. Hence, more aggressive treatments are recommended in patients with higher levels of cytokines. However, more studies on the mechanisms that contribute to CLL resistance and strategies to overcome them are required.

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¶These authors share their first co-authorship.

AUTHORS' CONTRIBUTIONS

MK: Data curation, Formal Analysis. **AR:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **AA:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **FLA:** Writing – original draft, Writing – review & editing. **NA:** Writing – original draft, Writing – review & editing. **AA:** Supervision.

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ORIGINAL ARTICLE

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Predictive value of oxidative, antioxidative, and inflammatory status for left ventricular systolic recovery after percutaneous coronary intervention for ST-segment elevation myocardial infarction

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SUMMARY

OBJECTIVE: This study aimed to evaluate the association between left ventricular ejection fraction recovery and the total oxidant status, total antioxidant capacity, and high-sensitivity C-reactive protein levels.

METHODS: A total of 264 ST-elevation myocardial infarction patients were classified into two groups according to baseline and 6-month follow-up left ventricular systolic function: reduced and recovery systolic function. Predictors of the recovery of left ventricular ejection fraction were determined by multivariate regression analyses.

RESULTS: Multivariable analysis indicated that oxidative status index, baseline left ventricular ejection fraction and peak creatine-kinase myocardial bundle level, and high-sensitivity C-reactive protein were independently associated with the decreased of left ventricular ejection fraction at 6-month follow-up.

CONCLUSION: Oxidative stress and inflammation parameters were detrimental to the recovery of left ventricular ejection fraction in patients with ST-elevation myocardial infarction.

KEYWORDS: Ventricular dysfunction. Oxidants. Antioxidants. ST elevation myocardial infarction.

INTRODUCTION

Primary percutaneous coronary intervention (p-PCI) is recommended as the preferred reperfusion strategy for acute ST-segment elevation myocardial infarction (STEMI) patients who are admitted within the first few hours after the initiation of symptoms[1]. Unfortunately, a significant proportion of patients undergoing STEMI remain with reduced left ventricular systolic function (LVSF)[2]. LVSF is the most important prognostic indicator of in-hospital and long-term mortality of patients with STEMI[3]. Therefore, early identification of these patients is vital because early interventions such as more intense anti-remodeling therapy, close follow-up, and implantation of automated cardioverter-defibrillator may be beneficial for these patients[3].

Several underlying mechanisms including local ischemia and myocardial cell death, oxidative stress and inflammation in the injured myocardial tissue, cardiodepressive effects of reactive oxygen species (ROS) and inflammatory cytokines, changes in the extracellular matrix in response to metalloproteinase activation, structural changes due to mechanical stress, and increased synthesis of collagen and myocardial fibrosis are responsible for the pathogenesis of LV remodeling[4-6]. These processes are interrelated and enable the advancement of the disease from acute to chronic. Furthermore, oxidative stress and inflammation play an essential role in the apoptotic and necrotic death seen in cardio-myositis[4,5]. In this study, we aimed to evaluate the association between total oxidative status (TOS), total antioxidative capacity (TAC), and high-sensitivity C-reactive protein (hs-CRP) in the development of left ventricular systolic dysfunction (LVSD) in patients presenting with STEMI.

METHODS

Study population

This cohort study initially recruited patients with a first STEMI. A total of 1980 adult patients presenting with STEMI between

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February 2010 and April 2016 were screened. Patients with a diagnosis of acute myocardial infarction (MI) based on clinical, electrocardiographic, and cardiac biomarker criteria[7] and an echocardiographic LVEF ≤0.40 were included. Transthoracic echocardiography was performed in each patient before randomization, and the echocardiographic LVEF was determined by the Simpson method. The inclusion criteria also included successful PCI (defined as Thrombolysis in Myocardial Infarction [TIMI] flow grade 3 and residual stenosis of the infarct-related artery 30%) performed 12 h after the onset of symptoms and informed consent to perform echocardiography at three predefined time points.

Exclusion criteria were defined as clinical signs of congestive heart failure or cardiogenic shock in the first week after infarction, other significant cardiac diseases, EF >0.40, life-limiting noncardiac disease, Killip class IV heart failure, prior MI, severe chronic obstructive pulmonary disease, and symptomatic peripheral arterial disease. Based on these criteria, 1716 patients were excluded: 1561 due to EF >0.40, 6 with Killip class IV heart failure, 15 due to the presence of a re-flow phenomena, 2 with severe chronic obstructive pulmonary disease, 10 because of prior heart failure, and 30 with previous MI. In addition, 5 patients died before randomization and 5 refused to participate, 47 patients were excluded due to noncompliance in the follow-up, 35 patients were excluded due to side effects or noncompliances of drugs. Therefore, a total of 264 patients (aged 23–91 years) were included in this study. At discharge, patients were administrated medical therapy according to contemporary guidelines[8,9]. Clinical and echocardiographic evaluations were repeated at 6 months according to the institutional guideline-based pre-hospital, in-hospital, and outpatient clinical care track protocol (MISSION!)[10]. Afterward, the patients were divided into two groups according to their LVEF at 6-month follow-up: LVEF ≤40% (nonrecovery) and LVEF >40% (recovery) (Figure 1). Clinical data were collected in the Cardiology Department (Microsoft access system) and hospital Information System (Enlilsoft®). The study complies with the Declaration of Helsinki of 1975, as revised in 1983. The Institutional Review Board approved the study, and written informed consent was obtained from all subjects.

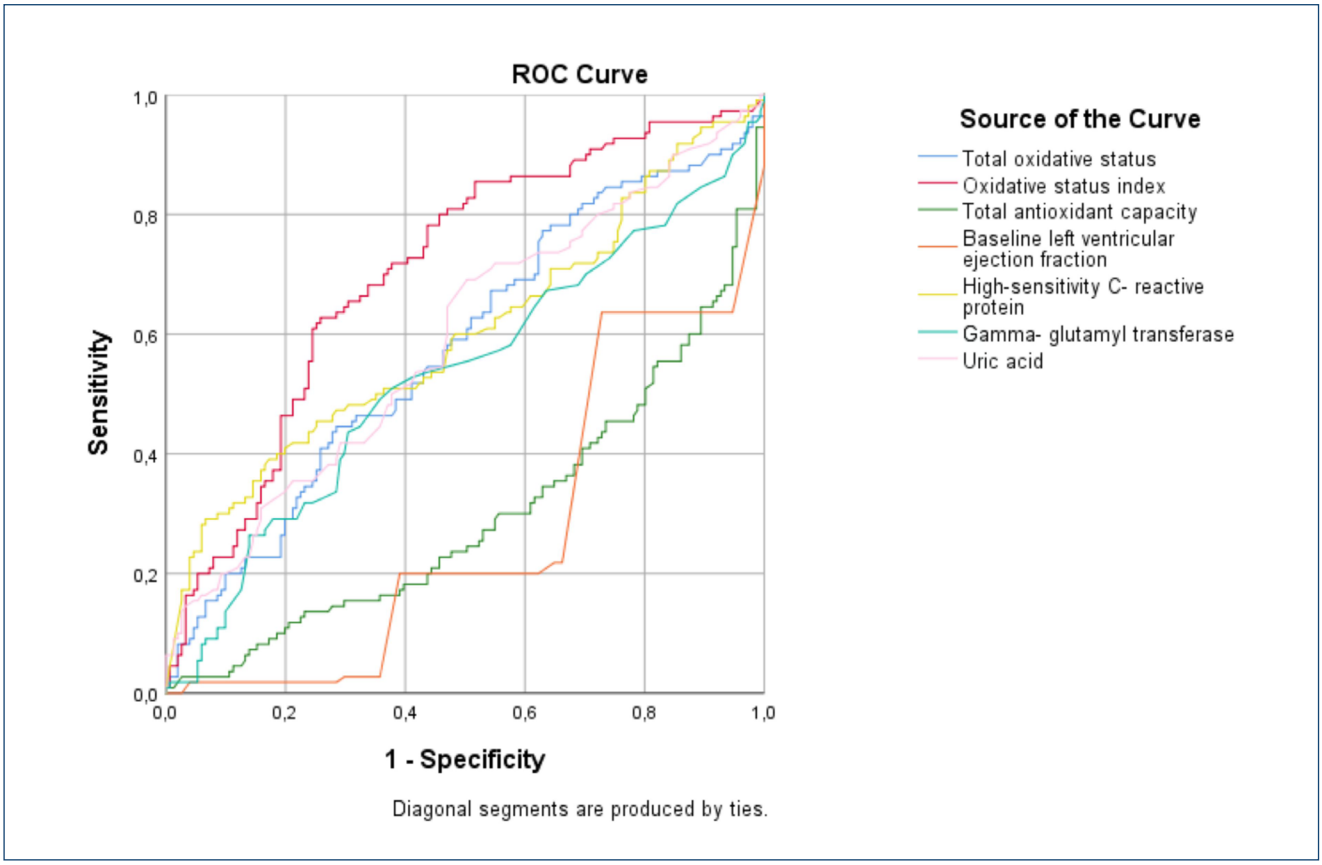

Figure 1. Receiver operating characteristic curve with calculated area under the curve and optimal cutoff point for oxidative status index, total antioxidant capacity, total oxidative status, baseline left ventricular ejection fraction, high-sensitivity C-reactive protein, gamma-glutamyltransferase, and uric acid to identify the recovery of left ventricular ejection fraction.

Transthoracic echocardiography protocol

Transthoracic echocardiography was performed within 12 h of admission and at the completion of the study procedures using a commercially available system (X5-1 probe, IE33, Philips, Andover, MA, USA) with a 3.5-MHz or M5S transducer from standard parasternal and apical transducer positions with two-dimensional frame rates of 60–100 frames/s and tissue Doppler frame rates >100 frames/s. Standard M-mode, 2D, color, pulsed, and continuous-wave Doppler images were acquired and stored digitally for subsequent off-line analysis (Xcelera, Phillips Healthcare) by two echocardiography specialists blinded to the study time point, treatment allocation, and oxidative and antioxidant status values. The LVEF was calculated in the apical four- and two-chamber views using Simpson's biplane method[11].

Laboratory analysis

Serum total oxidative stress (TOS), TAC levels, hs-CRP, gamma-glutamyltransferase (GGT), and uric acid (UA) levels were measured at baseline. Decreased TAC and increased TOS and UA levels were used as markers of oxidative stress, and increased hs-CRP was used as a marker of inflammation. Laboratory analysis was performed as stated in the previous study[12-14]. TAC and TOS levels were determined with a spectrophotometric kit (Rel Assay Diagnostics, Gaziantep, Turkey) and read in an auto-analyzer (Olympus AU2700; Olympus, Tokyo, Japan). The TAC and TOS levels were expressed as mmol Trolox equivalent/L and mmol H_2O_2 equivalent/L, respectively. The oxidative status index (OSI) is defined as the ratio of TOS to TAC levels, expressed as a percentage. For the calculation of OSI, TAC units were represented as mmol/L, and the OSI value calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol H_2O_2 equiv./L)/TAC (mmol Trolox equiv./L)[12-14].

Statistical analysis

The SPSS version 16.0 software package was used for statistical analyses in this study. Categorical variables were expressed as frequency (%) and compared using the χ^2 test. Kolmogorov-Smirnov test was used to test the distribution of numeric variables; those with normal distribution were expressed as mean±standard deviation and were compared with Student's t-test. Data without normal distribution were expressed as median (interquartile range of 25–75% percentiles) and were compared with the Mann-Whitney U-test. If groups were more than two, continuous variables were compared using one-way ANOVA or the Kruskal-Wallis test. In all statistical analyses, p-value <0.05 was considered as statistically significant. Regression and receiver operating characteristics (ROC) curve analysis was performed as stated in the previous study[14].

RESULTS

Of the 1980 patients with acute STEMI examined, 264 patients with an LVEF ≤40% in admission were included. The mean age of the patient population was 62.08±12.8 years (range 23–91) and 81% were males (Table 1). In 143 (47.7%) patients, the only culprit lesion was in the left anterior descending coronary artery. All patients received the treatment considered appropriate by the current guidelines at discharge. Echocardiographic data obtained within 24 h of admission are presented in Table 1. The mean initial LVEF was 30.6±4.3%, while the mean follow-up LVEF was 42±8.2%. Moderate-to-severe mitral regurgitation was observed in 59 (23%) patients. At 6-month follow-up, 129 (48%) patients did not show any recovery of LVSF, and the LVEF remained ≤40%. The remaining 135 (52%) patients showed LVSF recovery (Table 1).

Univariate and multivariate analyses were performed to evaluate the correlates of reduced LVEF (<40%) at 6-month follow-up. Univariate analysis showed that peak troponin T, peak CK-MB, blood urea nitrogen (BUN), TOS, TAC, OSI, UA, hs-CRP levels, age, initial heart rate, and baseline LVEF were significantly correlated with LVEF recovery. Multivariate analysis showed that OSI (odds ratio [OR] 1.12, 95% confidence interval (CI) (1.06–1.18); p<0.001), baseline LVEF (OR 0.85, 95%CI 079–0.91; p=0.006), and peak CK-MB level (OR 1.004, 95%CI 1.002–1.006; p<0.001) were independently associated with normalization of LVEF (>40%) at 6-month follow-up (Table 2). ROC curve analysis showed that OSI (C-statistic 0.723; 95%CI 0.66–0.77, p<0.001), TOS (C-statistic 0.579; 95%CI 0.52–0.63, p<0.001), TAC (C-statistic 0.719; 95%CI 0.66–0.76, p<0.001), initial LVEF (C-statistic 0.734; 95%CI 0.68–0.78, p<0.001), hs-CRP (C-statistic 0.59; 95%CI 0.52–0.67, p=0.006), and UA (C-statistic 0.59; 95%CI 0.52–0.66, p=0.012) were significant predictors of LVEF recovery following STEMI (Figure 1). We calculated the cutoff point of 20 for OSI, 1.3 for TAC, 25 for TOS, 30 for initial LVEF, 54 for hs-CRP, and 5.88 for UA to estimate the LVEF recovery following STEMI, with a sensitivity of 64, 56, 76, 78, 29, and 68% and a specificity of 75, 78, 39, 67, 93, and 49%, respectively.

DISCUSSION

The main finding of this study is the association of oxidant/antioxidant status and inflammation parameters with the recovery of LV functions in patients presenting with acute STEMI.

Table 1. Demographic and clinical characteristics of patients with and without depressed left ventricular ejection fraction.

	Group I (n=128)	Group II (n=136)	p-value
Baseline characteristics			
Female gender, n (%)	27 (21.1)	25 (18.4)	0.345
Diabetes mellitus, n (%)	31 (24.2)	39 (28.7)	0.248
Hypertension, n (%)	65 (50.8)	55 (40.4)	0.059
Hyperlipidemia, n (%)	33 (25.8)	31 (22.8)	0.336
Smoking, n (%)	74 (57.8)	88 (64.7)	0.153
Obesity, n (%)	46 (35.9)	52 (38.2)	0.398
Age (years)	64.8±12.7	61.1±12.7	0.017
Systolic blood pressure (mmHg)	123.00±26.6	123.6±29.0	0.849
Diastolic blood pressure (mmHg)	74.4±15.8	73.5±14.1	0.634
Heart rate (bpm)	79.6±19.0	75.9±16.9	0.96
Weight (kg)	73.2±13.5	76.3±12.4	0.056
Length (cm)	165.64±7.5	167.1±7.5	0.108
BMI	26.2±4.0	26.8±3.9	0.235
Waist circumference (cm)	91.6±8.3	93.5±7.6	0.06
Previous treatment, n (%)			
RAS blockers	45 (18.0)	8 (16.0)	0.458
β-Blockers	25 (19.5)	17 (12.5)	0.082
Statins	19 (14.8)	14 (10.3)	0.176
Medication at discharge, n (%)			
ACEi/ARBs			
β-Blockers	125 (98)	134 (99)	0.481
Statins	121 (95)	127 (94)	0.483
Antiplatelet	121 (95)	131 (97)	0.430
Aldosterone antagonists	128 (100)	136 (100)	0.622
Diuretics	40 (31)	13 (10)	<0.001
Device therapy, n (%)			
Cardiac resynchronization therapy	20 (15)	4 (3)	<0.001
Implantable-cardioverter defibrillator	8 (7)	2 (2)	<0.001
Localization of MI, n (%)			<0.001
Anterior	80 (62.5)	47 (37.6)	
Nonanterior	48 (37.5)	89 (65.4)	
Infarct-related artery, n (%)			<0.001
LAD	70 (66.0)	46 (38.0)	
Cx	13 (12.3)	19 (15.7)	
RCA	23 (21.7)	56 (46.3)	
Duration of CCU stay (day)	2.2±0.8	2.0±0.5	0.02
Laboratory findings			
Total antioxidant capacity	1.30±0.2	1.51±0.2	<0.001
Total oxidant capacity	29.55±5.72	27.69±4.99	0.005
OSI	23.51±6.32	18.99±5.50	<0.001
hs-CRP (mg/L), median (IQR)	38.5 (8.7–60.00)	12.7 (6.72–15.00)	<0.001
Uric acid (mg/dL)	6.5±1.6	5.9±1.3	0.001

Continue...

Table 1. Continuation.

	Group I (n=128)	Group II (n=136)	p-value
LDL (mg/dL)	108.2±36.5	107.3±34.4	0.843
HDL (mg/L), median (IQR)	43.00 (35.00–48.00)	39.00 (33.00–44.00)	0.006
Trigliserit (mg/dL)	123.2±62.9	152.6±126	0.017
Total cholesterol (mg/dL)	175.8±41.6	176.1±42.4	0.959
Initial creatinine (mg/dL)	1.1±0.3	1.0±0.2	0.004
BUN	21.08±8.62	17.8±5.8	< 0.001
Initial glucose (mg/dL)	179.3±100	167.4±77	0.280
Hemoglobin (mg/dL)	14.3±2.6	14.2±1.7	0.802
Platelet count	237±80	233±66	0.659
Initial CK-MB (U/L)	36.5 (22.00–69.75)	29.00 (20.00–50.00)	0.009
Initial troponin (ng/mL)	0.18 (0.40–0.58)	0.09 (0.01–0.44)	0.037
Peak CK-MB (U/L)	250.00 (100.00–368.00)	135.00 (81.00–234.00)	<0.001
Peak troponin (ng/mL)	5.5 (2.8–9.4)	3.1 (1.1–5.9)	< 0.001
Echocardiographic parameters			
LV diastolic diameter (mm)	50.8±4,5	48.5±3.5	<0.001
LV systolic diameter (mm)	36.6±4.9	32.2±5.0	<0.001
IVS (mm)	11.6±1.1	11.3±1.1	0.07
Left atrial diameter (mm)	40.3±3.2	39.4±3.7	0.04
LV ejection fraction at baseline (%)	28.9±4.0	32.2±4.0	< 0.001
LV ejection fraction in the first year (%)	34.8±4.3	48.8±4.5	<0.001
LV mass index (g/m)	117±33	104±32	<0.001

BMI: body mass index; RAS: renin–angiotensin system; ACEi/ARBs: angiotensin-converting enzyme inhibitors/ angiotensin ii receptor blockers; MI: myocardial infarction; LAD: left anterior descending artery; Cx: circumflex; RCA: right common artery; CCU: coronary care unit; OSI: oxidative status index; hs-CRP: high-sensitivity C-reactive protein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; IQR: interquartile range; BUN: blood urea nitrogen; CK-MB: creatine kinase myocardial bundle; LV: left ventricle; IVS: interventricular septum; GFR: glomerular filtration rate, BUN: blood urea nitrogen. Group I: patients with depressed left ventricle ejection fraction; Group II: Patients with recovery left ventricle ejection fraction after 1 year to the ST-segment elevation myocardial infarction. Data presented as mean±SD or number (%) of the patients.

Table 2. Univariate and multivariate regression analysis of predictors of left ventricular recovery in the study population.

	Unadjusted odds ratio	Confidence interval	p-value	Adjusted odds ratio	Confidence interval	p-value
TOS	1.05	1.00–1.09	0.017			
TAS	0.05	0.02–0.13	<0.001			
OSI	1.14	1.09–1.19	<0.001	1.12	1.06–1.18	<0.001
Age	1.03	1.01–1.04	0.002			
ACEi	3.48	1.39–8.67	0.007			
CK-MB peak	1.004	1.002–1.005	<0.001	1.004	1.002–1.006	<0.001
Troponin peak	1.06	1.01–1.12	0.014			
BUN	1.06	1.02–1.10	0.001			
Uric acid	1.27	1.07–1.50	0.005			
hs-CRP	1.01	1.01–1.02	<0.001	1.016	1.005–1.028	0.006
Heart rate	1.01	0.99–1.02	0.07			
LV ejection fraction (baseline)	0.80	0.75–0.85	<0.001	0.85	0.79–0.91	<0.001

TOS: total oxidative status; TAS: total antioxidative status; OSI: oxidative status index; ACEi: Angiotensin-converting enzyme inhibitors; CK-MB: creatine-kinase myocardial binding; BUN: blood urea nitrogen; hs-CRP: high-sensitivity C-reactive protein; LV: left ventricle.

The other findings of this study are OSI, baseline LVEF, and peak CK-MB level were independently associated with normalization of LVEF (>40%) at 6-month follow-up and ejection fraction alteration (ΔEF) at 6-month follow-up was positively correlated with TAC and negatively correlated with TOS, OSI, baseline LVEF, hs-CRP, and UA.

Consistent with our data, previous studies have suggested that oxidative stress, increased inflammation, and decreased antioxidant capacity are associated with poor cardiovascular outcomes[4]. In contrast, we evaluated a possible role of TAC, TOS, and inflammatory status in LV systolic recovery after a first STEMI. We found that increased inflammation and TOS and decreased TAC might pave the way for permanent myocardial dysfunction in patients with STEMI. This study results showed that depending on the underlying inflammation, oxidant and antioxidant status accompanying myocardial contractile dysfunction might contribute to LVSD in patients with STEMI.

Borekci et al.[15] have demonstrated that OSI, UA, and neutrophil-to-lymphocyte ratio were associated with spontaneous reperfusion in patients with STEMI. Similarly, Turan et al.[16] have reported that plasma TOS and OSI were associated with the complexity and severity of coronary artery disease in patients with acute coronary syndrome. Additionally, we have previously reported a positive association between the development of atrial fibrillation after STEMI and TAC, TOS, and OSI[17]. However, none of these studies addressed the relationship between LVSD and oxidative stress markers in patients with STEMI. Data from this study suggest that in STEMI patient population, plasma TAC, TOS, and OSI were increased in patients with LVSD when compared to those without LVSD. Thus, increased oxidative stress may contribute to pathogenesis in these patients.

LVSF is the most important predictor of in-hospital and long-term prognosis in patients with STEMI[3]; therefore, an estimate of which patients may develop LVSD is critical. Abou et al.[18] showed that smaller enzymatic infarct size, baseline LVEF, and absence of mitral regurgitation were independently associated with LVEF recovery at follow-up. Using the Korean Acute Myocardial Infarction Registry and Korean Myocardial Infarction Registry, Oh et al. showed that recovery of LVSD was observed in 51% of the subjects. The same study reported that moderate systolic dysfunction, Killip class I-II, lack of use of diuretics, non-STEMI, lower peak troponin I level, single-vessel disease, non-left anterior descending culprit lesion, and statin use were independent predictors of recovery of depressed LVEF[19]. In the PREDICTS study, Brooks et al. showed that EF >35% at presentation, length of stay, prior MI, lateral wall motion abnormality at presentation, and peak troponin were related to the recovery of LVSD[20]. Similarly, in this study, low EF at presentation, higher CK-MB, and hs-CRP levels were independently associated with depressed LVEF. Additionally, we report that oxidant and antioxidant parameters were associated with depressed LVEF. Additionally, it has been shown that other inflammatory parameters such as IL-6, IL-1RA, and resistin plasma levels at baseline have a good predictive value both as independent variables and as a group for the development of adverse LV recovery and major cardiovascular outcomes at 6-month follow-up after STEMI[21]. Our findings supported this study.

CONCLUSIONS

Studies have failed to clarify how guideline-based medications influence LVEF recovery at follow-up fully. Previous studies with STEMI patients undergoing p-PCI showed the beneficial effects of these therapies; however, these studies reported lower usage of ACEi, ARB-II, and β-blockers[22,23]. Furthermore, this study showed that some patients did not present with LVEF alteration even when they received contemporary guideline-based medications. Therefore, we suggest that the oxidant, antioxidant, and inflammatory status of the patient may also be implicated in the pathophysiology. This emphasizes the importance of a systematic approach in treatment regimens that includes lifestyle changes and antioxidant therapy.

AUTHORS' CONTRIBUTION

FA: Project administration, Writing – review & editing. **HAB:** Conceptualization, Data curation. **AB:** Investigation, Validation. **HBS:** Formal analysis. **MÖ:** Methodology, Project administration.

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ORIGINAL ARTICLE

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Teleconsultations in neurology in a universal health system amid COVID-19: a descriptive study

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SUMMARY

OBJECTIVE: This study aimed to characterize teleconsultations in neurology executed by Regula+Brasil project in Recife, a capital city in northeastern Brazil.

METHODS: A descriptive study carried out by four private hospitals, in a partnership with the Ministry of Health in Brazil. Teleconsultation was performed preferably in the video modality. Conditions eligible for teleconsultation were headache, epilepsy, and cerebrovascular disorders. Period of analysis was May to September 2020.

RESULTS: A total of 243 teleconsultations were analyzed, of which 76.95% was a first appointment. In 48.97% of cases, the teleconsultation represented the first opportunity for the patient to be consulted with the specialist. Among cases of first appointment, 20.16% were further referred to a face-to-face consultation and 21.81% could be redirected to primary health care. Headache disorders were the most predominant clinical conditions.

CONCLUSIONS: The implementation and development of telemedicine by Regula+Brasil during the COVID-19 pandemic represented an opportunity to assess the value of having teleconsultations added along the line of care from primary care to a medical specialty, promoting the coordination of care across different levels of complexity of care in the health system and improving access to specialized care.

KEYWORDS: Neurology. Referrals and consultation. Effective access to health services. Public Health.

INTRODUCTION

Brazil's unified health system (Sistema Único de Saúde, SUS) is one of the largest universal health systems worldwide[1]. Ever since its conception, primary health care (PHC) was designed to be the main form of access to the health services, to facilitate a structured and coordinated care[2]. Despite these efforts, there are still important gaps between primary and specialized care due to the fragmentation of health care networks and to the expressive demand for consultations in secondary and tertiary care, ultimately resulting in long waiting lists for many medical specialties, including neurology[3].

In 2020, the SARS-CoV-2 pandemic brought additional challenges to the referral of cases to specialized care, since consultations needed to be postponed or canceled as a strategy to promote social distancing or to relocate resources to manage the public health emergency imposed by the coronavirus, which made the access to secondary and tertiary care even more difficult to the general population[4,5]. Telemedicine was impressively catalyzed by COVID-19 as a viable alternative given the challenge of increasing access to health care while preventing agglomerations, especially after the unprecedented approval of telehealth in a wider context during the pandemic by the Brazilian Federal Government through publication of Federal Law no. 13.989 in April 15, 2020[6].

The Regula+Brasil project is a partnership between five Brazilian private hospitals and the Ministry of Health of Brazil aiming to improve the efficiency in the referral of cases from PHC to specialized care, by the employ of telehealth strategies, including the implementation of a protocol-oriented, centralized referral management system. To minimize the impact of the pandemic on the access of patients to specialized care, the project expanded its activities by initiating the offer of teleconsultations[7].

The aim of this study was to describe the characteristics of teleconsultations in neurology, carried out by the Regula+Brasil project as a contingency measure to the COVID-19 crisis, and to assess the proportion of averted face-to-face appointments.

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METHODS

A descriptive study carried out by the Hospital Sírio-Libanês, Hospital Alemão Oswaldo Cruz, Hospital do Coração, and Hospital Moinhos de Vento, in a partnership with the Ministry of Health in Brazil. The study report was structured in a way to adhere to the Strengthening the Report of Observational Studies in Epidemiology (STROBE) Statement[8].

The teleconsultation activities were directed to patients living in Recife, a capital city in northeastern Brazil, who were allocated in waiting lists for face-to-face appointments with a neurologist. Conditions eligible for teleconsultation were headache, epilepsy, and cerebrovascular disorder (CVD). These criteria were defined based on the high prevalence of such cases in waiting lists and the current evidence regarding the possibility of proper management of these conditions through telehealth[9]. Other eligibility criteria were patients aged 18 years or older; preserved cognitive function or the availability of a companion during the teleconsultation; and willingness to be assisted by the employ of telemedicine.

During the appointment, the physician would request laboratory or imaging examinations and/or prescribe medication through an electronic prescription platform. Follow-up appointments were scheduled according to the clinical assessment by the neurologist. In case of possibility of proper follow-up in PHC after the measures taken during teleconsultation, the patient was then redirected to the PHC unit. For all urgent situations, referral would be made to the correspondent emergency department for continuity of care. Cases requiring face-to-face evaluation by a neurologist would be approved, and adjustment of the priority level on the SISREG platform was performed (Figure 1).

Data were extracted from the database of the Regula+Brasil project for the period comprised between May and September 2020. The unity of analysis was the teleconsultation rather than the individual. Variables extracted from the database included information on gender, age, education, type of the platform employed for teleconsultation, type of consultations (first or follow-up appointment), attachments to previously performed

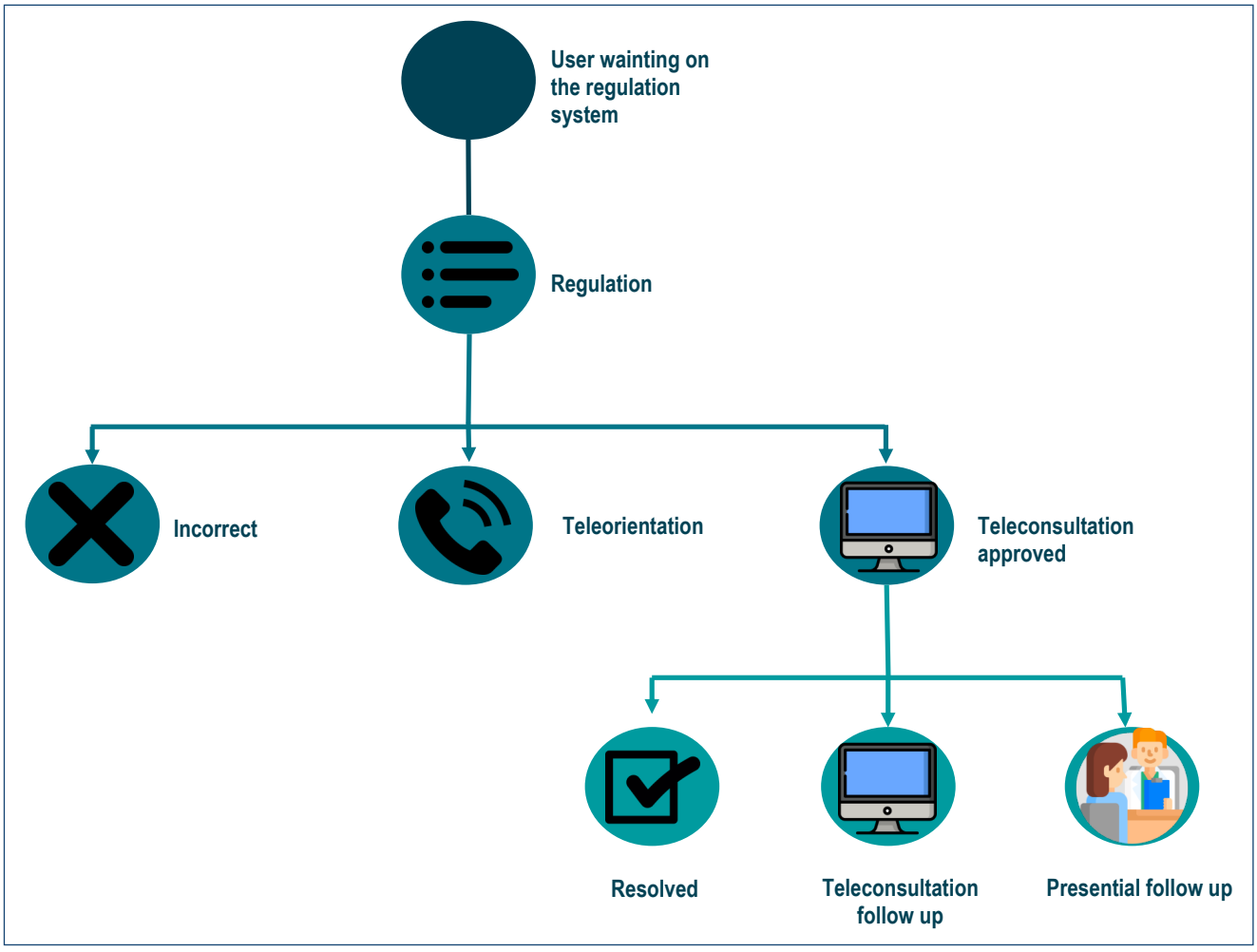

Figure 1. Neurology teleconsultation care process.

medical examinations, prescribed medications, requested medical tests, diagnosis, teleconsultation outcomes, previous consultations in neurology, and waiting time to the first consultation with a neurologist.

The study protocol was approved by the Institutional Research Ethics Committee of Hospital Sírio-Libanês, under the identifier CAAE 28453420.5.0000.5461, with waiver of informed consent.

RESULTS

A total of 243 teleconsultations were analyzed, of which 76.95% was a first appointment (Table 1). Mean age was 47 years (±18.31), 78.6% were female. Follow-up appointments were solicited in 57.2%. All patients accepted the teleconsultation after reading the consent form. If they do not agree, then patients would follow the municipal waiting list for neurology consultation. In most teleconsultations, a medical prescription was issued 69.54%, with medications for headache prophylaxis in 45.68%. In 48.97% of cases, the teleconsultation represented the first opportunity for the patient to be consulted with a neurologist. There was a neuroimaging request for 20.98% of cases, of which brain computed tomography corresponded to 68.62%. Laboratory tests were requested in 14.4%. Diagnosis of cases is presented in Table 2. Among the cases related to the first consultation, 20.16% were further referred to a face-to-face consultation and 21.81% could be followed up in PHC units (Table 3). The choice for the teleconsultation modality was made according to the availability of digital channels and the patient's preference. Among video teleconsultation, 10.65% were waiting list for in-person consultation with a neurologist and 18.93% could be followed up in PHC units.

The Net Promoter Score (NPS) is a methodology that uses research and classification tools to analyze the level of customer satisfaction and recommendation, and results above 90 demonstrate service promoters. Our NPS had a response rate of 22% and a result of 91[10].

DISCUSSION

To the best of our knowledge, this is the first study to address the characteristics of teleconsultations in neurology as part of a strategy to minimize the pent-up demand for specialized health care in SUS, as imposed by the COVID-19 pandemic. Our results demonstrate the importance of having implemented alternatives of care, since the median of waiting times until consultation was 270 days, and for 48.97% of cases, teleconsultation was the first access to a consultation with the neurologist.

Another important finding is the number of cases that could be redirected to PHC units after teleconsultation, without the need of face-to-face appointments with the specialist. Roughly

Table 1. Characteristics of teleconsultations.

Total*	n (%)
	243 (100)
Sex*	
Male	52 (21.40)
Female	191 (78.60)
Age (years)†	47.06 (±18,31)
Min–Max	16–107
Teleconsultation*	
First appointment	187 (76.95)
Follow-up appointment	56 (23.05)
Level of education*	
Illiterate	20 (8.23)
Elementary school	68 (27.98)
Secondary school	88 (36.21)
Higher education	22 (9.05)
No information	45 (18.52)
Teleconsultation follow-up plan*	
Yes	136 (55.97)
No	107 (44.03)
Number of medications in use*	
0	74 (30.45)
1	82 (33.74)
2	68 (27.98)
3	15 (6.17)
4	4 (1.65)
Modality of teleconsultation*	
Video	169 (69.55)
Telephone	74 (30.45)
Attachment of medical exams on the platform*	
Yes	16 (6.58)
No	227 (93.42)
Previous consultation with neurologist*	
Yes	77 (31.69)
SUS	61 (25.10)
Supplemental health care	15 (6.17)
No	119 (48.97)
No information	47 (19.34)

*Data are presented in absolute numbers and %. †Continuous variables without normal distribution are presented as medians and interquartile ranges.

Table 2. Variables.

Prescribed medications*	%
Amitriptyline	95 (26.69)
Anti-inflammatory	63 (17.7)
Analgesics	22 (6.18)
Prednisone	12 (3.37)
Propranolol	11 (3.09)
Fluoxetine	8 (2.24)
Others	70 (19.66)
None	75 (21.07)
Request for laboratory tests*	
No	208 (85.60)
Yes	35 (14.4)
Request for imaging tests*	
No	203 (83.54)
Yes	40 (16.46)
Imaging tests requested*	N 51 (100)
Computed tomography	35 (68.62)
Carotid and vertebral Doppler	5 (9.8)
Electroencephalogram (EEG)	5 (9.8)
Echocardiogram	2 (3.9)
Magnetic resonance	2 (3.9)
Computed tomography angiography	1 (1.9)
Electromyography (ENM)	1 (1.9)
Symptoms motivating teleconsultation (ICD-10)*	
Headache	187 (76.95)
Epilepsy	25 (10.28)
Stroke	24 (9.87)
Others	7 (2.88)
Diagnosis after consultation*	
Headache	178 (73.25)
Unspecified headache (CID R51)	126 (51.85)
Migraine (CID G43)	26 (10.70)
Tension-type headache (CID G44.2)	26 (10.70)
Epilepsy (CID G40)	18 (7.41)
Cerebrovascular disorder	16 (6.58)
Stroke, not specified as hemorrhage or infarction (CID I64)	9 (56.25)
Occlusion and stenosis of precerebral arteries (CID I65)	3 (18.75)
Transient ischemic attacks and related syndromes (CID G45)	3 (18.75)
Cerebral infarction (I63)	1 (6.25)
Syncope and collapse (CID R55)	4 (1.64)
Unspecified dementia (F03)	3 (1.23)
Others	24 (9.87)
Clinical decision*	
Follow up through teleconsultation	139 (57.20)
Follow up in PHC units	53 (21.81)
Waiting list for in-person consultation with a neurologist	47 (19.34)
Immediate in-person consultation	4 (1.65)
Waiting time for the neurologist's evaluation from the request by primary care to the date of teleconsultation (days)†	270 (±180)
Min-Max	5–750

*Data are presented in absolute numbers and %; †Continuous variables without normal distribution are presented as medians and interquartile ranges.

one-fifth of cases were deemed appropriate to be followed at the PHC unit, reducing the pressure on the in-person specialized service. Additionally, a minority of cases needed laboratory or imaging tests, which may be explained by the existence of previous results, or the possibility of managing the case based solely on the information provided by patients.

Among the conditions considered eligible for teleconsultation by neurologists in the project, the predominant condition in this series was headache (73.3% of cases), including migraine, tension-type, and nonspecified headache, followed by epilepsy and CVDs. This may explain the high predominance of females (78.6% of cases), since migraine and some other types of headache are more frequent among women[11]. The high frequency of headache cases among all health conditions imposing referrals to neurologists was reported by previous studies[7,11-13]. The profile of most prescribed medications could also be explained by the dominance of migraine cases. Most prescribed medications were prophylactic agents for chronic headaches, widely available in public health facilities, such as amitriptyline, as well as anti-inflammatory and analgesic agents.

Previous studies have also provided evidence of effectiveness of telemedicine for headache disorders, when compared to face-to-face consultations, as assessed by the Migraine Disability Assessment Score (MIDAS) and the Headache Impact Test (HIT-6)[14,15]. Furthermore, Muller et al. have estimated that the proportion between cases of missed secondary headache and total number of teleconsultations would be 1:20,000[15]. Kristoffersen reported that, during the COVID-19 pandemic in Denmark and Norway, there was a reduction in referrals due to headache and a shift to teleconsultation[16].

The literature has also demonstrated evidence supporting the successful use of telemedicine for patients with epilepsy[17-19], the second most prevalent condition in our series. Follow-up outpatient epilepsy visits rely characteristically on phenomenological interview, adherence, and counseling rather than physical examination[19]. In a study comparing telemedicine with face-to-face consultation, no differences were observed in relation to the number of seizures, emergency visits, or hospitalizations[17,19]. However, acceptability of telemedicine and its role in this context still need to be further explored, since a previous study suggests that patients presenting epilepsy may consider telemedicine a complementary service rather than a substitute for face-to-face consultation[20].

Finally, stroke was the reason for teleconsultation in 6.6% of cases. Although there are many studies assessing the role of telemedicine in the management of acute stroke[21-23], there are relatively fewer studies on the use of telemedicine in outpatients after stroke. Most of these studies suggest that telemedicine

Table 3. Teleconsultation outcomes.

n (total)	Video (n%)	Telephone (n%)
	169 (69.55)	74 (30.45)
Diagnosis after consultation*		
Headache		
Unspecified headache (CID R51)	87 (51.5)	39 (52.70)
Migraine (CID G43)	20 (11.83)	6 (8.10)
Tension-type headache (CID G44.2)	20 (11.83)	6 (8.10)
Epilepsy (CID G40)	11 (6.5)	7 (9.45)
Cerebrovascular disorder		
Stroke, not specified as hemorrhage or infarction (CID I64)	6 (3.55)	3 (4.05)
Occlusion and stenosis of precerebral arteries (CID I65)	2 (1.18)	1 (1.35)
Transient ischemic attacks and related syndromes (CID G45)	2 (1.18)	1 (1.35)
Cerebral infarction (I63)	1 (0.6)	0
Syncope and collapse (CID R55)	4 (2.36)	0
Unspecified dementia (F03)	1 (0.6)	2 (2.70)
Others	15 (8.87)	9 (12.16)
Clinical decision*		
Follow up through teleconsultation	118(69.82)	21 (28.37)
Follow up in PHC units	32 (18.93)	21 (28.37)
Waiting list for in-person consultation with a neurologist	18 (10.65)	29 (39.2)
Immediate in-person consultation	1 (0.59)	3 (4.05)

*Data are presented in absolute numbers and %.

may play a role in the improvement of care by, for example, improving the titration of anticoagulants and the management of modifiable risk factors[24,25].

Our results related to averted referral cases are also consistent with the one from a previous study. Constanzo et al., carried out in Chile, a teleneurology program has been showed to reduce the number of patients waiting for first appointments with a neurologist, as well as the waiting time from the referral made by PHC units and the consultation with a specialist[12].

Our data suggest a contribution of telemedicine to the reduction in the number of patients waiting for the first appointment with the neurologist, as well as the reduction in waiting times to specialized care, an important aspect in contexts with high demand and relatively limited resources, as it is the case in Brazil. A significant proportion of cases could be managed by specialists through telemedicine and redirected to PHC units, averting unnecessary face-to-face appointments, and helping the prioritization of cases that really needs in-person care.

Limitations

First, cases were selected based on predefined eligibility criteria, which makes results not generalizable to neurologic conditions that were not considered for inclusion in the study. Second, despite the efforts to provide a stable platform for video teleconsultation, almost 30% of consultations were carried out via phone, which is far from ideal for initial assessment of neurological patients. Therefore, technological access should be considered a barrier for the access to video teleconsultation in Brazil.

Our study did not assess the effectiveness of telemedicine since we did not assess clinical outcomes. Future studies are still needed to assess not only the acceptability and preferences of patients regarding telemedicine in this context but also the impact of telemedicine on health outcomes.

CONCLUSIONS

The implementation and development of telemedicine by Regula+Brasil during the COVID-19 pandemic represented an opportunity to assess the value of having teleconsultations added along the line of care from primary care to a medical specialty, promoting the coordination of care across different levels of complexity of care in the health system and improving access to specialized care.

AUTHORS' CONTRIBUTIONS

ERSA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **DLGR:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CEAB:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **JB:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SDG:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **KYK:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **DVP:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SS:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SCIS:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CEM:** 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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Impressions of the chronic 900-MHz electromagnetic field in the prenatal period on Purkinje cells in male rat pup cerebella: is it worth mentioning?

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SUMMARY

OBJECTIVE: The aim of this study was to examine the changes on the Purkinje cells in the cerebella of male rat pups born to pregnant dams that were exposed to an electromagnetic field in the prenatal period.

METHODS: The first stage of the study involved 12 Sprague-Dawley rats, 6 male and 6 female, weighing between 180 and 250 g. The female rats in the experimental group were exposed to a 900-MHz electromagnetic field for 1 h at the same time every day, and no procedure was performed on the control group. Following pregnancy, six male pups from each group were divided into experimental and control groups without any procedure on the pups. After 2 months, they were sacrificed and their cerebella were removed. Histopathologically, following routine processing and fixation procedures, the cerebella were embedded in the tissue blocks. The sections taken from these blocks were stained with cresyl violet. The Purkinje cells in the cerebella were then counted on sections using the optical dissector method on an image analysis system.

RESULTS: The estimation of number of the Purkinje cells in the groups revealed more cells in rats in the control group than in the experimental group. Histopathologically, Purkinje cells exhibited a normal morphological structure in the control group, while the cells in the experimental group showed damage.

