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ADDRESS: Rua São Carlos do Pinhal, 324
Bela Vista – São Paulo
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Humanization helps build a harmonious doctor–patient relationship

Haibo Xu[1], Baogui Wang[2*]

In recent years, based on the frequent occurrence of medical disputes, the doctor–patient relationship has gradually become tense[1], which not only seriously affects the medical industry but also becomes one of the social disharmony factors. A good doctor–patient relationship is a prerequisite for improving the compliance, satisfaction, and loyalty of patients and their families, and at the same time, doctors can reasonably take the best treatment measures to achieve satisfactory treatment results[2]. It shows that doctors have the key to establish a harmonious doctor–patient relationship.

Previously, in solving the doctor–patient relationship, through the formulation of various management policies and regulations, although it also played a partial effect, it is easy to cause over-reliance on the system of the rigid situation. As a matter of fact, the formulation of policies plays more of a constraining effect[3] but cannot change the human mindset. Therefore, from the perspective of doctors' social responsibility, the concept of humanized medical practice is the most effective way to solve the doctor–patient relationship.

Humanization is a comprehensive concept that encompasses several aspects. This study concludes that communication skills, service concepts, and medical ethics are the three most crucial aspects from a doctor's point of view. However, cultivating the concept of humanization should be implemented not only among professional doctors but also from the educational stage of medical students. The specifics are as follows.

1. Communication skills. Effective doctor–patient communication not only guides patients to accurately express their conditions and improves the accuracy of medical treatment but also establishes a trusting relationship between doctors and patients, reduces the degree of panic of patients about their diseases, and reflects more humanistic care.
2. Service consciousness. Doctors should adapt to the patient-centered diagnosis and treatment service consciousness, put the patient's needs and interests on the premise of diagnosis and treatment, give patients more humanistic care, so that patients get full attention and respect, and increase mutual trust.
3. Medical ethics education. Medical ethics is the behavioral principles and norms of those engaged in the medical profession. The education of medical ethics should be cultivated from the stage of medical students in school so that they can acquire the basic professional quality and establish correct values in advance.

AUTHORS' CONTRIBUTIONS

HX: Conceptualization, Formal Analysis, Writing – original draft. **BW:** Conceptualization, Supervision, Writing – review & editing.

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[1]Binzhou University, Department of Physical Education – Binzhou, China.
[2]Binzhou University, Department of Biological and Environmental Engineering – Binzhou, China.
*Corresponding author: wangbaogui168@163.com

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Comment on "Factors influencing neonatal outcomes in twin pregnancies undergoing cesarean section: a cross-sectional study"

Hua Tian[1], Jinjin Liu[1*]

Dear Editor,

We read with great interest the recent study[1] on neonatal outcomes in twin pregnancies undergoing cesarean section. The study's conclusion regarding the factors strongly associated with poor neonatal outcomes in twins delivered by cesarean section is particularly noteworthy. The findings highlight the significant impact of general anesthesia, emergency surgery, early gestational weeks, and birth weight below the 3rd weight percentile on neonatal well-being. Understanding these influential factors in twin pregnancies undergoing cesarean section is crucial for clinicians and healthcare providers. It not only aids in risk assessment but also allows for better-informed decision-making when planning and performing cesarean sections for twin pregnancies. The study's[1] emphasis on these specific factors provides valuable insights that can contribute to improving the overall care and outcomes for both mothers and their neonates in twin pregnancies. However, we believe that the following aspects require further clarification.

First, while the study's[1] conclusion indicates a strong association between poor neonatal outcomes and factors such as general anesthesia, emergency surgery, early gestational weeks, and birth weight below the 3rd weight percentile, it appears that the description provided in Table 3 of this study[1] somehow contradicts these findings. Specifically, Table 3 suggests a correlation between birth weight below the 3rd weight percentile and an increased risk of mortality (OR=5.263, 95%CI [1.934, 14.321], p=0.001), indicating an adverse association with neonatal outcomes. However, in the case of early gestational weeks, the reported relationship with mortality is presented as OR=0.591, 95%CI [0.510, 0.684], p=0.000, which could be easily misinterpreted as a decrease in mortality associated with early gestational weeks. It is plausible that an inadvertent choice of reference group may have led to this discrepancy in the data presentation. It appears that early gestational weeks may have been mistakenly set as the reference group, with non-early gestational weeks as the observation group, resulting in OR=0.591, p=0.000. Similar issues seem to exist in the context of neonatal intensive care unit (NICU) and mechanical ventilation (MV) outcomes. Thus, to avoid any misinterpretation of the study's conclusions, it is imperative to clarify the data description further and ensure that the reported findings align with the study's intended analysis.