CONCLUSIONS: It might be asserted that the exposure of mothers to an electromagnetic field in the prenatal period may affect the development of Purkinje cells in the pup cerebella.

KEYWORDS: Electromagnetic fields. Purkinje cells. Cerebellum. Anatomy. Anatomic pathology. Histopathology. Pathology. Surgery.

INTRODUCTION

The usage of wireless communication devices has become a ubiquitous part of daily personal and professional life[1], resulting in an augmented public exposure to radiofrequency electromagnetic radiation (RF-EMF)[2]. Long-term exposure to RF-EMF emitted by various electronic devices has been propounded to result in hazardous biological and health effects[3].

Despite the controversy, deleterious effects such as changes in intracellular calcium homeostasis, neuronal damage, and impairments in neurotransmitter systems have been reported in association with the biological effects of RF-EMF exposure on the central nervous system[4]. Population-based studies have revealed that the neurodevelopment of the offspring exposed to RF-EMF during pregnancy is adversely affected, with a greater risk of emotional and behavioral difficulties[5]. Several epidemiological studies have raised the question of the risk of increasing glioma and acoustic neuroma among these intensive users[6]. The International Commission on Non-Ionizing Radiation Protection recommends animal model evaluation to assess the health risks to humans caused by electromagnetic radiation[7].

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Adolescence in rodents has been classified as early adolescence (preadolescent, postnatal 21–34), mid-adolescence (periadolescent, postnatal 34–46), and late adolescence (young adults, postnatal 46–59)[8]. This study purposed to allow male rat pups to live to adulthood (postnatal 60) in order to investigate whether or not any change occurs in the Purkinje cells of male pups born to dams that were exposed to long-term, chronic EMF in the prenatal period. This time frame may be compared to the preadolescent period in humans. At the end of the study period, the Purkinje cells in the male rat pup cerebella would be subjected to histopathological examination, and the number of the Purkinje cells would be calculated using the stereological method.

METHODS

Ethical aspects

This study was approved by the Research and Ethics Committee of Experimental Animals linked to the Ordu University, Ordu, Turkey, under the approval number 82676388.3.16/2020.

Study design

All procedures in the experiment were performed in conformity with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. In all, 12 Sprague-Dawley rats, 6 male and 6 female, weighing 180–250 g, were used. Throughout the study period, the rats were housed in plastic transparent cages, in a 12:12 day:night cycle in a temperature-controlled environment (22±1°C) with 55% humidity. The rats were then allowed to mate for a 24-h period. The following morning, vaginal smears were collected, with the saline solution being injected into the vaginas using a Pasteur pipette. The solution was then collected back and examined under a light microscope.

The rats identified as pregnant on the first day were placed into the EMF group. The three rats in that group were then exposed to 900-MHz EMF for 1 h between 08:00 and 09:00 a.m. every day. The EMF applied to the rats in the EMF group was maintained throughout pregnancy. No sham group was established since the rats in both groups were housed under identical conditions. The G*Power 3.1.9.2 statistical software, the F-tests ANOVA, and the fixed-effects, omnibus, and one-way module were used to calculate the sample size. This analysis determined a sample size of six rats in each group in this study.

At the end of pregnancy, the rats in the EMF gave birth to 5, 7, and 4 pups, respectively (a total of 16), 7 of which were male. The rats in the control group gave birth to 5 and 8 pups (a total of 13), 6 of which were male. After 2 months, the rats included in the study were anesthetized through ketamine (60 mg/kg body weight, Sigma Chemical Comp, St. Louis, MO, USA)/xylazine (5 mg/kg body weight, Sigma Chemical Comp, St. Louis, MO, USA) injection at a 1/5 ratio. Then they were sacrificed and their cerebella were extracted and placed into 10% formaldehyde.

Electromagnetic field application

EMF application to the pregnant rats in the experimental group was performed according to previous studies. Briefly, the EMF application system consisted of an oscillator with an ultrahigh-frequency uninterrupted power source (1218-BV Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, MA, USA, Serial No. 1483). The study was performed with an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, Concord, MA, USA, Serial No. 903) (set to an approximate output of 300 mW and 900 MHz frequency) and a Plexiglas cage specially designed for the study (30×42×52 cm). The oscillator was attached to a half-wave antenna made from a copper rod of 1×150 mm in size through a coaxial cable. The antenna was placed in the center of the cage at a depth of 110 mm from the open top. The mean location intensity was calculated using a measurement device with a range of 100 kHz to 2.5 GHz (Chauvin Arnoux CA43 Isotropic Electric Field Intensity Measurement Device). This EMF represents the threshold values for a single source determined in Global System for Mobile Communications (GSM)-900 base stations.

Histopathological and stereological procedures

After the cerebella had been stored in 10% buffered formaldehyde for 1 week, they were removed. The surrounding tissues were dissected, and fixation procedures were performed. The cerebella were processed through a varying series of alcohol and xylene and embedded in paraffin blocks. Sections were next taken from these blocks using a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany). Samples cut to 30 µm thickness in the sagittal plane with 1/7 sampling were taken using single-use metal microtome blades (Type N35, Feather Company, Osaka, Japan) with a 5° blade angle. The sections were then placed onto gelatin-formaldehyde-covered slides and stained with cresyl violet. The stained slides were then covered with Entellan medium (Merck 107961 Entellan new for microscopy) and subjected to histopathological examination (Olympus, BX51, Japan).

Consistent with previous studies, the number of Purkinje cells in the cerebellum sections placed onto the slides was

estimated in an unbiased manner using the optical dissector method. A pilot study was conducted to determine the research strategy. Sampling was continued by taking every seventh section until the end of the procedure. The equipment used for cell counting with the optical dissector technique included a light microscope (Leica DM4000 B, Germany), a computer, a computer-controlled motorized joystick (Prior ProScan, USA), a digital camera (JVC, Japan), and an electronic microactuator (Heidenhain, Germany). The system was entirely controlled by the Stereo investigator software (version 9, MicroBrightField Inc., USA). The cell counting was conducted on a computer screen using a 100× Leica HCX Plan Apo lens (NA=1.135). Total number of neurons in the cerebellum was calculated using the formula $N=\sum Q.\frac{1}{ssf}.\frac{1}{asf}.\frac{1}{tsf}$ (N: total number of neurons; ΣQ: total number of dissector neurons; ssf; section sampling fraction; asf: area sampling fraction; and tsf: thickness sampling fraction). The number of cells sampled was validated by calculating the coefficient of error and coefficient of variation values which were obtained from previous reports in order to estimate the efficiency of the sampling strategy[9].

Statistical analysis

The normality assumption for all the research data (rat weights, rat cerebellum weight, and the number of rat cerebellar neurons) was tested using the Shapiro-Wilk test ($p>0.05$). Differences were therefore determined using the Student's t-test. The research findings were expressed as mean, standard deviation, minimum & maximum values and p-values <0.05 were regarded as statistically significant. All the statistical calculations were performed using the SPSS software version 22.0 V, demo version.

RESULTS

Histopathological evaluations estimated the Purkinje cell counts, and data concerning the rats' physical examinations and weights are given below.

Histopathological observations

The sections from the cerebella of the 2-month-old male rats from the control and EMF groups were subjected to histopathological examination. The Purkinje cells in the cerebella from the control group of rats exhibited a normal morphological appearance (Figure 1). However, the damage was histopathologically observed in the Purkinje cells from the EMF group of male rats (Figure 2) in the form of dark, pyknotic cytoplasm by cresyl violet staining.

Number of the Purkinje cells

The number of the Purkinje cells was significantly higher in rats in the control group compared to those in the EMF group ($p=0.001$). The relevant number of cells in the control and EMF groups is exhibited in Table 1.

Physical examination and the cerebellum weights

No pathological finding or abnormal external appearance was observed in either the EMF or control groups during the physical examination before sacrifice at the end of the 2 months.

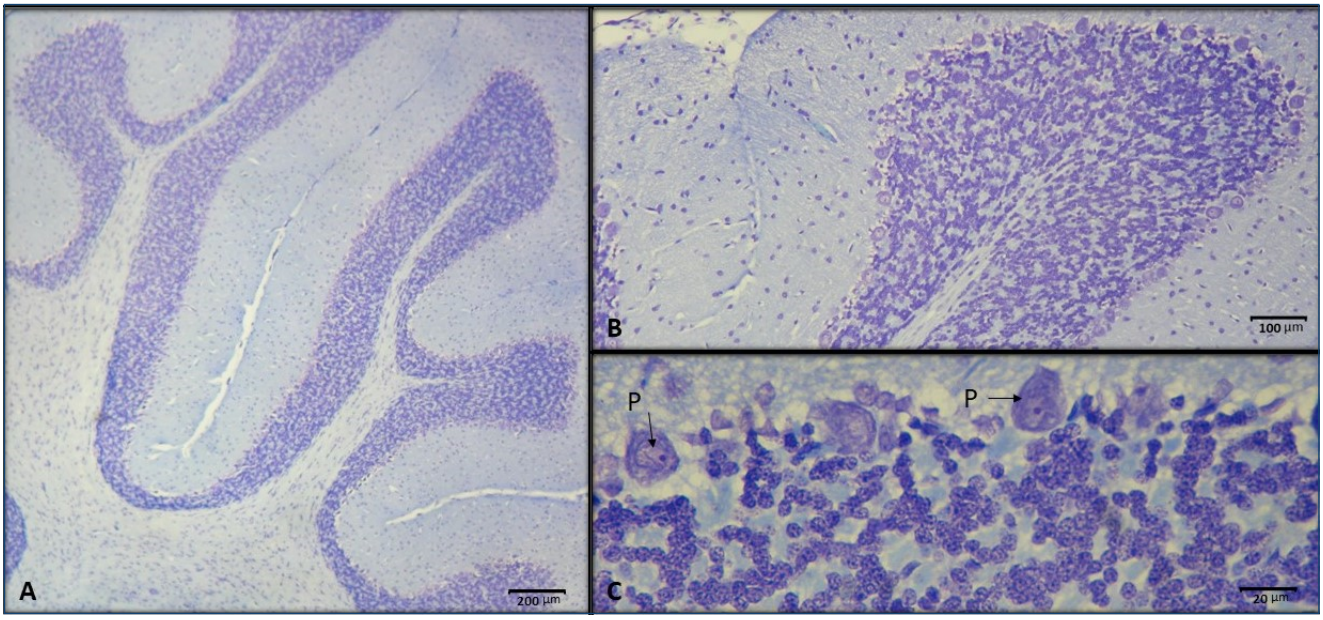

Figure 1. A normal morphological appearance, microphotograph of the cerebellum (cresyl violet staining; original magnifications, A: Bar=200 µm, B: Bar=100 µm, C: Bar=20 µm) P: Purkinje cell.

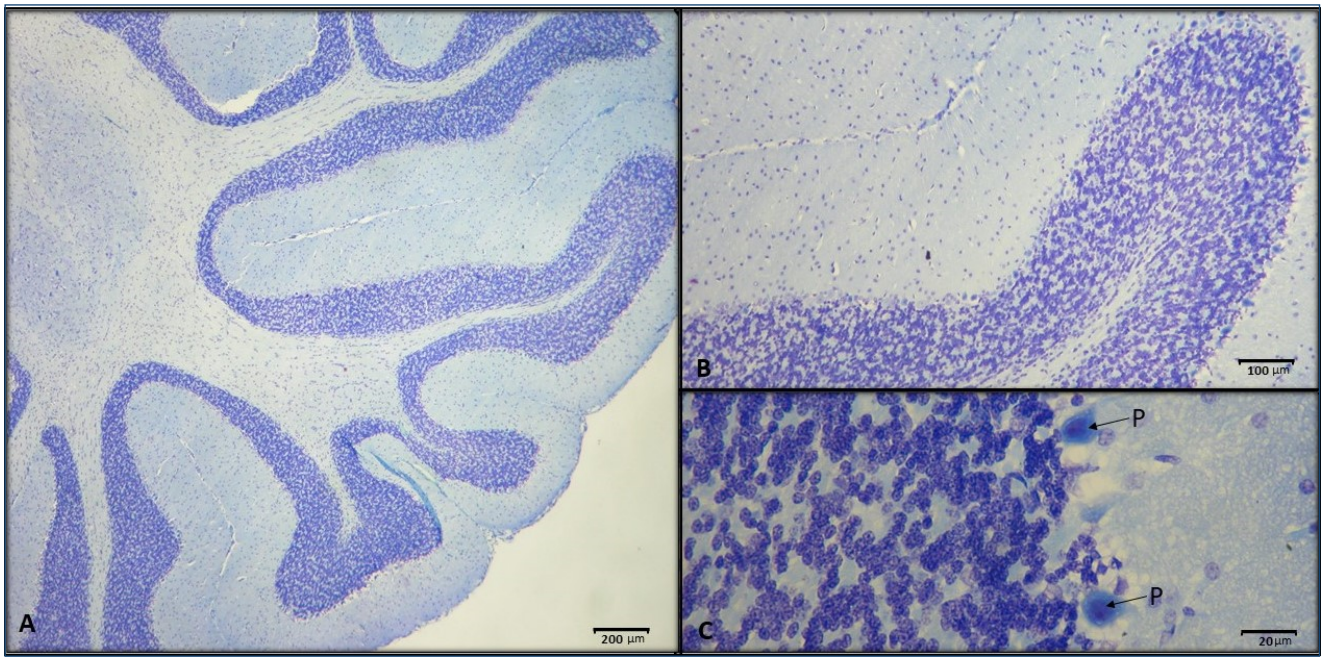

Figure 2. The damage, which was revealed in the Purkinje cells in the form of dark, pyknotic cytoplasm, microphotograph of the cerebellum (cresyl violet staining; original magnifications, A: Bar=200 µm, B: Bar=100 µm, C: Bar=20 µm) P: Purkinje cell.

At the end of the experiment, no statistically significant difference was observed in terms of the body weight or cerebellum weight between the two groups (p>0.05) (Table 1).

Table 1. Optical dissector analysis data for the estimation of total number of the Purkinje cells in the cerebella.

Stereological analysis parameters	Control (n=6)	Experimental (n=6)
Dissector particle number (mean)	184.83	165.47
Number of sampled section (mean)	17.81	18.11
Section thickness (mean) (µm)	19.87	20.01
Number of steps for counting (mean)	147.34	128.72
Section sampling fraction (ssf) (coronal)	1/7	1/7
Counting frame size (μm^2)	400	400
Area sampling fraction (asf) ($\mu m^2/\mu m^2$)	400/40,000	400/40,000
Thickness sampling fraction (tsf) (µm/µm)	10/19.87	10/20.01
Coefficient of error	0.7	0.7
Coefficient of variation	0.4	0.4

DISCUSSION

The biological effects of exposure to EMFs have been investigated in several studies, particularly in experimental mouse studies[10]. However, the biological effects of such exposure on the development of the brain and the underlying mechanisms have still not been completely elucidated[2].

Exposure to EMF both before and after birth has been reported to cause various potential impairments in the physiological and morphological structures and behavior of several animal species[11]. Haghani et al.[12] reported the adverse effect of EMF exposure on the central nervous system and investigated the effect of prenatal exposure on the Wistar rat pup cerebellum.

A study investigating the effect of exposure to an ultra-high frequency on occupational burnout syndrome enrolled 115 hospital central workers and 124 administrative personnel in the study group. Levels of oxidative stress biomarkers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity were measured. MDA, SOD, and CAT levels in the group exposed to low-frequency EMF were significantly lower than those in the nonexposed group. The prevalence of burnout syndrome and severity of depression were also higher in the former group[13]. These results suggest that oxidative stress induced by RF-EMF can lead to DNA damage in neurons during prolonged exposure to animals. Almost the same results have been found in several other studies[14].

Apoptosis was evaluated with caspase-3 activity in the ventral cochlear nucleus, which is the first hearing place in the brain stem, and neurons and oligodendrocytes at different stages of the postnatal period after 900-MHz EMF application in the prenatal period, and it was stated that shrunken apoptotic neurons and oligodendrocytes with fragmented nuclei were detected in the EMF group. In addition, it has

been reported that varying intensities of caspase-3 expression were observed in the neurons, oligodendrocytes in particular, in EMF study groups[15]. Furthermore, the effects of 900-MHz EMF on the cerebellum were investigated using histopathological and immunohistochemical methods and the results indicated, as a remarkable finding, that the Purkinje cells and granular layer cells in the cerebellum of the EMF study group had pycnotic nuclei, and a significant decrease in their cytoplasmic content was recognized. Caspase-3 induces apoptosis through chromatin condensation and degradation of DNA into nucleosomal subunits[16].

EMF affects not only the nervous system but also other systems. Borzoueisileh et al.[17] exposed rats to 900/1,800-MHz and 2,400-MHz EMF. Their histopathological examination revealed tubular cyst formation, tubular vacuolization, tubular dilatation, tubular atrophy, interstitial inflammation, interstitial bleeding, interstitial edema, lymph vessel dilatation, vascular wall thickening, and blood vessel inflammation in the kidneys.

CONCLUSIONS

Everyone is well aware that mobile devices are an inseparable part of daily life and is also concerned about the deleterious effects. This study investigated the potential deleterious effects of prenatal EMF exposure on individuals to be born subsequently. The effect of the EMF received by the dam in the prenatal period was found to affect the development of the Purkinje cells, a component of the central nervous system, in the pups, and was observed to persist in the postnatal period. Thus, this issue merits further investigation.

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AUTHORS' CONTRIBUTIONS

OB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **IS:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing – review & editing. **OFMB:** Investigation, Project administration, Resources, Validation, Visualization. **HH:** Investigation, Project administration, Resources, Validation, Visualization. **MD:** Investigation, Project administration, Resources, Validation, Visualization. **DS:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Visualization, Writing – review & editing. **EA:** Investigation, Project administration, Resources, Validation, Visualization. **USS:** Project administration, Resources, Validation, Visualization. **OFS:** Investigation, Resources, Validation, Visualization. **JMSJ:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Seroepidemiologic survey of the household contacts of leprosy patients

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SUMMARY

OBJECTIVE: Leprosy is a disabling infectious disease caused by *Mycobacterium leprae*. This study aimed to investigate the prevalence of leprosy among household contacts of leprosy patients.

METHODS: This study is a serological survey in household contacts of leprosy patients who had been treated or were undergoing treatment in the city of Presidente Prudente, São Paulo, Brazil, from 2006–2016, using clinical examination and screening for anti- Phenolic glycolipid-I antibodies with Mycobacterium leprae-flow serology.

RESULTS: A total of 263 index cases of leprosy were identified during the study period. Of these, 53 were approached, and among their household contacts, 108 were examined. The ML-flow test was positive in 2 (1.85%) individuals, but clinical examination revealed no signs or symptoms of leprosy in them. Therefore, they were considered to have a subclinical infection. Leprosy was not confirmed in any household contacts. In this study, a lower percentage of household contacts, when compared to that in the literature, had a positive Mycobacterium leprae-flow test result.

CONCLUSION: The use of Mycobacterium leprae-flow should be encouraged during the follow-up of at-risk populations, such as the household contacts of leprosy patients.

KEYWORDS: Hansen's disease. Leprosy. Mycobacterium leprae. Serologic tests.

INTRODUCTION

Leprosy is a chronic infectious and contagious disease caused by *Mycobacterium leprae*, which mainly affects the skin and peripheral nervous system[1]. According to the World Health Organization's (WHO) operational classification, multibacillary (MB) patients have more than five lesions or a positive bacilloscopic index, whereas paucibacillary (PB) patients have up to five lesions and a negative bacilloscopic index[1].

The main route of infection is the upper airway, with intimate and prolonged contact with the patient being the main risk factor for leprosy transmission[2]. This risk is 5–10 times higher if a family member has already presented with the disease[2].

In 2020, 127 countries reported 127,396 new cases to the WHO, the majority of which in India, with 65,147 cases[3]. Brazil ranked second, with 17,979 new cases[3]. The COVID-19 pandemic has disrupted program implementation and a reduction in new case detection by 37% in 2020 compared with 2019[3].

In Brazil, it is recommended that clinical examination of the household contacts (HHC) at the time of the diagnosis of the index case be conducted; if examination findings are normal, contacts are expected to receive the bacillus Calmette-Guérrin (BCG) vaccine[4]. However, due to its long incubation period (between 2 and 7 years), the disease may manifest later, thus requiring several years of monitoring[1].

Phenolic glycolipid-I (PGL-I), an *M. leprae*-specific membrane component, chemically comprises a trisaccharide, linked by a molecule of phenol to a chain of fatty acids[1]. It is present in the capsule of bacillus and can induce the production of antibodies, especially immunoglobulin M (IgM)[1]. Detection of these IgM antibodies in serum is suggestive of *M. leprae* infection[1]. Native and synthetic antigens are used in serological tests for leprosy diagnosis[1]. Enzyme-linked immunosorbent assay (ELISA) is the most common method used in these tests[5-8]. The rapid immunochromatographic and semi-quantitative lateral flow (ML-flow) tests have shown high sensitivity (97.4%) and specificity (90.2%) for the detection of MB leprosy, in addition to a 91% concordance rate with ELISA[9].

Clinical examination is insufficient for identifying infected individuals at an asymptomatic stage. As these individuals can disseminate *M. leprae* and have an increased risk of developing

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the disease, an active search for such individuals among the HHC of leprosy patients is required. Serological tests with PGL-I may assist with this search as they can contribute to early diagnosis[9].

Previous studies show the effectiveness of the ML-flow test in the detection of the disease among asymptomatic HHC of MB patients[9-11]. The method demonstrated concordance with the bacilloscopy, in addition to the ability to detect seropositive smear-negative patients[12].

We aimed to investigate the prevalence of leprosy among apparently healthy HHC of leprosy patients in Presidente Prudente, a city in the state of São Paulo, from 2006 to 2016, using clinical examination and the ML-flow test.

METHODS

This cross-sectional epidemiological study included HHC of leprosy patients who have been treated or are receiving treatment from 2006 to 2016 in Presidente Prudente, a city in the state of São Paulo, Brazil. It was a seroepidemiological survey in which clinical examination and the ML-flow test were applied. All procedures were approved by the Ethics Committee of the Universidade do Oeste Paulista, and the study was registered under the CAAE protocol (approval: 69516017.2.0000.5515) in October 2017.

For a simple random sampling scheme, assuming the probability of a type I error (α) of 5%, the value used in the formula to calculate the 95% confidence interval for normally distributed data was Z_{α}=1.96. Considering proportion p to be unknown, a value of 0.5 was used. Finally, the maximum margin of error allowed was ±2.5% (i.e., 5%). Thus, the ideal sample size for this study, considering a population of 263 leprosy patients, was 156.

The health department and primary and secondary care centers follow the guidelines of the Ministry of Health and WHO for the surveillance and elimination of leprosy and care of leprosy patients. Among the recommendations are tracking and monitoring HHC and conducting BCG vaccinations. Therefore, HHC were selected to participate in this study.

The HHC were defined as a person who either lives or has lived in the same house as a leprosy patient in the past 5 years before the patient's diagnosis.

Leprosy patients (index cases) were identified from the digital database of the health department. Information, such as telephone numbers, addresses, operational classification of the disease, and BCG vaccination status of the contacts, was extracted from their electronic records at the health department where the patients usually visited for their treatment and follow-up.

The researchers contacted the leprosy patients, who, in turn, identified their HHC and provided their names and contact information. All patients and their HHC were assured that their information would be kept safe, as described in the consent form they signed before study commencement. The inclusion criteria for HHC were that they were residents of Presidente Prudente and had no personal history of leprosy. Subjects were excluded if they presented with coexisting infection or any disease that could affect the peripheral nervous system, such as diabetes mellitus and alcoholism.

Mycobacterium leprae-flow Serological Test

The ML-flow test was performed during the household visit (using the blood obtained from pricking the index finger of the HHC) to detect circulating IgM antibodies against a semi-synthetic analog of PGL-I of *M. leprae* linked to bovine serum albumin (NT-P-BSA). The test was performed using a device containing a porous tape, marked at one end with the antibody (represented by the detection reagent, formed from mobile colloidal gold particles). It has a line in the center where the antigen is inserted, and a control line is marked with human IgM. Visual readings were taken by two independent readers, and a positive result was defined by visualization of both the control and test lines. The absence of the test line was considered a negative result, according to the manufacturer's specifications (IPTSP/UFG, GO, Brazil)[9].

Clinical examination

The HHC were clinically assessed at home, using a structured examination schema that contained the details of dermatological and neurological signs and symptoms of leprosy. This schema was designed to include even the nonspecific signs and symptoms of leprosy. The examination was performed by a specialist who had experience in diagnosing leprosy. The diagnosis of a case of leprosy was based on the presence of at least one of the following signs and symptoms:

1. lesion(s) and/or area(s) of the skin with altered sensation and
2. involvement of the peripheral nerve(s), with or without the thickening associated with sensory and/or motor and/or autonomic alterations[4].

Data were collected over 12 months (from November 2017 to November 2018).

The data were analyzed using Fisher's exact test to compare the frequencies between the groups. The significance level was set at $p<0.05$.

RESULTS

A total of 263 patients with leprosy were diagnosed during the study period. Of these index cases, 53 were enrolled in the study, and 210 were excluded for several reasons (Figure 1). Of the 53 participants, 108 household contacts were located.

A grouped bar chart representing the distribution of the index cases and examined HHC for each year of the study period is shown in Figure 2.

The grouped bar chart shows that the number of HHC examined in the later years was proportionally greater than that in the previous years. In contrast, the highest number of cases of leprosy was observed in the years at the beginning of the study period.

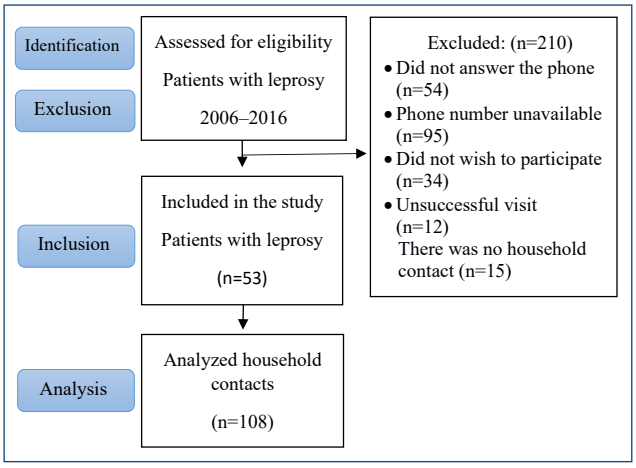

Figure 1. Flowchart of study participants.

The majority of the HHC were Caucasian women, who had been vaccinated with BCG at the time of the index case diagnosis and had a negative ML-flow test result (Table 1).

Table 1. Number and percentages of the examined contacts and the index cases according to the variables studied.

Data of the contacts examined (n=108)		
	Options	**n (%)**
Sex	Female	67 (62.04)
	Male	41 (37.96)
Age (years)	Mean±standard deviation	44.6±21.2
Ethnicity	Caucasian	65 (60.19)
	African American	6 (5.55)
	Mixed/Native	37 (34.26)
BCG vaccine	No	10 (9.26)
	Yes	98 (90.74)
ML-flow	Positive	2 (1.85)
	Negative	106 (98.15)
Case index data (n=53)		
Sex	Female	35 (66.04)
	Male	18 (33.96)
Age (years)	Mean±standard deviation	53.8±19
Ethnicity	Caucasian	34 (64.15)
	African American	2 (3.77)
	Mixed/Native	17 (32.08)
Operating classification	MB	27 (50.94)
	PB	26 (49.06)

BCG: bacillus Calmette-Guérrin; MB: multibacillary; PB: paucibacillary.

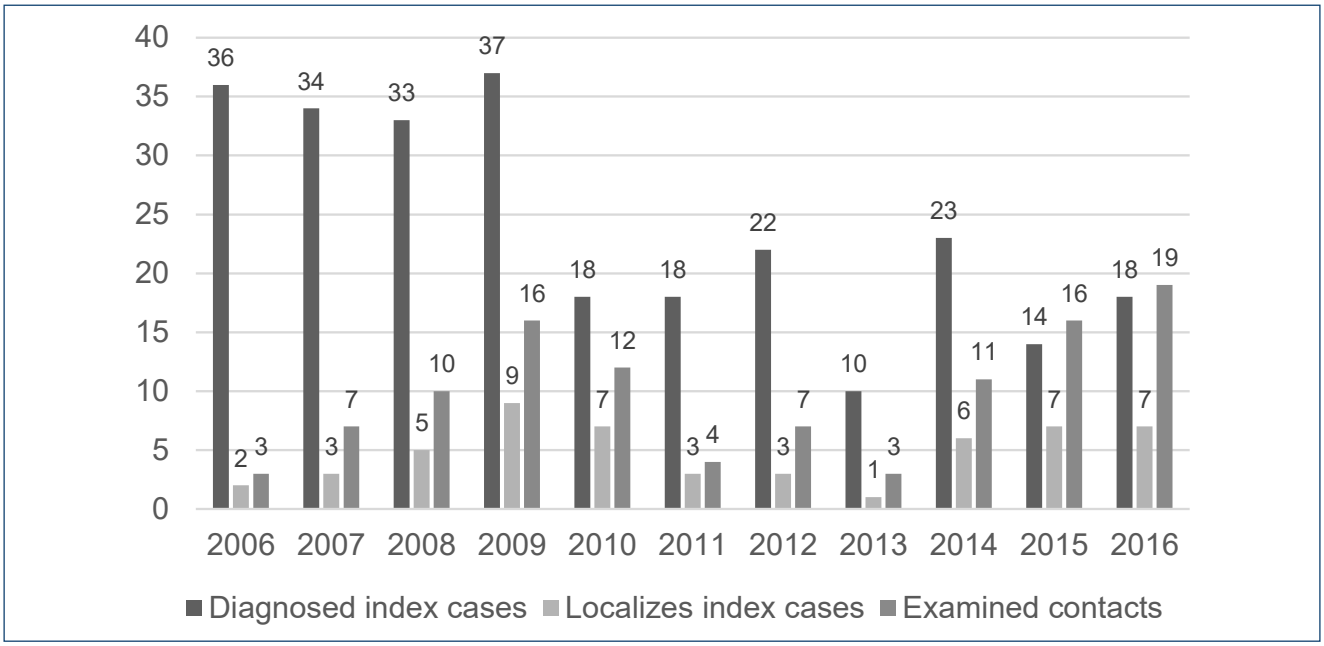

Figure 2. Distribution of the number of cases and contacts examined during the study period, from 2006–2016.

In two HHC (1.85%), the ML-flow test result was positive. One of them was a 100-year-old mixed-race woman who had received the BCG vaccine, and the index case related to her was a man with MB leprosy diagnosed in 2015. The second positive HHC was a 27-year-old mixed-race woman who had received the BCG vaccine, and the diagnosed case related to her was a man with PB leprosy diagnosed in 2009.

According to the operational classification, the HHC were almost equally related to the MB or PB leprosy cases. Of the 106 HHC who had a negative ML-flow test result, 54 (50.94%) were related to patients with MB leprosy and 52 (49.06%) to patients with PB leprosy (p=1).

Dermatological and neurological clinical examinations did not show leprosy symptoms or lesions in any of the HHC. Thus, the 2 (1.85%) HHC who had a positive ML-flow test were considered to have a subclinical infection and will be monitored annually, for 5 years, to enable early diagnosis if they eventually develop the disease. The other 106 (98.15%) HHC who had negative ML-flow test results and normal findings on clinical examination were considered normal. Thus, no new cases of leprosy were confirmed.

DISCUSSION

In this study, to improve early detection rates of patients with leprosy, anti-PGL-I antibodies were tested using ML-flow in the HHC of leprosy patients. A detection rate of 1.85% was observed, similar to that reported by Soares et al.[8] (1%). However, this rate is lower than the PGL-I seropositivity of up to 39% reported in most other studies[8,11].

Early detection of patients with leprosy is important in achieving disease control and elimination. For this, the most effective strategy is to monitor patients' HHC as a priority, especially if they have had prolonged exposure to untreated MB index cases. However, only clinical examination of HHC is recommended when the index case is diagnosed, which is not always sufficient to detect leprosy in the initial stage of infection as the diagnosis is only made when there are skin lesions and/or nerve damage. At this stage, transmission and incapacitating sequelae may have already occurred[5,7,11]. The use of other tools such as the ML-flow test may contribute to the identification of individuals with subclinical leprosy.

Positive serology in asymptomatic HHC not only means the transmission by the index case before treatment but also serves as a warning of the possibility that there are undiagnosed individuals living together with these HHC in the family nucleus or around.

Infected HHC who have good immunity against *M. leprae* or who will develop PB leprosy in the future may not have detectable anti-PGL-I antibody levels[6,9], and this may have been the case in this study. In addition, HHC of MB leprosy index cases who present with a high bacillary burden are more likely to become infected than those of PB leprosy index cases[8,9]. This could be another explanation for the low positivity in this study, as 49.06% of the index cases had PB leprosy.

Individuals who have had intimate and prolonged contact with untreated MB patients are at the greatest risk of transmitting leprosy[1]. Successful treatment of the index case will lead to cessation of *M. leprae* shedding within a few weeks of beginning multidrug therapy, reducing overall transmission and infection rates in HHC living with the case[1]. In this study, serological evaluation of the HHC was performed after the treatment of index cases; therefore, they were no longer exposed to the bacillus, and the period of exposure to the index case in the contagious phase may have been insufficient for disease transmission. Furthermore, in cases where an infection has occurred, the anti-PGL-I titer would likely be low-to-negative, particularly for those in the earlier years.

A vast majority of HHC received a prophylactic dose of the BCG vaccine at the time of the diagnosis of the index case. The BCG vaccine activates T-cell clones that recognize specific epitopes of *M. leprae*, conferring a protective effect against disease progression and leading to negative results for PGL-I tests that were previously positive[10,13].

This study has several limitations. The sample size was smaller than the calculated (ideal) size, which should have been 156, but 53 patients were included. Although we used strategies (including home visits, especially for the oldest cases) to reduce barriers and difficulties associated with accessing the index cases, we experienced difficulty in recruiting the participants because they either refused to participate or could not be located. Nevertheless, all registration data from the health sector database were considered for the initial screening of HHC, which probably preserved the sample representativeness. We also did not adopt any discriminatory criteria related to race, sex, and/or socioeconomic factors, which could have resulted in selection bias.

CONCLUSIONS

Our findings demonstrated no significant relationship between the studied variables. However, as only two HHC with a positive ML-flow test were observed, there was insufficient evidence to identify a relationship if any. Thus, future studies with larger sample sizes should be conducted to either rectify or ratify these

results. Because the ML-flow test is a quick, easy-to-perform, low-cost test that does not require laboratory structure, it can be easily used by health workers in field conditions and at different levels of care. Thus, it should be employed in screening at-risk populations, such as HHC, constituting an auxiliary tool for identifying an undiagnosed case, to sustain the elimination of leprosy in regions where this has already been achieved, as is the case in the region studied here.

AUTHORS' CONTRIBUTIONS

AMB: Conceptualization, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing. **SUS:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **ACCGT:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MAMMA:** Conceptualization, Formal Analysis, Data curation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Cardiac abnormalities in patients with nonalcoholic fatty liver disease

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SUMMARY

OBJECTIVE: This study aimed to evaluate the correlation between Nonalcoholic fatty liver disease and cardiac abnormalities.

METHODS: Patients with Nonalcoholic fatty liver disease who attended an outpatient clinic in Southern Brazil were prospectively evaluated. Patients should be older than 18 years and have steatosis.

RESULTS: A total of 174 patients were evaluated. The mean age was 63±12 years, 65% were women, 71% white, 82.2% hypertensive, 52.3% diabetic, 56.3% obese, and 30% dyslipidemic. There was no association between Nonalcoholic fatty liver disease and cardiac abnormalities, even after adjusting for age, sex, and metabolic syndrome.

CONCLUSIONS: The present study did not show a direct correlation between Nonalcoholic fatty liver disease and cardiac abnormalities, regardless of metabolic syndrome.

KEYWORDS: Nonalcoholic fatty liver disease. Cardiovascular disease. Cardiac disease. Cardiac arrhythmias.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most important causes of liver disease worldwide and, in other Western countries, it will be the main cause of indication for liver transplantation until 2030[1]. Its prevalence is around 20–25% of the world population[2,3]. In South America, it affects 30%[4] of the population, reaching 34.4% in Brazil[5].

Clinical evidence supports the hypothesis that NAFLD is a multisystem disease, involving a variety of extrahepatic organs, including the heart. Cardiovascular disease (CVD) is the main cause of death in these patients, even preceding the causes of death related to liver complications. Furthermore, the association of NAFLD with the presence of metabolic syndrome (MetS) reduces survival[6].

The current challenge is to discover the causal factor that directly relates NAFLD and CVD. Evidence suggests that the association of NAFLD and the occurrence of cardiovascular events is independent of traditional risk factors and MetS[7].

Therefore, the early and effective diagnosis of NAFLD in the population of patients at risk of developing this situation becomes increasingly relevant for prevention and effective therapeutic intervention as a public health measure, aimed at preventing or delaying the development of cardiometabolic complications[7].

The main objective of the present study was to evaluate the relationship between NAFLD and cardiac abnormalities through electrocardiographic changes and to evaluate the cardiac structure, function abnormalities, and valvular heart disease.

METHODS

Patients over 18 years of age at the outpatient clinics of Internal Medicine and Gastroenterology of Hospital Nossa Senhora da Conceição (HNSC), a tertiary hospital in southern Brazil, were prospectively evaluated from August 2018 to July 2019. They must have previously undergone electrocardiography (ECG) and abdominal ultrasound in the last 6 months.

The diagnosis of NAFLD was established according to the recommendations of the guidelines of the American Association for the Study of Liver Diseases (AASLD)[1] and the American Heart Association (AHA)[8]. There must be evidence of hepatic steatosis, either by imaging or histology, and a lack of secondary causes of hepatic fat accumulation, such as significant alcohol intake, long-term use of a steatogenic medication, or monogenic hereditary disorders.

Patients with excessive alcohol consumption (daily intake greater than 30 g/day for men and 20 g/day for women for more than 2 years), patients living with human immunodeficiency virus (HIV), hepatitis B or C virus, other causes of

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chronic liver disease, hepatocellular carcinoma, and other secondary causes of NAFLD were excluded.

All patients were evaluated with anamnesis and physical examination, and clinical information was collected about lifestyle habits (e.g., alcohol consumption, smoking, and physical activity), previous diseases, and their respective treatments, in addition to the measurement of weight, height, and waist circumference.

Total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), triglycerides (TyG), and glucose were evaluated. Total abdominal ultrasound was used in the diagnosis of NAFLD.

The diagnosis of atrial fibrillation (AF), long QT interval, increased PR interval, and left ventricular hypertrophy (LVH) was based on 12-lead ECG. The ejection fraction (EF) value using the Sympson method and the size of the left atrium (LA) and the left ventricle (LV) were obtained by echocardiography.

Obesity[9], systemic arterial hypertension (SAH)[10], type 2 diabetes mellitus (DM2)[11], and dyslipidemia[12] were defined according to international recommendations. MetS was defined by the National Cholesterol Education Program Adult Treatment Panel III[12,13]. Insulin resistance (IR) was assessed by calculating the lipid accumulation product (LAP), which is based on a combination of the abdominal circumference (cm) and TyG (mg/dl) [LAP in man: (waist circumference – 65) × TG; LAP in female: (waist circumference – 58) × TG][14].

To evaluate liver fibrosis, the NAFLD fibrosis score was calculated at the moment of inclusion in the study[15].

Ethical aspects

The research project was carried out in accordance with resolution 466 of 2012, which regulates the performance of research on human beings, and was approved by the local ethics committee. All patients were informed about the research and signed the Free and Informed Consent Form.

Statistical analysis

Data were presented as mean and standard deviation or frequency and percentage. Associations between categorical variables were tested using Pearson's $\chi 2$ test and between groups using McNemar's test. To compare continuous variables between groups, Student's t-test was used for variables with a normal distribution or the Mann-Whitney test for nonparametric distributions. For intragroup comparisons, according to the respective distributions, the paired t-test or Wilcoxon test was used. For the adjusted analysis of electrocardiographic and echocardiographic changes, logistic regression for categorical variables and analysis of covariance for continuous variables were used. The assumed significance level was 5%.

RESULTS

Initially, 184 patients were identified. After the exclusion criteria were applied, 8 (4.3%) patients with excessive alcohol consumption and 2 (1.1%) other patients with hepatitis C virus were excluded, leaving 174 patients. Of these, 94 (54%) presented with NAFLD and 80 (46%) without NAFLD.

The mean age was 63±12.0 years, with a predominance of women (65%), 71.3% white, 74.7% sedentary, 9.2% active smokers, 51.7% previously smoking, and 56.3% obese, with a mean BMI of 31.5±6.5 and mean waist circumference of 107±13.6 cm, with no statistical difference between the groups with and without NAFLD (Table 1).

MetS was present in 74% of patients, being higher in the NAFLD group when compared to patients without NAFLD [78 (83%) vs. 51 (64%); p=0.005]. The mean LAP index was 47±14.0, being higher in NAFLD patients when compared to the group without NAFLD (49.4±12.2 vs. 44.0±15.2; p=0.009).

In NAFLD patients, 30% did not have significant liver fibrosis and 16% met the criteria for advanced liver fibrosis based on the NAFLD score.

Regarding the alteration in the lipid profile, 30.0% of patients presented with dyslipidemia, 43.1% with altered HDL cholesterol, 42% with altered LDL cholesterol, and 40.8% with hypertriglyceridemia (Table 1).

In the assessment of cardiovascular risk using the Framingham score, most had an intermediate classification (38.5%), with no difference between groups (Table 1).

In the electrocardiographic findings, AF was present in 5.3% of patients with NAFLD and 11.3% without NAFLD (p=0.107). A long QT interval was identified in one patient without NAFLD. Prolongation of the PR interval was identified in two patients, one from each group. LVH was evidenced in 11 (11.7%) patients with NAFLD and 12 (15.0%) without NAFLD, with no statistical difference. When making the analysis adjusted for age, sex, and MetS, there was no change in the results between groups with and without NAFLD (Table 2).

In the echocardiographic findings, diastolic dysfunction was identified in 34 (65.4%) patients with NAFLD and aortic valve sclerosis in 26 (50%). In the evaluation of the combined analysis of the two parameters, there was no difference between the groups with and without NAFLD. NAFLD patients presented a mean ejection fraction of 61.1±11.5%, a mean LV size of 50.1±14.6 mm, and a LA size of 42.2±7.3 mm. Adjusted analyses by logistic regression or by the difference of means for age, sex, and MetS did not demonstrate statistical significance between groups with and without NAFLD (Table 3).

Obesity was equally prevalent in patients with and without NAFLD (32.2% vs. 24.1%, p=0.433). The LAP index

in nonobese with NAFLD reaches 47.5±12.0, a similar level observed in obese patients without NAFLD (46.0±15.2).

DISCUSSION

NAFLD is a growing public health problem due to its prevalence and its association with increased cardiovascular risk and metabolic changes[2,3]. It has been related to CVD[7], and patients with NAFLD have a 2 times higher risk of CVD[3]. However, the pathophysiological mechanisms that establish the relationship between these diseases are not fully understood[16].

Recently, an international consensus panel proposed a change in the nomenclature of NAFLD to metabolic-associated fatty liver disease (MAFLD), suggesting that positive criteria should be used for the diagnosis of MAFLD[17]. These criteria require the presence of hepatic steatosis in addition to one of the following: overweight/obesity, type 2 diabetes, or evidence of metabolic dysregulation. Because the change in nomenclature is new and has not yet been universally

Table 1. Basic characteristics (n=174).

	Total n=174	Without NAFLD n=80	With NAFLD n=94	p
Age (years), mean±SD	63±12.0	63.3±11.2	62.9±12.4	0.859
Sex, n (%)				
Female	113 (65.0)	56 (70.0)	57 (60.6)	0.258
Male	61 (35.1)	24 (30.0)	37 (39.4)	
White race, n (%)	124 (71.3)	52 (65.0)	72 (76.6)	0.129
Sedentarism, n (%)	130 (74.7)	57 (71.3)	73 (77.7)	0.540
Tabagism, n (%)				
Active	16 (9.2)	6 (7.5)	10 (10.6)	0.400
Not active	90 (51.7)	31 (38.8)	43 (45.7)	
Not tabagista	84 (48.3)	43 (53.8)	41 (13.6)	
Obesity, n (%)	98 (56.3)	42 (52.5)	56 (59.6)	0.433
BMI, mean±SD	31.5±6.5	31.2±7.2	31.6±5.9	0.701
WC, mean±SD	107±13.6	105±14.0	109±13.1	0.053
SAH, n (%)	143 (82.2)	66 (82.5)	77 (82.0)	> 0.99
DM2, n (%)	91 (52.3)	43 (53.8)	48 (51.1)	0.840
MetS, n (%)	129 (74.0)	51 (64.0)	78 (83.0)	0.005
LAP, mean±SD	47 ± 14.0	44 ± 15.2	49.4 ± 12.2	0.009
TC (mg/dL), mean±SD TC ≥200 mg/dL, n (%)	176±45.4 52 (30.0)	176±42.5 21 (26.3)	177±47.9 31 (33.0)	0.864 0.424
Cholesterol HDL (mg/dL), mean±SD *HDL^M<40/HDL^F<50 mg/dL, n (%)	49±13.7 75 (43.1)	51±15.2 29 (36.3)	47 ± 12.1 46 (48.9)	0.064 0.126
Cholesterol LDL (mg/dL), mean±SD LDL >100 mg/dL, n (%)	98±39.3 73 (42.0)	98±35.2 31 (38.8)	98±42.6 42 (44.7)	0.926 0.525
Tyg (mg/dL), median (95%CI) Tyg >150 mg/dL, n (%)	140 (151–186) 71 (40.8)	124 (91–166) 27 (33.8)	148 (109–234) 44 (46.8)	0.111
IC, n (%)	39 (22.4)	20 (25.0)	19 (20.2)	0.471
Heart failure, n (%)	44 (25.3)	22 (27.5)	22 (23.4)	0.657
Framingham, mean±SD Low risk (<7.4%), n (%) Intermediate (7.5 and 19.9%) High risk (≥20%), n (%)	13.55±1.2 62 (35.6) 67 (38.5) 45 (25.9)	13.5±10.7 30 (37.5) 32 (40.0) 18 (22.5)	14.3±11.4 32 (34.0) 35 (37.2) 27 (28.7)	0.646 0.645

NAFLD: nonalcoholic fatty liver disease; BMI: body mass index; WC: waist circumference; SAH: systemic arterial hypertension; DM: diabetes mellitus; LAP: lipid accumulation product; MetS: metabolic syndrome; Tyg: triglycerides; IC: ischemic cardiomyopathy. *HDL^M: High-density lipoprotein cholesterol in men; HDL^F: High-density lipoprotein cholesterol in women.

adopted[17-20], we decided to continue to use the term NAFLD for the present study.

In the present study, as in two other large cohort studies with long-term follow-up[21,22], there was no association between NAFLD and CVD. Lazo et al. evaluated prospectively 11,371 adults participating in the Third National Health and Nutrition Examination Survey (NHANES III), assessing liver steatosis and evaluating mortality from all causes. They concluded that NAFLD was not associated with an increased risk of death from all causes, CVD, cancer, or liver disease[21]. In the same way, Stepanova et al. evaluated patients from the same cohort, suggesting that NAFLD did not increase cardiovascular mortality over a 14-year period[22].

However, it is necessary to emphasize that CVD is claimed to be the major determinant of the prognosis of NAFLD patients[7]. It is estimated that 5–10% of NAFLD patients die from CVD. Abnormalities in cardiac structure and function, such as LV dysfunction and hypertrophy[23], LA enlargement[24], and heart failure[25], in addition to valvular heart disease such as aortic valve sclerosis[26] and arrhythmias, mainly AF[27], have been reported.

The patients evaluated in the present study presented a high number of classic cardiovascular risk factors, with 82.2% having SAH, 52.3% DM2, 56.3% obesity, 30% dyslipidemia, 74.7% sedentarism, and 60.9% active smokers or ex-smokers. According to the Brazilian Public Health System, the prevalence of SAH is 24.7%, DM2 is 7.7%, and obesity is 19.8% in the general population[28]. This difference can be explained by the fact that these are patients being attended at a tertiary hospital, proving to be a very comorbid population.

Among the 94 patients with NAFLD, obesity was present in 59.6%, DM2 in 51.1%, dyslipidemia in 33%, and SAH in 82%, with no statistical difference compared to patients without NAFLD. This can be considered quite high when compared to the findings of the meta-analysis of Younossi et al.[4], which included 86 studies evaluating patients with NAFLD. The authors observed the presence of obesity in 51%, DM2 in 22.5%, SAH in 39%, and MetS in 42.5% of cases. This finding further reinforces the morbid characteristics of the study population.

In the present study, most NAFLD patients (74%) met the diagnostic criteria for MetS, a prevalence considerably higher when compared to the literature[7].

Insulin resistance (IR) is present in both NAFLD and MetS and is the linking factor of disease to CVD through atherogenic dyslipidemia[7]. When assessing IR in the study population, the mean LAP index was higher in patients with NAFLD when compared to the group without NAFLD (p=0.009).

Table 2. Electrocardiographic findings (n=174).

	Without NAFLD n=80	With NAFLD n=94	Nonadjusted analysis		Adjusted analysis[a]	
			OR (95%CI)	p	OR (95%CI)	p
AF, n (%)	9 (11.3)	5 (5.3)	0.44 (0.14–1.38)	0.172	0.38 (0.11–1.24)	0.107
QT, n (%)	1 (1.3)	0 (0.0)	–	0.460	–	–
PR, n (%)	1 (1.3)	1 (1.1)	–	0.909	–	–
LVH, n (%)	12 (15.0)	11 (11.7)	0.75 (0.31–1.80)	0.654	0.70 (0.28–1.75)	0.451

NAFLD: nonalcoholic fatty liver disease; AF: atrial fibrillation; LVH: left ventricular hypertrophy. [a]Adjusted analysis for age, sex, and MetS.

Table 3. Echocardiographic findings (n=109).

	Without NAFLD n=57	With NAFLD n=52	Nonadjusted analysis		Adjusted analysis[a]	
			OR (95%CI)	p	OR (95%CI)	p
Diastolic dysfunction, n (%)	32 (56.2)	34 (65,4)	1.48 (0.68–3.20)	0.336	1.59 (0.70–3.64)	0.270
AoV sclerosis, n (%)	27 (47.4)	26 (50.0)	1.11 (0.52–2.35)	0.849	1.09 (0.50–2.37)	0.833
Combined analysis[b]	44 (77.2)	39 (75.0)	0.89 (0.37–2.14)	0.825	0.85 (0.34–2.14)	0.732
			Difference of means (95%CI)	p	Difference of means (95%CI)	p
EF, mean±SD	57.4±15.5	61.1±11.5	3.7 (-1.4–8.9)	0.154	3.4 (-1.9–8.6)	0.204
LV size, mean±SD	52.9±11.7	50.1±14.6	-2.8 (-7.9–2.2)	0.267	-2.4 (-7.5–2.7)	0.351
LA size, mean±SD	41.9±8.3	42.2±7.3	0.35 (-2.6–3.3)	0.815	0.1 (-02.9–3.2)	0.934

NAFLD: nonalcoholic fatty liver disease; AoV: aortic valve; EF: ejection fraction; LV: left ventricle; LA: left atrium. [a]Adjusted analysis by age, sex, and MetS. [b]Adjusted combined of diastolic dysfunction and AoV sclerosis.

NAFLD was stratified according to the presence of obesity. Nonobese NAFLD patients presented more criteria for MetS when compared to nonobese patients without NAFLD, and similar to obese NAFLD patients in general, suggesting that NAFLD is an obesity-independent IR marker.

The LAP index in nonobese patients with NAFLD reaches similar values to those observed in obese patients without NAFLD, denoting that NAFLD in nonobese patients can be a sensitive and early marker of metabolic dysfunction, as already described in the literature[29] and an independent factor for obesity.

Even though this is a population of patients with many comorbidities, it is possible to identify NAFLD as an important metabolic factor independent of obesity, demonstrating that it may be important to identify and monitor nonobese NAFLD patients to manage their metabolic profile in advance, thus avoiding future cardiovascular complications[29].

As possible limitations, we could highlight that the number of patients evaluated was smaller than expected, and this fact may have reduced the power of the study. Also, the duration of the disease may have been too short to observe a significant association between NAFLD and structural cardiac alterations.

We should emphasize that this is still an area of debate, and if NAFLD is really an independent factor for CVD or if the CVD is in fact a consequence of the NAFLD or the metabolic syndrome should be better investigated, as suggested by other authors[30,31].

CONCLUSIONS

This study did not show a direct correlation between NAFLD and cardiac abnormalities, regardless of MetS.

AUTHORS' CONTRIBUTIONS

AHM: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **DW:** Conceptualization, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing. **LEG:** Data curation, Writing – original draft, Writing – review & editing. **VJDAR:** Data curation, Writing – original draft, Writing – review & editing. **YCM:** Data curation, Writing – original draft, Writing – review & editing. **AAM:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing. **CVT:** Conceptualization, Formal Analysis, Project administration, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Molecular mechanism of benign biliary stricture inhibition by rosiglitazone-activated peroxisome proliferator-activated receptor gamma

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SUMMARY

OBJECTIVE: The aim of this study was to investigate whether rosiglitazone-activated peroxisome proliferator-activated receptor gamma can inhibit the occurrence of benign biliary stricture and further elucidate the relevant molecular signaling mechanism.

METHODS: The primary cultured rat biliary fibroblasts following experiments were performed using within the fifth generation cells, which were separated from the bile ducts of Sprague-Dawley rats. The primary cultured rat biliary fibroblasts were co-cultured with 10 ng/mL transforming growth factor-beta 1 for stimulating collagen formation. Competent cells were transfected with siRNA that specifically target Smad3 or connective tissue growth factor to inhibit the expression of the corresponding proteins. The cells were incubated with 10 μmol/L rosiglitazone to activate peroxisome proliferator-activated receptor gamma. The cells were incubated with 10 μmol/L GW9662 in the pretreatment session to inactivate peroxisome proliferator-activated receptor gamma. ELISA was used to determine the levels of connective tissue growth factor and type I collagen in the cell supernatant. Western blotting was used to detect the levels of intracellular p-Smad3/t-Smad3.

RESULTS: Rosiglitazone-activated peroxisome proliferator-activated receptor gamma inhibited the secretion of type I collagen induced by transforming growth factor-beta 1. Peroxisome proliferator-activated receptor gamma inhibitor GW9662 could significantly reverse the rosiglitazone-triggered inhibition of transforming growth factor-beta 1-induced type I collagen secretion by suppressing peroxisome proliferator-activated receptor gamma activation ($p<0.01$). Furthermore, we also found that the activation of peroxisome proliferator-activated receptor gamma was accompanied by the inhibition of transforming growth factor-beta 1-induced Smad3 phosphorylation ($p<0.01$), increased connective tissue growth factor expression ($p<0.01$), and production of type I collagen ($p<0.01$), all of which effects elicited by rosiglitazone could be reversed by peroxisome proliferator-activated receptor gamma inhibitor GW9662.

CONCLUSION: Peroxisome proliferator-activated receptor gamma activated by rosiglitazone inhibits the transforming growth factor-beta1 -induced phosphorylation of Smad3 and the increased connective tissue growth factor expression as well as inhibits the secretion of type I collagen in biliary fibroblasts.

KEYWORDS: TGF-beta1. PPARgamma. Signal pathway. Fibroblasts. Collagen.

INTRODUCTION

Benign biliary stricture (BBS) is a postoperative complication of biliary surgery and remains one of the most difficult problems encountered by hepatobiliary surgeons. BBS most often occurs after iatrogenic bile duct injury or reconstruction of the bile duct after liver transplantation[1]. Importantly, the occurrence of BBS severely diminishes the quality of life of patients and constitutes an economic burden to the country and the patient's family[2,3]. With the ultimate purpose of finding preventive and treatment approaches, the search for effective therapeutic targets from the molecular signaling mechanism of BBS formation has always been an ongoing research interest in recent years.

Proteins in the TGF-β/Smad signaling pathway have extensive involvement in the regulation of cell activation, proliferation, and synthesis of ECM such as collagen[4]. A previous study has also confirmed that the local tissues of biliary stricture have higher levels of expression of pro-fibrotic factors, such as TGF-β1, Smad4, and connective tissue growth factor (CTGF)[5]. Our previous studies have also confirmed that TGF-β1 induces the production of CTGF by activating Smad3, thereby promoting the synthesis of type I collagen in biliary fibroblasts[6].

As one of the nuclear hormone receptor super-family, PPAR-γ has been shown to have pleiotropic functions against tumorigenicity, CNS injury and repair, inflammatory response,

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tissue fibrosis and benefit to a series of diseases in animal models and clinic[7-10]. Studies also revealed that activated PPAR-γ inhibits liver fibrosis by inhibiting collagen production stimulated by the TGF-β signaling pathway[11]. However, the ability of PPAR-γ in inhibiting the TGF-β-induced collagen production in biliary fibroblasts so as to inhibit BBS and the underlying molecular signaling mechanism remains to be fully elucidated. In the present study, we determined whether PPAR-γ can inhibit the collagen production of biliary fibroblasts stimulated by TGF-β and delineate its molecular signaling mechanism in a cell culture experiment using primary cultured rat biliary fibroblasts.

METHODS

Culture of primary rat biliary fibroblasts

Sprague-Dawley rats, each weighed 150–200 g, were used in this experiment. The bile ducts were separated from rats and obtained on a sterile operating table. The inner membrane was scraped, and the remaining tissue was cut into small pieces and incubated in a mixture containing 1 mg/mL collagenase (Sigma) and elastase (Sigma) for 20 min. After centrifugation of the suspension, the cells were collected and then cultured in DMEM high glucose supplemented with FBS.

Identification of cultured primary rat biliary fibroblasts by immunofluorescence

The purified primary rat biliary fibroblasts were incubated with vimentin (Sigma) labeled with fluorescein isothiocyanate. Prior to any interventions, the cells were cultured in a FBS-starved medium (10 mL/L) for 12 h. TGF-β1 (Sigma) was used to stimulate biliary fibroblasts to induce the secretion of type I collagen. PPAR-γ inhibitor GW9662 (Sigma) was utilized to inhibit the activation of PPAR-γ, whereas rosiglitazone was used to activate PPAR-γ.

Enzyme-linked immunosorbent assays

The experiment was carried out in accordance with the manufacturer's instructions for the CTGF Detection Kit and Type I Collagen Detection Kit (MyBioSource, San Diego, CA, USA).

Determination of protein levels using Western blotting

The total concentration of intracellular protein in the collected supernatant was determined using the BCA Detection Kit (Pierce). The protein was loaded into each well of SDS-PAGE gel. After electrophoresis, the protein bands were transferred to the nitrocellulose membrane. Subsequently, blocking on the membrane was performed. Rabbit-derived antibodies against p-Smad3, t-Smad3, and GAPDH were used as primary antibodies, and HRP-labeled goat anti-rabbit IgG was used as secondary antibody. Scion NIH image analysis software was used to detect the expression level of the target proteins.

siRNA transfection

Liposome™ 2000 (Invitrogen) was used to transfect specific siRNAs targeting Smad3 and CTGF into the cultured competent primary fibroblasts. When the cell confluence reached about 50%, a transfection reagent that was prepared by mixing Lipofectamine™ 2000 with siRNA at a ratio of 1:1 was used to transfect the cells prior to intervention.

Detection of peroxisome proliferator-activated receptor gamma activation

PPAR-γ Transcription Factor Assay Kit (Cayman Chemical), based on the ELISA detection method, was adopted as per the supplied protocol.

Statistical analysis

The obtained data are expressed as mean±standard deviation. Statistical Package for Social Sciences (SPSS) data analysis software, version 17, was used for data analysis. One-way analysis of variance (one-way ANOVA) was used to compare the data between different groups. $p<0.05$ indicates that the difference is statistically significant.

RESULTS

Transforming growth factor beta1 stimulates collagen production in primary cultured rat biliary fibroblasts in a dose-dependent manner

At 48 h after incubation with TGF-β1, the results showed that TGF-β1 induced the secretion of Col I from biliary fibroblasts in a dose-dependent manner (Table 1A).

Connective tissue growth factor mediates the secretion of Col I from biliary fibroblasts induced by transforming growth factor beta1

The p-Smad3 level was significantly higher than that of the control group (Figure 1A, $p<0.01$). Next, the cells were transfected with siRNA specifically targeting Smad3. The cells were collected 24 h later and the t-Smad3 level was detected, suggesting that Smad3-specific siRNA significantly inhibited the expression of the target protein (Figure 1B, $p<0.01$).

Biliary fibroblasts were transfected with Smad3- or CTGF-specific siRNA for 24 h before stimulation by TGF-β1, and the culture supernatant was collected after 24 or 48 h of TGF-β1 incubation for the detection of CTGF or Col I level. Our results showed that the specific inhibition of Smad3 expression can significantly suppress the production of CTGF protein (Table 1B, p<0.01). We also found that CTGF-specific siRNA interference of target cells significantly inhibited the expression of the target protein (Table 1C, p<0.01). Our results further showed that Smad3- and CTGF-specific siRNAs can inhibit the secretion of Col I stimulated by TGF-β1 to the same extent (Table 1D, p<0.01). This indicates that TGF-β1 upregulates the expression of CTGF by activating Smad3, thereby stimulating the secretion of Col I in biliary fibroblasts.

Activated peroxisome proliferator-activated receptor gamma inhibits Col I production in rat biliary fibroblasts stimulated by transforming growth factor beta1 in a dose-dependent manner

The results showed that rosiglitazone can significantly activate the PPAR-γ signal, and its activity increased by 4.02 times compared with the control group (Table 1E, p<0.01), while GW9662 diminished the activity of the rosiglitazone group by 1.74 times (Table 1E, p<0.01). The results demonstrate that rosiglitazone-activated PPAR-γ can be inhibited by GW9662. We further found that TGF-β1-stimulated secretion of Col I in biliary fibroblasts in a dose-dependent manner and pre-incubation of cells with 10 μmol/L rosiglitazone can reduce the secretion of TGF-β1-stimulated Col I to a level of 1.44 times over control group (Table 1F, p<0.01).

Molecular mechanism of inhibition of transforming growth factor beta1-induced Col I secretion in biliary fibroblasts by activated peroxisome proliferator-activated receptor gamma

To delineate the molecular mechanism of activated PPAR-γ inhibiting the TGF-β1-induced secretion of Col I in biliary fibroblasts, the cells were transfected with Smad3- or CTGF-specific siRNA, pretreated with rosiglitazone (10 μmol/L), or pretreated with PPAR-γ inhibitor GW9662 (10 μmol/L) simultaneously at 24 h before TGF-β1 treatment. The results suggest that TGF-β1 significantly activates p-Smad3 (Figure 2, p<0.01), induces CTGF expression (Table 1G, p<0.01), and induces the secretion of Col I from biliary fibroblasts (Table 1H, p<0.01). PPAR-γ activated by rosiglitazone significantly reduced the activity of Smad3 (Figure 2, p<0.01) and inhibited CTGF expression (Table 1G, p<0.01) and Col I secretion

Table 1. Pharmacological activity of various treatment groups (fold of increase over control)[a].

A	
Treatment group	**Collagen I (fold of increase)**
Control	1.0
TGF-β1 (1 ng/mL)	2.2±0.24*
TGF-β1 (3 ng/mL)	3.1±0.32*
TGF-β1 (10 ng/mL)	4.3±0.65*
TGF-β1 (30 ng/mL)	4.6±0.74*
B	
Treatment group	**CTGF (fold of increase)**
Control	1.0
TGF-β1 (10 ng/mL)	2.28±0.36*
Smad3 SiRNA+TGF-β1 (10 ng/mL)	1.42±0.27#
C	
Treatment group	**CTGF (percentage of control)**
Control	100
Con SiRNA	97.03±3.45
CTGF SiRNA	36.38±2.46*
D	
Treatment group	**Collagen I (fold of increase)**
Control	1.0
TGF-β1 (10 ng/mL)	2.80±0.76*
Smad3 SiRNA+TGF-β1 (10 ng/mL)	1.49±0.33#
CTGF SiRNA+TGF-β1 (10 ng/mL)	1.41±0.17#
E	
Treatment group	**PPAR-γ (fold of increase)**
Control	1.0
Rosi (10 μmol/L)	4.02±0.65*
Rosi (10 μmol/L)+GW9662 (10 μmol/L)	1.74±0.52△
F	
Treatment group	**Collagen I (fold of increase)**
Rosi (0 μmol/L)+TGF-β1 (0 ng/mL)	1.0
Rosi (10 μmol/L)+TGF-β1 (0 ng/mL)	0.97±0.12
Rosi (0 μmol/L)+TGF-β1 (10 ng/mL)	2.74±0.53*
Rosi (1 μmol/L)+TGF-β1 (10 ng/mL)	2.11±0.42
Rosi (3 μmol/L)+TGF-β1 (10 ng/mL)	1.73±0.33#
Rosi (10 μmol/L)+TGF-β1 (10 ng/mL)	1.44±0.23#
Rosi (30 μmol/L)+TGF-β1 (10 ng/mL)	1.42±0.26#
G	
Treatment group	**CTGF (fold of increase)**
Control	1.0
TGF-β1 (10 ng/mL)	3.18±0.65*
Rosi (10 μmol/L)+TGF-β1 (10 ng/mL)	1.52±0.27#
GW9662 (10 μmol/L)+Rosi (10 μmol/L)+TGF-β1 (10 ng/mL)	2.23±0.45δ
H	
Treatment group	**Collagen I (fold of increase)**
Control	1.0
TGF-β1 (10 ng/mL)	2.63±0.76*
Rosi (10 μmol/L) + TGF-β1 (10 ng/mL)	1.46±0.31#
GW9662 (10 μmol/L)+ Rosi (10 μmol/L)+TGF-β1 (10 ng/mL)	1.97±0.69δ

TGF-β1: transforming growth factor beta; CTGF: connective tissue growth factor. *p<0.01 compared with Con; #p<0.01 compared with TGF-β1 group; △p<0.01 compared with Rosi group; δp<0.01 compared with Rosi+TGF-β1 group. Here a is n=3 in each group.

(Table 1H, p<0.01). However, GW9662 inhibited the activation of PPAR-γ, thereby significantly reversing the abovementioned effects of rosiglitazone (Figure 2, Table 1G,H, p<0.01). This suggests that rosiglitazone-activated PPAR-γ can inhibit the activation of Smad3 stimulated by TGF-β1 and inhibit the expression of its downstream protein CTGF, thereby suppressing the secretion of Col I in biliary fibroblasts.

DISCUSSION

The preferred treatment for BBS is endoscopic retrograde cholangiopancreatography (ERCP) under X-ray combined with biliary stent placement[12]. Based on the clinical guidelines issued by the American Society for Gastrointestinal Endoscopy, the incidence of bile duct restenosis within 2 years after biliary stent placement is as high as 30%, and restenosis should be treated using repeated or multiple ERCP combined biliary stent placement operations[13]. Compared with endoscopic biliary stent placement, biliary reconstruction surgery after surgical management of biliary stenosis is associated with higher difficulty and surgical risk[14]. Therefore, it is particularly important to study the molecular mechanism of BBS formation so as to explore therapeutic targets and drugs that inhibit the formation of BBS. We have developed a degradable polymer stent with good supporting properties as a treatment for BBS[15]. In view of this, we aimed to search for new drugs that inhibit BBS.

As an important pathogenic factor of BBS, specific inhibition of the expression of CTGF is considered to be a potential therapeutic approach to preventing benign stricture of the bile duct[5]. This study further confirmed that CTGF specifically mediates the activation of the TGF-β1/Smad3 signaling pathway and induces the secretion of type I collagen in biliary fibroblasts. The anti-fibrotic effect of activated PPAR-γ has been proven[16,17]. Using biliary fibroblasts, the current study confirmed that the activation of PPAR-γ can inhibit Smad3 phosphorylation and the expression of downstream protein CTGF and suppress TGF-β1-induced type I collagen secretion in biliary fibroblasts, suggesting that activated PPAR-γ may prevent the occurrence of BBS by specifically suppressing the secretion of type I collagen in biliary fibroblasts. Our previous studies also confirmed that PPAR-γ exerts an anti-proliferative effect on biliary fibroblasts, and activated PPAR-γ

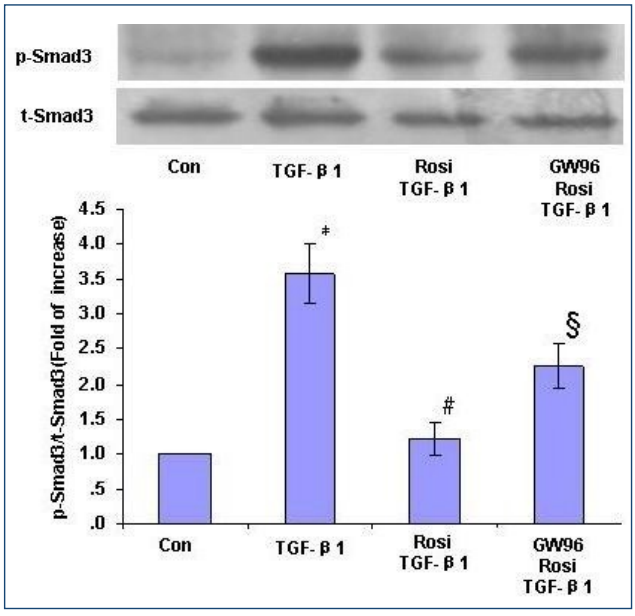

Figure 2. *p<0.01 compared with Con. #p<0.01 compared with the TGF-β1 group. §p<0.01 compared with the Rosi+TGF-β1 group. Activated PPAR-γ suppresses the secretion of TGF-β1-stimulated Smad3 phosphorylation. n=3 in each group. Con, control group; Rosi, rosiglitazone; TGF-β1.

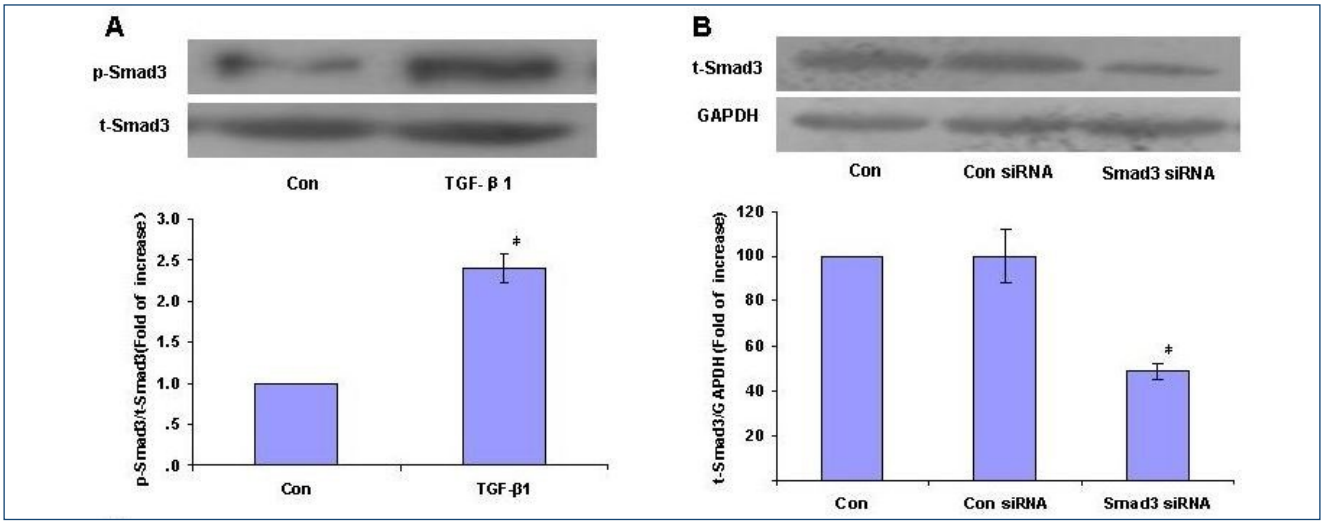

Figure 1. (A and B) TGF-β1 upregulates p-Smad3 activation. n=3 in each group. *p<0.01 compared with Con.

inhibits platelet-derived growth factor (PDGF)-stimulated PI3K/AKT/Skp2 signaling axis to influence AKT phosphorylation and its downstream effects[18].

As a specific PPAR-γ activator, rosiglitazone has been utilized to treat diabetes in the clinic. Many animal or clinic studies have suggested that enhancing the function of PPAR-γ might benefit a lot of other diseases, such as polycystic ovary syndrome in women by affecting insulin-like growth factor-binding protein-3, insulin-like growth factor 1, and insulin resistance[19]. A number of studies including this one further indicate that PPAR-γ activation could inhibit remolding of biliary and has a potential value in the treatment of BBS. Yet, a series of clinical trials are further needed to assure that the treatment mode is effective and safe in the management of BBS[11,20].

CONCLUSIONS

Activated PPAR-γ can inhibit the proliferation of biliary fibroblasts and the deposition of type I collagen by manipulating different molecular signaling mechanisms, indicating the potential of PPAR-γ activator rosiglitazone and other drugs in preventing BBS. This study is expected to provide ideas on new therapeutic approaches for the prevention of BBS.

AUTHORS' CONTRIBUTION

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

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ORIGINAL ARTICLE

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Cutaneous melanoma diagnosis delay: socioeconomic and demographic factors influence

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SUMMARY

OBJECTIVE: Malignant cutaneous melanoma is the most aggressive type of skin cancer, and its early detection and prompt initiation of treatment play an important role in reducing disease-associated morbidity and mortality. Many factors influence the diagnosis of melanoma, and its recognition is essential for the development of strategies for its early detection. This study was carried out to Identify the main variables related to the delay in diagnosis of Malignant Cutaneous Melanoma and correlate them with the time interval for making the definitive diagnosis.

METHODS: Retrospective analysis of 103 patient records from January 2015 to December 2020 correlating social, economic, demographic, and cultural factors with the time elapsed between the onset of symptoms and the diagnosis of malignant cutaneous melanoma.

RESULTS: The average time to seek medical services from the onset of symptoms was 29.54 months. The mean time for a referral from the primary to the referral service was 1.35 months, and the factors that contributed to a faster diagnosis were lesion Breslow (>1 mm), lesion growth, income range (≤1.5 minimum wages), lower phototypes (I and II), not having gone to the Basic Healthcare Units, profession (household), smoking, and type of housing.

CONCLUSIONS: Our findings demonstrate that there is still a great delay in the recognition of signs and symptoms related to the diagnosis of malignant cutaneous melanoma in our country, influenced by several socioeconomic and demographic factors.

KEYWORDS: Melanoma. General practice. Public health. Late diagnoses. Epidemiology.

INTRODUCTION

Skin cancer accounts for 30% of all cancer diagnoses in Brazil. Malignant cutaneous melanoma (MCM) is the most aggressive tumor, representing about 5% of malignant cutaneous tumors and being responsible for most skin cancer-related deaths[1]. However, if diagnosed in its early stages, complete resection of the lesion is associated with favorable survival rates.

Some signs and symptoms are often undervalued, which can contribute to a delay in diagnosis[2]. The main warning sign regarding melanoma is the change in clinical features of pre-existing nevus lesions or the occurrence of a new pigmented lesion. Changes such as variation in color, diameter, height, or shape (asymmetry) are reported by 80% of patients at the time of diagnosis[3,4].

Due to its high potential to produce metastasis, early recognition is extremely important. This represents, however, a challenge for dermatologists, cancerologists, and surgeons, since they must perform the differential diagnosis with several other pathologies without delaying the definitive diagnosis and its therapy, which could interfere with the prognosis since excisional removal is often curative[5]. For this, the instruction received during their professional training is essential.

Patient education regarding attention to early signs of the disease is also essential for early diagnosis and successful treatment of melanoma. The level of education and socio-economic-cultural factors of patients can influence the early recognition of complaints associated with melanoma. Restricting access to specialized centers in our country with continental dimensions can also contribute to delayed diagnosis.

The aim of the present study was to identify the main variables related to the delay in diagnosis of MCM and to correlate them with the time interval for making the definitive diagnosis in a center specialized in the treatment of skin cancer.

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METHODS

After approval by the institution's Research Ethics Committee, medical records of 103 patients followed in our institution by the Brazilian Unified Health System, without age restriction, diagnosed with melanoma skin cancer and treated between 2015 and 2020 were retrospectively analyzed. Cases that lost follow-up in the service were excluded. All necessary information was obtained from 89 medical records and, in 14, socioeconomic and demographic variables could not be collected.

Socioeconomic and cultural variables were evaluated, such as occupation, family income (in minimum wages and per capita income), employees living in the same household, physical housing conditions, and education. Aspects related to the diagnosis, such as initial signs and symptoms, presence of metastasis, and type of treatment given were also evaluated.

The time elapsed between the initial perception of symptoms and the first contact with the medical service, as well as between this contact and the first consultation at the referral hospital, where the diagnosis was made, were analyzed. The first contact with the health service was considered when the patient was seen for the first time with symptoms related to the final diagnosis. These time intervals were used to define diagnostic delay.

To verify the hypothesis of agreement between two dichotomous categorical variables, checking the symmetry between lines and columns, the McNemar test was used. The Wilcoxon-Mann-Whitney test was used to compare measures of central tendency between two groups. This test is an alternative non-parametric method of the two-independent sample t-test that is used to test whether the (median) positions of population measurements are equal, considering the magnitude of differences between the pairs. To verify the association or compare proportions, the chi-square test or Fisher's exact test was used, when necessary.

RESULTS

Through descriptive analysis, it was found that the mean age of patients at diagnosis was 66.34±13.5 years; 44.7% were male and 55.3% were female; 1% were phototype I, 40.8% phototype II, 55.3% phototype III, 1% phototype IV, and 2% phototype V. Metastasis at initial diagnosis was reported in 5.82% of cases and tumors with Breslow ≤1 cm in 76.7%. Other nonmelanoma skin cancers were found concomitantly in 31.06% of patients.

The analysis of variables related to socioeconomic, cultural, and demographic conditions revealed that 68.53% had a monthly income of at least 1.5 minimum wages; 1.12% were illiterate; 20.22% had incomplete primary education; 34.83% complete primary education, 8.98% incomplete secondary education, 21.34% complete secondary education, and 12.8% complete higher education. Regarding profession, 56.31% of the patients worked outside the home and 43.68% were housewives or retired. Regarding housing conditions, 80.9% lived in brick and 19.1% in wood houses; 83.1% had basic sanitation and 16.9% used a septic tank.

Among the initial symptoms reported, lesion growth was present in 33% of cases, bleeding in 2.9%, lesion color change in 27.18%, pruritus in 8.73%, and pain in 3.88%. In total, 39.8% of patients did not observe changes in the lesions.

The average length of time between the first symptom observed by the patients and the first contact with the medical service was 29.54 months (ranging from 0 to 240 months). The total time between the first symptom and diagnosis was 30.9 months (range 0–240 months). The relationship of these times with the histological tumor subtype can be seen in Table 1.

Simple linear regression analysis was used to identify variables that could influence the time interval between the appearance of the first symptoms and the first medical contact. Significant associations ($p<0.05$) were found between the variables: Breslow less than or equal to 1 mm ($p=0.024$),

Table 1. Descriptive analysis and comparisons with histological type.

	Superficial spreading melanoma (n=46)	Lentigo maligna melanoma (n=8)	Acral lentiginous melanoma (n=2)	In situ superficial spreading pattern (n=10)	In situ Lentigo maligna pattern (n=31)
Time period 1 (mean±SD) Time period 1 (mean min–max)	22.54±27.69 12.0 (0.0–120.0)	26.25±40.3 15.0 (0.0–120.0)	5.1±2.96 5.1 (3.0–7.2)	52.83±74.79 24 (0.36–240.0)	38.57±53.6 24.0 (0.0–240.0)
Time period 2 (mean±SD) Time period 2 (mean min-max)	0.77±2.2 0.03 (0.0–12.0)	0.31±0.37 0.26 (0.0–1.0)	2.5±3.53 2.5 (0.0–5.0)	0.62±0.95 0.15 (0.0–3.0)	1.92±6.34 0.26 (0.0–36.0)
Time period T (mean±SD) Time period T (mean min-max)	23.28±28.35 12.03 (0.0–126.0)	26.52±40.17 15.5 (0.0–120.0)	7.6±6.5 7.6 (3.0–12.2)	53.45±74.71 24.1 (1.06–240.0)	39.29±52.86 24.5 (0.0–240.0)

Total time period (T): Sum of time periods 1 and 2. Time period 1: Time elapsed from the onset of symptoms and the first medical contact. Time period 2: From the first medical contact to the first consultation at the reference hospital.

Stage 0 (neoplasms in situ) (p=0.028), perception of lesion growth (p=0.032), income range lower than 1.5 minimum wages (p=0.016), phototypes I and II (p=0.024), and search for BHU (p=0.008). Among these, the variable perception of lesion growth and income range were negative in time for seeking medical care, while Breslow less than or equal to 1, Stage 0, phototypes I and II, and the search for the BHU were positive, contributing to a longer time until the first contact with the health services (Table 2).

Furthermore, statistically significant associations for some variables with the time interval between the first contact with the medical service until the definitive diagnosis in the reference service, namely, were found: smoking and household profession proved to be significantly negative for the delay in referral to the reference service (p=0.034 and p=0.005, respectively), while housing condition (living in a brick house) was found to be a positive association for the delay in referral (p=0.043) (Table 2).

Finally, significant associations were evidenced between some variables and the time interval between the onset of the first symptom and the definitive diagnosis of melanoma in the reference service. The presence of tumors whose Breslow is less than or equal to 1 (p=0.037), Stage 0 tumors (in situ, p=0.039), phototypes I and II (p=0.021), and the search for the BHU before arrival at the service reference (p=0.009) presented as positive correlation factors for the greatest delay in time to definitive diagnosis since the presentation of the first symptom; while income range lower than 1.5 minimum wages (p=0.026) and residence in a wooden house (p=0.036) were significantly negative for the same time interval (Table 2).

DISCUSSION

The signs and symptoms associated with MCM are often common to other more prevalent dermatological pathologies, making it difficult for both patients and general practitioners/dermatologists to recognize them, leading to a delay in its diagnosis and influencing both the immediate management and the prognosis of the disease.

Metzger described in 1998 that diagnostic delay in half of the patients diagnosed with MCM (and in 25% of patients with palmoplantar melanoma) was due to initial diagnostic errors. In most cases, the incorrect clinical diagnosis was made by non-dermatologists, specialists to whom patients usually initially report for dermatological complaints, and who often lack knowledge or diagnostic tools such as the

Table 2. Results of linear regression analysis to identify variables that discriminate the different times (statistically significant).

	Average time (months)	Etimated parameter	p
Time period 1			
Breslow (mm) ≤1 x >1	33.03 x 17.85	2.301	0.024
Lesion growth n x y	34.52 x 18.65	2.184	0.032
Stage lesion 0 x III and IV	41.16 x 16.8	2.271	0.028
Income (minimum wages) <1.5 x ≥1.5	14.13 x 31.56	-2.471	0.016
Phototypes I and II x III, IV, and V	43.08 x 20.13	2.328	0.024
Search BHU n x y	16.28 x 37.35	-2.729	0.008
Time period 2			
Household profession x others	0.069 x 1.485	-2.875	0.005
Smoking n x y	1.445 x 0.32	2.155	0.034
Home conditions masonry x wooden	1.721 x 0.4035	2.054	0.043
Total time period (T)			
Breslow (mm) ≤1 x >1	34.12 x 20.12	2.112	0.037
Stage lesion 0 x III and IV	42.9 x 18.86	2.139	0.039
Income (minimum wages) <1.5 x ≥1.5	16.46 x 32.69	-2.273	0.026
Phototypes I and II x III, IV, and V	44.71 x 21.3	2.381	0.021
Search BHU n x y	17.97 x 38.5	-2.655	0.009
Home conditions masonry x wooden	30.04 x 15.87	2.138	0.036

n x y: no x yes; BHU: basic healthcare units.

dermatoscope, thus interfering with the patient's prognosis, according to Robsahm et al.[6].

Regarding the association between clinical parameters of the lesions and the time to diagnosis, our study showed that for lesions with Breslow >1 mm and more advanced stages (III and IV), the time between the onset of symptoms and the patient's search for medical assistance (time 1) as well as the time between the onset of symptoms and the final diagnosis was significantly shorter. Although it seems logical to assume that a tumor will grow more deeply over time, once its invasive growth begins, there may not be a simple linear correlation between the time to diagnosis and Breslow's thickness, as demonstrated by data in the literature and in our study[7,8]. This delay in the diagnosis of initial lesions can be explained by the perception of physicians and patients who consider the hypothesis of malignant skin tumors only in more advanced stage presentations.

In a large French prospective multicenter study, a negative correlation was found between Breslow thickness and time to seek medical care. In this study, thicker tumors were responsible for a surprisingly shorter delay[9]. Furthermore, the biological behavior of the tumor may be the most important determinant of tumor thickness. As changes in slow-growing MCM tend to gradually settle down over a period of several months, it is possible that they are not noticed by the patient[7].

Symptoms other than the growth of the lesion were not decisive in motivating the early search for medical care. In our study, we found that the average time between the appearance of the first symptom and seeking medical care was 29.54 months, which highlights the difficulty in recognizing signs and symptoms related to this neoplasm.

Regarding the phototype, our study revealed a delay in the diagnosis of patients with lower phototypes, I and II of Fitzpatrick, in relation to those with higher phototypes (III and IV). This is in contrast to literature data showing that these neoplasms in patients with higher phototypes are more difficult to be detected, as 60–75% appear in less pigmented areas of the skin, generally not exposed to the sun, which may go unnoticed or be misdiagnosed as warts, fungi, or dark nails[10]. However, despite this contrast with findings in the literature, the data found in our study can be explained by the greater attention given by the population with higher phototypes to the appearance of new lesions, as they present a certain protective factor against skin neoplasms.

Surprisingly, by correlating the socioeconomic status of patients with the time between the first symptom of MCM and seeking medical care, as well as the definitive diagnosis in the oncology reference service, we observed a negative association between them, such that less favored patients had shorter times compared with more economically advantaged ones. These data go against findings in the literature that higher levels of education, generally associated with better socioeconomic conditions, would be associated with higher rates of self-detection and a faster diagnosis of melanoma[8]. The result leads us to believe that possibly skin cancer awareness campaigns may be reaching the neediest population more effectively.

A greater diagnostic delay was found in patients who sought primary health services before being referred to the tertiary hospital. Some studies have evaluated the role of physicians in delaying the diagnosis of cutaneous melanoma[6,11], noting that the difficulty in diagnosis and the performance of inadequate treatments significantly contribute to the delay in the early diagnosis of a patient[12]. This fact becomes more serious in melanoma[8,13].

The average time between the first medical appointment and the final diagnosis in this study was 1.35 months. Differences in times evidenced in the literature (1.3 months in South Africa; 1.5 months in Italy; 2 months in Germany; 3 months in the United States; and 3.9 months in Canada)[8] can be explained by the presence of a private and bureaucratic health system in other countries, compared with the Brazilian Unified Health System, which offers universal care to the entire population. The delay in referring the patient to the tertiary referral hospital interferes with the early definitive diagnosis may be attributed to the inexperience, insecurity, or insufficient training of physicians to make the diagnosis of melanoma.

Therefore, all physicians involved in primary or secondary health care need to be aware of the possibility of Melanoma, especially in those with a positive family history, report of long exposure to the sun, skin with photodamage, or other changes evidenced by the patient, as a change in the clinical characteristics of a pre-existing nevus lesion (changes such as variation in color, diameter, height, or change in shape – asymmetry), and the occurrence of a new pigmented lesion.

CONCLUSIONS

We observed that there is a significant lack of knowledge about melanoma and the recognition of its first signs, both by patients and by health professionals, especially in primary care, significantly affecting the early diagnosis of this pathology, with consequent delay in the treatment of the disease. It is of great importance that strategies to raise awareness of patients and health professionals are implemented to reduce the time for diagnosing these tumors.

AUTHORS' CONTRIBUTIONS

IOG: Conceptualization, Formal analysis, Data curation, Project administration, Writing – review & editing. **ACZ:** Conceptualization, Formal analysis, Data curation, Project administration. **DPA:** Conceptualization, Formal analysis. **DASA:** Conceptualization, Project administration, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of anesthesia type on outcome measures in cesarean section in the presence of fetal macrosomia

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SUMMARY

OBJECTIVE: The aim of this study was to compare the effects of general and spinal anesthesia on maternal and neonatal outcomes during cesarean section in pregnancies with macrosomia.

METHODS: This retrospective cohort study included 1043 patients who delivered by cesarean section between May 2018 and December 2021 and had a baby born with a birth weight of 4000 g or greater. Maternal and neonatal outcomes were compared according to the type of anesthesia performed in the spinal anesthesia group (n=903; 86.6%) and general anesthesia group (n=140; 13.4%). The Apgar score was categorized into <7 and ≥7.

RESULTS: Neonates with an Apgar score of <7 at the first minute (11.4 vs. 0.4%; p<0.001) and the fifth minute (2.9 vs. 0.3%; p=0.004) were significantly higher in the general anesthesia group. The preoperative and postoperative hematocrit difference was significantly lower in patients who received spinal anesthesia than those who received general anesthesia [2 (1.1–3.1) vs. 4.05 (2.8–5.35); p<0.001]. The number of patients transfused was higher in the general anesthesia group (9.3 vs. 2.7%; p<0.001). In the regression model, general anesthesia, birth weight, and emergency conditions were significant independent factors related to the preoperative and postoperative hematocrit decrease (p<0.001, p=0.005, and p=0.034, respectively).

CONCLUSIONS: Apgar scores of <7 at the first and fifth minutes are higher in macrosomic neonates who received general anesthesia than in neonates who received spinal anesthesia. Performing cesarean section under general anesthesia in mothers of macrosomic neonates results in a greater decrease in hematocrit value and a greater need for blood transfusion than under spinal anesthesia.

KEYWORDS: Cesarean section. Fetal macrosomia. General anesthesia. Pregnancy outcomes. Spinal anesthesia.

INTRODUCTION

Macrosomia is defined as birth weight (BW) of 4000 g or higher, regardless of the gestational age, and accounts for 8% of all births. Although not a disease in the strict sense, it is of clinical importance because of the potential risks to mother and neonate and the difficulties in delivery planning. The feared complications of macrosomia are shoulder dystocia, clavicular fracture, brachial plexus injury, and maternal third- and fourth-degree perineal lacerations, which most commonly occur with vaginal delivery[1].

The goal of obstetric anesthesia is to ensure the safety and well-being of the mother, deliver a healthy baby, and provide appropriate surgical conditions. The choice of anesthesia type depends on factors such as the urgency of the cesarean section (CS), patient's current systemic problems, experiences of the anesthesiologist and surgeon, and size of a hospital[2]. Although general anesthesia (GA) has rapid application in emergencies, regional anesthesia is the most commonly used and widely accepted method for elective CS, even for emergencies without contraindications. Spinal anesthesia (SA) prevents the possibility of aspiration pneumonia, failed tracheal intubation, maternal and neonatal respiratory complications, and maternal awareness when CS is performed under GA and allows for early maternal-neonatal bonding and improved postpartum pain management[3].

Cesarean section, which appears to reduce the risk of birth trauma in macrosomic fetuses compared with vaginal delivery and is performed more frequently than in nonmacrosomic fetuses, does not eliminate all the risks associated with maternal and neonatal morbidity despite improved anesthetic techniques[1]. Previous studies that compared maternal and neonatal outcomes between GA and SA for CS reported controversial results regarding neonatal well-being[4,5]. Pre-postoperative hematocrit reduction was reported to be greater in those receiving GA due to the uterine-relaxing effects of inhalation anesthetics[6,7]. Although fetal macrosomia is a proven obstetric risk factor for postpartum hemorrhage due to uterine overdistension and uterine atony associated with prolonged labor[8], there is limited

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evidence regarding the effects of anesthetic technique on CS procedures performed in the presence of fetal macrosomia.

In this study, we aimed to compare the effects of SA and GA on maternal and fetal outcomes and determine which anesthesia type is safer during CS surgeries for the mother and neonate with a BW of 4000 g or greater.

METHODS

After approval by the Local Ethics Committee (2011-KAEK-25 2022/01-02), a retrospective cohort study was performed by reviewing the medical records of 1102 cesarean deliveries with a baby BW of 4000 g or greater between May 2018 and December 2021. A total of 5 (0.5%) intrauterine fetal deaths, 4 (0.4%) fetal anomalies, 4 (0.4%) placental abnormalities, 37 (3.3%) combined spinal-epidural anesthesia, and 9 (0.8%) SA to GA conversions were excluded from the study. A total of 1043 subjects who met the inclusion criteria were divided into the SA group (n=903; 86.6%) and GA group (n=140; 13.4%) and then compared.

In the urgency of CS classification by Lucas et al., category 1 CS is defined as immediate threat to life of woman or fetus, category 2 CS is defined as maternal and fetal compromise, which is not immediately life-threatening, category 3 CS is defined as requiring early delivery but not maternal or fetal compromise, and category 4 CS is defined as the time that suits the mother and maternity team[9]. In this study, categories 1 and 2 were grouped as emergency CS, while categories 3 and 4 were grouped as elective CS.

Preoperative hematocrit values were obtained from the complete blood count (CBC) within 1 week before surgery for elective CS and immediately before surgery in emergency CS.

In our clinic, which is a tertiary referral hospital, anesthetic procedures are performed by an experienced anesthesia team according to the same protocol. After preloading, single-shot SA was administered with a 25-gauge spinal needle, at the level of lumbar vertebrae 3-4 or 4-5 interspinously. About 8–10 mg of 0.5% hyperbaric bupivacaine combined with 20 µg fentanyl was injected intrathecally to achieve adequate sensorial block (T4-T5) and then the surgery was initiated. If hypotension occurred, the intravenous (IV) fluid infusion rate was increased, and if hypotension persisted, 5–10 mg IV ephedrine was administered.

After preoxygenation, GA was induced with 2–2.5 mg/kg propofol and 0.6–1 mg/kg rocuronium. After endotracheal intubation, GA was maintained with 50% oxygen in air until delivery of the neonate. After delivery, IV administration of 2 µg/kg fentanyl and 0.15 mg/kg rocuronium was initiated, and 1% sevoflurane in 50% oxygen was continued to be administered. At the end of the surgery, the residual neuromuscular block was resolved with 2–4 mg/kg sugammadex.

Cesarean section was performed in the same manner and with a standard technique in all cases. After the delivery of the neonate, 5 IU of oxytocin was routinely infused to induce uterine contractions. A pediatrician evaluated the neonates for Apgar, BW, and the need for neonatal resuscitation. The criteria for blood transfusion are strictly applied to patients with symptoms of anemia and non-massive bleeding is defined as a hemoglobin value of <7 g/dL[10].

A CBC was performed 12 h after surgery to determine the hematocrit levels. If the patient had received a transfusion of blood products, then the CBC was referred to before discharge to determine the mean difference in hematocrit values. The length of hospital stay was calculated as days from the 24th hour after CS.

Data were analyzed using the IBM SPSS Statistics 18© Copyright SPSS Inc. 1989, 2010 software. The fit of continuous variables to normal distribution was examined using the Kolmogorov-Smirnov test. Nominal variables were expressed as frequencies and percentages (%), whereas continuous variables were expressed as mean, standard deviation (SD), or median and interquartile range (IQR) for the non-normally distributed variables.

In the analysis of categorical variables, Pearson's chi-square test was used. Mann-Whitney U test was applied when the assumptions of the parametric test were not met while comparing the means of two groups, and Student's t-test was used when provided. In addition, the variables and confounding factors that might affect the preoperative and postoperative hematocrit difference according to the literature were analyzed using a linear regression model[1,6,8]. The level of statistical significance was assumed to be 0.05.

RESULTS

Of the 1043 patients who underwent elective or emergency CS during the study period, 903 (86.6%) received SA and 140 (13.4%) received GA. The rate of emergency CS was 58.9% in the SA group and 73.6% in the GA group (p=0.001). Preoperative hematocrit values were similar in both groups (34.98±3.06 vs. 34.73±3.60; p=0.396), but postoperative hematocrit values were lower (32.74±3.20 vs. 30.58±3.82; p<0.001) and the mean hematocrit difference was higher [4.05 (2.8–5.35) vs. 2 (1.1–3.1); p<0.001] in patients in the GA group. The general characteristics of the groups are summarized in Table 1.

In the distribution of CS indications, a suspected macrosomic fetus was more frequent in the SA group, while fetal distress and umbilical cord prolapse were observed in the GA group (p<0.001).

Neonates with an Apgar score of <7 at the first minute (11.4 vs. 0.4%; p<0.001) and the fifth minute (2.9 vs. 0.3%; p=0.004) were significantly higher in the GA group. Approximately 7.3% of neonates in the SA group and 15.0% of neonates in the GA group were admitted to the neonatal intensive care unit (NICU) (p=0.002) (Table 2).

There were no differences in intraoperative and postoperative complications between the groups (p=0.983 and p=0.205, respectively). Approximately 2.7% of the patients in the SA group required transfusions, while this rate was higher in the GA group at 9.3% (p<0.001). The length of hospital stay was significantly longer in the GA group than in the SA group [2 (2–3) vs. 3 (2–3); p=0.002] (Table 2).

Analysis of cases by emergency (n=637) or elective (n=408) surgery showed that the mean hematocrit difference [2.4 (-3.40–13.60) vs. 2.0 (0.10–8.10); p=0.001] was significantly higher in the emergency CS cases. While the need for NICU admission (9.1 vs. 7.1%; p=0.248) did not differ by type of CS surgery, Apgar scores of <7 at the first minute (2.5 vs. 0.7%; p=0.035), on the one hand, were higher in emergency CS cases, with no difference in Apgar scores at the fifth minute (0.09 vs. 0.02%; p=0.177). On the other hand, those who received SA for both elective and emergency CSs had a higher Apgar score of ≥7 at the first minute (99.7 vs. 96.4%, p<0.001).

A regression model was created for the variables and confounding factors that might affect the preoperative and postoperative hematocrit difference according to the literature[1,6,8]. The use of GA caused an increase in hematocrit difference by 1.102 units (p<0.001), elective surgery caused a decrease in hematocrit difference by -0.124 units (p=0.034), and an increase in BW by 1 unit caused an increase in hematocrit difference by 0.0834 units (p=0.009). GA, BW, and emergency CS were found to be significant independent risk factors for a decrease in hematocrit. Anesthesia type was the parameter that best explained the variation in pre-postoperative hematocrit difference in the model (t=13,204) (Table 3).

Table 1. General characteristics according to the type of anesthetic technique applied.

	Spinal anesthesia (n=903)	General anesthesia (n=140)	Total (n=1043)	p
Maternal age (years)	29 (16–45)	30 (18–46)	29 (16–46)	0.024µ
Gestational age (weeks)	39 (35–42)	39 (34–43)	39 (34–43)	0.590µ
BMI (kg/m²)	35.10 (27.80–44.30)	35.00 (27.80–44.30)	35 (27.80–44.30)	0.568µ
Parity number	2 (1–11)	2 (1–8)	2 (1–11)	0.007µ
Previous cesarean number	1 (1–5)	1 (1–5)	1 (1–5)	0.327µ
Birth weight (g)	4190 (4000–5530)	4250 (4000–5520)	4200 (4000–5530)	0.045µ
Maternal DM	23 (2.5)	11 (7.9)	34 (3.3)	0.002ˣ
Maternal GDM	46 (5.1)	7 (5.0)	53 (5.1)	0.999ˣ
Pre-op Hct	34.98±3.06	34.73±3.60	34.94±3.13	0.396ᵗ
Post-op Hct	32.74±3.20	30.58±3.82	32.45±3.37	<0.001µ
Hct difference (pre-postoperative Hct)	2 (1.1–3.1)	4.05 (2.8–5.35)	2.2 (1.2–3.4)	<0.001µ
Infant gender				
Female	294 (32.6)	37 (26.4)	331 (31.7)	0.147ˣ
Male	609 (67.4)	103 (73.6)	712 (68.3)	
Type of cesarean section				
Emergency	532 (58.9)	103 (73.6)	635 (60.9)	0.001ˣ
Elective	371 (41.1)	37 (26.4)	408 (39.1)	

BMI: body mass index; DM: diabetes mellitus; GDM: gestational diabetes mellitus; Hct: hematocrit. Results are given as mean±SD, median (IQR), or n (% column). µMann-Whitney U test; ᵗStudent's t-test; ˣPearson's χ^2test. Bold indicates significant values.

Table 2. Neonatal and maternal outcomes by type of anesthesia.

	Spinal anesthesia (n=903)	General anesthesia (n=140)	Total (n=1043)	p
Apgar first minute				
<7	4 (0.4)	16 (11.4)	20 (1.9)	<0.001ˣ
≥7	899 (99.6)	124 (88.6)	1023 (98.1)	
Apgar fifth minute				
<7	3 (0.3)	4 (2.9)	7 (0.7)	0.004ˣ
≥7	900 (99.7)	136 (97.1)	1036 (99.3)	
NICU admission	66 (7.3)	21 (15.0)	87 (8.3)	0.002ˣ
Intraoperative complications				
No	894 (99.0)	138 (98.6)	1032 (98.9)	0.642ˣ
Yes	9 (1.0)	2 (1.4)	11 (1.1)	
Uterine rupture	1	1		
Bladder laceration	2	1		
Uterine atony	5	0		
Bowel laceration	1	0		
Postoperative wound infection				
No	897 (99.3)	137 (97.9)	1034 (99.1)	0.078ˣ
Yes	6 (0.7)	3 (2.1)	9 (0.9)	
Blood transfusion requirement				
No	879 (97.3)	127 (90.7)	1006 (96.5)	<0.001ˣ
Yes	24 (2.7)	13 (9.3)	37 (3.5)	
Pre-postop hematocrit difference	2.23±1.43	4.15±2.18	2.49±1.68	**<0.001 μ**
Length of hospital stay (day)	2 (1–15)	3 (1-14)	2 (1-15)	**0.002 μ**

NICU: neonatal intensive care unit. Results are given as median (IQR), or n (% column). μMann-Whitney U test;ˣPearson; χ^2 test. Bold indicates significant values.

Table 3. Regression model for factors affecting pre-postoperative hematocrit difference.

Predictor	Estimate	SE	95% confidence interval		t	p	Stand. estimate
			Lower	Upper			
Intercept[a]	-4.04754	2.04259	-8.05562 – -0.0395		-1.982	0.048	
Birth weight	0.55511	0.19549	0.17151–0.9387		2.840	0.005	0.08340
Maternal age (years)	0.01087	0.00807	-0.00496–0.0267		1.348	0.178	0.03849
Gestational age (weeks)	0.02312	0.04206	-0.05941–0.1056		0.550	0.583	0.01580
BMI (kg/m^2)	0.0040	0.01610	-0.02756–0.0356		0.250	0.802	0.00727
Type of anesthesia GA–SA	1.85994	0.14086	1.58353–2.1364		13.204	<0.001	1.10263
Preoperative Hct	0.07551	0.01519	0.04571–0.1053		4.972	<0.001	0.14046
Type of CS Elective-Emergency	-0,20968	0.09883	-0.40362 – -0.015		-2.122	0.034	-0.12431

GA: general anesthesia; SA: spinal anesthesia; Hct: hematocrit. Model coefficients: pre-postoperative hematocrit difference. [a]Reference level. Bold indicates significant values.

DISCUSSION

Our results show that GA is associated with Apgar scores of <7 at the first and fifth minutes, increased NICU admission rates, higher pre-postoperative hematocrit difference, increased number of transfused patients, and increased length of hospital stay in CS procedures with macrosomia. GA, BW, and emergency conditions were independent risk factors for decreased hematocrit pre- and postoperatively.

Afolabi et al. reported that Apgar scores were significantly lower at the first and fifth minutes in emergency CS cases who received GA compared with SA[4]. Another study found that despite faster delivery in neonates born under category 1 CS according to the Lucas classification[9], an Apgar score of <7 at the fifth minute and NICU hospitalization were significantly higher in the GA group compared with the SA group[11].

Al-Husban et al. evaluated both elective and emergency CS procedures and concluded that the Apgar scores were higher in emergency category cases who received SA than those who received GA, with no significant difference in the elective category[12]. In contrast, Mancuso et al[5]. and Saygı et al[7]. reported that SA had a better effect on Apgar scores in elective CS than GA.

On the one hand, our study found that neonates exposed to GA were more likely to have Apgar scores of <7 at the first and fifth minutes and more likely to be admitted to the NICU than those exposed to SA. When the same neonates were evaluated according to whether they were delivered as an emergency or electively, Apgar scores of <7 at the first minute were higher in emergency CS cases. On the other hand, neonatal morbidity was not classified according to the indication for CS. Therefore, our results suggest that most GA-related adverse neonatal outcomes may be influenced by the emergency nature of the procedure and the indication of the CS surgery.

In this study, we focused on neonates with macrosomia and found that postoperative hematocrit values were lower and the pre-postoperative hematocrit difference was greater in the GA group than in the SA group. The need for blood transfusion was also higher in the GA group than in the SA group. Sung et al.[6] found that the mean difference between preoperative and postoperative hematocrit levels was greater in the GA group. However, in agreement with the results of Heesen et al.[13], the proportion of transfused patients did not differ between the groups.

Our results are consistent with the study by Aksoy et al., who examined elective cesarean deliveries in uncomplicated term pregnancies and concluded that SA was associated with lower blood loss during CS than GA. Blood transfusions were required in 4 (2%) patients in the GA group and 2 (1%) patients in the SA group, although whether this difference was statistically significant was not stated[14]. In our regression model, GA, BW, and emergency conditions were independent factors associated with the decrease in hematocrit values. In emergency CS cases, the mean hematocrit difference increased, but the need for blood transfusion did not increase. It can be concluded that in CS procedures performed in the presence of a fetus with macrosomia, GA is associated with a clinically significant reduction in the hematocrit level.

The main limitation of this study was its retrospective design. The focus on macrosomic fetuses and the large sample size are the strengths of our study. The fact that the same surgical team managed the patients and infants were cared for in the same center also contributes to the strengths of the study.

CONCLUSIONS

The incidence of fetal macrosomia is steadily increasing and poses potential obstetric and fetal risks at birth. In CS procedures performed in the presence of a fetus with macrosomia, Apgar scores of <7 at the first and fifth minutes and NICU hospitalization were significantly higher in the GA group than in the SA group. GA was associated with a greater decrease in hematocrit values during CS and a greater need for blood transfusions.

AUTHORS' CONTRIBUTIONS

FNT: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **NK:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing.

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Self-medication practices with conventional and herbal drugs among ear, nose, and throat patients

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SUMMARY

OBJECTIVE: This study evaluates the self-practices with conventional and herbal drug use among ear, nose, and throat outpatients.

METHODS: A cross-sectional survey-based study was carried out among all ear, nose, and throat outpatients on their first visit to the otorhinolaryngology department at a tertiary care hospital. The survey comprised a total of 14 questions with 4 different sections, including demographic characteristics, self-medication of conventional medicines, herbal medication usage, and perception regarding herbal medicines.

RESULTS: Overall, 255 questionnaires were distributed among patients, of which 183 completed the questionnaire (response rate=71.7%). Respondents reported self-medication (44.8%) with conventional drugs before visiting a hospital. The most commonly used medicine was analgesics (31.7%) and antibiotics (21.9%). Nearly half of the patients (49.2%) used at least one herbal drug. The most commonly used herbal medications were *Tilia cordata* (78.8%), *Zingiber officinale* (62.2%), and *Camellia sinensis* (45.5%). According to the International Union for Conservation of Nature Red List, most of the medicinal herbs were considered as data deficient/least concern. About 36.6% of the participants perceived that herbal drugs are effective for ear, nose, and throat problems. Moreover, 22.9% of the patients did not know about herbal-drug interaction with other medications.

CONCLUSIONS: This study observed a considerable prevalence of self-based practices with conventional and herbal medications. Strict national regulations on conventional and herbal medication access and long-term actions should be implemented to discourage inappropriate drug use.

KEYWORDS: Self medication. Herbal medicine. Antibiotics. Analgesics. Otolaryngology. Turkey.

INTRODUCTION

Self-medication (SM) is a major public health issue around the world[1]. It is a frequent practice in both developing and developed countries[2]. Several studies have shown that inappropriate SM leads to wastages of health resources, raises pathogen resistance, and poses major health risks such as adverse drug reactions (ADRs), extended suffering, and dependency[1,2]. It is reported that a higher number of medications in developed countries were obtained without a prescription[2]. The use of herbal and nonherbal medication practices to meet healthcare needs was also prevalent in developing countries[3]. Moreover, it is also reported that more than 70% of people in developing countries utilize herbs for various illnesses[4].

It is estimated that more than 75 and 50% of the populations in developing and developed countries, respectively, use natural plant products to treat lifestyle-related illnesses[5]. It is the widespread belief that plant-based products are risk free and can be consumed without restriction. It should be noted that plant derivatives, like any other medication, have adverse and toxic side effects[6]. They might contain toxins or other contaminants that increase the chance of adverse effects. More research is needed to clarify and determine the clinical significance of herb-drug interactions. It is critical for health professionals, patients, regulatory authorities, and herbal medicine suppliers to be aware of the potential ADRs and drug interactions that can occur when herbal medicines are used either alone or in combination with conventional drugs[7]. Additionally, many of them have unknown long-term effects, necessitating appropriate use, education, and research[6]. Therefore, it is important to conduct rigorous scientific procedures and clinical trials to guarantee the consistency and quality of herbal products[5,7].

Ear, nose, and throat (ENT) diseases cause major disruption in patients' daily lives[1]. The prevalence of ENT diseases varies by geography and patient age[8]. Self-practices with conventional and herbal drugs are common in ENT patients. Recent studies reported that the ENT patients' self-practice with conventional medication ranged from 79.1–83%[8,9]. It is also reported that the rate of herbal drug utilization among ENT patients ranges from 2–63% in different nations[4]. Individuals may suffer serious consequences as a result of SM practices without consulting a medical expert[1,2,8].

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The self-practices of over-the-counter (OTC) drugs and nonpharmaceutical products (e.g., vitamins, herbal products, and dietary supplements) are frequently used by the Turkish population[10]. These medications are available without a prescription, and people perceived that they are safe and effective. However, these medications can have adverse effects like prescription drugs and be misused or abused because of their active ingredient[10,11]. Herbal and conventional medicine's concomitant use can also cause dangerous herb-drug interactions[12].

In Turkey, ENT illnesses impact people of all ages and are an important public health issue[13-15]. However, literature searches revealed that previously no research examined the self-practices with conventional and herbal drug use among ENT patients in our study healthcare setting as well as in Turkey. Periodic studies are critical for assessing the usage of self-based medication practices in ENT patients and to give current insight to healthcare policy makers. Therefore, this study was carried out to assess the self-practices with conventional and herbal drug use among ENT patients at a tertiary care hospital in Turkey.

METHODS

A cross-sectional questionnaire-based survey was carried out among all ENT outpatients on their first visit to the otorhinolaryngology department at the tertiary care hospital in Adana, Turkey, in November 2017. This study was approved by the university's Ethical Review Committee, and the research was carried out in accordance with the Helsinki Declaration (meeting no: 69; date: October 6, 2017; Supplementary file 1).

Patients aged 17 years or older who attended an ENT department were included in the study, while respondents under the age of 17 and not willing to participate were excluded during the study period. This study used a convenience sampling technique. As per hospital data, an average of 750 patients visited the ENT department within 1 month. According to the Raosoft sample size calculator, the minimum calculated sample size was 255. The response distribution was assumed to be 50%, the margin of error was set at 5%, and the confidence level for the sample estimate was set at 95% (http://www.raosoft.com/samplesize.html).

A self-reported questionnaire was developed for this study based on a review of prior literature[1,4,8,12]. Three researchers reviewed the questionnaire instrument before the execution of the study to determine the appropriateness and validity (internal and external), along with the confirming adequacy of the questions. Data collection was conducted on a daily basis via a face-to-face approach. Informed consent was obtained from all participants.

The final 14-item questionnaire was divided into four sections:

1. demographic,
2. SM of conventional medicines,
3. herbal medication usage, and
4. perception regarding herbal medicines.

Demographic characteristics included age, gender, and education level. SM of conventional medicines included four questions regarding self-practices of conventional medication for ENT problems before visiting a hospital, types of medications, source of information, and their action regarding extra medications after the completion of treatment. The third section comprised four items regarding herbal medication use, types of herbal drugs, purpose of use, and source of information. The last section contained three questions related to the patient's perception of herbal medicines. We also classify the herbal drugs according to the International Union for Conservation of Nature (IUCN) Red List (https://www.iucnredlist.org/).

The final data were collected and transferred to Microsoft Excel 365 (version 2108, Microsoft Corp., USA), and the findings were recorded in number and percentage form. The final results are presented in tabulated form.

RESULTS

Of the 255 questionnaires distributed among the patients who visited the ENT department during the study period, 183 completed the questionnaire, and the response rate was 71.7%. The reason given for not completing the questionnaire was lack of time in the clinic (n=69), while 13 questionnaires were excluded owing to incomplete data. Finally, this study included 183 patients with a mean age of 34.96 (SD ±15.81) years (Table 1). Notably, 82 (44.8%) patients reported SM before visiting a hospital. The most commonly used medicine was analgesics (31.7%) and antibiotics (21.9%). The participants also reported the combined use of different medication classes. Further details are listed in Table 1.Nearly half of the patients (49.2%, n=90) used at least one herbal drug. Most of the participants indicated the usage of more than one herbal drug. The most commonly used herbal medications were *Tilia cordata* (78.8%), *Zingiber officinale* (62.2%), and *Camellia sinensis* (45.5%). The IUCN Red List classified the majority of the therapeutic herbs in this study as data deficient/least concerning (Table 2).

Among herbal users, 52 (57.8%) patients reported the purpose of usage. The most cited purposes of herbal drug use were common cold (26.9%) and general well-being (11.5%). None of the patients reported a qualified healthcare professional as a source of information for herbal drugs in this study. Additional information is provided in Table 3.

Table 1. Demographic characteristics and self-practices with conventional drugs (n=183).

Age	Frequency (n)
Average age	34.96
Minimum age	17
Maximum age	86
Standard deviation	15.81
Gender	**n (%)**
Male	65 (35.5)
Female	118 (64.5)
Education	
Primary school	29 (15.8)
Middle school	18 (9.8)
High school	52 (28.4)
University or above	84 (45.9)
None	0 (0)
Self-medication before visiting a hospital for ENT diseases (n=183)	**n (%)***
No	101 (55.2)
Yes	40 (21.9)
Sometimes	42 (22.9)
If yes/sometimes, which drugs (n=82)	
Analgesic	26 (31.7)
Antibiotic	18 (21.9)
Flu/cold medicine	9 (10.1)
Nasal spray	4 (4.8)
Throat spray	3 (3.6)
Ear drops	2 (2.4)
Lozenge	2 (2.4)
Inhalers	1 (1.2)
Antibiotic and analgesic	6 (7.3)
Antibiotic and cold medicine	2 (2.4)
Antibiotic and nasal spray	2 (2.4)
Antibiotic and lozenge	1 (1.2)
Antibiotic and mouth wash	1 (1.2)
Pain and fever reducer drug	2 (2.4)
Analgesic and nasal spray	1 (1.2)
Cough syrup and analgesic	1 (1.2)
Analgesic, cold medicine, and nasal spray	1 (1.2)
Total	**82**
Information about the medicine used during your treatment of ENT	
Pharmacist	84 (45.9)
Doctor	75 (40.9)
Neighbor/relative/friend	54 (29.5)
Internet	25 (13.6)
The prospectus of the drug	6 (3.2)
Other	3 (1.6)
What do you do with the extra medications after your treatment?	
Kept in home	87 (47.5)
Throw it in the bin	58 (31.7)
Give to the pharmacy	25 (13.6)
Give to my relatives/friends/neighbor	6 (3.3)
Give to a family doctor	4 (2.2)
Give to an emergency department in the hospital	2 (1)
My medication does not increase	1 (0.5)

*n (%):is the number and percentage indicating that more than one response was permitted. ENT: ear, nose, and throat.

DISCUSSION

Self-medication practices are widespread among otorhinolaryngological patients[1,4,8,9]. In this study, the prevalence of SM with conventional drugs was 44.8% among ENT patients before visiting a hospital. A study conducted in Nepal among ENT outpatients revealed similar results (47.3%)[16]. A higher prevalence (99%) of self-practices with conventional medicine was revealed in a study conducted in Lebanon[9]. However, a lower percentage (31%) of SM practices among patients attending the ENT department was observed in Nigeria[17]. The variation in the reported prevalence rate in this study compared to previous studies may be due to the differences in study methodology, cultural factors, populations, study design, and sample size. A considerable proportion of the population uses self-practice with conventional medication, and this is a concerning issue and may have the potential risks of adverse effect[4].

The most commonly used medicine was analgesic and antibiotics. Participants also reported a combined usage of different medication classes. This finding was also supported by the studies conducted in Brazil and Lebanon[8,9]. According to a meta-analysis of analgesics, cough medicines, dermatological products, nutritional supplements, and antibiotics were the most commonly self-practiced therapeutic drugs[18]. It is reported that if analgesics, cold drugs, cough syrups, and other OTC drugs are not used properly, they might harm the patient's quality of life and intensify the symptoms[8,10,11]. According to the World Health Organization, antibiotic resistance is currently one of the most serious threats to global health, food security, and development. Antibiotic resistance can affect anyone, regardless of age or place[19]. Antibiotic SM is a major contributor to the current antibiotic resistance dilemma[19]. Proper public health education is required to enhance public awareness about the dangers of SM of antibiotics, as well as legislation restricting access to OTC drugs[10,11].

In this study, a substantial proportion of the patients (49.2%) stated the use of at least one herbal drug for ENT illnesses before visiting the hospital. A previously published study in Kenya also reported substantial levels of herbal drug use (37.3%) among ENT patients[20]. A recent small-scale study conducted among patients suffering from chronic tinnitus in Turkey also reported the use of herbal medicines[21]. It is reported that the frequency of herbal medicine practices varies greatly among regions, countries, and around the world due to societal, traditional, and disease types[4,21].

Linden (*T. cordata*), ginger (*Z. officinale*), and green tea (*C. sinensis*) were the most frequently reported herbal medication approaches in this study. Recent review-based articles stated the effectiveness of Linden in anxiety, colds, cough, cardiovascular,

Table 2. Herbal medication use among ear, nose, and throat outpatients (n=183)

Use of herbal medicines	Frequency (n)	Percentage
No	93	(50.8)
Yes	60	(32.8)
Sometimes	30	(16.4)
If yes/sometimes, which herbal drugs (n=90)		
Herbal name (local name/scientific name)	**n (%)***	**IUCN Red List**
Linden (Ihlamur/*Tilia cordata*)	71 (78.8)	Least concern
Ginger (Zencefil/*Zingiber officinale*)	56 (62.2)	Data deficient
Green tea (Yeşil çay/*Camellia sinensis*)	41 (45.5)	Data deficient
Mint (Nane/*Mentha longifolia*)	17 (18.8)	Least concern
Lemon (Limon/*Citrus limon*)	17 (18.8)	Least concern
Garlic (Sarımsak/*Allium sativum*)	14 (15.5)	Least concern
Turmeric (Zerdaçal/*Curcuma longa*)	11 (12.2)	Data deficient
Fennel (Rezene/*Foeniculum vulgare*)	8 (8.8)	Least concern
Nettle (Isırgan/*Urtica dioica*)	8 (8.8)	Least concern
Grape seeds (Üzüm çekirdeği/*Vitis vinifera*)	5 (5.5)	Least concern
Sage (Adaçayı/*Salvia officinalis*)	2 (2.2)	Least concern
Liquorice (Meyan kökü/*Glycyrrhiza glabra*)	2 (2.2)	Least concern
Perforate St John's-wort (Sarı kantaron/*Hypericum perforatum*)	2 (2.2)	Least concern
Daisy (Koyungözü/*Bellis perennis*)	1 (1.1)	Data deficient

*n (%) is the number and percentage indicating that more than one response was permitted. IUCN: The International Union for Conservation of Nature.

and sore throat[22]. A Turkish study also found that linden (*T. cordata*) and sage (*Salvia officinalis*) were the most often utilized herbal medications[23]. Another study reported that linden was among the 10 most frequently used medicinal plants for health problems in Spain[24]. The effectiveness and use of *Z. officinale* in allergic rhinitis and oral cavity health problems were also reported[25]. It is reported that *C. sinensis* is a valuable natural source of antibacterial phytochemicals with multiple applications[26]. The effectiveness of medicinal herbs is dependent on proper use and preparation. The inappropriate use of herbal drugs may be responsible for adverse effects[24,25]. It is also important to highlight that the IUCN Red List classifies some of the medicinal plants consumed by the ENT patients in this study as data deficient/least concern. None of the herbal medications utilized by the participant in this study included the threatened/vulnerable/endangered category. Moreover, rigorous scientific proof of the effectiveness of phytotherapy in otorhinolaryngology is still lacking, and it is critical to be aware of the interactions with conventional treatment. However, further in-depth research, national and international legislation, and strict regulation are necessary for the controlled use, safety, and efficacy evaluation of these herbal drugs[5,7,24].

Table 3. The purpose of use, source of information, and patients' perception of herbal drugs.

Purpose of use (n=52)	n (%)*
Common cold	27 (51.9)
General well-being	10 (19.2)
Relaxation	4 (7.6)
Disease prevention	2 (3.8)
Fever	2 (3.8)
Throat pain	2 (3.8)
Shortness of breath	1 (1.9)
Cough	1 (1.9)
Immunity	1 (1.9)
Headache	1 (1.9)
Weight problem	1 (1.9)
Sources of information	
Internet	114 (62.3)
Environment (neighbor/relative/friend)	57 (31.1)
Media (TV, newspaper)	30 (16.4)
Book magazine	24 (13.1)
Qualified healthcare professionals (doctor/pharmacist/nurse, etc.)	0 (0)
What do you think about herbal treatment for ENT treatment?	
Noneffective	105 (57.4)
Effective	67 (36.6)
Uncertain	11 (6)
Did you know that herbal products/drugs may interact with other medicines?	
Yes	125 (68.3)
No	42 (22.9)
Sometimes	16 (8.7)
Did you know that herbal products/drugs also have side (adverse) effects?	
Yes	121 (66.1)
No	34 (18.6)
Sometimes	28 (15.3)

*n (%) is the number and percentage indicating that more than one response was permitted. ENT: ear, nose, and throat.

Interestingly, in this study, the participants reported that pharmacists and doctors are the main sources of information for conventional therapy compared to neighbors/relatives/friends and the Internet. In contrast, the Internet, neighbors/relatives/friends, and media were the most cited sources for herbal medication usage. None of the patients reported a qualified healthcare professional as a source of information for herbal drugs in this study. A Turkish study revealed similar findings and indicated that media and social circles (neighbors/relatives/friends) were the primary sources of information for herbal medication[21]. Another study conducted in Spain also reported family/friends and the Internet as a frequently cited source by the participants[24]. According to the most recent 2020 estimate, Turkey has 54 million social media users, which could explain why so many respondents in our survey acquired knowledge regarding herbal use via the Internet[26]. Herbal products are commonly used to treat clinical illnesses and are routinely purchased online without the supervision of a healthcare provider. Due to widespread inflated claims of clinical efficacy and safety, the use of herbals is still controversial[4]. The information may be more conveniently available on Internet social media sites; however, there is a risk of misinformation and may lead to safety issues[27].

Similar to other studies, this study also has limitations. The present findings may not be generalizable, especially since our study was based on a convenience sample of ENT patients recruited from a single hospital in Turkey. This may have biased the findings, such as an overestimation or underestimating of total conventional and herbal drug usage. This is a cross-sectional study with the limitation of only collecting data on all variables at one point in time, so no causal relationship could be inferred. Additionally, we did not inquire in depth about the participants' demographic details, such as religion or income level, due to ethical concerns, which could have resulted in the omission of some confounding variables.

Despite these limitations, this study has some strengths. This is the first study to evaluate SM practices with conventional and herbal drugs among ENT patients in our healthcare setting. This study targeted both conventional and herbal medicines to self-medicate, and these results may serve as an excellent starting point for further investigation. This study also provides a baseline of local data regarding SM practices (conventional and herbal medicines), and the results may be beneficial for practical clinics, healthcare professionals, and policy makers.

CONCLUSIONS

This study observed a considerable prevalence of self-based practices with conventional and herbal medications in ENT patients who attended the general otolaryngology clinic. Strict national regulations on conventional and herbal medication access and long-term actions should be implemented to discourage inappropriate drug use. A patient education program concerning the effects of SM practices is also urgently needed.

AUTHORS' CONTRIBUTIONS

OK: Conceptualization, Project administration, Writing – review & editing. **FB:** Conceptualization, Project administration, Writing – review & editing. **ZK:** Conceptualization, Project administration, Writing – review & editing. **MD:** Project administration, Writing – review & editing. **YK:** Project administration, Writing – review & editing.

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ORIGINAL ARTICLE

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Are inflammatory parameters an independent predictor of hip osteoarthritis severity? A prospective cross-sectional study

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SUMMARY

OBJECTIVE: This study aimed to investigate the relationship between the presence of hip osteoarthritis and the neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, monocyte-lymphocyte ratio, and neutrophil-monocyte ratio.

METHODS: Participants with hip osteoarthritis and healthy controls aged 45–75 years were recruited in the study. The participants with hip osteoarthritis were divided into two groups: mild/moderate hip osteoarthritis and severe hip osteoarthritis. Complete blood parameters of the participants were recorded, and neutrophil-lymphocyte ratio, neutrophil-monocyte ratio, monocyte-lymphocyte ratio, and platelet-lymphocyte ratio were calculated. Pain severity was evaluated using a visual analog scale.

RESULTS: A total of 76 participants with hip osteoarthritis and 59 healthy controls were included in the study. The mean age of the participants was 57.6±6.11 years. Mean neutrophil-lymphocyte ratio and neutrophil-monocyte ratio values were statistically significantly different between the hip osteoarthritis group and healthy control group ($p<0.05$). Platelet-lymphocyte ratio, monocyte-lymphocyte ratio, erythrocyte sedimentation rate, and C-reactive protein values were not significantly different between the groups. Also, there was no difference between all inflammatory parameters and hip osteoarthritis severity ($p>0.05$).

CONCLUSIONS: Neutrophil-lymphocyte ratio and neutrophil-monocyte ratio values were higher in patients with hip osteoarthritis than in healthy controls. Mean platelet-lymphocyte ratio, monocyte-lymphocyte ratio, erythrocyte sedimentation rate, and C-reactive protein values did not change according to the presence of hip osteoarthritis. Not all hematological indices give valuable information regarding the severity of hip osteoarthritis.

KEYWORDS: Hip osteoarthritis. Inflammation. X-rays.

INTRODUCTION

The hip joint, one of the largest weight-bearing joints in the body, is most affected by osteoarthritis (OA), along with the knee joint[1]. Although the articular cartilage is damaged in the foreground in hip osteoarthritis (HOA), the entire joint is affected. Articular cartilage loss, subchondral cysts, osteophyte formation, periarticular ligament laxity, muscle weakness, and possible synovial inflammation are seen in the process of OA[2]. Synovial inflammation, age, genetic factors, trauma, joint dysplasia, sex, and obesity are included in the etiology of HOA[3]. Although there are often different causes in the pathophysiology of OA, similar culminating processes occur during the development of the disease, and this situation creates the typical progression of OA[4]. Studies have shown that inflammation and the immune system play important role in the development of OA[5]. Various studies investigating the role of inflammation in the pathogenesis of OA have shown the effects of cytokines on disease progression, duration, and severity[6].

The neutrophil-lymphocyte ratio (NLR), which shows the two main functions of the immune system, i.e., inflammation and adaptive immune balance in peripheral blood, has been used as an effective and inexpensive biomarker for the past 20 years[7]. In the literature, NLR has been associated with conditions such as ischemic stroke[8] and cardiovascular diseases[9].

In addition, the platelet-lymphocyte ratio (PLR) and monocyte-lymphocyte ratio (MLR) are also accepted as markers to show inflammation in different diseases[10,11]. Furthermore, the neutrophil-monocyte ratio (NMR) has also been indicated as a potential marker that can be used to identify diseases such as skin cancer, breast cancer, knee OA, or predict prognosis, similar to PLR[12].

This study aimed to investigate the relationship between the presence of HOA and NLR, PLR, MLR, and NMR, which was previously shown to be an independent predictor of knee OA severity[12,13]. Using the information obtained from this study, it was aimed to better understand the role of inflammation in the pathogenesis of HOA and determine the points that could be used in treatment.

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METHODS

This was a prospective, cross-sectional study. Ethical approval was granted by the Abant İzzet Baysal University Medical Faculty clinical research ethical committee (2020/329) in conformity with the Declaration of Helsinki. Participants invited to take part in the study were informed in writing and verbally about the aim of the study. A signed Informed Volunteer Consent Form was obtained from each participant before inclusion in the study.

Participants

A total of 415 consecutive outpatients with HOA who were admitted to the Physical Medicine and Rehabilitation Outpatient Clinic between February and December 2021 were evaluated.

The power analysis of the study was estimated using the G*Power 3.1.9.4 software package (Franz Faul, Christian-Albrechts University of Kiel, Germany). We calculated that to achieve $\alpha<0.05$, $\beta=80\%$; according to the PLR, the minimum sample size required to be included in the study is 71 for the patient group and 53 for the healthy control group in order to identify a statistically significant difference between the two groups in terms of repeated measurements according to Taşoğlu et al.[14].

The inclusion criteria were ages 45–75 years and the presence of a radiologic and clinical diagnosis of HOA. Patients with secondary HOA, avascular necrosis, rheumatologic or autoimmune disease, chronic inflammatory disease, corticosteroid use, malignancy, hematological disease, and active viral and bacterial infection were excluded. Clinical and demographic characteristics of the participants were recorded.

Assessment methods

Radiologic Assessment

Grading of HOA was performed radiologically according to the Kellgren-Lawrence (KL) classification. The KL classification system is a radiologic grading method in OA. According to this system, narrow joint space exists in grade 1, joint obliteration and possible osteophytic lipping occur in grade 2, definite osteophytes, sclerosis, and cyst formation are seen in grade 3, and gross loss in joint space with sclerosis, cysts, and large osteophytes are present in grade 4[15].

Pain measurement

Participants were asked about mean pain experienced during the day using a visual analog scale (VAS) in order to measure hip pain. The patient was asked to indicate the severity of pain on a 10-cm line marked from 1 to 10, with 0 indicating no pain and 10 the worst possible pain[16].

Laboratory parameters

Peripheral venous blood specimens were collected using standard surgical procedures during presentation and were investigated in the Abant İzzet Baysal University Medical Faculty central laboratory. Complete blood count parameters (i.e., hemoglobin, leukocytes, erythrocytes, platelets, and leukocyte subtypes) were analyzed using a Syxmex XN-1000 (Kobe, Japan) automatic analyzer. Biochemical parameters [C-reactive protein (CRP)] were measured on an automatic biochemical analyzer (Abbott Architect C8000, USA). In addition, NLR (absolute neutrophil count/absolute lymphocyte count), NMR (absolute neutrophil count/absolute monocyte count), MLR (absolute monocyte count/absolute lymphocyte count), and PLR (absolute platelet count/absolute lymphocyte count) were calculated.

Statistical analysis

Statistical analysis was performed using the SPSS software version 23.0 (MacOs, IBM Corp., Armonk, NY, USA). The normality of the distribution of the variables was examined using the Shapiro-Wilk test. Descriptive statistics are presented as mean (standard deviation), median, and minimum and maximum values. For intergroup comparisons, the Mann-Whitney U test was used for nonparametric variables, and independent t-test was used for parametric variables. Spearman's correlation analysis was used to determine the relationship between the variables for ordinal or non-normally distributed variables.

RESULTS

A total of 76 participants with hip OA and 59 healthy controls aged 45–75 years were included in the study. Of these participants, 32 (23.7%) were male and 103 (76.3%) were female. The mean age of the participants was 57.6±6.11 years. The mean body mass index of the patients was 28.7±4.0. Demographic and clinical characteristics were similar between the HOA group and control group ($p>0.05$). Mild-to-moderate HOA was present in 37 (48.7%) patients, and 39 (51.3%) had severe HOA.

The mean NLR ($p=0.005$) and NMR ($p<0.001$) values were statistically significantly different between the hip OA group and healthy control group. In contrast, the mean PLR, MLR, erythrocyte sedimentation rate (ESR), and CRP values were not significantly different between the two groups (Table 1).

When the HOA group was separated into two groups – mild/moderate OA and severe OA, there was no significant

difference in terms of hemocytometric parameters between the two groups (Table 2).

According to the correlation analysis of the variables in HOA, there was no relationship between laboratory and hemocytometric parameters with the KL classification (severity of the HOA) and VAS (pain intensity) (Table 3).

Table 1. Intergroup analysis for HOA group and control group according to laboratory parameters.

	HOA group (n=76)	Control group (n=59)	p
NLR	2.1±1.3	1.7±0.7	0.005*
NMR	9.3±3.1	7.2±2.3	<0.001*
PLR	122.6±45.9	117.4±46.2	0.338
MLR	0.2±0.1	0.2±0.1	0.346
CRP (mg/L)	3.5±4.0	2.4±1.6	0.605
ESR (mm/L)	12.2±10.2	11.8±7.3	0.447

HOA: osteoarthritis; NLR: neutrophil-lymphocyte ratio; NMR: neutrophil-monocyte ratio; PLR: platelet-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate. *p<0.05 is considered significant for Mann-Whitney U test.

Table 2. Intergroup analysis for mild/moderate and severe osteoarthritis groups according to hemocytometric parameters.

	Mild-moderate HOA group (n=37)	Severe HOA group (n=39)	p
NLR	2.1±1.5	2.1±0.9	0.716
NMR	8.9±3.1	9.6±3.1	0.383
PLR	122.9±45.5	122.3±46.9	0.685
MLR	0.2±0.1	0.2±0.1	0.589
CRP (mg/L)	3.1±2.7	3.8±5.0	0.693
ESR (mm/L)	11.8±9.3	12.7±11.1	0.888
Neutrophil	1.8±2.9	1.6±0.2	0.697
Lymphocyte	2.2±0.8	2.2±0.7	0.647
Monocyte	0.2±0.0	0.1±0.0	0.693
Platelet	249.5±62.5	250.5±69.9	0.967
VAS	6.4±1.3	7±1.4	0.081

HOA: osteoarthritis; NLR: neutrophil-lymphocyte ratio; NMR: neutrophil-monocyte ratio; PLR: platelet-lymphocyte ratio; MLR: monocyte-lymphocyte ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; VAS: visual analog scale. p<0.05 is considered significant for Mann-Whitney U test.

DISCUSSION

The relationship between the presence of HOA and inflammatory parameters has not yet been demonstrated[17]. In this study, the relationship between the presence of HOA and NLR, NMR, PLR, and MLR, which are indicators of inflammation, was evaluated. According to the results of the study, it was revealed that there is a relationship between NLR and NMR and the presence of HOA. Although there are few studies on this subject, it has been shown in recent studies that there is a relationship between hematologic indices and OA[12,18]. NLR, an inflammatory marker, was used to determine the prognosis of systemic inflammation[18,19]. Only one study has investigated the levels of NLR in HOA. Barbosa et al. found that NLR values were higher in unilateral coxarthrosis than that in the bilateral group[17]. Many studies investigated NLR levels in knee OA in the literature. In a retrospective, cross-sectional study, NLR was reported as an inflammatory marker that predicted the radiographic severity of knee OA[18]. In another study, a blood NLR cutoff value of ≥2.1 was reported as a predictor of knee OA by Tascioglu et al[14]. Similarly, in this study, the mean NLR value was significantly higher in participants with HOA than in healthy controls, with the mean value of 2.1. However, the mean value did not change significantly with respect to the severity of HOA. The aforementioned study reported a cut-off value for knee OA, and there is no cutoff value for HOA, which may have affected the results.

Similar to NLR, NMR is a biomarker for chronic inflammation in some diseases[19,20]. Shi et al. reported that NMR was correlated with KL grades in knee OA[12]. In this study, NMR was found to be significantly higher in participants with HOA than in healthy controls. In contrast, there was no relationship between disease severity and NMR in the HOA group.

Several studies reported that PLR and MLR levels were higher in systemic inflammatory processes such as rheumatologic disease[11,21]. A retrospective study reported that there was a relationship between PLR values and the radiographic severity of HOA[14]. Conflictingly, no relationship was found between PLR and the severity of HOA in this study. The mean age of our

Table 3. Correlation between the pain intensity, disease severity of hip osteoarthritis, and laboratory and hemocytometric parameters.

	NLR	NMR	MLR	PLR	Neutrophil	Lymphocyte	Monocyte	Platelets
VAS	r=-0.085 p=0.468	r=0.064 p=0.583	r=-0.145 p=0.211	r=0.092 p=0.429	r=-0.045 p=0.701	r=0.158 p=0.172	r=-0.145 p=0.211	r=0.041 p=0.727
KL grade	r=0.042 p=0.719	r=0.101 p=0.386	r=-0.0.62 p=0.592	r=-0.047 p=0.688	r=-0.045 p=0.699	r=0.053 p=0.650	r=-0.046 p=0.695	r=-0.005 p=0.967

NLR: neutrophil-lymphocyte ratio; NMR: neutrophil-monocyte ratio; MLR: monocyte-lymphocyte ratio; PLR: platelet-lymphocyte ratio; VAS: visual analog scale; KL: Kellgren-Lawrence. p<0.05 is considered significant.

participants was lower than that in the aforementioned study. It is known that the inflammatory process increases with age[22]. The nonsignificance of the PLR values may be related to the lower mean age of the participants in this study. In one study, MLR was found to be a reliable potential predictor for knee OA[23]. In this study, MLR values were not significantly different between the HOA group and healthy controls. However, no study has evaluated the level of MLR in HOA in the literature. Accordingly, it would be wrong to generalize the results of knee OA to HOA.

In several studies, elevated serum ESR and CRP levels were found in patients with knee OA[14,24]. No study has evaluated the relationship between these parameters and hip OA. In this study, there was no difference in ESR and CRP levels between participants with HOA and healthy controls. Additionally, no relationship was found between ESR and CRP levels and the severity of hip OA. ESR and CRP levels may increase in disease activity and correlate with clinical findings such as tenderness, patellar ballottement, and swelling[24]. The lack of difference between the groups in ESR and CRP levels may be related to the fact that some of the participants were not in an exacerbation period of OA.

The presence of inflammatory mediators in OA can cause a greater response to painful stimuli[25]. In this study, there was no correlation between pain intensity and inflammatory parameters. The exacerbation period in hip OA is not easy to detect through clinical examinations, so this situation can be explained by the fact that some of the participants were not in an exacerbation period. This is an important finding because this is the first study to evaluate the relationship between clinical findings such as pain intensity and inflammatory markers in HOA.

Being a prospective study and evaluating the relationship between most hematologic indices (NLR, NMR, PLR, MLR) and clinical findings, such as pain intensity, can be considered the strengths of the study. Additionally, this is the first study to compare inflammatory parameters of patients with HOA and healthy controls.

There are some limitations to the study. First, larger participant groups should be studied to determine the relationship between the severity of HOA and hematologic indices. Second, a cross-sectional design could be allowed for an instant evaluation.

CONCLUSION

This study showed that NLR and NMR values were higher in patients with HOA than in healthy controls. Mean PLR, MLR, ESR, and CRP values did not change according to the presence of HOA. Not all hematological indices give valuable information about the severity of HOA.

AUTHORS' CONTRIBUTIONS

MDK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **AKM:** Conceptualization, Formal Analysis, Investigation, Supervision, Visualization, Writing – original draft, Writing – review & editing. **EY:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Visualization.

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ORIGINAL ARTICLE

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Risk of premature coronary atherosclerosis in patients with nonalcoholic fatty liver disease

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SUMMARY

OBJECTIVE: In the current literature, there are few studies investigating the relationship between premature coronary atherosclerosis and nonalcoholic fatty liver disease. We aimed to evaluate the relationship between nonalcoholic fatty liver disease and premature coronary atherosclerosis.

METHODS: In this cross-sectional study, female patients aged <55 years and male patients aged <50 years were enrolled. Both male and female patients underwent coronary angiography and abdomen ultrasonography between 2014 and 2019. A stepwise binary logistic regression analysis was carried out to evaluate the independent variables related to premature coronary atherosclerosis and nonalcoholic fatty liver disease. A p-value<0.05 was considered statistically significant.

RESULTS: nonalcoholic fatty liver disease was present in 44% of patients (n=377). Notably, 62% of the patients were female and the mean age was 44.5 (39–49) years. In a multivariate analysis, nonalcoholic fatty liver disease was shown to be an independent risk factor of premature coronary atherosclerosis (OR 1.438; 95%CI, 1.050–1.969; p=0.024).

CONCLUSIONS: The presence of nonalcoholic fatty liver disease is an important independent risk factor for the development of premature coronary atherosclerosis.

KEYWORDS: Atherosclerosis. Coronary artery disease. Nonalcoholic fatty liver disease.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is well known to affect one in every four adults in the developed countries, potentially emerging as a public health concern across the globe[1]. Furthermore, its prevalence is expected to increase substantially in the upcoming decades. In particular, the prevalence of NAFLD is even higher in patients with type 2 diabetes (T2D) and obesity (up to 70–80%)[2]. Interestingly, the most common cause of mortality in patients with NAFLD appears to be the cardiovascular disease (CVD) (accounting for 40–45% of deaths) rather than liver disease[3,4]. Experimental evidence clearly demonstrates that NAFLD (particularly its severe forms) might release a variety of pro-inflammatory, procoagulant, and profibrogenic mediators, potentially leading to a variety of cardiovascular complications mostly through exacerbation of systemic/hepatic insulin resistance with consequent atherogenic dyslipidemia. Based on these findings, patients with NAFLD might significantly benefit from intensive surveillance and, where necessary, earlier therapeutic interventions in an effort to reduce the risk of premature coronary atherosclerosis (PCA) and associated cardiovascular complications[5-8].

Traditionally, PCA has been defined as the presence of coronary artery disease (CAD) in females aged <65 years and males aged <55 years[9]. Clinically, PCA is strongly associated with acute myocardial infarction (AMI) that might, in turn, lead to increased risk of heart failure (HF) and mortality along with a substantial cost due to necessary therapeutic interventions, including myocardial revascularization strategies in adults. Interestingly, it has been reported that 50–66% of all NAFLDs have been encountered in patients with AMI[10]. This might also suggest the evaluation of PCA in patients with NAFLD through highly applicable and predictive methods[11,12]. In the current literature, NAFLD has been suggested to be associated with the presence and severity of CVD across different populations largely through its association with various markers of subclinical atherosclerosis (for instance, increased arterial stiffness and carotid atherosclerotic plaques)[13-15]. To the best of our knowledge, there has been no single study particularly investigating the relationship between PCA and NAFLD. Accordingly, we aimed to investigate the relationship between PCA and NAFLD.

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METHODS

This is a single-center, cross-sectional study. Approval was obtained from the Institutional Ethics Committee prior to the study (TÜTF-BAEK 2018/332). Between 2014 and 2019, consecutive patients (comprising women aged 18–55 years and men aged 18–50 years) who underwent coronary angiogram (CAG) and abdominal ultrasonogram (USG) were recruited. Not to affect the results due to the COVID-19 pandemic, the records of patients after 2019 were not included. Evaluation of NAFLD by abdominal USG in patients with CAD was part of our institutional protocol based on the fact that it has been proven to be associated with traditional CAD and MI in previous studies[1-4]. Patients who did not undergo CAG and abdominal USG evaluation in the same year, cancer patients, pregnant women, and re-MI were excluded from the study. The remaining cases were recorded on case report forms (CRFs). Based on these data, basic clinical and echocardiographic (TTE) features, therapeutic strategies (including drugs), and laboratory results (total cholesterol [TC], low-density lipoprotein [LDL], triglycerides [TG], high-density lipoprotein [HDL]) were evaluated.

In TTE, patients were monitored by using Vivid 7 Pro (General Electric Medical System, Milwaukee, WI, USA) echocardiography device to obtain parasternal long axis, parasternal short axis, apical four spaces, and apical two spaces images obtained by a 2.5- to 3.5-MHz transducer. Left ventricular ejection fraction (EF) was measured using the Simpson method.

Abdominal USG was planned after 12 h of fasting as per our institutional protocol and was performed by radiologists using the device Toshiba Aplio 500. Right kidney echogenicity was used as a comparative marker to determine the grade of liver parenchymal echogenicity as follows:

- Grade 0 (normal): No difference in echogenicity between renal cortex and liver parenchyma (normal liver parenchyma)
- Grade 1 (mild): Mildly enhanced echogenicity defined in at least three regions of the parenchyma
- Grade 2 (moderate): Diffuse enhancement in liver echogenicity along with normal contours of intrahepatic vessels and diaphragm
- Grade 3 (severe): Slight deterioration in the appearance of the diaphragm and intrahepatic vessels along with a widespread increase in hepatic echogenicity

As per our institutional protocols, CAGs were evaluated by expert cardiologists. CAD was defined as a stenosis degree of >50% in at least one coronary artery on CAG. Subsequently, the participants were divided into two groups, namely, patients with CAD and those with normal coronary arteries. The participants were also categorized into two groups, namely, patients with NAFLD and those without NAFLD. The relationship between NAFLD and PCA was investigated between the two groups.

Statistical analysis

Shapiro-Wilk test was harnessed to analyze the normal distribution. Regarding the comparison of the groups, the Student's t-test was harnessed for variables within the normal distribution, along with the use of Mann-Whitney U test for those out of the normal distribution. Regarding multi-group comparisons, one-way analysis of variance was harnessed for those that were in accordance with the normal distribution, along with the use of Kruskal-Wallis test for those out of the normal distribution. Regarding the association between the quantitative variables, the Pearson's correlation coefficient was implemented for variables concording with the normal distribution along with the use of the Spearman's correlation coefficient for those out of the normal distribution. Pearson's χ^2 test was harnessed to assess the potential association among qualitative variables.

Stepwise binary logistic regression analysis was harnessed to uncover risk factors for PCA. Bland-Altman graphs identified the potential inter-intra observer concordance. The mean and standard deviation were harnessed for variables within the normal distribution along with the use of median and quarters for those out of the normal distribution. A p-value of <0.05 served as significant in all statistical assessments. Statistical software: Turcosa Analytics Ltd. Co., Turkey, www.turcosa.com.tr.

RESULTS

The demographic features of participants are summarized in Table 1. HDL-C levels were significantly lower and TC, TG, and LDL levels were significantly higher in the PCA group as compared with controls. In addition, the incidence of traditional risk factors, including DM, HT, smoking, obesity, hyperlipidemia, and gender, was significantly different between the two groups. Furthermore, the incidence and severity of NAFLD was found to be significantly higher in the PCA group (Table 1).

When the angiographic outcomes between those with and without NAFLD were compared, the NAFLD group was found to undergo elective CAG in a more frequent manner and have a higher incidence of PCA (Table 2). In addition, the mean LVEF value in the NAFLD group was found to be significantly higher as compared with the control group (Table 2).

Clinical factors, including HDL-C, TC, DM, HT, age, smoking, and the presence of NAFLD (potentially associated with PCA), were also evaluated in the multivariate regression analysis. NAFLD was found to serve as an independent risk factor for PCA evolution (p=0.024) (Table 3).

DISCUSSION

In the present study, we were able to demonstrate an increased frequency (and severity) of NAFLD along with a male gender predominance in patients with PCA as compared with controls. Specifically, we have also demonstrated characteristic biochemical findings of metabolic syndrome, including changes in specific lipoproteins (decreased HDL, increased TG) in patients with NAFLD. NAFLD is associated with cardiovascular risk factors, such as insulin resistance, diabetes, obesity, and dyslipidemia[16]. These conditions are well known to serve as components of metabolic syndrome. Therefore, NAFLD might be construed as a hepatic manifestation of metabolic syndrome. Interestingly, we have also demonstrated an increased mean LVEF value in patients with NAFLD in comparison to those without NAFLD. Even though the exact mechanism of this finding remains obscure, HT, being an important component of metabolic syndrome, might have elicited a relatively hyperdynamic ventricle (potentially due to left ventricular hypertrophy) in patients with NAFLD.

Importantly, NAFLD was found to serve as an independent risk factor for the evolution of PCA. We demonstrated this result by performing a stepwise binary logistic regression analysis (Table 3). In particular, this is the first study in which NAFLD was identified as an independent risk factor for the development of PCA, possibly due to its adverse effects on insulin resistance. The potential association between NAFLD and CAD was also demonstrated in previous studies[17-19]. Assy et al. demonstrated the relationship between coronary plaques and NAFLD through CT angiography (a noninvasive test)[17]. In contrast, we have confirmed the presence of PCA with CAG, which is accepted as the gold standard for the diagnosis of CAD[17]. Similarly, NAFLD, on top of genetic and environmental factors, was also reported to be a risk factor for the evolution of CAD in other studies[18-19]. In addition, it has been reported that nonalcoholic steatohepatitis (NAS), an advanced stage of

Table 1. Baseline demographic parameters of the study population.

	PCA group (n=408)	Control group (n=448)	p
Age	45 (40–50)	44.5 (39–49)	0.146
Gender (%)			
Female	211 (51.71)	319 (71.20)	<0.001
Male	197 (48.28)	129 (28.79)	
HT (%)	288 (70.58)	227 (50.66)	<0.001
DM (%)			
Tip 1	30 (7.37)	18 (4.01)	<0.001
Tip 2	143 (35.13)	77 (17.18)	
Smoking (%)	181 (44.36)	91 (20.53)	<0.001
Hyperlipidemia (%)	176 (43.13)	130 (29.01)	<0.001
Obesity (%)	28 (6.86)	17 (3.79)	0.045
NAFLD (%)	204(50)	173(38.61)	<0.001
NAFLD grade (%)			
0	204 (50)	275 (61.38)	0.001
1	115 (28.18)	116 (25.89)	
2	76 (18.62%)	48 (10.71%)	
3	13 (3.18%)	9 (2.00%)	
LVEF (%)	55 (50–60)	60 (55–63)	<0.001
LDL (mg/dL)	155 (129–188)	146 (119–175)	0.002
Triglycerides (mg/dL)	220 (148–343)	168 (123–260)	<0.001
Total cholesterol (mg/dL)	231 (197–270)	218 (185–259)	0.002
HDL (mg/dL)	49 (42–58)	54 (44–64)	<0.001

PCA: premature coronary atherosclerosis; HT: hypertension; DM: diabetes mellitus; n: patient number; NAFLD: nonalcoholic fatty liver disease; LVEF: left ventricular ejection fraction; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

NAFLD, is also of greater risk for the development of CAD[20]. However, previous studies did not specifically focus on PCA[18-19]. Inci et al. reported through USG that moderate-to-severe NAFLD might have the potential to predict CAD, but not its severity[21]. In our study, we found a significant relationship between NAFLD and PCA, particularly due to the increased frequency of PCA in moderate-to-severe (grades 2–3) NAFLD patients. This might also indicate that patients with moderate-to-severe NAFLD might be particularly prone to the evolution of PCA and should be under close supervision in terms of adverse cardiovascular events. In other words, detection of NAFLD on USG, which is a safe and noninvasive imaging

Table 2. Angiographic parameters of the study population.

	NAFLD group (n=377)	Control group (n=479)	p
Indications for angiography Generally (%)			
Elective	241 (63.9)	271 (56.78)	0.034
ACS	136 (36.07)	207 (43.21)	
CCS angina	93 (24.66)	114 (23.79)	
MPS positivity	76 (20.15)	74 (15.44)	
Exercise test positivity	72 (19.09)	82 (16.70)	
Low LVEF	3 (0.79)	6 (1.25)	
Anterior MI	19 (5.03)	27 (5.63)	
Inferior MI	14 (3.71)	22 (4.59)	
Lateral MI	1 (0.26)	3 (0.62)	
NSTEMI	36 (9.54)	46 (9.60)	
USAP	42 (11.14)	47 (9.81)	
Premature CAD (%)	204 (54.11)	204 (42.58)	<0.001
Coronary artery lesions (%)			
LMCA	17 (4.50)	25 (5.21)	0.822
LAD	185 (49.07)	212 (44.25)	
CX	126 (33.42)	144 (30.06)	
RCA	125 (33.10)	156 (32.56)	
SB	95 (25.19)	126 (26.30)	
Coronary collateral flow (%)			
0–1	349 (92.57)	439 (91.64)	0.856
2	27 (7.16)	39 (8.14)	
3	1 (0.26)	1 (0.20)	
Coronary ectasia (%)	8 (2.12)	7 (1.46)	0.465
Coronary calcification (%)	2 (0.53)	2 (0.41)	0.810
Coronary anomaly (%)	4 (1.06)	5 (1.04)	0.983
Coronary slow flow (%)			
0–1	4 (1.06)	5 (1.04)	0.825
2	18 (4.77)	23 (4.80)	
3	355 (94.16)	451 (94.15)	
LVEF (%)	59 (54.5–61.5)	57 (51–61)	0.036

NAFLD: nonalcoholic fatty liver disease; ACS: acute coronary syndrome; CCS: Canadian Cardiovascular Society; MPS: myocardial perfusion scintigraphy; LVEF: ejection fraction; MI: myocardial infarction; NSTEMI: non-ST-elevation myocardial infarction; USAP: unstable angina pectoris; CAD: coronary artery disease; LMCA: left main coronary artery; LAD: left anterior descending coronary artery; CX: circumflex coronary artery; RCA: right coronary artery; SB: side branch coronary artery.

Table 3. Stepwise binary logistic regression analysis of risk factors on premature coronary atherosclerosis.

Variable	OR	95%CI	p
Gender			
Male	–	–	<0.001
Female	0.334	0.223–0.500	
Age	1.058	1.034–1.083	<0.001
DM			
Type 1	2.387	1.222–4.665	0.011
Type 2	2.206	1.521–3.201	<0.001
HT	2.01	1.436–2.837	<0.001
Smoking	3.179	2.192–4.610	<0.001
Total cholesterol	1.005	1.002–1.008	<0.001
HDL	0.978	0.967–0.991	<0.001
NAFLD	1.438	1.050–1.969	0.024

DM: diabetes mellitus; HT: hypertension; HDL: high-density lipoprotein; NAFLD: nonalcoholic fatty liver disease; OR: odds ratio; CI: confidence interval.

modality, might allow further cardiovascular risk prediction[22]. Taken together, there exists a significant gap in the early diagnosis and management strategies of PCA. To date, most studies have generally focused on traditional cardiovascular risk factors and their management in an effort to combat this inauspicious phenomenon. However, an existing NAFLD (as demonstrated with USG) might possibly be taken into consideration when evaluating relatively young patients with a high cardiovascular risk for the early diagnosis of PCA.

Study limitations

This is a single-center study and has a retrospective design. Additional detection methods (e.g., magnetic resonance imaging and biopsy) are not evaluated in USG for NAFLD. Finally, we were not able to evaluate other inflammation markers that might also have important implications in this setting.

CONCLUSIONS

These data suggest the strong and independent association of NAFLD with PCA, regardless of atherosclerotic risk factors and components of metabolic syndrome. Therefore, an existing NAFLD might serve as an adjunct to cardiovascular diagnostic tests in the early diagnosis of PCA. However, further studies are still warranted to suggest NAFLD as a routine test in the setting of PCA diagnosis.

AUTHORS' CONTRIBUTIONS

GT: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **ME:** Data curation, Methodology, Validation, Writing – review & editing. **SS:** Data curation, Software, Validation, Writing – original draft. **ÇK:** Data curation, Methodology, Software, Writing – review & editing. **KY:** Data curation, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Breastfeeding success in the first 6 months of online breastfeeding counseling after cesarean delivery and its effect on anthropometric measurements of the baby: a randomized controlled study

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SUMMARY

OBJECTIVE: The aim of this study was to determine the effect of online breastfeeding counseling after cesarean section on breastfeeding success and anthropometric measurements of the baby in the first 6 months.

METHODS: The study was conducted with single-blind randomized controlled experimental research design and performed with 151 primiparous women as intervention (n=76) and control (n=75) groups. The mothers were given training in the first 24 h postpartum by applying the "Data Collection Form," "Breastfeeding and Infant Follow-up Form," and "Breastfeeding Self-Efficacy Scale – Short Form," who followed up at the first and sixth months, and further again for 6 months.

RESULTS: Although there was no difference and homogeneity at the beginning of study among the participants in the intervention group compared with the control group, it was observed that the breastfeeding rates at the first and sixth months were higher and significant. When the anthropometric measurements of the participants in both the groups were compared, it was found that there was a significant difference between the measurements of height and weight at discharge, first, and sixth months. Breastfeeding self-efficacy scores in the intervention group were significantly higher at discharge, 4 weeks postpartum, and 6 months postpartum than those in the control group ($p<0.05$).

CONCLUSIONS: Breastfeeding training and online counseling given to mothers who give birth by cesarean section during the early postpartum period increased breastfeeding rates and self-sufficiency, and the anthropometric measurements of babies were found to be higher at healthy limits.

KEYWORDS: Breastfeeding. Self-sufficiency. Cesarean section. Counseling.

INTRODUCTION

Breast milk is the most important food source that protects the health of the children and helps them to survive[1,2]. Given its short- and long-term benefits, it should be considered that breastfeeding is not only a feeding option but also a priority for public health[3,4]. But mothers can face with premature cessation of breastfeeding for various reasons[5,6]. In particular, the idea that milk is not enough itself, improper placement in the breast, and nipple problems are among the most important reasons that negatively affect breastfeeding success[7,8]. Also, the pain experienced by women who delivered by cesarean section negatively affects their breastfeeding abilities and the adverse effects of breastfeeding in turn results in a failed perception of self-sufficiency related to breastfeeding[9-11] . Breastfeeding is a behavior that needs knowledge, skills, support, and self-confidence of the mother. In a study, it was reported that the presence of social support in breastfeeding mothers increased breastfeeding self-efficacy[12]. Breastfeeding training and counseling provided by health professionals plays an important role in the effective initiation and maintenance of breastfeeding[13,14]. Studies report that those who had breastfeeding training and counseling during the postpartum period have less breastfeeding difficulties and higher and longer breastfeeding rates[11,13].

In the consultation held by health professionals, it is important to observe and evaluate breastfeeding and to place the baby in the breast with the right technique. Furthermore, although breastfeeding self-sufficiency is lower in the studies of mothers who delivered by cesarean[12], there is limited research in the literature evaluating the effect of counseling on mothers delivered by cesarean section on breastfeeding success, self-sufficiency, and neonatal anthropometric outcomes. The aim of this study was to determine the effect of online breastfeeding counseling after

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cesarean section on breastfeeding success and anthropometric measurements of the baby in the first 6 months.

METHODS

This study is a single-blind randomized controlled trial conducted on mothers who delivered by cesarean section research design. Before starting data collection, necessary permissions were obtained from the Non-Interventional Clinical Research Ethics Committee (ethics number: 199).

Population

The sample size of this study was determined using the G*Power (version 3.1.7) program. Franco-Antonio et al.[13] in a randomized controlled study that evaluated the effect of motivational intervention in the postpartum period on breastfeeding time and self-worth in the first 6 months, and examined a difference of 0.08% between the groups (80% strength and α=0.50%), found that a minimum of 79 mothers in each group should be included in sampling.

Inclusion criteria

1. The mother's cognitive level is sufficient to understand and answer the questions
2. 18–35 years old
3. Being primipar
4. Having a single pregnancy
5. Gestational age at birth between 37 and 42 weeks
6. Birth by cesarean section
7. Birth to a live newborn

Exclusion criteria

1. Mother pumping or having problems with the breast

Extraction criteria

1. Inaccessible in follow-ups

Randomization and blinding

During the study process, 467 mothers were interviewed and then 158 eligible and volunteered mothers who participated in the study were included in the randomization. The mothers included in the study were randomized into two groups (group A and group B) using the https://randomizer.org/ site. As a result, the research was completed with a total of 151 mothers, including 76 in the intervention group and 75 in the control group. The registration, assignment, follow-up, and analysis steps of the study were shown using CONSORT 2018 (Figure 1).

In the study, mothers were blind to each other in terms of practice, and blind to the groups involved in the study in terms of statistical analysis and reporting. Training and consultancy intervention and follow-up were carried out by researchers (FSB) who could not be blind to applications. Randomization and data collection were carried out by another researcher (AYK). Data collection forms were applied face-to-face. To prevent bias, the researcher (FSB) did not participate in any steps of the pre-evaluation, final test, and statistical evaluation process.

Data collection tools

The data were obtained using "Data Collection Form," "Breastfeeding and Infant Monitoring Form," and "Breastfeeding Self-Proficiency Scale – Short Form."

Data collection form

It was created by scanning literature of the researchers[14-19]. The design for the form consisted of 14 questions.

Breastfeeding and baby monitoring form

It was created by scanning literature of the researchers[13,14,17]. The form consists of 11 questions, including for breastfeeding problems and for aiming the anthropometric measurements of the baby.

Breastfeeding self-proficiency scale – short form

It was developed by Bandura[20] and Tokat et al.[21] and consists of a total of 14 questions evaluating breastfeeding self-sufficiency in Turkish validity and reliability breastfeeding. The scale is a 5-point Likert scale and the minimum score from the scale is 14 and the maximum score is 70. Higher scores indicate higher levels of self-sufficiency in breastfeeding. The Cronbach's alpha value of the scale was found to be 0.86 and was determined to be a reliable scale. In this study, Cronbach's alpha value was found to be 0.92.

Data collection method

Stage I: All primiparous mothers who delivered by cesarean section were randomized into intervention and control groups in the first 24 h after birth. The randomized researcher assigned the distribution of mothers into the control and intervention groups and did not share them with the other researcher. Data Collection Form and Breastfeeding Self-Proficiency Scale – Short Form was applied to mothers who met the sample selection criteria.

Stage II: In addition to the routine care of the clinic, breastfeeding techniques were applied to the mothers in the intervention group using demonstration method to develop breastfeeding skills after the importance of breastfeeding and breast milk was explained verbally with a face-to-face interview technique. Mothers were given a breastfeeding training manual developed by researchers at the end of the training. The women in the control group received routine care at the clinic.

Stage III: Breastfeeding counseling was provided to the mothers in the intervention group by asking about

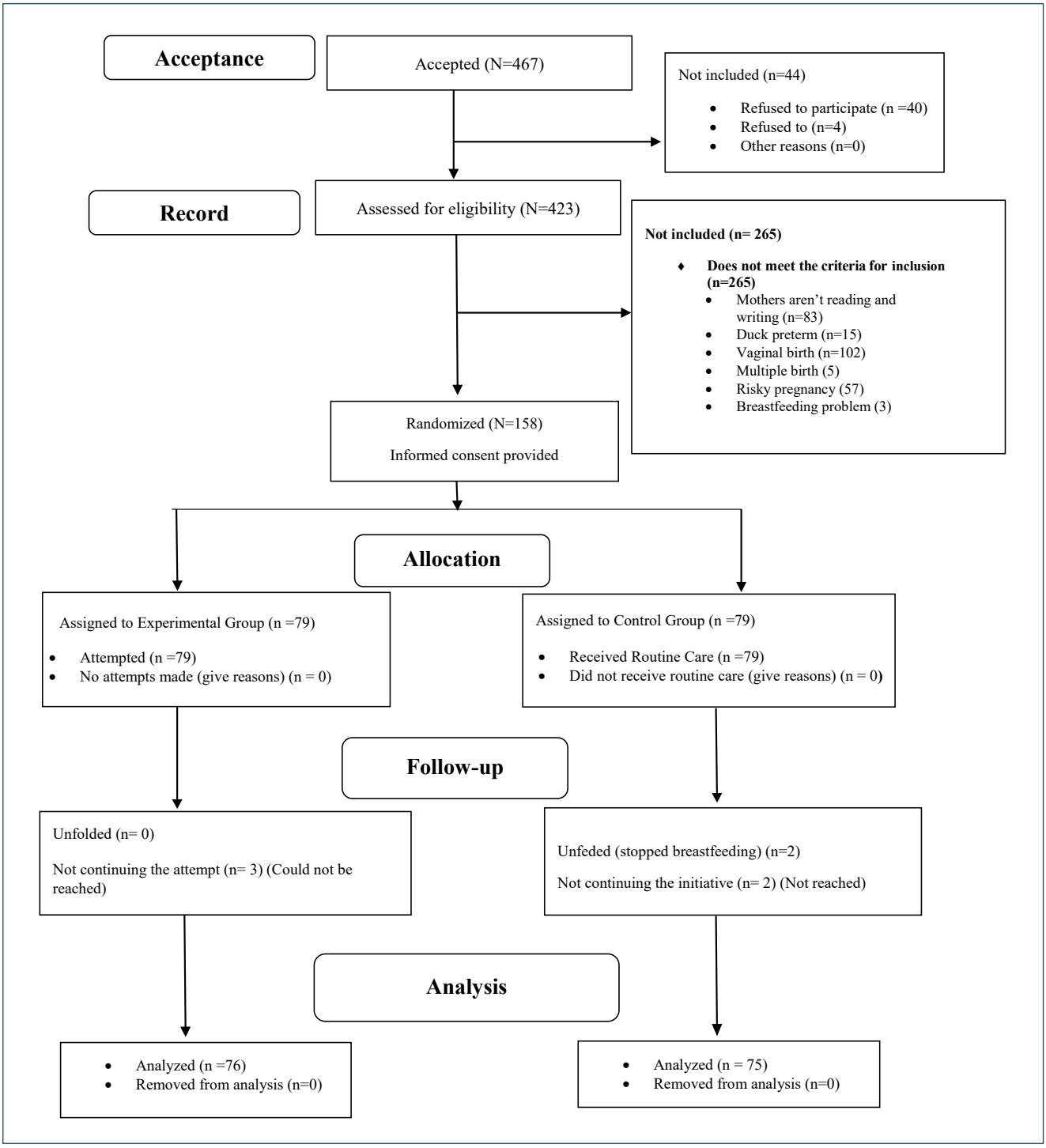

Figure 1. Consort flowchart was created to show compliance.

breastfeeding problems via an online interview method for 6 months. Mothers were given three follow-ups and repeated data measurements were collected at discharge and postpartum at 1 and 6 months.

Data analysis

The SPSS software (version 24) was used for data analysis. Sociodemographic characteristics, breastfeeding attitudes, and behaviors of both groups were compared using independent t-test and ANOVA, paired t-test, and variance analysis test in repeated measurements. A p-value <0.05 was considered statistically significant.

RESULTS

The mean age of the participants was 26.56±6.36 years in the control group and 25.56±7.36 years in the intervention group; the number of high school graduates was 48 (63.3%) in the control group and 47 (62.6%) in the intervention group, and the number of planned pregnancy was 52 (68.4%) in the control group and 55 (73.3%) in the intervention group. It was observed that the first breastfeeding time was 46 min (60.5%) in the control group and 39 min (52.0%) in the intervention group, for the first 30–60 min, and there was no significant difference between the two groups in terms of sociodemographic and postpartum characteristics (p>0.05).

Compared with the breastfeeding status of the mothers, it was found that there was no significant difference in the rates of breastfeeding after childbirth, the time of the first breastfeeding, and the breastfeeding in the discharge. Breastfeeding rates in the first and sixth months in the control group were higher and more significant than mothers in the intervention group. Compared with anthropometric measurements of infants in both the groups, the difference between discharge, height, and weight measurements in the first and sixth months was significant (p<0.000), while the difference between head measurements was not significant (p>0.05) (Table 1).

Breastfeeding self-sufficiency scores in the intervention group were significantly higher in discharge, 4 weeks after birth, and 6 months after birth than in the control group (p<0.05). The breastfeeding self-sufficiency scores of the women in the intervention group after discharge first increased, then somewhat decreased, and tended to rise as a whole. In the control group, the breastfeeding self-sufficiency score increased first, then decreased, and tended to decline as a whole (Table 2).

Table 1. Comparison of the characteristics and babies' anthropometric measurements of control and intervention group mothers regarding breastfeeding at 6 months (n=151).

Parameters	Control group (n=75)	Intervention group (n=76)	F/p-value
	n (%)	n (%)	
Breastfeeding only status			
At discharge			
Yes No	69 (92.19) 6 (7.81)	76 (100) –	-1.351 0.181
In the first month			
Yes No	54 (71.87) 21 (28.12)	73 (96.87) 3 (3.12)	-3.142 ***0.002***
In the sixth month			
Yes No	19 (25.28) 56 (74.72)	40 (52.25) 36 (47.75)	-1.528 ***0.000***
t***** p	0.518 0.097	-3.327 ***0.001***	
Stop breastfeeding			
At discharge			
Yes No	0 75 (100)	0 76 (100)	0.413 0.098
In the first month			
Yes No	4 (5.19) 71 (94.81)	0 76 (100)	75.00 ***0.000***
In the sixth month			
Yes No	24 (31.81) 51 (67.19)	0 76 (100) 100	68.00 ***0.000***
t***** p	0.345 0.067	-2.127 ***0.000***	
Pacifier/Bottle use			
At discharge			
Yes No	19 (25.28) 56 (74.72)	4 (4.68) 72 (95.32)	-6.278 ***0.000***
In the first month			
Yes No	39 (52.25) 36 (47.75)	19 (25.28) 57 (74.72)	-3.465 ***0.000***
In the sixth month			
Yes No	50 (67.19) 25 (31.81)	21 (28.25) 55 (71.75)	-1.758 ***0.000***
t***** p	0.218 0.045	-1.276 ***0.001***	
	$\bar{X}$±SD (n=75)	$\bar{X}$±SD (n=76)	
Weight			
At discharge	3276.84±392.134	3255.17±422.40	.318 ***0.001***
In the first month	4023.26±469.96	3878.81±457.18	2.240 ***0.000***
In the sixth month	8545.24±239.324	9245.37±332.221	1.187 ***0.000***
t p	-2.082 ***0.037***	-0.840 ***0.001***	

Continue...

Table 1. Continuation.

Parameters	Control group (n=75)	Intervention group (n=76)	F/p-value
	n (%)	n (%)	
Height			
At discharge	49.62±2.01	49.62±2.01	4.324 ***0.000***
In the first month	52.51±2.11	56.82±1.23	-3.842 ***0.000***
In the sixth month	65.89±2.32	69.89±2.32	-2.765 ***0.000***
t p	-1.034 ***0.004***	-0.402 ***0.001***	
Head			
At discharge	35.22±1.02	35.22±1.02	-0.371 0.078
In the first month	36.24±1.15	36.82±1.23	0.184 0.084
In the sixth month	39.45±1.22	39.65±1.33	0.174 0.074
t p	-3.026 0.065	-1.645 0.075	

SD: standard deviation. Bold italics: p<0.05.

DISCUSSION

This research was carried out as a single-blind, randomized controlled study to examine the effect of breastfeeding training and counseling on breastfeeding time, attitude and self-sufficiency, and anthropometric measurements of the baby in the first 6 months. As a result of the study, breastfeeding training and online counseling increased breastfeeding self-proficiency scores, longer breastfeeding times, and higher anthropometric measurements. According to the results of the study, it is possible to say that breastfeeding education given to primiparous mothers in the early postpartum period positively affects breastfeeding.

After discharge, the mother's professional support for breastfeeding will increase its success[12]. In this study, breastfeeding rates were higher in the first and sixth months compared with the control group of the mothers in the mid-first and sixth months, and there was a significant difference between them. According to a systematic review and methane study, it has been reported that three or more training and counseling interventions in the prenatal and postpartum period, both through face-to-face training and telephonic follow-up, can only be effective in increasing breastfeeding and partial breastfeeding for 6 months[18]. It is observed that the results of the research are parallel with the literature. This suggests that mothers need breastfeeding training and post-discharge counseling. It can be said that it will be possible to increase breastfeeding rates by providing the necessary training and counseling. In a study by Chan et al.[19] in Hong Kong, it was reported that postpartum did not affect breastfeeding rates of mothers who received telephonic counseling once after 2 weeks. The fact that this finding differs from the research finding, cultural differences, and the small number of consultations can be associated.

Breastfeeding and human milk are known to protect the child's health and ensure the best nutrition of the baby, maintain immunity, and regulate growth, development, and metabolism[1,13]. In this study, it was found that the discharge of infants in the midge's group was higher in height-weight measurements in the first and sixth months compared with those in the control group, and there was a significant difference between them. It has been reported that the intervention of mobile phone assisted breastfeeding counseling in Nigeria is associated with the weight of babies in only the sixth month of anthropometric characteristics[19]. In another study, it was reported that the height and weight after breastfeeding education intervention were higher in the intervention group in the second postpartum month, while in the 12th month, when the difference decreased in 6 months, there was almost no difference[20]. The results of the literature differ in themselves and with the findings of the research. This suggests that nutrition alone cannot be a parameter in infant and child development. The difference seen in infants receiving breast milk, especially in the first months, may be associated with breastfeeding reducing morbidity.

Breastfeeding self-sufficiency is related to how satisfied the mother feels about breastfeeding. The higher the self-sufficiency, the healthier the initiation and maintaining the breastfeeding[21]. In this study, breastfeeding self-proficiency scores of mothers in the intervention group were higher in discharge, in the first month and sixth month after birth, and there was a significant difference between them. Shi et al.[21] reported in a systematic review of 15 intervention studies conducted in developing countries that effective health education interventions can improve breastfeeding knowledge level and help improve better breastfeeding behaviors. In a study with mothers with gestational diabetes, You et al.[7] reported that the breastfeeding self-sufficiency scores of mothers in the intervention group were significantly higher than those of mothers in the control group at discharge, sixth week, fourth month, and sixth month after childbirth[11]. It is seen that the studies are consistent with the research findings. This

Table 2. Comparison of breastfeeding self-efficiency scores of control and intervention group babies (n=151).

Group	n	BSES – discharge $\bar{X}$±SD	BSES – first month $\bar{X}$±SD	BSES – sixth month $\bar{X}$±SD	t/F p-value
Intervention	76	59.49±8.46	57.74±7.32	67.56±6.49	-3.142 0.002
Control	75	54.29±6.36	56.38±6.49	52.53±5.56	-1.351 0.181
F p	151	-2.082 0.037	-0.840 0.401	5.237 0.001	

SD: standard deviation; BSES: breastfeeding self-efficiency scores. Bold italics: p<0.05.

may be thought to be related to knowledge and encouragement in the education and counseling process of breastfeeding self-sufficiency in mothers.

Limitations

While the fact that the research is carried out in a single center reflects a certain sociocultural segment applying to the institution, the inclusion of cesarean mothers prevents them from generalizing to vaginal-born pregnant women.

CONCLUSIONS

It was found that early breastfeeding education and online breastfeeding counseling given to mothers who gave birth by cesarean section extended the breastfeeding period, increased the breastfeeding self-efficacy score, and increased the anthropometric measurements of the babies at healthy levels.

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AUTHORS' CONTRIBUTIONS

AYK: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **FSB:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of cardiac-electrophysiological balance according to National Institutes of Health Stroke Scale score at admission and discharge in acute ischemic stroke patients: A pilot study

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SUMMARY

OBJECTIVE: The main objectives of this investigation were to determine whether there were any relationships between corrected cardiac-electrophysiological balance value and National Institutes of Health Stroke Scale scores at admission and discharge in patients with acute ischemic stroke and to assess whether cardiac-electrophysiological balance value was an independent predictor of high National Institutes of Health Stroke Scale scores (National Institutes of Health Stroke Scale score ≥5).

METHODS: In this retrospective and observational study, 231 consecutive adult patients with acute ischemic stroke were evaluated. The cardiac-electrophysiological balance value was obtained by dividing the corrected QT interval by the QRS duration measured from surface electrocardiography. An experienced neurologist used the National Institutes of Health Stroke Scale score to determine the severity of the stroke at the time of admission and before discharge from the neurology care unit. The participants in the study were categorized into two groups: those with minor acute ischemic stroke (National Institutes of Health Stroke Scale score=1–4) and those with moderate-to-severe acute ischemic stroke (National Institutes of Health Stroke Scale scores ≥5).

RESULTS: Acute ischemic stroke patients with National Institutes of Health Stroke Scale score ≥5 had higher heart rate, QT, corrected QT interval, T-peak to T-end corrected QT interval, cardiac-electrophysiological balance, and cardiac-electrophysiological balance values compared with those with an National Institutes of Health Stroke Scale score of 1–4. The cardiac-electrophysiological balance value was shown to be independently related to National Institutes of Health Stroke Scale scores ≥5 (OR 1.102, 95%CI 1.036–1.172, p<0.001). There was a moderate correlation between cardiac-electrophysiological balance and National Institutes of Health Stroke Scale scores at admission (r=0.333, p<0.001) and discharge (r=0.329, p<0.001).

CONCLUSIONS: The findings of this study demonstrated that the cardiac-electrophysiological balance value was related to National Institutes of Health Stroke Scale scores at admission and discharge. Furthermore, an elevated cardiac-electrophysiological balance value was found to be an independent predictor of National Institutes of Health Stroke Scale score ≥5.

KEYWORDS: Cardiac Arrhythmia. Ischemic stroke. Cardiac electrophysiologic study. Acute ischemic stroke.

INTRODUCTION

Acute ischemic stroke (AIS) is a complex clinical entity associated with major cardiovascular complications as a result of autonomic dysfunction and disruption of neurohormonal pathways[1]. Remarkably, the risk of developing cardiac complications increases with the severity of AIS[2]. Similarly, worsened cardiac functions as a consequence of AIS may result in poor neurological outcomes[3].

The National Institutes of Health Stroke Scale (NIHSS) is a simple, effective, and reliable tool for measuring acute stroke-related neurologic impairments[4]. The NIHSS score is a useful scale for clinical evaluation in patients with AIS since it allows for the identification of appropriate therapy, prediction of lesion size, measurement of stroke severity, and prediction of patient prognosis.

The electrocardiographic (ECG) measures of ventricular repolarization, such as QT, Tp-e, and Tp-e/QTc, were studied in AIS patients to predict the probability of ventricular arrhythmia[5]. The cardiac-electrophysiological balance (iCEB), which is determined on surface ECG by dividing the QT interval by QRS duration, is a noninvasive index that can be used to determine malignant ventricular arrhythmias[6]. Since iCEB is considered the cardiac wavelength analog, the iCEB values have been linked to ventricular arrhythmia risk. Nevertheless,

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there is a scarcity of data in the existing medical database on the values of iCEB based on NIHSS scores at admission and discharge in patients with AIS. As a result, we aimed to determine whether there were any relationships between iCEBc values and NIHSS scores at admission and discharge in patients with AIS and whether high iCEBc values were an independent predictor of high NIHSS scores.

METHODS

Participants and design of the study

A total of 231 consecutive patients from a single stroke center who were diagnosed with an AIS between January 2018 and November 2020 were included in the study. This was a retrospective and observational study. The exclusion criteria of the study were as follows: patients with moderate-to-severe heart valve disease, a history of cardiac valve surgery, electrolyte imbalance, a history of a pacemaker or implantable cardioverter-defibrillator implantation, and those with bundle branch block and using drugs that affect the cardiac conduction system (n=38). The diagnosis of AIS was determined based on a neurologic examination and cranial imaging. All patients underwent a computed tomography scan and magnetic resonance imaging after being admitted to the emergency service. The Ethics Committee authorized the study, and the study was carried out in conformity with the principles of the Helsinki Declaration (decision number: HNEAH-KAEK-2021/KK/55).

Echocardiographic examination

According to the American Society of Echocardiography guidelines, all patients had a complete transthoracic echocardiographic examination within the first 24 h. The usual biplane Simpson approach was used to assess the left ventricular ejection fraction (LVEF). The end-systolic, end-diastolic, and left atrial anteroposterior diameters were recorded from the parasternal long-axis view. The left atrial volume index (LAVI) was calculated using the modified biplane area-length method, which was then corrected for body surface area.

Analysis of electrocardiography parameters

The standard 12-lead ECG was recorded at 25 mm/s speed and 10 mm/mV gain upon admission to the hospital. All ECG records were digitally scanned at 300 dpi scanning and 10× amplification. The ECG records were evaluated by two expert cardiologists. The EP Calipers application (EP Studios, USA) was applied to calculate the amplitudes and time periods in ECG recordings. The QT interval was determined from the onset of the QRS to the end of the T wave. The end point of the T wave was recognized as the point of return to the isoelectric line. To remove the effect of heart rate on the QT interval, the QT interval was corrected using Bazett's approach (QTc=square root of the QT/RR interval). T-peak to T-end (Tp-e) was measured from the peak to the end of the T wave. To compute iCEB, the QT/QRS ratio from ECG recordings was utilized, and iCEBc was obtained by dividing the QTc by the QRS.

Assessing the severity of stroke

AIS was defined as a neurological impairment caused by a cerebral, spinal, or retinal infarction on imaging. The NIHSS score was utilized by an expert neurologist to identify the severity of the stroke at the time of admission and before discharge from the neurology care unit. Patients were divided into two subgroups: those with minor AIS (NIHSS score=1–4) and those with moderate-to-severe AIS (NIHSS score ≥5).

Statistical analysis

The statistical analysis was carried out using the SPSS statistics program (version 12.0; SPSS Inc., Chicago, IL, USA). Qualitative variables were represented using frequencies and percentages. To analyze categorical data between groups, the chi-square analysis was applied. The distribution of all continuous variables was non-normal. As a result, these variables were reported as medians [interquartile ranges (IQR)]. To assess the normality of variable patterns, the Kolmogorov-Smirnov test was chosen. To compare continuous variables between groups, the Mann-Whitney U test was employed. The independent predictors for NIHSS score ≥5 were determined using univariable and multivariable logistic regression analysis (backward entry method). Parameters having a p-value of 0.05 in univariable analysis were included in the multivariate logistic regression analysis. The association between iCEBc levels and NIHSS scores at admission and discharge was determined using Spearman's correlation analysis. All findings were presented as odds ratios (OR) and 95% confidence intervals (95%CI). The level of statistical significance was specified at $p<0.05$. The goodness-of-fit test presented adequate calibration for the multivariate model (Hosmer-Lemeshow goodness-of-fit=10.158, p=0.412). The effect size (Cohen's d) and power value (1–β) were calculated using the G*Power software (version 3.1.9.2). The effect size and power value were 0.82 and 0.94, respectively.

RESULTS

This investigation involved 231 patients with AIS. Participants of the study were divided into two groups: those with an NIHSS score of 1–4 (n=94) and those with NIHSS ≥5 (n=137).

Clinical characteristics and echocardiographic findings are summarized in Table 1. Patients with NIHSS ≥5 were older and smokers. The other clinical characteristics of the groups were similar in terms of gender, hypertension, diabetes, insulin dependency, hyperlipidemia, chronic obstructive pulmonary disease, cancer, and dementia. With regard to echocardiographic data, LVEF, left ventricular end-diastolic dimension, left ventricular end-systolic dimension, left atrial anteroposterior diameter, and LAVI were similar in both groups.

With regard to laboratory measurements, both groups were similar (Table 2). When ECG recordings were analyzed, patients with NIHSS ≥5 had higher heart rate, QT, QTc, Tp-e/QTc, iCEB, and iCEBc values compared with those with an NIHSS score of 1–4.

To find potential predictors of NIHSS ≥5, both univariable and multivariable logistic regression analyses were conducted. Age, smoking, LAVI, heart rate, QRS, QTc, and iCEBc were predictors of NIHSS ≥5 according to univariable logistic regression analysis. After incorporating all of the aforementioned variables into a multivariable logistic regression analysis, age, smoking, LAVI, and iCEBc (OR 1.102; 95%CI 1.036-1.172; p<0.001) were shown to be independently related to NIHSS ≥5. There was a moderate correlation between iCEBc and NIHSS scores at admission (r=0.333; p<0.001) and discharge (r=0.329; p<0.001) (Figure 1).

DISCUSSION

The study findings revealed that the iCEBc value calculated at admission was associated with NIHSS scores at admission and discharge. Furthermore, a high iCEBc value was an independent predictor of NIHSS ≥5.

Both ischemic and pro-arrhythmic ECG changes are common in the first 24 h after AIS[7]. One of the primary mechanisms involved in stroke-heart mixing is the hypothalamic-pituitary-adrenal axis (HPA). When a central regulatory region in the brain is affected, several pathways are activated based on the affected area and the severity of the lesion. Stimulation of the frontal lobe's orbital surface and the cingulate gyrus, for example, affects blood pressure and heart rate regulation; ischemic lesions of the insular cortex affect blood pressure control; and trigger serious cardiac complications, such as arrhythmias and autonomic dysfunction[7,8]. Furthermore, cerebral infarction in the left hemisphere is linked to a higher risk of negative cardiac outcomes and long-term mortality[9].

Table 1. Baseline clinical characteristics and echocardiographic findings of all patients according to the National Institutes of Health Stroke Scale score.

	National Institutes of Health Stroke Scale score 1–4 (n=94)	National Institutes of Health Stroke Scale score ≥5 (n=137)	p-value
Age, years	63 (54–74)	72 (63–78)	0.006
Gender, male, n (%)	65 (69.1)	85 (62.0)	0.266
Risk factors			
Hypertension, n (%)	65 (69.1)	105 (76.6)	0.204
Diabetes mellitus, n (%)	36 (38.3)	53 (38.7)	0.952
Insulin dependency, n (%)	10 (10.6)	10 (7.3)	0.379
Hyperlipidemia, n (%)	40 (42.6)	57 (41.6)	0.886
Smoker, n (%)	12 (12.8)	35 (25.5)	0.015
Chronic renal failure, n (%)	11 (11.7)	14 (10.2)	0.722
Coronary artery disease, n (%)	19 (20.2)	39 (28.5)	0.155
Chronic obstructive pulmonary disease, n (%)	9 (9.6)	15 (10.9)	0.736
Cancer, n (%)	4 (4.3)	9 (6.6)	0.446
Dementia, n (%)	9 (9.6)	18 (13.1)	0.403
Echocardiographic data			
Left ventricular ejection fraction, %	58 (58–59)	58 (57–58)	0.114
Left ventricular end-diastolic dimension, mm	46 (45–48)	46 (44–49)	0.601
Left ventricular end-systolic dimension, mm	25 (24–29)	25 (24–32)	0.117
Left atrial anteroposterior diameter, mm	36 (35–40)	37 (35–40)	0.912
Left atrial volume index, mL/m^2	22 (21–26)	23 (21–27)	0.113

Continuous variables are presented as median (IQR), and nominal variables are presented as frequency (%).

In 60–90% of AIS patients, ECG abnormalities can occur[10]. T-wave inversion (35%), ST depression (33%), a longer QTc interval (29%), and U waves (28%) are the most common findings[11]. The most frequent arrhythmias after AIS consist of atrial fibrillation, supraventricular tachycardia, ventricular ectopic beats, ventricular tachycardia, and sinus tachycardia[11].

Table 2. Laboratory variables and electrocardiography findings of all patients according to the National Institutes of Health Stroke Scale score.

	NIHSS score 1–4 (n=94)	NIHSS score ≥5 (n=137)	p-value
Laboratory data			
Hemoglobin, g/dL	13.8 (11.9–14.8)	13.1 (12.0–14.0)	0.120
Red cell distribution width, %	12.9 (12.5–13.5)	13.3 (12.36–14.4)	0.191
White blood cell count, cells/μL	8.7 (6.9–10.7)	8.4 (6.3–9.3)	0.113
Platelet count, mm^3	237 (192–278)	226 (181–286)	0.232
Mean platelet volume, fL	9.6 (8.7–10.1)	9.2 (8.2–10.8)	0.838
Creatinine, mg/dL	1.0 (0.8–1.2)	0.9 (0.8–1.1)	0.171
Urea, mg/dL	37 (30–48)	36 (26–47)	0.136
Thyroid-stimulating hormone, mIU/L	1.2 (0.7–2.0)	1.2 (0.5–2.3)	0.770
Aspartate aminotransferase, U/L	22 (19–29)	21 (16–44)	0.267
Alanine aminotransferase, U/L	23 (15–37)	25 (15–34)	0.870
Albumin, mg/dL	3.8 (3.6–4.2)	3.8 (3.4–4.4)	0.812
Glucose, mg/dL	110 (91–156)	105 (87–145)	0.150
Electrographic data			
Heart rate, beats/min	77 (63–88)	82 (72–94)	0.006
QRS, ms	101 (87–110)	96 (78–108)	0.057
QT, ms	399 (365–436)	455 (404–478)	<0.001
QTc, ms	452 (420–479)	511 (455–581)	<0.001
Tp-e, ms	88 (69–94)	90 (78–95)	0.118
Tp-e/QT	0.21 (0.17–0.24)	0.20 (0.16–0.23)	0.058
Tp-e/QTc	0.19 (0.16–0.20)	0.17 (0.14–0.20)	0.015
iCEB (QT/QRS)	4.0 (3.4–4.5)	4.6 (4.2–5.5)	<0.001
iCEBc (QTc/QRS)	4.5 (4.0–5.0)	5.6 (4.9–6.2)	<0.001
NIHSS score at admission	3 (2–4)	8 (6–10)	<0.001
NIHSS score at discharge	2 (1–3)	9 (6–11)	<0.001

Continuous variables are presented as median (IQR), and nominal variables are presented as frequency (%). QTc: corrected QT; Tp-e: T-peak to T-end; iCEB: cardiac-electrophysiological balance; NIHSS: National Institutes of Health Stroke Scale.

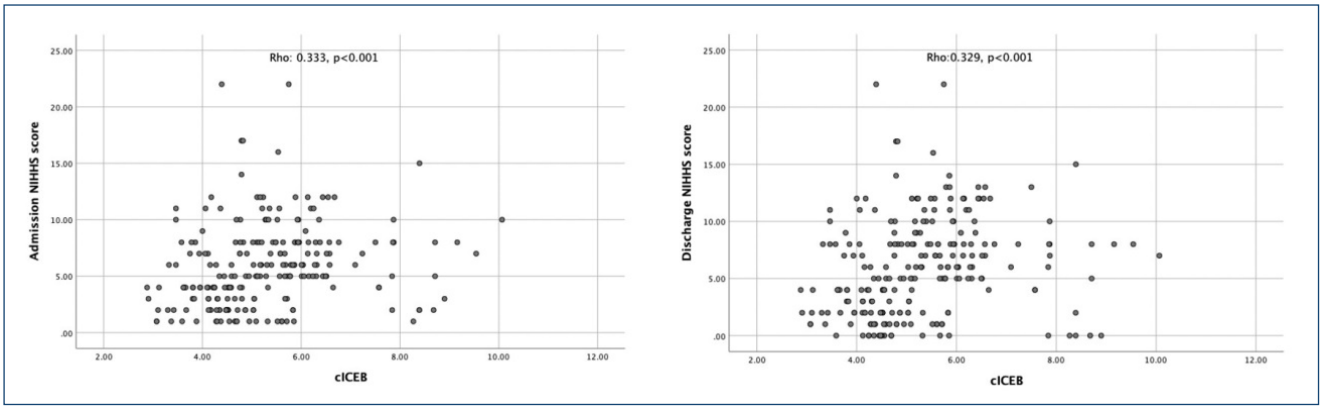

Figure 1. A correlation analysis between and NIHSS scores at admission and discharge. corrected cardiac-electrophysiological balance; National Institutes of Health Stroke Scale.

Acute hemodynamic instability is frequently associated with conduction abnormalities, which is linked to higher morbidity and death following AIS[12]. Moreover, a history of heart failure, the severity of the AIS, the QTc interval, and ventricular extrasystoles might be the risk factors for major cardiac events following an AIS[13]. Villa et al. discovered that patients with AIS with a longer QTc interval had a considerably higher mortality rate than patients with a normal QTc interval[14]. A prior study discovered a link between increases in QTd and the severity of stroke as measured by the NIHSS[15]. In our study, we revealed that both QT and QTc were substantially longer in patients with NIHSS ≥5. However, none of them were independent predictors of high NIHSS scores.

The iCEBc has been proposed as a novel noninvasive index for predicting lethal arrhythmia risk[6]. It was proposed that maintaining the electrical stability of the ventricles requires a careful balance between depolarization (QRS duration) and repolarization (QT interval), in which the deterioration of this balance may lead to arrhythmias. Sivri et al. revealed that after hemodialysis, elevated iCEBc values suggested a higher risk of TdP-mediated cardiac arrhythmia in patients with end-stage renal diseases[16]. Asoğlu et al. showed that iCEBc could be a new noninvasive and simple biomarker to detect the increased risk of pro-arrhythmia in COVID-19 patients[17]. In our study, we discovered that patients with AIS with a high NIHSS score had significantly higher iCEB values. No study showed a link between iCEB values at admission and NIHSS scores at discharge of patients with AIS when we searched through the literature. In addition, we found that high iCEBc levels were independently correlated with high NIHSS scores.

Our results, we believe, would be valuable in a clinical setting. Because patients with a high NIHSS are at a higher risk of arrhythmia, the iCEBc levels can be implemented to identify high-risk individuals. Furthermore, iCEBc levels, which are cheaply and quickly acquired, might be employed for risk assessment and follow-up plans for AIS with NIHSS ≥5 after discharge from hospital.

In our study, although iCEBc is found to be an independent predictor of higher NIHSS scores, their clinical importance of them appears to have a need to be supported with larger cohorts. As a result, new clinical scoring systems, including iCEBc, should be formed to increase the precision of future predicting systems.

Limitations

There were several limitations to our investigation. First, the study was conducted retrospectively, which might be considered a major weakness. Second, because consecutive AIS patients from a single institution were enrolled, the results may be limited in their relevance to a larger population and produce a susceptibility to selection bias. This limitation, however, might be minimized by the relatively large number of individuals in our study. Third, we were unable to assess in-hospital and long-term arrhythmias among patients with high iCEBc and NIHSS scores. However, we believe that our study may be a pilot study in this field. Finally, even though ECG recordings were anonymously evaluated by two experienced cardiologists, computer analyses of the traces were not employed, which might have resulted in human error in the results.

CONCLUSIONS

This study concluded that iCEBc levels were an independent predictor of NIHSS ≥5 and were correlated with both the NIHSS scores at admission and discharge.

AUTHORS' CONTRIBUTION

YK: Conceptualization, Writing – original draft, Writing – review & editing. **MİH:** Conceptualization, Formal Analysis, Writing – review & editing. **MS:** Conceptualization, Data curation, Writing – review & editing. **VÇ:** Data curation, Funding acquisition, Writing – review & editing. **SD:** Data curation, Funding acquisition, Writing – review & editing. **MMA:** Supervision, Writing – review & editing. **TÇ:** Conceptualization, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Time of clerkship rotations' interruption during COVID-19 and differences on Progress Test's scores

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SUMMARY

OBJECTIVES: The transition from face-to-face to remote teaching is yet to be fully understood. In clinical training, traditional teaching must prevail because it is essential for the acquisition of skills and professionalism. However, the responses of each school to the pandemic and the decision on when to resume clerkship rotations were mixed. In this study, we aimed to analyze whether the time to resume clerkship rotations was associated with the performance of the students by using a multi-institutional Progress Test.

METHODS: This is a cross-sectional study conducted at nine different Brazilian medical schools that administer the same annual Progress Test for all students. We included information from 1,470 clerkship medical students and analyzed the time of clinical training interruption as the independent variable and the student's scores as the dependent variable.

RESULTS: The comparisons of the students' scores between the schools showed that there are differences; however, they cannot be attributed to the time the clerkship rotations were paused. The correlation between the schools' average scores and the time to resume clerkship rotations was not significant for the fifth year (r= -0.298, p=0.436) and for the sixth year (r= -0.440, p=0.240). By using a cubic regression model, the time to resume clerkship rotations could explain 3.4% of the 5-year students' scores (p<0.001) and 0.9% of the 6-year students, without statistical difference (p=0.085).

CONCLUSIONS: The differences between the students' scores cannot be attributed to the time when the schools paused the clerkship rotations.

KEYWORDS: COVID-19. Knowledge assessment. Medical students.

INTRODUCTION

The COVID-19 pandemic has led many medical schools to interrupt their activities to keep their students safe and minimize the spread of infection[1]. For medical schools, this decision was more challenging because the students, especially those in the final years of their undergraduate training, need to be trained in real scenarios. However, many just-graduated physicians would eventually look after patients with COVID-19 without adequate training[2,3].

In the beginning of the pandemic, less was known about the clinical and epidemiologic characteristics of the disease and, therefore, most of the schools and universities stopped giving classroom lectures and initiated remote teaching. Conventional lectures were replaced by synchronous or asynchronous online classes, and clinical teaching was replaced by case-based online discussions. Upon gaining some understanding about the causes and methods of prevention of the disease, some schools and universities decided to reopen, provided that social distancing and personal protective equipment were adopted[4]. The decision for reopening was not unanimous across countries and even within the same geographical region[5]. In Brazil, the lack of national guidelines for health and sanitary measures led the

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states and cities' leaders to decide the most appropriate time to reopen scholar activities. Accordingly, the universities resumed their presential classes at different times[6,7].

The effects of online teaching on the students' knowledge are yet to be properly evaluated. Although several studies show that faculty members and students have adapted to the new situation, the literature is scarce on objective demonstration of equivalence between traditional and online teaching for clinical training[8-10]. In a previous study, we showed that knowledge acquisition was impaired among final-years medical students during the pandemic. A possible explanation is that even after resuming clerkship, the students had less exposure to real clinical scenarios due to the closure of outpatient clinics and reduced variety of diseases in the wards (that were allocated to COVID-19 patients) [*unpublished data*].

Considering that different Brazilian medical schools resumed their clerkship rotations at different times and that clinical teaching is pivotal for knowledge acquisition, we aimed to investigate whether the time to resume clerkship rotations was associated with the performance of the students on a Progress Test.

METHODS

An annual Progress Test was conducted at nine medical schools of Brazil for all enrolled students. In this cross-sectional study, we included information of the 5- and 6-year medical students (clerkship years). We analyzed the time of clinical training interruption as the independent variable and the students' scores as the dependent variable.

Ethical considerations

Since we dealt with secondary data and no student was identified, ethics committee approval was not necessary, according to Brazilian legislation.

Settings and progress test information

The participating schools are Universidade Estadual Paulista (UNESP), Universidade Estadual de Campinas (UNICAMP), Universidade de São Paulo (USP), Universidade Federal de São Paulo (UNIFESP), Universidade Federal de São Carlos (UFSCAR), Faculdade de Medicina de Marília (FAMEMA), Faculdade de Medicina de São José do Rio Preto (FAMERP), Universidade Estadual de Londrina (UEL), and Universidade Regional de Blumenau (FURB). The undergraduate program lasts 6 years and the clerkship rotations take place in the fifth and sixth years.

These 9 schools are joint in a 16-year-old consortium for the annual administration of the Progress Test. Except for USP, all the schools stopped clerkship rotations in March 2020.

The Progress Test consists of 120 multiple-choice items equally divided into 6 content areas: basic sciences, internal medicine, pediatrics, surgery, obstetrics and gynecology, and public health. The items are clinical vignette-based aiming for applied knowledge rather than knowledge recall. In 2020, the exam was administered in November and was computerized with a safe exam browser instead of the traditional paper-based question book. The test has only formative purposes (i.e., the student's score does not have high-stake implications for progression in the educational program)[11].

Data analysis

The scores of all clerkship rotations were eligible to enter in the analysis. However, tests with less than 30 correct answers (casual mark) were excluded. The data of students who did not apply for the test were also excluded.

To compare the performances between the schools, the Kruskal-Wallis test was used. It was followed by Dunn's test for post-hoc analysis.

After verifying whether there was a difference in scores between the schools, we analyzed the correlation between the time of clinical interruption and the schools' average scores and the students' individual scores. For parametric and non-parametric data, the Pearson's and Spearman's correlation were used, respectively.

In the second step, we analyzed if a linear model could explain the relationship between the variables. The correlation between the residuals was tested with the Durbin-Watson test and acceptable values should range between 1.5 and 2.5. In the final step, we analyzed which equation model could better explain the relationship and provide the coefficient of determination, i.e., how much of the time of interruption would explain the students' scores. We set the statistical significance level at a p-value of 0.05.

The statistical analyses were performed using the IBM Statistical Package for the Social Sciences (SPSS), version 24.0 (IBM SPSS Statistics for MacBook, IBM Corp., Armonk, NY, USA).

RESULTS

Scores of 1,470 students (728 and 742 from the fifth and sixth years, respectively) were included in the study. The mean time of clerkship interruption was 141.6±52.0 and 111.4±49.1 days for the fifth and sixth years, respectively. Table 1 summarizes

the number of students and the time of interruption for each school.

The comparisons of the students' scores between the schools showed that there are differences for both clerkship years (i.e., fifth and sixth years), with p<0.001 (Figure 1). Post-hoc analysis showed significant differences for some pairwise comparisons.

Table 1. Number of students(n) and days of clerkship interruption by school.

School	Fifth year		Sixth year	
	n	Days of interruption	n	Days of interruption
UNESP	76	164	89	115
UNICAMP	113	168	104	125
USP	92	0	81	0
UNIFESP	117	154	126	63
UFSCAR	35	180	32	168
FAMEMA	73	199	89	171
FAMERP	74	80	76	70
UEL	77	140	78	119
FURB	71	47	67	47

The correlation between the schools' average scores and the time to resume clerkship rotations was not significant both fifth year (r= -0.298, p=0.436) and sixth year (r= -0.440, p=0.240).

The correlation between the students' score and the time to resume clerkship rotations was significant for the fifth year (r= -0.082, p=0.026), though not for the sixth year (r= -0.064, p=0.084). A linear model showed R^2 values of 0.8 and 0.5% for the fifth and sixth years, respectively. However, it was unable to explain the relationships between the variables (score and time) because of the lack of homoscedasticity (constant variance of errors). Durbin-Watson values were 0.268 and 0.223 for the fifth and sixth years, respectively.

A curve estimation analysis showed a better fit of model using a cubic regression (higher R^2 values and lower p values) for both the clerkship years. The model summary and parameters estimates are presented in Table 2. The R^2 values for the fifth and sixth years are 3.4 and 0.9%, respectively, with nonsignificant value for the sixth year. The parameters estimates are too low, demonstrating that the dependent variable (students' scores) is poorly explained by the independent variable (time).

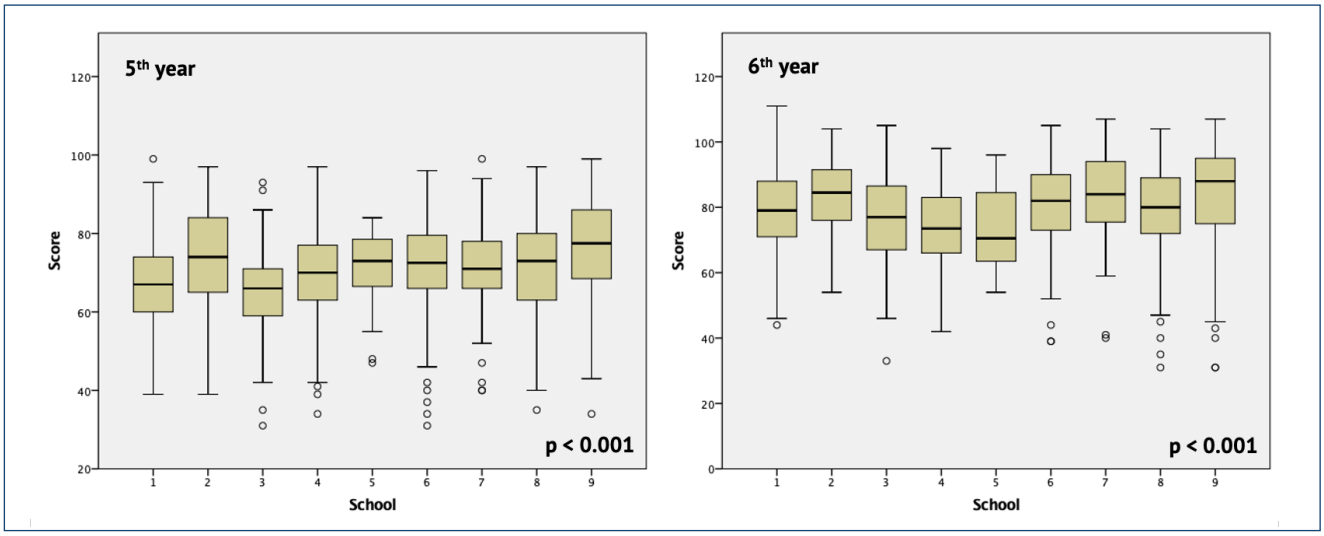

Figure 1. Boxplots of students' scores according to school. The identification of schools is not presented due to secrecy agreements of the Progress Test consortium policy.

Table 2. Regression model for students' scores in function of the time to resume clerkship rotations.

	Model summary						Parameter estimates			
Year	Equation	R^2	F	Df_1	Df_2	Sig.	Constant	B_1	B_2	B_3
5	Cubic	.034	8.526	3	724	.000	75.551	-271	.003	$-1.003E^{-5}$
6	Cubic	.009	2.211	3	738	.085	82.728	-136	.002	$-6.216E^{-6}$

For both the years, a cubic regression has the better fit. The equation is $y=k+b_1*x+b_2*x^2+b_3*x^3$, where y: score (dependent variable); k: constant (intercept); x: time (independent variable); and $(b_1*x+b_2*x^2+b_3*x^3)$: slope.

DISCUSSION

The COVID-19 pandemic has changed several aspects of human life, especially the social interactions. Health and education were affected and, due to the unprecedented situation, some decisions were difficult to make, such as the interruption and resumption of clinical and educational activities of medical undergraduates[11,12].

The effects of the pandemic on the medical students' learning are yet to be understood properly. Initial observations concluded that the transition from face-to-face to remote activities did not decrease the students' knowledge. An American survey with 19 students enrolled in a third-year surgical clerkship during COVID-19 concluded that scores of students did not change in comparison with those who studied in the year before the outbreak of the pandemic[13]. However, this sample size is too small to provide unequivocal and generalizable conclusions. Another American study, with 335 first-year students, also showed in differences on the students' scores before and during the COVID-19 pandemic[14].

An Indonesian cross-sectional study with 270 fourth- and fifth-year medical students in surgery clerkship found no differences between the multiple-choice questions examinations conducted before and during the pandemic. However, in the objective-structured clinical examination, the students evaluated during the pandemic achieved higher scores than those evaluated before the pandemic[15].

Importantly, none of the aforementioned studies described equating methods to compare different tests administered to different students reliably. Equating is an important tool to guarantee a reliable comparison[16,17]. By linking and equating different tests, we have previously demonstrated that the clerkship students' knowledge acquisition was impaired during the pandemic [*unpublished data*].

It is possible that pre-clerkship and clerkship students have been affected differently by the pandemic and the transition to remote teaching[18]. Much of the educational material developed during the pandemic was helpful and replaced some traditional face-to-face activities, such as lectures and tutorial sessions, quite well[19,20]. In some instance, the pandemic induced a positive effect on developing such material.

However, the scenario may be a little different for clerkship students who need direct contact with patients and scenarios to fully develop their professional competence with knowledge, skills, and positive and empathic attitudes toward the patients[21]. Therefore, the clerkship students were the first to resume their educational programs (even in the *new normal* context). Even so, the decisions on the most appropriate time to resume the clerkship rotations were different across many schools.

We hypothesized that the students who were away from clerkship rotations for a longer period would have lower scores on the test. Our findings do not support this hypothesis. Since there are differences in the median scores of the schools, we believe that other factors such as the nature and quality of how remote teaching was employed play more important roles in determining the students' knowledge scores. Besides, we cannot discard the possibility of previous differences of the students' scores across the schools before the pandemic.

A limitation of this study is that we are unable to provide information regarding the direct impact of the social distancing and remote teaching for each school individually, as no comparison with the pre-pandemic scores was done.

Another concern to be discussed is the possible interference of the computer-based exam on students' performance, which could have changed the difficulty of the exam. However, there are several previous studies demonstrating that there is no difference on students score by using either computer- or paper-based exams, including for Progress Test[22,23]. Besides, as our Progress Test has no high-stake implications, cheating behavior is minimal and, if present, might be homogeneous across the schools.

CONCLUSIONS

We found that the students' scores in the Progress Test were different across the schools. However, these differences cannot be attributed to the time during which the students were kept away from the clerkship rotations.

AUTHORS' CONTRIBUTION

PTHF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Methodology, Writing – original draft. **JCM:** Conceptualization, Data curation, Investigation, Writing – review & editing. **ZMTR:** Data curation, Investigation, Writing – review & editing. **LD:** Conceptualization, Data curation, Investigation, Writing – review & editing. **RDL:** Data curation, Investigation, Writing – review & editing. **UCA:** Data curation, Investigation, Writing – review & editing. **ARAL:** Data curation, Investigation, Writing – review & editing. **RCO:** Data curation, Investigation, Writing – review & editing. **MCA:** Data curation, Investigation, Methodology, Writing – review & editing. **AMB:** Conceptualization, Data curation, Funding acquisition, Project administration, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Demonstration of kinesio taping effect by ultrasonography in neck pain

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SUMMARY

OBJECTIVE: This study aimed to demonstrate the effectiveness of kinesio taping in nonspecific neck pain and to assess whether ultrasonographic parameters of the upper trapezius muscle can be used in the follow-up of kinesio taping treatment.

METHODS: This was a single-blind, prospective, randomized controlled trial study involving 60 participants with nonspecific neck pain. The participants were randomly assigned into two groups. Kinesio taping group (n=29) received a 4-week neck exercise program, with kinesio taping applied twice a week for a total of four times, and the exercise group (n=28) received a 4-week neck exercise program. Participants were evaluated according to pain intensity (Visual Analog Scale), cervical range of motion, and disability (Neck Disability Index). Also, trigger point diameter and upper trapezius muscle thickness were evaluated with ultrasonography. Before and after the therapy, as well as the first month, all measures were taken by an investigator other than the practitioner of the treatment program.

RESULTS: The results showed that the Visual Analog Scale and Neck Disability Index scores in the kinesio taping group were statistically significantly improved when compared to the exercise group ($p<0.05$). In addition, the thickness of the upper trapezius muscle and the diameter of the trapezius muscle trigger point were statistically significantly improved in the kinesio taping group compared to the exercise group ($p<0.05$). In the kinesio taping group, there was no statistical significance in cervical range of motion as compared to the exercise group.

CONCLUSION: The combination of kinesio taping and exercise therapy was effective in reducing nonspecific neck pain and neck disability. Also, this study showed that ultrasonographic evaluation of the trapezius muscle could be used in the follow-up of kinesio taping therapy.

KEYWORDS: Kinesio taping. Ultrasonography. Neck pain. Trigger point. Trapezius muscle.

INTRODUCTION

Nonspecific neck pain is a pain without structural pathology that progresses between the first thoracic vertebra and the superior nuchal line. There are no specific pathologies, and often, a diagnosis cannot be made due to its multifactorial etiology[1]. One of the reasons for nonspecific neck pain is myofascial pain syndrome (MPS), which is relatively widespread in the general population[2]. MPS is caused by myofascial trigger points and is classified as a common local musculoskeletal pain syndrome. The incidence can be up to 54% in women and 45% in men[3].

The diagnosis of MPS is mainly based on manual palpation and clinical experience[4]. Today, although there are no objective criteria, the diagnosis is made by clinical features defined by Simons et al.[5]. Treatments of MPS consisted of oral medicine, physical therapy modalities, soft-tissue release, manual therapies, and needling therapy. There have been a few studies recently looking into the therapeutic impact of the kinesio taping (KT) technique, which is a new MPS therapy[6].

Dr. Kenzo Kase created the KT technique in 1979. With its wavy design, it can change the proprioception and somatosensory inputs. The purpose of KT is soft-tissue support, edema control, joint protection, and reduction of active inflammation[6-8].

To explain the processes of KT, theories such as improving blood and lymphatic circulation, aiding in postural alignment, and relaxing overused muscles have been offered[9,10]. In addition, increased afferent feedback on the skin stimulates the door-control mechanism, resulting in a reduction in pain[9-11]. Many studies demonstrate that KT can help with sports injuries, but there is not enough evidence that it can help with MPS[12-14]. The purpose of this study was to demonstrate the effect of KT in MPS and to show that ultrasonographic evaluation of the trapezius muscle can be used in the follow-up of KT treatment.

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METHODS

This single-blinded randomized controlled trial was approved by the Kanuni Sultan Süleyman Research Training Hospital Ethics Committee (KAEK/2021.03.87). All participants were informed, and a formal agreement was obtained for this study, which followed the ethical principles outlined in the Declaration of Helsinki. A total of 262 patients with non-specific neck pain were evaluated. Sixty participants who met the inclusion criteria and were diagnosed with MPS according to Travell and Simons[5] criteria were enrolled in the study and were randomly assigned to one of two groups: exercise group (n=30) or KT group (n=30).

Pain with VAS, extension, flexion and cervical lateral flexion with goniometer, Neck Disability Index (NDI), trigger point diameter, and trapezius thickness were evaluated before treatment, after treatment, and after the first month of treatment.

Following the initial evaluation of the KT group, "I" strip kinesio tape was applied to the upper trapezius muscle using the space correction technique[15] for a total of four sessions, 2 days a week, and the participants were included in a 1-month home exercise program (Figure 1). Participants were assessed before treatment, after treatment, and after the first month of treatment. Participants in the exercise group were required to participate in a 1-month home exercise program.

Figure 1. The application of the "I" strip kinesio tape to the upper trapezius muscle using the space correction technique.

The participants had to be between the ages of 18 and 65 years and have had symptoms for at least 3 months. A history of acute neck injury or cervical surgery, psychiatric disorders, a diagnosis of cervical radiculopathy or myelopathy, recent trigger-point injection or participation in a physical treatment program within the past 6 months, any allergies to the tape, and having previously received treatment with KT were all exclusion criteria.

The exercise program included a cervical range of motion (ROM), isometric chin-in in sitting, upper trapezius stretching, upper trunk extension with chin-in in prone, scapular retraction, bent over row, and reverse flies. Each exercise was asked to be performed in three sets of 10 repetitions, three times a week for 4 weeks. Participants filled out a daily exercise diary.

Participants were evaluated according to pain, the active ROM of the cervical spine, and disability. Active ROM of the cervical spine was measured with the goniometer.

Neck pain was evaluated with VAS. Activity, rest, and night pain were questioned. VAS is a 10-point scale that evaluates pain, with score ranging from 0–10[16].

NDI, which was validated and reliable in Turkish, was used to assess disability. The total score ranges between 0 and 50[17].

Trigger point diameter and trapezius muscle thickness were evaluated with ultrasonography. The ultrasonographic evaluation of the trapezius muscle in MPS was found to have high sensitivity and specificity, and hence, this assessment was considered a useful tool to diagnose MPS[18].

All treatments were performed by an investigator, and evaluations and measurements were made by a different investigator.

The IBM SPSS version 23.0 software was used for statistical analysis. The Shapiro-Wilk test was used to determine the normality of data. The chi-square test was used for categorical variables. For within-group analysis, the Friedman test was utilized. Bonferroni-corrected Wilcoxon signed-rank test was used, if a significant difference was discovered in within-group comparisons. The Kruskal-Wallis test was used to perform between-group analysis. Bonferroni-corrected Mann-Whitney U test was applied, when a significant difference was discovered in between-group analysis. A p-value of <0.05 was considered statistically significant, and for Bonferroni-corrected results, $p<0.0167$ was considered significant.

The G*power version 3.1.2 program was used to calculate study power and sample size. A total of 52 patients were required for the primary VAS, with a minimum of 26 participants in each group, 80% power, 5% type I error, and an effect size of 0.79[19].

RESULTS

A total of 57 participants were evaluated [KT group (n=29) and exercise group (n=28)]. One participant in the KT group was excluded from the evaluation due to an allergic reaction of KT. Two participants in the exercise group were excluded from the evaluation due to a lack of data in the follow-up period. In terms of all outcome variables, there was no significant difference in the baseline assessment between the two groups (Table 1).

Table 1. Differences in terms of demographic and clinical characteristics.

	KT n=29)	Exercise (n=28)	p
Age	34.4 (8.5)	32.7 (7.2)	0.251
Gender, n (%)			0.159
Women	24 (82.8)	21 (75)	
Men	5 (17.2)	7 (25)	
Occupation, n (%)			0.191
Not working	17 (58.6)	10 (35.7)	
Desk worker	6 (20.7)	7 (25)	
Physically demanding	6 (20.7)	11 (39.3)	
Marital status, n (%)			0.322
Married	20 (69)	21 (75)	
Single	9 (31)	7 (25)	
Education, n (%)			0.584
Primary education	15 (51.7)	12 (42.9)	
High school	5 (17.3)	2 (7.1)	
University	9 (31)	14 (50)	
VAS rest, mean (SD)	4.1 (1.1)	3.7 (1.5)	0.064
VAS activity, mean (SD)	6.1 (1.4)	6.1 (1.3)	0.991
VAS night, mean (SD)	2.8 (1.0)	2.3 (1.2)	0.685
Trapezius thickness, mean (SD)	11.2 (1.9)	10.9 (2.1)	0.430
Trigger point diameter, mean (SD)	4.9 (1.1)	5.1 (1.5)	0.160
NDI, mean (SD)	15.1 (4.4)	14.4 (5.6)	0.344
Right lateral flexion, mean (SD)	39.1 (4.8)	38.2 (4.1)	0.291
Left lateral flexion, mean (SD)	37.7 (4.1)	37.5 (3.2)	0.137
Flexion, mean (SD)	44.3 (6.7)	44.8 (6.0)	0.330
Extension, mean (SD)	55.0 (6.8)	54.6(5.8)	0.319

KT: kinesio taping; VAS: Visual Analog Scale; NDI: Neck Disability Index; SD: standard deviation. $p<0.05$ was considered significant for homogeneity of variances.

Compliance with the exercise program was 87% for the KT group and 86% for the exercise group. In terms of adherence rates, there was no significant difference.

The results showed that there was a statistically significant improvement in the VAS, NDI, trapezius thickness, and trigger point diameter scores in the KT group compared to the exercise group after treatment and at the first month. Changes in the outcome measure values are summarized in Table 2.

DISCUSSION

As a result of the study, there was an improvement in ultrasonographic parameters of the upper trapezius muscle, pain, and disability with KT. KT has not been shown to be superior to exercise in ROM evaluation. In addition, it was demonstrated that ultrasonographic parameters of the upper trapezius muscle can be used in the follow-up of KT treatment in MPS.

There are many studies in the literature that include evaluations of the trapezius muscle with ultrasonography in chronic nonspecific neck pain and MPS[18,20,21]. In light of these data, for diagnosing MPS, ultrasonography can be a useful tool. However, Korkmaz et al. showed that ultrasonographic evaluations of the upper trapezius muscle can be used in the follow-up of dry needle therapy in addition to diagnosis[22]. This study showed that ultrasonographic evaluations of the trapezius muscle can be used to demonstrate the effectiveness of KT and different exercise protocols. All the scales evaluating the results of treatments for MPS are patient-based and based on subjective data. Although the ROM measurements are objective data, they are not specific for MPS and are affected by many diseases such as inflammatory, degenerative, or mechanical diseases. Therefore, the trigger point size is important and is specific for the treatment follow-up of MPS.

In this study, pain, neck disability, and cervical ROM improved with KT and exercise therapy, and the improvement in pain and neck disability was higher in the KT group. There are studies in the literature that support the results of this study with an increase in cervical ROM and a decrease in pain and NDI with KT. Hernandez et al. compared the efficacy of KT and cervical confidence manipulation in neck pain and observed similar reductions in disability and pain intensity and increases in ROM in both treatment groups[23]. Azatcam et al. showed an increase in ROM and a decrease in pain with KT and exercise treatment, and a decrease in disability assessed by NDI[24]. In a study comparing KT to sham KT, Ay et al. discovered an increase in ROM and a decrease in pain, which were found similar to these results[19]. They did not detect a decrease in disability in the short term, but in

Table 2. Comparison of data between and within groups.

	KT (n=29)	p[a]	p[b]	Exercise (n=28)	p[a]	p[b]	p[c]
VAS rest (mm)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p=0.001	p=0.168
BT			AT-1M			AT-1M	AT-1M
	4.1±1.2		p=0.001	3.7±1.5		p=0.002	p=0.824
AT	1.5±1.1		BT-1M	3.1±1.6		BT-1M	BT-1M
1M	0.9±1.1		p<0.001	2.4±1.0		p<0.001	p<0.001
VAS activity (mm)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p<0.001	p<0.001
BT			AT-1M			AT-1M	AT-1M
	6.1±1.4		p<0.001	6.1±1.3		p<0.001	p<0.047
AT	3.5±1.6		BT-1M	5.2±1.6		BT-1M	BT-1M
1M	2.2±1.5		p<0.001	4.3±1.5		p<0.001	p<0.001
VAS night (mm)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p=0.004	p<0.001
BT			AT-1M			AT-1M	AT-1M
	2.8±1.0		p=0.008	2.3±1.2		p=0.022	p=0.985
AT	0.7±1.1		BT-1M	1.8±1.6		BT-1M	BT-1M
1M	0.3±0.7		p<0.001	1.3±1.2		p<0.001	p<0.001
Trapezius thickness (mm)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p<0.001	p=0.008
BT			AT-1M			AT-1M	AT-1M
	11.2±1.9		p=0.001	10.9±2.1		p=0.391	p=0.02
AT	9.4±1.1		BT-1M	10.2±1.9		BT-1M	BT-1M
1M	9.1±1.1		p<0.001	10.1±1.8		p<0.001	p=0.006
Trigger point diameter (mm)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		0.003	p=0.006	p<0.001
BT			AT-1M			AT-1M	AT-1M
	4.9±1.1		p<0.001	5.1±1.5		p=0.196	p=0.075
AT	3.5±1.2		BT-1M	4.6±1.4		BT-1M	BT-1M
1M	3.2±1.1		p<0.001	4.5±1.2		p=0.004	p<0.001
NDI			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p<0.001	p=0.002
BT			AT-1M			AT-1M	AT-1M
	15.1±4.4		p<0.001	14.4±5.6		p=0.003	p=0.199
AT	7.2±2.8		BT-1M	10.0±4.2		BT-1M	BT-1M
1M	5.4±2.2		p<0.001	8.8±4.1		p<0.001	p=0.002
Right lateral flexion (°)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p<0.001	p=0.480
BT			AT-1M			AT-1M	AT-1M
	39.1±4.8		p<0.317	38.2±4.1		p<0.004	p=0.005
AT	44.4±1.5		BT-1M	42.5±2.9		BT-1M	BT-1M
1M	44.6±1.3		p<0.001	44.3±1.8		p<0.001	p=0.609

Continue...

Table 2. Continuation.

	KT (n=29)	p^a	p^b	Exercise (n=28)	p^a	p^b	p^c
Left lateral flexion (°)			BT-AT			BT-AT	BT-AT
		<0.001	p<0.001		<0.001	p<0.001	p=0.102
BT			AT-1M			AT-1M	AT-1M
	37.7±4.1		p=0.083	37.7±4.1		p<0.003	p<0.045
AT	44.5±1.5		BT-1M	42.7±3.5		BT-1M	BT-1M
1M	45.0±0.0		p<0.001	44.3±1.8		p<0.001	p=0.501
Flexion (°)			BT-AT			BT-AT	BT-AT
		<0.001	p=0.007		0.009	p=0.053	p=0.527
BT			AT-1M			AT-1M	AT-1M
	44.3±6.7		p=0.011	44.8±6.0		p=0.026	p=0.748
AT	45.9±6.3		BT-1M	46.1±4.8		BT-1M	BT-1M
1M	47.2±4.9		p=0.002	47.8±2.9		p=0.009	p=0.882
Extension (°)			BT-AT			BT-AT	BT-AT
		0.020	p=0.015		0.006	p=0.068	p=0.783
BT			AT-1M			AT-1M	AT-1M
	55.0±6.8		p=0.705	54.6±5.8		p=0.493	p=0.367
AT	57.1±5.1		BT-1M	56.8±4.3		BT-1M	BT-1M
1M	56.8±4.5		p=0.061	57.1±4.4		p=0.053	p=0.482

BT: before treatment; AT: after treatment; 1M: 1-month; VAS: Visual Analog Scale; KT: kinesio taping; NDI: Neck Disability Index. p^a and p^b values show the results of the within-group analysis (Friedman test and Bonferroni-corrected Wilcoxon test). p^c value shows the between-group analysis results (Kruskal-Wallis test). $p^a<0.05$ were considered significant. p^b and $p^c<0.0167$ were considered significant for Bonferroni correction.

this study, a decrease in disability after treatment and at the first month was found. In this study, an improvement was found in the ROM in the KT group, but no difference was found between the exercise group and the KT group in the improvement in the ROM. The KT group received exercise therapy in addition to KT. The improvement in ROM is an improvement due to exercise therapy. No additive effect was observed in the increase of KT on ROM. In the study by Noguera-Iturbe et al., no improvement was found in the ROM with the KT treatment applied with the space correction technique[25]. Similar to this study, Ptaszkowski et al. showed improvement in pain with KT treatment applied with the space correction technique[14]. The effect of exercise and KT therapy on pain and disability may be an additive effect on exercise in this study. Few systematic reviews comparing KT with sham banding or other interventions have shown no significant benefit or small effect of KT. However, the sample sizes of these studies were small, and the quality of these studies was low to moderate[9-11].

In light of these data, higher sample sizes and longer follow-up periods are required to demonstrate the utility of KT in MPS. In this study, KT was performed only for the upper trapezius, and other muscles of the participants were not evaluated in terms of nonspecific neck pain, which is one of the study's limitations. In this study, there was no group that did not receive treatment to show the true effect of KT. Evaluations of ultrasonography in MPS have only been made for the trapezius muscle, and it is unclear whether it can be used in other muscles. In addition, there is a need for studies showing that ultrasonographic evaluation of the trapezius muscle is usable with different treatment approaches.

CONCLUSIONS

In this study, a greater improvement in neck pain, disability, and trigger point diameter and trapezius muscle thickness was found with 2 weeks of KT treatment added to exercise. This study provides information that ultrasonographic assessment for trapezius muscle can be used in the diagnosis and treatment follow-up of MPS. This could give clinicians a dependable and objective method to evaluate treatment response in myofascial pain.

AUTHORS' CONTRIBUTIONS

CMC: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Software, Writing – original draft. **MDK:** Data curation, Formal analysis, Investigation, Methodology, Resources. **MC:** Data curation, Software, Writing – original draft. **FNK:** Conceptualization, Software, Writing – review & editing.

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ORIGINAL ARTICLE

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Temporal progression of sepsis on critical care COVID-19 patients: a retrospective cohort study

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SUMMARY

OBJECTIVE: This study aimed to describe sepsis progression in critical COVID-19 patients using the SOFA score and investigate its relationship with mortality.

METHODS: Three researchers collected and analyzed retrospective clinical and laboratory data found in electronic health records from all patients admitted to a severe COVID-19 exclusive intensive care unit from March 2020 to October 2020. Mixed-effect logistic regression was used to evaluate SOFA (Sepsis-3) score variables as mortality prediction markers, while Kaplan-Meier survival curves were used to compare mortality between groups of patients. Cox proportional hazard models were used to further stratify mortality association between variants.

RESULTS: A total of 73 patients were included. Temporal COVID-19-related sepsis progression analysis indicates difference in degrees and timing between different organ dysfunction over time. Sepsis-3 Cardiovascular Dysfunction characterized by severe hypotension added to the use of any vasopressor drugs was the only parameter associated with in-hospital death during the first 5 days of hospital admission (OR 2.19; 95%CI 1.14-4.20; p=0.01).

CONCLUSION: Increased Sepsis-3 Cardiovascular Dysfunction score, characterized as hypotension associated with the use of vasopressor drugs in the first days of intensive care unit stay, is related to higher mortality in COVID-19 patients and may be a useful prognostic prediction tool.

KEYWORDS: Sepsis. COVID-19. SaRS-CoV-2. Critical care.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, which started in December 2019, has claimed over 6 million lives in more than 192 countries[1] and yet its physiopathology is poorly understood. Acute respiratory distress syndrome was described as a major complication[2], but symptoms may present as a spectrum ranging from asymptomatic to multisystemic, and some patients do not even develop respiratory distress – instead, they develop other symptoms unrelated to respiratory distress, which may be valuable prognostic markers[3].

In these critical patients, multiorgan damage, manifested as sepsis, has been described since early reports[4,5]. The most recent systematic review and meta-analysis about the prevalence of sepsis in COVID-19[6] have explored the hypothesis of COVID-19 as the direct cause of viral sepsis and described a prevalence as high as 77.9% in affected patients.

However, after more than 2 years of the beginning of the COVID-19 pandemic and a wide description of multiple variants, as of April 2022, as few as nine systematic reviews could be found on the PubMed indexing website referring to a search combining "sepsis" and "covid-19" as MeSH terms, which suggests that the correlation between sepsis and COVID-19 is yet underestimated.

Given the lack of studies focusing on the multi-organic aspect of COVID-19 critical care, the continuous spread of the pandemic, and overall sub-notification of sepsis cases, it is necessary to describe and analyze the temporal evolution of systemic organ failure in intensive care patients, in order to accurately identify risk factors, hallmarks, and evidence of prognosis that can help the development of better treatment and reduce morbimortality.

This study aimed to describe sepsis progression in critical COVID-19 patients using the SOFA score and investigate its relationship with mortality.

METHODS

This is a retrospective cohort study based on the analysis of a COVID-19-positive sample of patients from Santa Clara Hospital, Porto Alegre, Brazil, admitted between May 2020 and

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October 2020 with available data on the local electronic health records system. Ethical approval for this study was obtained from Irmandade da Santa Casa de Misericórdia de Porto Alegre Review Board (approval number 4.237.991).

Eligibility criteria for data inclusion were as follows:

1. having an RT-PCR method confirmed diagnostic of COVID-19;
2. having 2 and more days of intensive care unit (ICU) stay; and
3. availability of minimal data for patient identification, including age, sex, and comorbidities.

Sample size calculation using a 95% of confidence level with a margin of error of 5% and considering a 3% mortality rate for COVID-19 cases yielded a minimum necessary of 45 measurements to meet statistical constraints. This was exceeded using a convenience sampling drawn from the hospital setting chosen.

Our choice comprises the total number of patients in a COVID-19 exclusive ICU from the beginning to the end of the so-called the first wave of the pandemic in Brazil. After that, as disease treatment progressed and mortality was reduced as a result of vaccination and public health efforts, fewer examinations became available. Therefore, our sample displays abundant data for qualitative analysis and stratification while also readily representing disease progression with minimal confounding factors.

Data were collected by an independent researcher and checked by two researchers, while a fourth settled on differences in interpretations. Right censored data included patients who were

1. lost to follow-up;
2. lost due to evasion from healthcare complex facilities, and
3. transferred to another healthcare complex.

The sample was divided and compared between groups of deceased versus recovered patients. The primary outcome under analysis was death. The predictors of mortality were SOFA (Sepsis-3) score[7] values, which progressively rate organ dysfunction from 1 to 4, with 4 being the most severe, as follows:

- Respiratory system (PaO_2/FiO_2 ratio) from <400 (1) to <100 (4)
- Coagulation system (serum platelets in cell/mm^3) from <150,000 (1) to <20,000 (4)
- Liver function (serum bilirubin in mg/dL) from 1.2–1.9 (1) to >12.0 (4)
- Cardiovascular system (mean arterial pressure in mmHg) <70 (1) or use of vasopressor drug >15 mcg/kg/min (4)
- Renal system (serum creatinine in mg/dL) from 1.2–1.9 (1) to >5.0 (4)

Exposure was considered as RT-PCR confirmed SARS-CoV-2 infection. Tertiary medical care by ICU staff was considered a major potential confounder and modifier. Since it is impossible to distinguish its effects from those of usual COVID-19 progression, our time frame was defined as a 10-day analysis after ICU admission and then subdivided into two 5-day analyses, to reduce confounding effects.

Statistical analysis

We verified normality in data distribution using Shapiro-Wilk test. Our plot (Figure 1) was generated by using LOWESS (Locally Weighted Scatterplot Smoothing) to fit a smooth curve to nonparametric data points. LOWESS weight function gives the most weight to the data points nearest the point of estimation and the least weight to the data points that are furthest away.

To analyze the association between increasing sepsis score and mortality, a mixed-effect logistic regression model was applied by using the lme4 package, available for R Studio software. The odds ratio was calculated by taking the exponential coefficient output. Our model aimed to estimate binary outcome variables (death vs. survival) ratios, with SOFA score variables as patient-level continuous predictors, days since admission as a patient-level categorical predictor (0–10), and a random intercept by patient ID. Cox proportional hazard models were used to further stratify mortality association between variants.

A p-value <0.05 was adopted as a cutoff value for statistical significance. All data were extracted and cleaned using R Studio version 4.1.2 for macOS.

RESULTS

Our sample yielded a total of 73 patients, of which 37 (50.3%) were deceased. The majority were 60 years or older (53%), and 38 (52%) were females. Hypertension was the most common comorbidity (58%), while kidney disease was the least common (4%). On admission, dyspnea was the most common symptom (67.5%), while tachycardia was present in only 1 patient. Median time from symptom onset until ICU admission was 10.17 days. Detailed clinical characteristics during admission can be observed in Table 1.

Figure 1 shows the LOWESS plot for our data. Detailed results for mixed-effect logistic models and hazard models can be observed in Table 2. Complete data have been submitted as Supplementary file. On the first 4 days of ICU admission, the

Table 1. Patient characteristics on admission.

	Total (n=73)	Survivors (n=36)	Fatal (n=37)
Median age in years (IQR)	61.5 (47.25–73.0)	52.0 (43.0–66.0)	66.0 (56.0–79.0)
<40	7	6	1
40–60	28	17	11
≥60	38	13	25
Sex			
Female	38	17	21
Male	35	19	16
Weight in kg (IQR)	80.0 (70.0–95.0)	83.5 (70.0–100)	74.50 (66.75–90.5)
Previous hospitalization	40	21	19
Comorbidities			
Hypertension	41	17	24
Diabetes	29	12	17
Obesity	19	10	9
Smoking history	9	3	6
Respiratory diseases	9	4	5
Cardiovascular disease	5	2	3
Gastrointestinal diseases	5	2	3
Central nervous system diseases	11	4	7
Liver diseases	7	2	5
Chronic kidney diseases	3	0	3
Surgery history	10	4	6
Chronic heart disease	14	6	8
Cancer	9	5	4
Signs and symptoms on ICU admission			
Fatigue	10	4	6
Fever	29	15	14
Dyspnea	50	23	27
Tachycardia	1	0	1
Cough	32	18	14
Coryza	7	2	5
Myalgia	11	7	4
Chest pain	6	3	3
Pharyngalgia	5	2	3
Diarrhea	9	2	7
Nausea and vomiting	6	2	4
Median (IQR) time from onset of symptom to hospital admission, days	10 (2–16)	12 (6–15)	8 (2–16)
Median (IQR) time from hospital admission to outcome, days	9 (2–16)	8 (2–15)	10 (3.0–16.5)
Mean SOFA scores			
Respiratory System Score	1.48	1.53	1.42
Coagulatory System Score	0.25	0.1	0.42
Liver Function Score	0.02	0.4	0
Cardiovascular System Score	0.89	0.21	1.54
Renal Function Score	0.53	0.26	0.78
Total SOFA score	2.71	1.94	3.45
Other			
Days from symptom onset until ICU admission (mean)	9.0	10.0	5
Days hospitalized (IQR)	12	8.5	10.0
Sepsis diagnostic by ICU team (total)	14	4	10

IQR: interquartile range; ICU: intensive care unit; SOFA: systemic organ failure.

odds of in-hospital death was associated with increased cardiovascular dysfunction (OR 2.19; 95%CI 1.14-4.20; p=0.01). On days 5–10, no statistically significant relationship was detected. Cox proportional hazard model adjusted for multivariate analysis demonstrated increased risk for Cardiovascular Dysfunction score = 3 (OR 2.87; 95%CI 1.05–7.8; p=0.04), but reduced risk for Respiratory Dysfunction score = 1 (OR 0.3; 95%CI 0.092–1.0; p=0.04) when compared to score = 0 (no organ dysfunction).

DISCUSSION

Our study demonstrates that the progression of dysfunction has a different timing of worsening for each system evaluated. Regarding risk factors, cardiovascular dysfunction, characterized by Sepsis-3 as hypotension added to the use of vasopressor drugs[7], is the only factor evaluated with a positive correlation for mortality in the first 5 days of hospitalization. Respiratory dysfunction, characterized by a low PaO_2/FiO_2 ratio ranging from <400 – <100, showed no positive association.

There are three reasons for our choice of a time frame of 10 days of analysis. First, we considered that after 5 days, too many confounding factors could be included in the patient's profile after intensive care symptomatic management. Second, we found no previous studies in which a time series analysis had been done in ICU patients for sepsis progression. One of the first reports pointed to a median time from disease onset to death of 16 days but did not stratify this number as a subset of ICU admission time[8]. Third, our data displayed evident inflection points in which the parameter would behave differently, i.e., organ dysfunction would decrease.

The difference in observable trends implies that the primary mechanism behind clinical symptoms in critical patients does not involve only the lung, although it does not explain when the adjacent organ damage initially happens. Current evidence[9] implies that the exudative and proliferative phases of alveolar

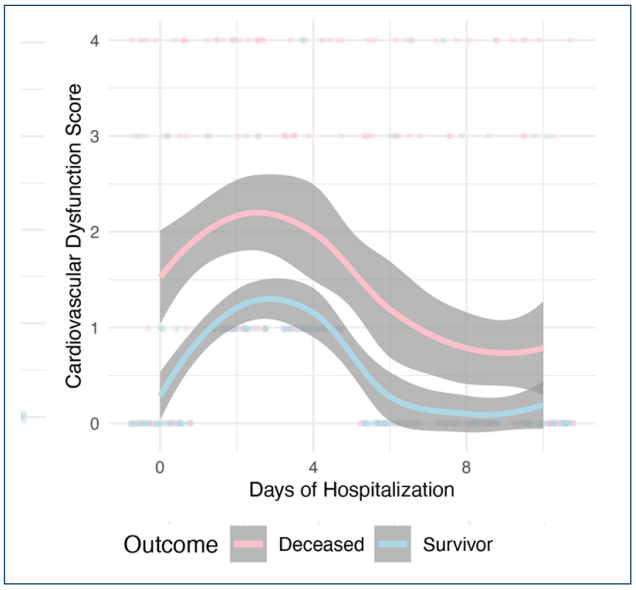

Figure 1. SOFA Cardiovascular Dysfunction Score progression over time comparing deceased and recovered patients in a COVID-19 exclusive intensive care unit.

Table 2. Sepsis-3 score hazard models and mixed-effect logistic regression.

Cox proportional hazard models		
Cardiovascular Dysfunction score	**Odds ratio (95%CI)**	**p-value**
3	2.87 (1.05–7.8)	0.04*
4	1.69 (0.65–4.4)	0.283
Coagulation Dysfunction score		
1	2.34 (0.48–11.5)	0.295
2	2.42 (0.67–8.8)	0.178
3	1.18 (0.15–9.6)	0.875
Respiratory Dysfunction score		
1	0.30 (0.092–1.0)	0.049*
2	0.92 (0.332–2.5)	0.867
3	0.81 (0.217–3.0)	0.751
4	0.30 (0.062–1.5)	0.135
Mixed-effect logistic regression		
Days 0–4		
Respiratory	1.08 (0.37–3.34)	0.83
Coagulation	3.62 (0.08–152.55)	0.5
Cardiovascular	2.19 (1.14–4.20)	0.01*
Liver	2.31 (0.27–19.44)	0.44
Renal	1.20 (0.44–3.29)	0.71
Days 5–10		
Respiratory	1.28 (0.3–5.3)	0.73
Coagulation	0.21 (0.01–3.65)	0.63
Cardiovascular	1.73 (0.31–9.48)	0.52
Liver	NA	NA
Renal	1.12 (0.45–2.74)	0.8

Cox proportional hazard model adjusted for multivariate analysis for each category of systemic organ failure score and odds for in-hospital death associated with an increase of 1 point in systemic organ dysfunction score (systemic organ failure-3) using mixed-effect logistic regression using a generalized linear mixed model fit by maximum likelihood (Adaptive Gauss-Hermite Quadrature) and Sepsis-3 reference values. The data show that a greater Cardiovascular Dysfunction score, which is characterized by the use of any vasopressor drug added to increased hypotension, is related to overall increased mortality. The odds ratio for liver dysfunction is nonavailable (NA) due to insufficient data from patients in a 10-day time frame.

damage should happen within the first 10 days. However, respiratory dysfunction peaks around day 6 in our data, and platelets still seem to be within normal levels in our sample (although COVID-19 has been described as a coagulopathy[10,11].

A negative correlation of respiratory system distress also corresponds to that reported for the first wave in China, where the use of invasive mechanical ventilation ranged could go as low as 13.46%[12], strengthening the hypothesis that multiple organ damage, which in turn may evolve into sepsis, does not derives uniquely from lung damage.

These data also confirm considerably reliable evidence, suggesting that patients with higher risk can be identified early in the hospitalization process. Cardiovascular disease and hypertension were already described as strong predictors[13] since cardiac involvement is 13 times higher in critical patients[14]. The same dynamic applies to kidney injury[15], a major correlate with ICU mortality[16]. Many biomarkers have been pointed out as predictors in previous systematic reviews[17], as well as genetic factors, such as overexpression of ADAM9 metalloprotease, which have also been suggested as a "signature" of critically ill patients[18], since it directly influences the uptake and replication of SARS-CoV-2 in lung intraepithelial cells.

In terms of novelty and study similarity, to the best of our knowledge, our study is the first to analyze sepsis progression over a defined time frame by using the SOFA (Sepsis-3) score as a prognostic prediction tool for sepsis in intensive care COVID-19 patients. Our study adds further knowledge to the field of critical care by pointing out which parameters should be observed with additional attention on COVID-19 patients during intensive care admission to precociously differentiate which patients have a higher chance of worsening. Such knowledge is crucial considering each day in ICU may decrease mean survival probability by 3.27% per day[19].

Sepsis is still a major mortality cause in a hospital setting and further studies are necessary to increase survival rates through early detection and treatment. Nonetheless, our data provide evidence that supports the idea that cardiovascular system dysfunction might be an important parameter to be observed during the hospital admission process to distinguish between patients with good or bad chances of recovery before a major adverse event occurs.

Limitations

This study has some limitations. A larger sample could be used in the same study design to yield undetectable results. Also, the use of retrospective data from electronic health records in a hospital setting may result in a lack of uniformity of clinical data availability subjected to each patient's profile. This may have impaired further clinical analysis by underestimating SOFA score results or impairing data analysis, such as in the case of renal system function, which had scarce data.

Finally, this is a study with data from patients of an exclusive ICU during the second half of the year 2020, and since then, multiple variants have been described and may display slightly different disease severity onset.

CONCLUSIONS

Increased Sepsis-3 Cardiovascular Dysfunction score, characterized as hypotension associated with the use of vasopressor drugs in the first days of ICU stay, is related to higher mortality in COVID-19 patients and may be a useful prognostic prediction tool.

AUTHORS' CONTRIBUTIONS

PL: Conceptualization, Data curation, Writing – original draft. **FBN:** Supervision, Writing – review & editing. **GB:** Supervision, Writing – review & editing. **JAH:** Data curation, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of the Rho-kinase gene expression and polymorphisms in adult patients with acute appendicitis: a differential impact of gender

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SUMMARY

OBJECTIVE: Acute appendicitis represents one of the most common causes of acute intra-abdominal emergencies worldwide. In this case-control study, we aimed to investigate associations of Rho-kinase gene expression and polymorphisms with acute appendicitis in a Turkish population. We also aimed to study the effects of gender on these parameters.

METHODS: A total of 93 unrelated patients with acute appendicitis and 93 healthy controls in the Department of Emergency Medicine, Erciyes University, between June 2019 and June 2021 were included in this study. Genomic DNA was isolated from peripheral leukocytes, and the LightCycler 480 II real-time polymerase chain reaction was utilized to detect Rho-kinase1 gene rs35996865 and Rho-kinase2 gene rs2230774 (Thr431Asn) polymorphisms. Quantitative real-time polymerase chain reaction was applied to determine Rho-kinase1 and Rho-kinase2 gene expressions.

RESULTS: There was a marked increase in Rho-kinase1, but not in Rho-kinase2, mRNA expression, and this increase was evident only in male patients (p=0.0008). No significant differences were found in allele and genotype frequencies for Rho-kinase1 gene rs35996865 and Rho-kinase2 gene rs2230774 polymorphisms between the patients with acute appendicitis and the control group.

CONCLUSIONS: Our data imply that Rho-kinase1 (rs35996865) and Rho-kinase2 (rs2230774) gene variants are not risk factors for the development of acute appendicitis in the Turkish population. However, increased mRNA expression of the Rho-kinase1 gene in males indicated that Rho-kinase1 is involved in the pathogenesis of acute appendicitis in a gender-specific way.

KEYWORDS: Appendicitis. Pharmacogenomic variants. Gene expression. Inflammation. Rho kinase.

INTRODUCTION

Acute appendicitis (AA) resulting from inflammation of the appendix is a leading cause of abdominal surgical emergency. Despite its classic signs and symptoms being well known, it is still difficult to diagnose. Any delay in diagnosis or untreated appendicitis is linked with perforation and increased complications, including abscess, ileus, and peritonitis[1]. Therefore, timely diagnosis is necessary to reduce morbidity and mortality. However, since symptoms of AA overlap with many gynecologic, abdominal, and urologic conditions, reaching a definitive diagnosis is a clinical challenge. In fact, after clinical diagnosis, a negative appendectomy rate of 5.8% and missed perforated appendicitis rate of 3.4% have been described in the previous studies[2]. An estimated 17.7 million cases (incidence 228/100,000) with over 33,400 deaths (0.43/100,000) have been reported in 2019[3].

Rho-kinase (ROCK) is a serine/threonine protein kinase with a molecular mass of ~160 kDa, which has been identified as the first downstream effector of the Rho family of small GTPases. Ubiquitously expressed and highly homologous ROCK1 and ROCK2 isoforms have been identified[4]. The Rho/ROCK signaling pathway regulates cellular migration, adherence, and proliferation through control of the cell contraction and actin-cytoskeletal assembly[4]. Experimental

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data suggest that ROCK activity regulates sepsis-induced systemic inflammation and organ injury[5]. There are no studies investigating the role of *ROCK* gene expression or polymorphisms in AA. We hypothesized that *ROCK* gene expressions and single-nucleotide polymorphisms (SNPs) contribute to the risk of AA development. Thus, the goal of this study was to assess an association between *ROCK* gene expressions/SNPs and AA in a Turkish population. Another objective of this study was to identify the effects of gender on *ROCK* gene expression or polymorphisms.

METHODS

Study design and patients

This prospective case-control study investigated 93 patients admitted to the Erciyes University Department of Emergency Medicine with suspected cases of AA. Of all admitted patients, only those with the intraoperatory diagnosis of AA, aged 18 years and older, were included. Clinical and surgical diagnoses were confirmed postoperatively by histopathological examination. Approval of this research was granted by the Erciyes University Clinical Research Ethics Committee (decision no.: 2019/374) and was performed in accordance with the principles of the Declaration of Helsinki. All participants submitted written informed consent to blood sampling, genotyping, and inclusion in the study. All genetic studies were carried out in the Erciyes University Genome and Stem Cell Center (GENKOK).

The control group was composed of 93 healthy, gender-matched, and age-matched volunteers who had no recent surgery, history of medical illness, or diagnosis of genetic, neurologic, psychiatric, liver, infectious, or chronic inflammatory disease. The volunteers for the healthy control group were formed from resident doctors, employees of the hospital, and their families. Patients who had known or apparent systemic diseases such as heart failure, ischemic heart disease, malignancies, hypertension, autoimmune diseases, chronic pulmonary, renal, or liver diseases, pregnancy or breastfeeding, drug addiction, and receiving immunosuppressive therapy were excluded.

AA patients were classified before surgery using the Alvarado score, which is determined by the signs, symptoms, and diagnostic tests of suspected patients and is composed of a 10-point clinical scoring system for the AA diagnosis. Routine radiological imaging consisted of ultrasonography, computed tomography, or both. Appendectomies were performed via videolaparoscopy or laparotomy. Surgically removed all the appendix specimens were submitted to histopathological analysis according to routine protocols.

Blood samples and DNA isolation

Venous blood samples (6 mL) were drawn into EDTA-containing tubes from all individuals preoperatively in the emergency department and divided into two parts. One part of the samples was used for the measurements of complete blood count (CBC) and other biochemical parameters. Another part of the blood samples was quickly transferred to the GENKOK. Genomic DNA was extracted from peripheral leukocytes using a commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Germany) in accordance with the instructions of the manufacturer. The final DNA concentration was measured using a micro-volume UV-Vis spectrophotometer (BioSpec-nano, Shimadzu, Kyoto, Japan). An absorbance ratio of 1.8 at 260–280 nm was taken as an indicator of DNA purity. DNA samples were then stored at –20°C for further studies. Clinical parameters including pulse rate, mean arterial pressure, respiratory rate, and imaging data were recorded and analyzed as a routine evaluation at the emergency department.

Single-nucleotide polymorphisms selection and genotyping

The preliminary screening criteria for *ROCK1* and *ROCK2* gene SNPs were as follows:

1. minor allele frequency (MAF) and
2. on the basis of previously published studies.

This led to selection of one SNP in *ROCK1* (rs35996865 T>G MAF=0.26) and one SNP in *ROCK2* (rs2230774 G>T MAF=0.40) for inclusion in this study. A total of 15 µL mix was prepared using LightCycler Fast Start DNA Master Hyprobe, $MgCl_2$ stock solution, SNP Primer/Probe, and PCR-Grade water. To identify *ROCK1* gene rs35996865 and *ROCK2* gene rs2230774 (Thr431Asn) polymorphisms, genotyping was done using commercially synthesized primers and fluorescently labeled probes and the LightCycler 480 II real-time polymerase chain reaction (RT-PCR) system (Roche Diagnostics GmbH, Mannheim, Germany). Gene variants were detected by analyzing the detailed melting curve of the PCR product obtained.

RNA isolation and gene expression analysis

PureZol was applied to extract total RNA from patient whole blood samples (Bio-Rad, CA, USA) according to the manufacturer's recommendations. The quantity (absorbance at 260 nm) and quality (ratio of absorbance at 260 and 280 nm) of the RNA were evaluated using a NanoDrop spectrophotometer. RNA was stored at -80°C until use. An iScript cDNA Synthesis kit (CA, USA) was used to reverse transcribe 1g of RNA as stated by the manufacturer's instructions. In a 20-µl reaction volume, quantitative RT-PCR (qRT-PCR) test reactions were

performed. Initial denaturation at 95°C for 10 min was followed by 45 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 60 s. The LightCycler 480 II instrument was used to perform qRT-PCR on duplicate reactions for *ROCK1* and *ROCK2* gene expressions (Roche, Germany). β-Actin (*ACTB*), as a housekeeping gene, was used. The $2^{-\Delta\Delta Ct}$ method of relative quantification was used to evaluate changes in gene expression.

Statistical analysis

The results are presented as mean (SD) for parameters with parametric distribution and median (IQR) for nonparametric data. The normal distribution of numerical variables was analyzed using the Kolmogorov-Smirnov normality test. For the normally distributed data, an unpaired Student's t-test was applied. Mann-Whitney U test was used for data with nonparametric distribution, or for comparing gene expression data. Categorical data were analyzed using the chi-square test. Hardy-Weinberg distribution was tested using the chi-square test by comparing the observed and expected genotype frequencies. Differences in allele and genotype frequencies among the controls and cases were compared using chi-square or Fisher's exact test. Analysis of data was carried out using GraphPad Instat version 3.05 (GraphPad Software Inc., San Diego, CA, USA). All tests were two-sided, and significance was considered at $p<0.05$.

RESULTS

After matching the exclusion and inclusion criteria, all cases of surgically and clinically diagnosed AA were taken for this study. Histopathological analysis was used to confirm the preoperative diagnosis.

A total of 93 patients with AA and 93 healthy volunteers were enrolled in this study. The demographic, laboratory, and clinical characteristics of the study population are given in Table 1. Compared with the controls, the average age, gender, systolic and diastolic blood pressure, respiratory and pulse rates, platelet and lymphocyte counts, glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, and alanine aminotransferase in AA group were similar. Neutrophil and white blood cell (WBC) counts, total bilirubin, lactate dehydrogenase, and C-reactive protein (CRP) levels were found to be increased in the AA group when compared with the controls (Table 1). Our data showed a slight male predominance, and the male/female ratio was 2.6:1.

All patients underwent a videolaparoscopic or laparotomic appendectomy, and no incidences of complications were reported during their hospitalization with an average stay of 1.5 days. A total of 54 patients were evaluated with ultrasonography, 28 patients with computed tomography, and 11 patients with both methods. Using the Alvarado system, 2 (2.2%) patients had a score of 5 or 6, 84 (90.3%) had a score of 7 or 8, and 7 (7.5%) had a score of 9 or 10.

Both the control (*ROCK1,* p=0.9998; *ROCK2,* p=0.4621) and patients (*ROCK1,* p=0.3013; *ROCK2,* p=0.9271) groups were found to be in Hardy-Weinberg equilibrium. For the *ROCK1* gene rs35996865 polymorphism, no marked differences in both genotype (T/T, 60.2; T/G, 37.6; G/G, 2.2%) and allele (T, 79.0; G, 21.0%) frequencies in the AA group were detected when compared with controls (T/T, 59.1; T/G, 35.5; G/G, 5.4; T, 76.9; G, 23.1%, p>0.05) (Table 2). For the *ROCK2* gene rs2230774 (Thr431Asn) polymorphism, genotype (AA:

Table 1. Demographic, clinical, and laboratory characteristics of the study cases.

	Patients with AA (n=93)	Controls (n=93)	p
Age (years)	33.7 (9.4)	34.0 (8.9)	0.8228
Gender, n (%)			
Male	67 (72.0)	67 (72.0)	
Female	26 (28.0)	26 (28.0)	1.0000
Systolic BP (mmHg)	120.6 (13.3)	119.1 (10.8)	0.3775
Diastolic BP (mmHg)	74.2 (8.1)	75.4 (10.0)	0.4010
Pulse rate (beats/min)	87.3 (9.1)	90.1 (11.0)	0.0627
Respiratory rate (beats/min)	18.2 (2.4)	18.4 (7.3)	0.8218
Platelet (10^3/μL)	269.3 (87.8)	273.6 (45.1)	0.6741
Neutrophils (10^3/μL)	11.0 (4.2)	7.0 (0.9)	<0.0001
Lymphocytes (10^3/μL)	2.1 (1.3)	2.0 (0.9)	0.2662
WBC (10^3/μL)	14.1 (4.3)	9.9 (2.0)	<0.0001
Glucose (mg/dL)	109.1 (26.3)	106.9 (18.7)	0.5138
BUN (mg/dL)	14.2 (5.6)	13.9 (9.6)	0.8088
Creatinine (mg/dL)	0.9 (0.2)	0.9 (0.6)	0.6384
Na^+ (mmol/L)	139.8 (2.8)	–	
K^+ (mmol/L)	4.4 (0.4)	–	
AST (U/L)	23.2 (13.2)	20.8 (11.5)	0.1827
ALT (U/L)	19.2 (10.2)	21.5 (12.7)	0.1863
Total bilirubin (mg/dL)	0.7 (0.5)	0.5 (0.2)	0.0013
LDH (U/L)	277.5 (84.0)	254.9 (62.3)	0.0386
C-reactive protein (mg/L)	43.6 (9.2)	0.7 (0.3)	<0.0001

AA: acute appendicitis; BP: blood pressure; WBC: white blood cells; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; MAP: mean arterial pressure. All results were parametric and are presented as mean (SD), except gender which is shown as n (%).

Table 2. Genotype and allele frequencies of Rho-kinase1 gene rs35996865 and Rho-kinase2 gene rs2230774 (Thr431Asn) polymorphisms among cases and controls.

Genotypes/ alleles	Patients with AA (n=93) n (%)	Controls (n=93) n (%)	p
ROCK1 rs35996865			
T/T	56 (60.2)	55 (59.1)	
T/G	35 (37.6)	33 (35.5)	0.8946
G/G	2 (2.2)	5 (5.4)	0.4392
T	147 (79.0)	143 (76.9)	
G	39 (21.0)	43 (23.1)	0.7075
ROCK2 rs2230774 (Thr431Asn)			
G/G	30 (32.3)	25 (26.9)	
G/T	44 (47.3)	52 (55.9)	0.3890
T/T	19 (20.4)	16 (17.2)	0.9808
G	104 (55.9)	102 (54.8)	
T	82 (44.1)	84 (45.2)	0.9169

AA: acute appendicitis; ROCK: Rho-kinase.

G/G, 32.3; G/T, 47.3; T/T, 20.4%, and controls: G/G, 26.9; G/T, 55.9; T/T, 17.2%) and allele (AA: G, 55.9; T, 44.1%, and controls: G, 54.8; T, 45.2%) frequencies were not significantly associated with AA (Table 2).

We found significant differences in leukocytes' *ROCK1* and *ROCK2* gene mRNA expressions in healthy controls and in patients with AA (Table 3). *ROCK1*, but not *ROCK2*, gene expression was markedly elevated in the AA group (p=0.0027). This marked increase in *ROCK1* gene expression was observed in males (p=0.0008), but not in females (p=0.5252).

DISCUSSION

In our study, we showed insignificant associations between AA and *ROCK1* gene rs35996865 and *ROCK2* gene rs2230774 (Thr431Asn) polymorphisms in the Turkish population. However, we demonstrated a marked increase in *ROCK1* gene mRNA expression in male AA patients. To the best of our knowledge, this is the first work to assess the link of the

Table 3. Comparison of mRNA content in leukocytes for the control and patient with acute appendicitis.

		Control Median (min–max)	Patients with AA Median (min–max)	Fold	p*
ROCK1					
Total (n=93)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.22 (0.02–7.01)	2.11 (0.01–42.08)	$\Delta\Delta C_T$: 1.22–2.11= -0.89	0.0027
	Content=$2^{-\Delta CT}$	0.43 (0.01–0.98)	0.23 (0.00–0.99)	Fold: 0.43/0.23=1.87	
Male (n=67)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.19 (0.02–7.01)	2.01 (0.01–42.08)	$\Delta\Delta C_T$: 1.19–2.01=-0.82	0.0008
	Content=$2^{-\Delta CT}$	0.44 (0.01–0.98)	0.25 (0.00–0.99)	Fold: 0.44/0.25=1.76	
Female (n=26)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.79 (0.02–4.66)	2.18 (0.01–6.75)	$\Delta\Delta C_T$: 1.79–2.18= -0.39	0.5252
	Content=$2^{-\Delta CT}$	0.29 (0.04–0.98)	0.22 (0.01–0.99)	Fold: 0.29/0.22=1.32	
ROCK2					
Total (n=93)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.40 (0.23–21.93)	1.20 (0.15–40.09)	$\Delta\Delta C_T$:1.40–1.20=0.20	0.0570
	Content=$2^{-\Delta CT}$	0.38 (0.00–0.85)	0.43 (0.00–0.90)	Fold: 0.38/0.43=0.88	
Male (n=67)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.49 (0.24–21.93)	1.12 (0.15–40.09)	$\Delta\Delta C_T$:1.49–1.12=0.37	0.0719
	Content=$2^{-\Delta CT}$	0.36 (0.00–0.85)	0.46 (0.00–0.90)	Fold: 0.36/0.46=0.78	
Female (n=26)	$\Delta C_T = C_t(\text{target}) - C_t(\text{housekeeping})$	1.20 (0.23–4.61)	1.29 (0.15–3.85)	$\Delta\Delta C_T$:1.20–1.29= -0.09	0.4671
	Content=$2^{-\Delta CT}$	0.43 (0.04–0.85)	0.41 (0.07–0.90)	Fold: 0.43/0.41=1.05	

ROCK: Rho-kinase. The results are presented as median (IQR). *Mann-Whitney U test.

ROCK gene polymorphisms with AA susceptibility. This is also the first research reporting that there was a gender-dependent effect on AA in terms of gene expression. The findings of this study indicate that rs35996865 and rs2230774 polymorphisms are unlikely to play a role in AA development.

The rs35996865 polymorphism is located in the *ROCK1* promoter region, about 2 kb upstream of the transcription start site. However, it is not known whether this polymorphism is able to alter the expression level of the *ROCK1* gene[6]. The *ROCK1* gene rs35996865 variant mapping to the 5′-UTR has been reported to be markedly associated with obesity-related metabolic syndrome[7], respiratory distress syndrome[8], and nonsyndromic cleft palate[6], but not with sepsis[9] or primary open-angle glaucoma[10]. The result of our study showed that there was no association between the rs35996865 variant and AA.

The rs2230774 polymorphism is located in the exon 10 of the *ROCK2* gene and causes amino acid change (Thr431Asn). This polymorphism is markedly associated with breast cancer metastases[11] and obesity-related metabolic syndrome[7]. In contrast, there are several reports showing that this polymorphism is not associated with respiratory distress syndrome of the newborn[8], mantle cell lymphoma[12], and primary open-angle glaucoma[10]. The result of the present study demonstrated that there was no association between rs2230774 polymorphism and AA.

Reactive oxygen species (ROS) and imbalance in the oxidant/prooxidant defense system may play an important role in the pathology and progression of AA[13]. ROS have been indicated to have a relationship with the RhoA/ROCK pathway[14]. These reports may support our findings, showing that upregulation of *ROCK1* gene expression in male AA patients could be related to increased oxidative stress. Estrogens are capable of diminishing oxidative stress and increasing antioxidative cell potency[15]. This may explain the result of the present study, showing that there was no upregulation of *ROCK* gene expression in female AA patients.

We observed increased neutrophil and WBC counts and CRP levels in AA patients. CRP can activate RhoA/ROCK to elevate endothelial plasminogen activator inhibitor-1 expression, which may lead to atherothrombogenesis[16]. Serum levels of interleukins (IL-1, IL-6, and IL-8), INF-γ, and TNF-α were markedly elevated in patients with appendicitis[17]. TNF activates different Rho GTPases, enhances filamentous actin, remodels endothelial cell morphology, and induces actin stress fibers through RhoA and ROCK. A rise in Rho-ROCK activity and F-actin promotes morphological changes in the endothelium on TNF exposure[18]. Thus, increased CRP or TNF levels may contribute to the elevated *ROCK1* gene expression in the present study.

The main limitation of this study is related to the small sample size in polymorphism studies. This could be the source of potential bias or imprecision. Therefore, further large population studies are needed to demonstrate the contribution of ROCK gene polymorphisms.

CONCLUSIONS

This study identified that AA has a genetic background and is influenced by the *ROCK* gene. We suggest that AA can be influenced by gene expressions in a gender-specific manner. These findings can improve understanding of the genetic factors influencing AA, which may also result in more accurate diagnosis, more targeted therapy, and eventually personalized treatment of AA.

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AUTHORS' CONTRIBUTIONS

NEG: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review & editing**. EB:** Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review & editing. **SD:** Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – review & editing. **EFŞ:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **RT:** Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **NG:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **ATD:** Formal analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship between measures of thoracic diameter and cardiopulmonary resuscitation-induced thoracoabdominal injury

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SUMMARY

OBJECTIVE: We investigated the relationship between thoracic diameters and chest compression-related thoracoabdominal injury in patients with non-traumatic out-of-hospital cardiac arrest who had a return of spontaneous circulation after cardiopulmonary resuscitation.

METHODS: A total of 63 consecutive adult non-traumatic out-of-hospital cardiac arrest patients were enrolled in this prospective study. Computed tomography was performed on each patient and the anteroposterior diameter, skin-to-skin anteroposterior diameter, and transverse diameter of the chest were measured. Patients were divided into two groups based on the presence or absence of cardiopulmonary resuscitation-related thoracoabdominal injury. Age, sex, and duration of cardiopulmonary resuscitation, anteroposterior diameter, skin-to-skin anteroposterior diameter, and transverse diameter were compared between the groups. The primary outcome was the relationship between thoracic diameters and cardiopulmonary resuscitation-induced thoracoabdominal injuries.

RESULTS: Thoracoabdominal injuries were detected in 46% (n=29) of the patients and consisted of rib fractures in 22 (34.9%) patients, pulmonary contusion in 7 (11.1%), and sternal fracture in 3 (4.8%) patients. There were no significant differences in cardiopulmonary resuscitation duration between patients with and without thoracoabdominal injuries (p=0.539). Similarly, there were no significant differences in anteroposterior diameter, skin-to-skin anteroposterior diameter, or transverse diameter between patient groups (p=0.978, p=0.730, and p=0.146, respectively) or between patients who died within the first 28 days and those who survived for longer than 28 days (p=0.488, p=0.878, and p=0.853, respectively).

CONCLUSION: The iatrogenic thoracoabdominal injuries caused by cardiopulmonary resuscitation performed according to the cardiopulmonary resuscitation guidelines were independent of thoracic diameters. Therefore, the cardiac compression depth of 5–6 cm recommended by the current cardiopulmonary resuscitation guidelines is reliable for patients with different thoracic diameters.

KEYWORDS: Cardiopulmonary resuscitation. Out-of-hospital cardiac arrest.

INTRODUCTION

Cardiopulmonary resuscitation (CPR) involves a set of basic and advanced vital support procedures conducted to recover spontaneous pulse, respiration, and cardiac function in patients with cardiac arrest (CA)[1]. It is carried out based on the international guidelines, and the 2021 CPR Guidelines of the European Resuscitation Council (ERC) and 2020 American Heart Association (AHA) Guidelines for CPR and Emergency Cardiovascular Care emphasize that the depth of chest compression should be 5–6 cm[2-4]. However, chest diameter varies between countries, and there is some debate whether chest compression depth should be adjusted for thoracic diameter in patients undergoing CPR[5,6]. A reasonable hypothesis is that CPR-related injuries may be more common in patients with smaller thoracic diameters.

We investigated the relationships between measures of thoracic diameter and chest compression-related thoracoabdominal injury in patients with non-traumatic out-of-hospital cardiac arrest (OHCA) who had a return of spontaneous circulation (ROSC) after CPR according to the recommendations of the current AHA and ERC guidelines for CPR.

METHODS

Study Design

This prospective, single-centre, observational study was conducted in accordance with the 1989 Declaration of Helsinki and was approved by the Institutional Review Board of Institutional Review Board of Haseki Research and Training Hospital, Istanbul, Turkey (approval no. 03-2021). From March 2021 through September 2021, 63 consecutive adult patients (aged 20–95 years) who were brought by Emergency Medical Services (EMS) ambulance to our tertiary care hospital due to non-traumatic OHCA were enrolled.

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Patients were provided Advanced Cardiac Life Support by the code blue team upon presenting to the hospital, according to the current 2021 ERC and 2020 AHA Guidelines for CPR[2-4]. After the initiation of vital function monitoring, written informed consent was obtained from the authorized representative of each patient for participation in the study. The primary outcome was the relationship between thoracic diameters and CPR-induced thoracoabdominal injuries. Therefore, thoracoabdominal computed tomography (CT) was performed to detect CPR-related thoracoabdominal injury.

The CT scans in axial view with a slice thickness of 4 mm (Ingenuity Elite 128 Slice Philips CT Scan Machine; BMEC Imaging Pvt. Ltd., Chennai, Tamil Nadu, India) were analysed by a senior radiology physician. Thoracic diameter, including anteroposterior diameter (APD), skin-to-skin anteroposterior diameter (SSAPD), and transverse diameter (TD) values, was measured at the midpoint of the lower half of the sternum on axial view images. APD was measured from the anterior side of the vertebral corpus to the posterior side of the sternum. SSAPD was measured as the anteroposterior distance from the skin on the anterior chest wall to the skin on the back in transverse sections on the midsagittal line. TD was measured from the inner surface of the rib to the inner surface of the counter rib (Figure 1).

Data Collection

All cases meeting the eligibility criteria during the study period were included to reduce selection bias. Accordingly, we enrolled a total of 87 patients who were admitted to the emergency department (ED) due to non-traumatic OHCA. Patients with a history of cardiothoracic surgery (N=8), placement of a central venous catheter in the subclavian and/or internal jugular veins (N=11), and patients resuscitated with an automatic chest compression device (N=5) were excluded from the study to clearly detect iatrogenic injuries associated with CPR. Therefore, possible pneumothorax and other complications related to these procedures were excluded. Finally, we included 63 patients who were brought to ED due to non-traumatic OHCA.

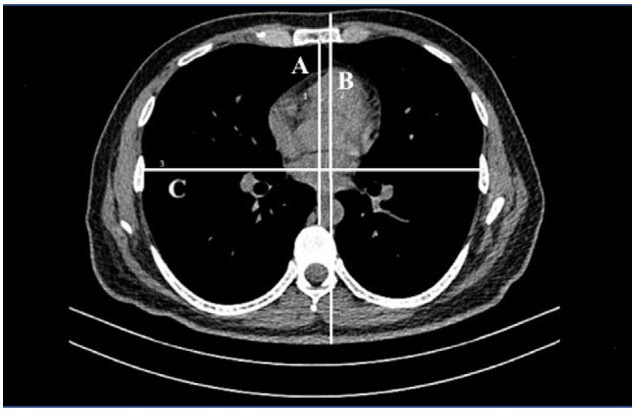

Figure 1. A diagram of the thorax showing each of the thoracic diameter measurements. (A) Anteroposterior diameter (APD). (B) Skin-to-skin anteroposterior diameter (SSAPD). (C) Transverse diameter (TD).

We assessed patients' demographic information (age and sex), arrest rhythm on admission, comorbidities (hypertension [HT], diabetes mellitus [DM], chronic renal failure [CRF], chronic heart failure [CHF], chronic obstructive pulmonary disease [COPD], and coronary artery disease [CAD]), thoracic diameters (including APD, SSAPD, and TD values), duration of CPR, and clinical outcome (death within the first 28 days and survival for longer than 28 days). Patients were divided into two groups based on whether they did or did not have CPR-related thoracoabdominal injury. In addition, APD, SSAPD, and TD were compared between two subgroups stratified as patients who died within the first 28 days and patients who survived for longer than 28 days.

Data analysis

The required sample size was calculated by power analysis prior to data collection based on information from previous studies[5,7]. It was estimated that at least 63 participants would be required with a power of 95% and an alpha error of 5%. All analyses were conducted using SPSS (version 15.0 for Windows; IBM Corp., Armonk, NY, USA). Numerical data (i.e., APD, SSAPD, TD values and duration of CPR) are expressed as the mean (standard deviation [SD]), minimum, maximum, median, and interquartile range (IQR) values; categorical variables (sex and age) are presented as number (N) and percentages (%). Intragroup (patients with vs. without CPR-related thoracoabdominal injury) and subgroup (patients who died within the first 28 days vs. those who survived longer than 28 days) comparisons were conducted using Pearson's chi-square test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. In all analyses, $p<0.05$ was taken to indicate statistical significance.

RESULTS

The study population consisted of 41 male (65.1%) and 22 female (34.9%) patients, with a mean age of 63.8±16.20 (median=64) years. The mean duration of CPR was 19.00±14.30 (median=15) min. The heart rhythms associated with OHCA were asystole in 47 (74.6%) patients, pulseless electrical activity in 5 (7.9%), ventricular fibrillation in 10 (15.9%), and pulseless ventricular tachycardia in 1 (1.6%) patient. Of the patients with ROSC, 48 (76.2%) died within 28 days and 15 (23.8%) survived for

longer than 28 days after ROSC. The determined CA causes were acute coronary syndrome in 12 (19%) patients, stroke in 5 (7.9%), COPD in 3 (4.8%), CHF in 4 (6.4%), and malignancy in 6 (9.5%) patients. In 33 (52.4%) patients, the CA cause was not determined.

Of the whole patient population, 87.3% (55) had one or more comorbidities: HT (39.7%, 25), DM (31.7%, 20), CAD (30.2%, 19), CHF (15.9%, 10), COPD (22.2%, 14), and CRF (9.5%, 6).

The mean chest APD, SSAPD, and TD values of the patients with ROSC were 126.40±18.80, 234.30±26.90, and 245.70±20.10 mm, respectively.

The rate of thoracoabdominal injury associated with CPR was 46% (29): chest wall injury in 25 (39.7%) patients, lung parenchymal injury in 8 (12.7%), and abdominal injury in 3 (4.8%) patients. The most common thoracoabdominal injuries were rib fractures in 22 (34.9%) patients, followed by pulmonary contusion in 7 (11.1%), sternal fracture in 3 (4.8%), costochondral separation in 3 (4.8%), pneumothorax in 2 (3.2%), pneumomediastinum in 2 (3.2%), liver pericapsular haematoma in 2 (3.2%), and pneumoperitoneum in 1 (1.6%) patient.

There were no significant differences in median age between patients with and without CPR-related thoracoabdominal injury (p=0.424). Similarly, there were no significant differences in sex distribution (p=0.550) or in CPR duration between the patient groups (p=0.539). In addition, there were no significant differences in APD, SSAPD, or TD values between these two groups (p=0.146) (Table 1).

There was no significant difference in median age or sex distribution between the patients with ROSC who died within the first 28 days and those who survived for longer than 28 days (p=0.488 and p=0.636, respectively). In addition, there was no significant difference between these two groups in terms of frequency of thoracoabdominal injury (p=0.955). However, the median duration of CPR was shorter in patients who survived for longer than 28 days (p=0.006). Finally, there were no significant differences in thoracic diameters between these two groups (p=0.488, p=0.878, and p=0.853, respectively) (Table 2).

DISCUSSION

This study investigated the relationships between measures of thoracic diameter and chest compression-related thoracoabdominal injury in patients with non-traumatic OHCA who had a return of ROSC after CPR in accordance with the recommendations of the current AHA and ERC guidelines for CPR.

It is important to resuscitate patients in accordance with the most current resuscitation guidelines, which are constantly updated and require theoretical and practical training[2-4,8]. The 2020 AHA and 2021 ERC guidelines limit the depth of each compression in CPR to 5–6 cm[2,4]. However, recent studies suggested that CPR may be associated with severe thoracoabdominal injuries[9-11]. Thoracic injuries due to chest compressions cause significant mortality and morbidity[6,7,12]. Previous studies have indicated that CPR-related injuries are more common in older and female patients[13]. In a study including 223

Table 1. Comparison of age, sex, cardiopulmonary resuscitation duration, and thoracic diameters including anteroposterior diameter, skin-to-skin anteroposterior diameter and transverse diameter values in patients with and without cardiopulmonary resuscitation-related thoracoabdominal injury.

Characteristic	Thoracoabdominal injury		p*
	Presence	Absence	
	Median (IQR 25–75)	Median (IQR 25–75)	
Age (year)	63.50 (52–75.25)	65 (58–78)	0.424
CPR duration (min)	15 (8.75–20)	15 (10–30)	0.539
Thoracic diameter (mm)			
APD	126 (111.50–143.75)	127 (118–137)	0.978
SSAPD	235 (212.00–258.50)	238 (213.5–247)	0.730
TD	247 (224.75–255.50)	252 (233.50–264.50)	0.146
Characteristic	**n (%)**	**n (%)**	
Sex			
Male	21 (61.8)	20 (69.0)	0.550
Female	13 (38.2)	9 (31.0)	

CPR: Cardiopulmonary resuscitation; APD: anteroposterior diameter; SSAPD: skin-to-skin anteroposterior diameter; TD: transverse diameter. Data are expressed as number (n); percentage (%); median, and interquartile range (IQR). *Intragroup comparisons (patients with vs. without Cardiopulmonary resuscitation-related thoracoabdominal injury) were conducted by Pearson's χ^2 test or Mann-Whitney U test, as appropriate.

Table 2. Comparison of age, sex, cardiopulmonary resuscitation duration, thoracoabdominal injuries, and measures of thoracic diameter, including anteroposterior diameter, skin-to-skin anteroposterior diameter and transverse diameter, between patients who died within the first 28 days and those who survived for longer than 28 days.

Characteristic	Clinical outcome		p*
	Died within 28 days	Survived >28 days	
	Median (IQR 25–75)	Median (IQR 25–75)	
Age (year)	63.5 (55–79)	65 (53–68)	0.488
CPR duration (min)	20 (10–30)	10 (5–15)	0.006
Thoracic diameter (mm)			
APD	127 (116.25–140.50)	124 (103–139)	0.488
SSAPD	238.50 (210–254.25)	236 (217–247)	0.878
TD	249.50 (229.75–261)	247 (225–261)	0.853
Characteristic	**n (%)**	**n (%)**	
Sex			
Male	32 (66.7)	9 (60.0)	0.636
Female	16 (33.3)	6 (40.0)	
Thoracoabdominal injury	22 (45.8)	7 (46.7)	0.955

CPR: Cardiopulmonary resuscitation; APD: anteroposterior diameter; SSAPD: skin-to-skin anteroposterior diameter; TD: transverse diameter. Data are expressed as number (n), percentage (%), median, and interquartile range (IQR). *Subgroup comparisons (patients who died within the first 28 days vs. those who survived for longer than 28 days; patients with vs. without CPR-related thoracoabdominal injury) were conducted by Pearson's χ^2 test or Mann-Whitney U test, as appropriate.

patients, Kashiwagi et al.[13] reported no correlation between CPR-related injury and demographic data, such as age or sex. Similar to Kashiwagi et al.[13], we observed no significant differences in age or sex distribution between groups with and without CPR-related thoracoabdominal injury.

In a study regarding CPR-related injuries, Oya et al.[5] reported a significantly smaller mean APD of the chest in patients with than without pneumothorax and rib fractures. They reported an average chest SSAPD of 253 mm in men and 235 mm in women of European descent and 175 mm in patients of both sexes of Japanese descent. As the SSAPD for individuals of Japanese descent is smaller by more than 50 mm compared to those of European descent, the current international guidelines may allow fatal adverse events associated with CPR. The mean SSAPD value in our study population was similar to individuals of European descent. However, there were no significant differences in median chest APD, SSAPD, or TD between patients with and without CPR-related thoracoabdominal injury. Furthermore, there were no significant differences in median chest APD, SSAPD, or TD between patients who died within the first 28 days and those who survived for longer than 28 days. Conversely, in a study with 246 non-traumatic in-hospital CA patients who received CPR, Hokenek et al.[14] reported that lower thoracic diameters were associated with an increased prevalence of CPR-related thoracic injury.

The most commonly detected CPR-related injuries are chest wall injuries and pulmonary contusions[6,9]. Rib fractures are the most common CPR-related chest wall injuries[9,15]. The rate of iatrogenic chest trauma was reported to be significantly higher in patients following CPR performed per the 2010 AHA compared to the 2005 AHA[5,7]. In a study of 82 OHCA patients, Choi et al.[7] reported that the most common iatrogenic injuries after CPR were skeletal chest wall injuries followed by lung contusions. Krischer et al.[12] reported lung parenchymal injuries at a rate of 13% in an autopsy series among patients with non-traumatic CA. Similarly, the most common iatrogenic complications after CPR in non-traumatic OHCA patients in the present study were chest wall injuries and rib fractures, and 12.7% of patients had lung parenchymal injuries.

Abdominal organ injuries associated with CPR are less common than thoracic wall and lung parenchymal injuries[16]. Spoormans et al.[17] reported gastric perforation after CPR in a series of 67 cases, which they associated with conditions such as misplacement of the hand applying CPR and esophageal intubation. In addition, liver injury was detected at rates of 0.6–3% in their studies, which was attributed to trauma to the left lobe of the liver by the xiphoid process of the sternum. In addition, sporadic cases of pneumoperitoneum related to CPR have been reported as a rare complication of gastric rupture[9,18,19].

Consistent with the literature[16-18], our cases showed an abdominal injury rate of 4.8%, including liver pericapsular haematoma and pneumoperitoneum in 2 and 1 of 29 patients with thoracoabdominal injury associated with CPR.

This study had some limitations; the most important of which were the small sample size, single-centre design, and lack of measurement of chest compression depth using an accelerometer. In addition, we did not evaluate the possible association between body mass index and CPR-related thoracoabdominal injury. Finally, since our study evaluated OHCA cases who underwent CPR, we could not obtain thorax CT images of the patients before CPR. However, we excluded patients with a history of cardiothoracic surgery, placement of a central venous catheter in the subclavian and/or internal jugular veins, and patients resuscitated with an automatic chest compression device to clearly detect iatrogenic injuries associated with CPR. Thus, we assumed that the existing lung pathologies are related to CPR.

CONCLUSIONS

The most commonly detected CPR-related thoracoabdominal injuries were chest wall injuries and pulmonary contusions. The iatrogenic thoracoabdominal injuries associated with CPR performed according to the current guidelines were independent of thoracic diameters. Therefore, a cardiac compression depth of 5–6 cm for CPR is reliable for individuals of different thoracic diameters. In addition, neither CPR-related thoracoabdominal injury nor thoracic diameter is useful for predicting the short-term prognosis of patients with ROSC.

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AUTHORS' CONTRIBUTIONS

TBÜ: Conceptualization, Formal Analysis, Writing – original draft. **OS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **AA:** Data curation, Formal Analysis, Writing – original draft. **SC:** Formal Analysis. **ID:** Formal Analysis.

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ORIGINAL ARTICLE

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Comparison of clinical characteristics of wild-type SARS-CoV-2 and Omicron

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SUMMARY

OBJECTIVE: This study aimed to investigate the effect of mutations by comparing wild-type SARS-CoV-2 and Omicron regarding clinical features in patients with COVID-19. It also aimed to assess whether SARS-CoV-2 cycle threshold value could predict COVID-19 severity.

METHODS: A total of 960 wild-type and 411 Omicron variant patients with positive results in SARS-CoV-2 real-time reverse transcriptase polymerase chain reaction test from oropharyngeal and/or nasopharyngeal samples during their hospital admissions were included in this retrospective study. The reference symptoms of the patients were obtained from the hospital database. The correlation between chest computed tomography findings and the "cycle threshold" of patients with wild-type SARS-CoV-2 was assessed.

RESULTS: Cough, fever, shortness of breath, loss of taste and smell, and diarrhea were found to be statistically significantly higher (p=0.001; 0.001; 0.001; 0.001; and 0.006; respectively) in the wild-type cohort, while in the Omicron cohort, sore throat and headache were found to be statistically significantly higher (p=0.001 and 0.003, respectively). An inverse relationship was found between chest computed tomography findings and viral load.

CONCLUSION: This study revealed that the Omicron variant tended to infect predominantly the upper respiratory tract and showed decreased lung infectivity, and the disease progressed with a milder clinical course. Therefore, the study showed that the tropism of the virus was changed and the viral phenotype was affected. It was also found that SARS-CoV-2 viral load did not predict COVID-19 severity in patients with wild-type SARS-CoV-2.

KEYWORDS: COVID-19. SARS-CoV-2 variants. Coronavirus. Viral load.

INTRODUCTION

The massive spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enabled the rapid evolution of the virus, resulting in the emergence of numerous variants. The World Health Organization (WHO) classified these as "variant of concern" (VOC) that gained increased contagiousness, worsening of the clinical features, and affecting diagnosis and vaccine performance. Wild-type SARS-CoV-2, which started the pandemic, was replaced by variants, and five VOCs, Alpha, Beta, Gamma, Delta, and finally Omicron, have been identified so far[1]. Omicron is the most highly mutated one with 50 mutations accumulating in its genome. Studies comparing wild-type SARS-CoV-2 and other variants have shown that these mutations increase the contagiousness and infectivity of Omicron and facilitate its escape from immunity[2].

Timely and accurate diagnosis of COVID-19 is critical to the successful management of the pandemic. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) is the gold-standard test for diagnosing SARS-CoV-2. The rRT-PCR "cycle threshold" (Ct) – a semi-quantitative measure of viral load – is the number of cycles required for the fluorescent signal, resulting from amplification of the target gene, to cross the threshold. Because of the length of time and lack of sensitivity as well as false-negative results for rRT-PCR tests, chest computed tomography (CT) is recommended for the diagnosis of viral pneumonia[3].

COVID-19 can progress with different clinical features, ranging from asymptomatic or mild clinical course to severe respiratory failure. Since variants of the virus have emerged, the virus-host relationship may also vary depending on these[2].

This retrospective study aimed to investigate the effect of mutations by comparing wild-type SARS-CoV-2 and Omicron regarding clinical features in patients with COVID-19 who applied to Ankara City Hospital (Türkiye). The study also

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evaluated the relationship between SARS-CoV-2 Ct values detected by rRT-PCR and chest CT findings of the patients in the wild-type cohort to assess whether the SARS-CoV-2 Ct value could predict COVID-19 severity.

METHODS

This retrospective study included 1371 patients with positive results in the SARS-CoV-2 rRT-PCR test from oropharyngeal and/or nasopharyngeal samples (OP/NP) during their hospital admissions, 960 with wild-type SARS-CoV-2 between April 1, 2020, and June 30, 2020, and 411 with Omicron between March 1, 2022, and March 31, 2022. The patients' demographic features and symptoms of admission to the emergency department were obtained from the hospital database.

At the beginning of the pandemic, a chest CT scan was performed for patients with suspected wild-type SARS-CoV-2 in our hospital. However, this approach was abandoned in patients infected with Omicron, and a chest CT scan was performed only in the elderly and in patients with low oxygen saturation and comorbidities. Patients aged ≥18 years diagnosed with COVID-19 by rRT-PCR and having a chest CT scan time interval of less than 72 h after obtaining OP/NP swab were included in this study. A correlation analysis was performed on the rRT-PCR Ct values and chest CT findings of a total of 960 patients with wild-type SARS-CoV-2 diagnosed by rRT-PCR and simultaneous chest CT.

Detection of SARS-CoV-2

OP/NP samples from patients were placed in transfer tubes containing vNAT (Viral Nucleic Acid buffer/various manufacturers) and sent to the Molecular Microbiology Laboratory. Detection of SARS-CoV-2 in samples was performed by the rRT-PCR method with two commercial kits according to the manufacturer's instructions: the BioSpeedy COVID-19 RT-qPCR Detection Kit (Bioeksen, Türkiye) targeting the *RdRp* (RNA-dependent RNA polymerase) gene was used for the detection of the wild-type, and the SARS-CoV-2 Plus Omicron Variant Detection Kit (Gensutek, Türkiye) targeting SARS-CoV-2 specific "Orf1ab" and "N" genes as well as Omicron-specific genome regions was used for the detection of the Omicron variant. Both kits target the human RNaseP (Ribonuclease P) gene as an internal control to evaluate sample-based inhibition control and kit reagent control. The PCR reaction was performed on the Rotor-Gene Q (Qiagen, Germany) device. Ct values <40 in the detection of wild-type SARS-CoV-2 and ≤38 Ct values in the detection of Omicron were considered positive.

Imaging technique and imaging interpretation

Thin-section, noncontrast, chest CT (Revolution, GE Medical System, Germany) examinations were performed. The tomography protocol was as follows: 100 kV, 110–400 mA, and a slice thickness of 2.5 mm in all cases. Images with a slice thickness of 0.625 mm were obtained by reconstruction. The sections were evaluated by two specialist radiologists.

According to the Radiological Society of North America (RSNA) Expert Consensus Statement, parenchymal pneumonic involvement was divided into four groups:

1. typical appearance,
2. indeterminate appearance,
3. atypical appearance, and
4. negative for pneumonia[4].

In this study, disease severity is evaluated according to the RSNA classification.

Statistical analysis

The statistical analysis was carried out using the SPSS software version 23.0 (IBM Corp.). The normality analysis of numerical data was evaluated by histogram and Kolmogorov-Smirnov tests. The difference between the groups was calculated by chi-square, Fisher's exact test, Student's t-test, and Mann-Whitney U test as appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

A total of 1371 SARS-CoV-2 patients were evaluated in this study, involving 960 wild-type and 411 Omicron variants. The percentage distributions of male and female patients in the wild-type and Omicron groups were 0.92 and 1.4, respectively (p=0.001). The median age was 40 [interquartile range (IQR), 24] and 38 [IQR, 27] in the wild-type and Omicron groups, respectively. There was no statistically significant difference between the two groups regarding median age (Mann-Whitney U test, p=0.292).

When admission symptoms were compared, cough (52.9%), fever (34.6%), shortness of breath (21.1%), loss of taste and smell (5.8%), and diarrhea (4.5%) were found to be statistically significantly higher in the wild-type group (p=0.001; 0.001; 0.001; 0.001; and 0.006; respectively). In the Omicron group, symptoms of sore throat (17.5%) and headache (7.6%) were found to be statistically significantly higher (p=0001 and 0.003, respectively). The clinical symptoms detected in the wild-type and Omicron groups are given in Table 1.

A chest CT was performed in all wild-type infected patients. The distributions of chest CT findings were found as follows: typical appearance 40.8%, negative for pneumonia 38.6%, indeterminate appearance 17.1%, and atypical appearance 3.4%. The comparison of chest CT findings classified according to RSNA and rRT-PCR Ct values of the wild-type infected patients is given in Table 2.

Chest CT was performed in only 3.9% (411/16) of Omicron-infected patients. The chest CT results were found as follows in the Omicron cohort: typical appearance 1.7%, atypical appearance 0.7%, negative for pneumonia 1%, and indeterminate appearance 0.5%.

When chest CT findings and Ct values were compared, the mean Ct value of the "negative for pneumonia" group was statistically significantly lower than the mean Ct value of those with "typical appearance" group (p=0.001). The mean Ct value of those with "indeterminate appearance" was statistically significantly lower than the mean Ct value of those with "typical appearance" (p=0.043).

DISCUSSION

COVID-19 has a very broad clinical spectrum, ranging from mild to severe and critical course. In this study, the most common symptoms found in the wild-type cohort were cough, fatigue/muscle-joint pain, fever, and shortness of breath as consistent with the literature[5-8]. The symptoms of sore throat, fatigue/muscle-joint pain, cough, and fever were detected in the Omicron cohort, as consistent with the literature[9,10].

Omicron carries a large number (32) of mutation on the spike (S) protein, which is the main antigenic target of antibodies. The focus of mutations has been the receptor-binding domain (RBD) of the S protein due to its potential impact on infectivity and resistance to antibodies. This is because the RBD located on the S protein facilitates the binding between the S protein and the host angiotensin-converting enzyme 2 (ACE2). The S-ACE2 binding helps SARS-CoV-2 enter the host cell and initiate the infection process. Vaccine or natural infection-induced antibodies that bind strongly to RBD neutralize the virus directly. Therefore, this mutation in the RBD has led to new inquiries about the efficacy of current vaccines and the reinfection potential of the virus, thereby increasing the global panic[11].

Results of early clinical studies show that the rapidly spreading Omicron variant is less dangerous than previous variants. A study with cell culture reported that compared to Delta, the Omicron variant may have lower replication capacity in the lungs[12].

In a study, three-dimensional modeling of respiratory organs was used to demonstrate the entry of SARS-CoV-2[13], and it was shown that Omicron exhibited less severe infection than Delta and Wuhan/D614G strain. Therefore, less access to the lower respiratory tract may mean milder symptoms when compared to other variants[13,14].

Syrian golden hamsters suffering from weight loss and pneumonia following COVID-19 infection provide a robust model to study SARS-CoV-2 disease in humans. In a study, hamsters were infected with WA1/2020, Alpha, Beta, Delta, and Omicron variants, and weight loss occurred in variants other than Omicron[15]. In contrast to WA1/2020 infection, Omicron-infected hamsters had higher viral loads in the nose and lower viral loads in the lungs[14].

Table 1. Distribution of clinical symptoms in wild-type and Omicron groups.

Symptoms	Wild-type (n=960)	Omicron (n=411)	p
Cough (%)	508 (52.9)	136 (33.1)	0.001
Fatigue/muscle-joint pain (%)	376 (39.2)	175 (42.6)	0.238
Fever (%)	332 (34.6)	84 (20.4)	0.001
Shortness of breath (%)	203 (21.1)	27 (6.6)	0.001
Sore throat (%)	168 (17.5)	186 (45.3)	0.001
Headache (%)	73 (7.6)	52 (12.7)	0.003
Loss of taste and smell (%)	56 (5.8)	4 (1)	0.001
Diarrhea (%)	43 (4.5)	6 (1.5)	0.006
Nausea-vomiting (%)	38 (4)	9 (2.2)	0.099

Table 2. Comparison of rRT-PCR cycle threshold values of chest computed tomography findings in wild-type infected patients.

Chest CT imaging findings	Typical appearance	Negative for pneumonia	Indeterminate appearance	Atypical appearance
rRT-PCR Ct value mean (SD)	26.5 (5.3)	23.9 (5.9)	25.4 (5.5)	28.3 (5.4)
Typical appearance	1	p=0.001	p=0.043	p=0.060
Negative for pneumonia		1	p=0.004	p=0.001
Indeterminate appearance			1	p=0.008
Atypical appearance				1

Ct: cycle threshold; CT: chest computed tomography.

When comparing admission symptoms, this study found that cough, fever, and shortness of breath, which indicate wild-type lower respiratory tract infection, were replaced by sore throat and headache, which indicate upper respiratory tract infection in the Omicron cohort. This result showed that Omicron tended to infect the upper respiratory tract, as discussed in previous studies[13-15].

Suzuki et al., investigating the effect of mutations in the S protein on the viral phenotype, showed that Omicron in the hamster model caused lower infectivity and less pathogenicity in the lungs compared to Delta and wild-type SARS-CoV-2[16]. In our study, taste-smell loss and diarrhea were found to be statistically significantly lower in the Omicron cohort, suggesting that viremic activity is reduced in organs other than the respiratory system.

Due to the high contagiousness of the COVID-19, rapid diagnosis and isolation are critical for the struggle against the pandemic. It has been suggested that since rRT-PCR test results in several hours, it may be insufficient for rapid triage and that chest CT can be an alternative to the rRT-PCR test in the diagnosis of pneumonia[4]. In this study, "typical appearance" tomography findings supporting COVID-19 pneumonia radiologically were detected in 40.8% of wild-type and 1.7% of Omicron variant cases. These results support that the lung infectivity of Omicron is decreased and has a milder clinical course. In addition, the patients with SARS-CoV-2 positive on rRT-PCR and "negative for pneumonia" in chest CT were as high as 38.6% in the wild-type cohort. According to this result, only the chest CT scan for COVID-19 may cause misdiagnosis.

Liu et al. reported that viral load is crucial in determining disease severity[17]. Our study found an inverse relation – viral load being higher in the group "negative for pneumonia" – between chest CT findings and Ct values of wild-type infected patients, as consistent with previous studies[3]. The viral load of the group with pneumonia was low while the viral load of the group without pneumonia was found to be high, which may have been caused by the use of upper respiratory tract samples in diagnosis. Therefore, we evaluated that it would be more appropriate to detect the viral load in lower respiratory tract samples in patients with pneumonia and to investigate the relationship between pneumonia severity and Ct values.

This study has some limitations. In previous studies, it has been reported that vaccinated individuals have a milder disease[18]. In our study, since data regarding vaccination status was not available, the milder clinical course may have been caused not only by mutations but also by immunity. The data provided by the Republic of Türkiye Ministry of Health show that the first dose of vaccination rate is as high as 93.20% and the second dose rate is as high as 85.51% (as of June 15, 2022)[19]. Although these rates are not directly applicable to this study's patient population, they can still be generalized for our results too.

CONCLUSIONS

This study compared the clinical features of wild-type SARS-CoV-2 and the Omicron variants to investigate the clinical effect of mutations and revealed that Omicron tended to infect predominantly the upper respiratory tract, showed decreased lung infectivity, and the disease progressed with a milder clinical course. As a result, this study showed that the tropism of the virus was changed and the viral phenotype was affected. It was also found that SARS-CoV-2 viral load did not predict COVID-19 severity in patients with wild-type SARS-CoV-2.

AUTHORS' CONTRIBUTIONS

FK: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **SA:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **AG:** Formal Analysis. **AEK:** Data curation. **FAEÖ:** Formal Analysis. **YTT:** Data curation. **İOB:** Data curation. **PG:** Data curation. **RSÖ:** Formal Analysis. **BD:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison of the outcomes of flexible ureteroscopy and mini-percutaneous nephrolithotomy for the treatment of kidney stones: a matched-pair analysis

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SUMMARY

OBJECTIVE: Mini-percutaneous nephrolithotomy is a recent advancement in the field of kidney stone treatment; however, its role has not been completely established. We aimed to compare the outcomes of initial Mini-percutaneous nephrolithotomy and flexible ureteroscopy.

METHODS: A retrospective review of consecutive mini-percutaneous procedures was performed. Inclusion criteria were as follows: all percutaneous nephrolithotomy procedures performed with an access sheath up to 24Fr, kidney stone burdens up to 1550 mm^3; and the presence of postoperative computed tomography (for control). The data collected for Mini-percutaneous nephrolithotomy procedures were paired 1:2 with patients treated with flexible ureteroscopy for stones between 100 and 1550 mm^3, and with postoperative computed tomography for control. A 14Fr Mini-percutaneous nephrolithotomy set was used. The stone-free rate was defined as the absence of fragments on the control computed tomography, whereas success was limited to 2-mm residual fragments. Statistical analysis was performed using SPSS version 19.

RESULTS: A total of 63 patients met the inclusion criteria (42 with flexible ureteroscopy and 21 with mini-percutaneous nephrolithotomy). Demographic data were comparable. The stone-free rate and success were similar between the groups (76.2 vs. 66.7%, p=0.42 and 90.5 vs. 85.7%, p=0.57). The complication rate was also similar (26.1 vs. 9.6%, p=0.188), but Mini-percutaneous nephrolithotomy had longer hospitalization and fluoroscopy time (p=0.001 in both).

CONCLUSIONS: Our initial study of Mini-percutaneous nephrolithotomy showed that it is a promising procedure, with outcomes similar to flexible ureteroscopy, but with higher inpatient numbers and fluoroscopy times. A larger study population size and better equipment may improve the outcomes of mini-percutaneous nephrolithotomy.

KEYWORDS: Kidney stone. Percutaneous ultrasonic lithotripsy. Nephrolithotomy, percutaneous. Ureteroscopy. Lithotripsy, laser.

INTRODUCTION

Percutaneous nephrolithotomy (PNL) is the gold-standard treatment for kidney stones >20 mm, according to the most recent guidelines. Smaller stones may be treated by external shockwave lithotripsy (ESWL) or by flexible ureteroscopy (FURS), despite suboptimal results for stones between 15 and 20 mm[1,2].

Recently, mini-percutaneous nephrolithotomy (MiniPNL), a modification of the regular PNL, has been introduced. Routine kidney stone procedures use catheters between 24 and 30Fr; however, the MiniPNL utilizes a smaller sheath, 22Fr or less, with the aim that a smaller tract could inflict less parenchymal damage and bleeding. Furthermore, the MiniPNL could potentially improve clinical outcomes when compared to FURS owing to its higher capacity for aspiration and fragment removal. However, the reduction in the diameter of the access sheath used for MiniPNL, compared to that of the PNL, could result in a lower success rate. Even though the technique of MiniPNL is similar to PNL, the diameter of access is not standardized, and there are divergences in the literature regarding the size of the access sheath and the instrument to be utilized[3,4].

As MiniPNL is a relatively new procedure, there is no consensus in the literature about the best application for this technique, as currently stones <20 mm are commonly treated with FURS, and stones >20 mm with PNL. Thus, our aim was to evaluate the initial results obtained with this technique and compare them with similar cases that underwent FURS.

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METHODS

After ethics committee approval, we retrospectively reviewed the electronic database of all MiniPNL cases performed between May 2015 and April 2020. In this group, the inclusion criteria were all patients who underwent a MiniPNL (<24Fr) in the supine position. The exclusion criteria were incomplete data and the absence of preoperative and postoperative CT scans. The registry protocol was 34540620.3.0000.0068 at the National Registry site.

Data collected for MiniPNL cases were as follows: demographic: age, sex, BMI, and American Society of Anesthesiologists (ASA) score; and perioperative information: volume, localization, density of the stone, preoperative and postoperative creatinine, surgery and fluoroscopy time, total radiation in mGy, access sheath diameter, site and number of punctures, method of fragmentation, nephrostomy placement, surgeon, hospitalization time, residual stone size, and complications (classified according to the Clavien-Dindo score).

After defining the MiniPNL group, cases were chosen from a prospective database of FURS[5] performed by the same group between August 2016 and August 2017, in a proportion of 2 FURS:1 MiniPNL. The cases were paired according to the size and stone burden. The inclusion criteria for the FURS group were patients with stone burden between 100 and 1550 mm^3 (calculated, the same as in MiniPNL group, using a formula to ellipsoids = (Diameter 1) (Diameter 2) (Diameter 3) π 0.166666667) treated with FURS, with preoperative and postoperative CT scans. Exclusion criteria were stone burden less than 100 mm^3 or larger than 1550 mm^3, kidney malformation, ureteral stenosis, previous ipsilateral surgery (endoscopically or open), large hydronephrosis, double-J stent placement, or any contraindication for endoscopic flexible surgery.

The data collected in the FURS group were age, body mass index (BMI), ASA score, and perioperative information (i.e., volume, localization, stone density, preoperative and postoperative creatinine, surgery and fluoroscopy time, radiation used in mGy, hospitalization time, residual stone size, ureteral lesions, and complications).

Regarding outcomes, stone-free rate (SFR) was defined as the absence of any stone fragment on the control CT, realized up to 3 months, while success rate (SUC) was defined as the presence of stone fragments up to 2 mm in the CT.

The primary objective of this study was to evaluate the MiniPNL outcomes. The secondary objective was to compare MiniPNL with FURS in terms of results and complication rates.

The surgical technique of MiniPNL was described by Lipsky et al.[3] and FURS technique by Danilovic et al.[5]

Statistical analysis was performed using SPSS version 19 (Chicago, IL, USA). Continuous variables were analyzed using the two-sample t-test assuming nonequal variances, and for noncontinuous samples, the Pearson's χ^2 test was used. The data were analyzed using the median (min–max) value due to the small number of cases and a non-normal distribution.

RESULTS

Overall, 21 cases met the criteria for the MiniPNL group and were paired with 42 FURS cases (Figure 1). The demographic data and the stone characteristics are presented in Table 1.

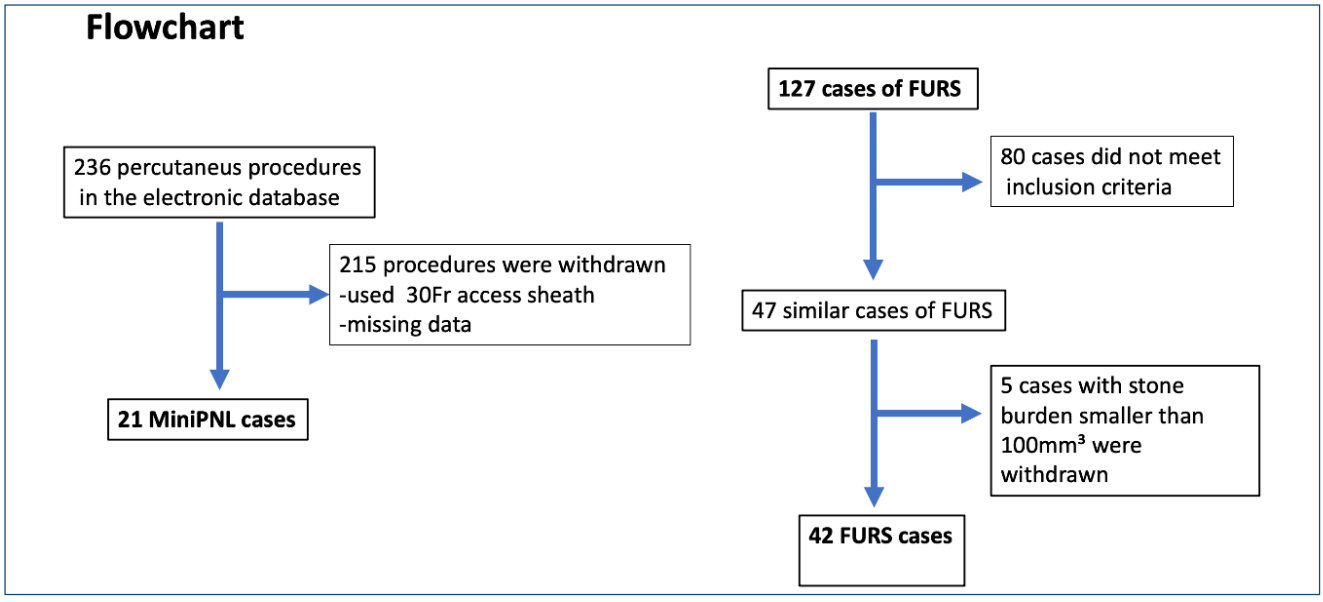

Figure 1. Flowchart of cases selected.

Patients in the FURS group had a significantly higher BMI (28.6 vs. 25.25%, p=0.003). The groups were similar in terms of stone burden and density.

The stone location in the FURS group was 21.4% (9) in the upper pole, 9.5% (4) in the middle pole, 50% (21) in the lower pole, and 19.1% (8) in the renal pelvis; in the MiniPNL group, stone location was 0% in the upper pole, 4.8% (1) in the middle, 28.6% (6) in the lower pole, and 66.6% (14) in the renal pelvis (p=0.001; Table 1).

With regard to the outcomes, the median residual stone diameter on postoperative CT was 0 mm (0–6.3) in the FURS group and 0 mm (0–5) in the MiniPNL group (p=0.682). The SFR was 76.2% (32) in the FURS group and 66.7% (14) in the MiniPNL group (p=0.422), while the SUC was 90.5% (38) in the FURS group and 85.7% (18) in the MiniPNL group (p=0.571). The complication rate was 26.1% for FURS and 9.6% for MiniPNL (p=0.188). No Clavien-Dindo score >3 was observed. Complications are described in Table 2.

The median fluoroscopy time and radiation dosage was, respectively, 0.43 min (0.13–2.6) and 0.83 mGy (0.14–8.16) for the FURS group and 4.12 min (0.46–21.35) and 27.1 mGy (1.61–175) for the MiniPNL group, both with p<0.001. The median inpatient time was 16 h (12–168) in the FURS group and 44 h (18.49–79.33) in the MiniPNL group (p<0.001). Other perioperative parameters are shown in Table 2.

DISCUSSION

The MiniPNL technique was developed with the aim to reduce the complication rates associated with treatment of renal stones. The concept behind this novel procedure is that by using a smaller access sheath, there would be less dilation required and therefore lower morbid than when using the standard PNL size access sheath. Moreover, when compared to FURS, the MiniPNL utilizes a larger working channel compared to ureteral diameter, which facilitates the fragmentation and extraction of renal stone remnants.

Thus far, MiniPNL does not have a definitive indication for its use. Some studies have attempted to clarify the capacities and limitations of this procedure; however, the results are still controversial. Li et al.[6] concluded that the SUCs of MiniPNL and FURS were not significantly different; however, FURS incurs lower costs and hospitalization time than MiniPNL. Other studies have reported similar results[7-9].

In this study, the initial MiniPNL outcomes were demonstrated to be a safe and effective procedure, with good SFR and lower complication rates, which is in agreement with other studies[6,10]. Moreover, we found a comparable SFR and SUC between initial MiniPNL results and FURS performed by an expert, with similar residual stone size, showing that MiniPNL may be as effective as FURS for a similar stone burden, but with the potential for better outcomes.

Table 1. Pre-op data.

	FURS	MiniPNL	p
Number of cases	42	21	
Age – median (min–max)	56 (19–72)	46 (25–83)	0.114
Gender – female % (n)	57.1 (24)	76.1 (16)	0.12
BMI (median)	28.6 (19–45.4)	25.25 (19.9–32.3)	0.003
ASA %			
I	33.3 (14)	30 (6)	0.826
II	52.4 (22)	60 (12)	
III	14.3 (6)	10 (3)	
Largest stone diameter (mm)	11.4 (5.9–17.7)	16 (9–23)	0.001
Stone volume, mm^3 (median)	508 (68–2653)	710 (292–2725)	0.233
Stone density (median)	1007 (260–1409)	1200 (400–1500)	0.028
Laterality (%)	Left (16–38.1) Right (26–61.9)	Left (18–85.7) Right (2–9.5) Transplanted kidney (1–4.8)	0.001
Stone location (%)	Upper pole (9–21.4) Middle pole (4–9.5) Lower pole (21–50) Renal pelvis (8–19.1)	Upper pole (0) Middle pole (1–4.8) Lower pole (6–28.6) Renal pelvis (14–66.6)	0.001

Pre-op: preoperative, FURS: flexible ureteroscopy, Mini-PNL: mini-percutaneus nephrolithotomy, BMI: body mass index, ASA: Store -american society of anaesthesiologists score.

Table 2. Intra- and postoperative information.

	FURS	MiniPNL	p
Pre-op creatinine (mg/dL)	0.73 (0.47–1.56)	0.91 (0.67–1.36)	0.028
Minutes of fluoroscopy (median)	0.3 (0.13–2.6)	4.12 (0.46–21.35)	0.001
Emitted radiation, mGy (median)	0.83 (0.14–8.16)	27.1 (1.61–175)	0.001
Post-op creatinine (mg/dL)	0.83 (0.54–2.2)	0.9 (0.66–1.66)	0.244
Hospitalization time (median)	12 (12–168)	43 (18.49–79.33)	0.001
Residual stone size in mm (median)	0 (0–6.3)	0 (0–5)	0.682
Stone-free rate %	76.2 (32)	66.7 (14)	0.422
Success % (residual <2 mm)	90.5 (38)	85.7 (18)	0.571
Complications, n (%)	11 (26.1)	2 (9.6)	0.188
Clavien-Dindo score %			
0	73.9 (31)	90.4 (19)	
1	7.1 (3)	4.8 (1)	
2	11.9 (5)	0 (0)	
3	7.1 (3)	4.8 (1)	0.158
Surgical complications	2 – Analgesia	1 – Moderate bleeding 1 – Conversion PNL	
	5– Antibiotic		
	2 – PULS 3		
	3 – Placement JJ post-op		
Blood transfusion	0	0	

FURS: flexible ureteroscopy, Mini-PNL: mini-percutaneus nephrolithotomy, mGy: unidade de medida de radiação-miligray, PULS: post ureteroscopy lesion scale, PNL: percutaneus nephrolithotomy.

The hospitalization time and amount of radiation used in fluoroscopy were significantly higher in the MiniPNL group, which was also observed in other studies[6-9]. However, technical improvements such as a higher ultrasound frequency for performing the puncture and more studies with proper patient selection may transform MiniPNL in an outpatient procedure, as well as FURS. This study used a 270-nm laser fiber for MiniPNL, but potentially a larger fiber of 400 or 600 nm could improve success. In addition, the 7Fr mini-nephroscope used for MiniPNL in this study, provided limited visualization when bleeding occurred due to reduced irrigation. Newer 12Fr nephroscopes are available, which could enhance visualization in order to improve outcomes. The best setting is still a matter of debate.

The safety profile is an important aspect in deciding between two similar procedures. A higher rate of infection-associated complications was observed for FURS than MiniPNL (26.1 vs. 9.6%, p=0.188). This may be due to higher intrarenal pressure in FURS than in MiniPNL, which may be more significant in a larger study population and could also be clinically important as most mortality cases in FURS are due to uncontrolled sepsis[11]. Bleeding is a potentially serious complication when the kidney is punctured. During the initial phase, there was an isolated MiniPNL case where significant bleeding that obscured the vision occurred and regular PNL had to be substituted for MiniPNL. These data contradict the concept that endoscopic procedures such as FURS would be less harmful than percutaneous procedures such as MiniPNL. There is no consensus in the literature regarding this issue, since in some studies the complication rates were lower for FURS[12,13], some were not different[6,14],and some were higher[15]. Different MiniPNL sets and surgical techniques may also impact the outcomes.

In both procedures, all patients were considered to be stone-free by the surgeon at the end of the procedure. This was found to be incorrect since 23.8% of FURS cases and 33.3% of MiniPNL cases displayed residual fragments on the control CT scan. This indicates that technical refinement may be required in order to render the patient stone free and suggests that the use of a retrograde flexible ureteroscope at the end of the procedure may improve the outcomes, as was demonstrated by Gökce et al.[16]

Despite this, the current study had several limitations. First, the retrospective study design restricted the potential for comparison, and the limited number of procedures reduced

the precision of the statistical analysis. Second, there was variation in the clinical presentation of certain variables between groups. BMI was higher in FURS cases, which possibly highlights a selection bias toward lower BMI patients being treated with MiniPNL, as they would typically be treated with PNL; in MiniPNL, cases were typically situated in the lower and middle poles and renal pelvis, while in FURS cases, there was no pattern. Conversely, MiniPNL cases had larger kidney stone diameters, which was not reflected in the volume that presented; therefore, the significance of the lower SFR and SUC was potentially subestimated. Larger studies could clarify the best use of MiniPNL in the treatment of renal stones and the rate of surgical complications associated with this procedure.

Regardless of this, the initial MiniPNL outcomes are promising and encouraging. A cost analysis of MiniPNL is recommended, since the use of disposable materials appears to be lower in MiniPNL, when compared to other procedures. Technical improvements may also improve outcomes and inpatient time may be reduced with the implantation of an ambulatory surgery program, similar to FURS protocols. Further studies may help define the role of MiniPNL in kidney stone treatment.

CONCLUSIONS

Our initial series study showed that MiniPNL was safe and effective for treating kidney stones, with similar outcomes to FURS but with a longer inpatient time.

AUTHORS' CONTRIBUTIONS

JECMR: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **FCV:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **AD:** Data curation, Formal analysis, Investigation. **GSM:** Formal analysis. **EM:** Writing – review & editing. **FCMT:** Visualization, Writing – review & editing. **CAB:** Visualization, Writing – review & editing. **WCN:** Visualization, Writing – review & editing.

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