Second, we would like to emphasize another critical factor that plays a significant role in neonatal outcomes in twin pregnancies undergoing cesarean section, namely, anemia. Anemia is a multifaceted issue deserving careful consideration in the context of this study. A retrospective study[2] involving 427 participants found that the anemic group had higher rates of low 1-minute Apgar scores (4.4% vs. 1.8%, p=0.028), perinatal death (1.9% vs. 0.2%, p=0.012), and NICU admissions (27.2% vs. 20.2%, p=0.017). It also discovered that after adopting oral iron therapy, the recovered group showed a lower NICU admission rate (13.5% vs. 30.3%, p=0.006), along with higher gestational week and birth weight (β, 0.954 week and β, 171.01 g; respectively). This evidence strongly suggests a correlation between anemia and neonatal outcomes in twin pregnancies and the presence of anemia is evidently associated with adverse neonatal outcomes. Although the current study[1] provides some information about anemia, it does not explore the relationship between anemia and neonatal outcomes in twin pregnancies comprehensively. Given that anemia is a reversible pathological condition, further investigation into its connection with neonatal outcomes in twin pregnancies, along with the implementation of appropriate strategies to rectify anemia, has the potential to significantly improve neonatal outcomes in this context.

AUTHORS' CONTRIBUTIONS

HT: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **JL:** Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

[1]Shiyan Maternal and Child Health Hospital, Department of Maternity – Shiyan, China.
*Corresponding author: liujingjing3414@163.com

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Performance of ChatGPT-4 in answering questions from the Brazilian National Examination for Medical Degree Revalidation

Somsri Wiwanitkit[1], Viroj Wiwanitkit[2*]

Dear Editor,

We follow the topic entitled "Performance of ChatGPT-4 in answering questions from the Brazilian National Examination for Medical Degree Revalidation[1]." The purpose of this study was to evaluate ChatGPT-4's performance in answering the 2022 Brazilian National Examination for Medical Degree Revalidation (Revalida) and its potential as a tool for providing feedback on the examination's quality. Two independent physicians entered all examination questions into ChatGPT-4 and compared their responses to the test solutions, determining whether they were adequate, inadequate, or indeterminate. Consensus was used to resolve disagreements. The study also used statistical analysis to evaluate performance across medical themes and to eliminate queries.

In the Revalida examination, ChatGPT-4 correctly answered 71 (87.7%) of the questions and mistakenly answered 10 (12.3%). The proportion of correct responses did not change statistically significantly across medical themes. However, in nullified questions, the model had a lower accuracy of 71.4%, and there was no statistical difference between the non-nullified and nullified groups. The reliance on the judgments of only two independent physicians to evaluate the accuracy of ChatGPT-4 is a potential weakness of this study. This raises the likelihood of subjective bias in their evaluations. Furthermore, the study does not provide extensive information on the criteria used to categorize the model's replies as adequate, inadequate, or uncertain, which may impair the evaluation's credibility.

Furthermore, the study does not provide extensive information on the criteria used to categorize the model's replies as adequate, inadequate, or uncertain, which may impair the evaluation's credibility. Furthermore, the study does not investigate the reasons for ChatGPT-4's wrong answers, which could have provided useful insights for enhancing the model's performance. Furthermore, the study does not address the potential constraints or obstacles of evaluating a medical examination utilizing a broad language model like ChatGPT-4. Overall, while the study gives some insights into ChatGPT-4's competence in answering the Revalida examination, the study's small number of evaluators and insufficient information on evaluation criteria are significant weaknesses. More studies with a larger and more diverse sample are needed. Modern approaches and a large training set are needed to remove bias and errors from chatbots[2,3]. This is due to the possibility of issues arising when relying solely on a huge data source. Employing chatbots poses ethical questions since it could lead to unforeseen or undesirable effects. To prevent the dissemination of harmful ideas and incorrect information, ethical controls and restrictions must be put in place as artificial intelligence language models advance[4].

AUTHORS' CONTRIBUTIONS

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

[1]Private Academic and Editorial Consultant – Bangkok, Thailand.
[2]Saveetha University, Saveetha Institute of Medical and Technical Sciences, Saveetha Medical College – Chennai, India.
*Corresponding author: wviroj@yahoo.com

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Response to "Analysis of possible risk predictors in patients with coronavirus disease 2019: a retrospective cohort study"

Marium Amjad[1]*

Dear Editor,

I have read with great sincerity the article entitled "Analysis of possible risk predictors in patients with coronavirus disease 2019: a retrospective cohort study" by Beatriz Nienkotter et al[1]. It was a pleasure for me to read the concisely written article, and I congratulate the authors for their excellent efforts. I agree with the ultimate findings of the study that age older than 65 years and a lung involvement extension greater than 50% are predictors of a poor prognosis for coronavirus disease 2019 (COVID-19), as well as the need for high-flow oxygen therapy.

Based on varied research[2,3], I agree that different clinical and computed tomography (CT) findings are associated with critical illness and a poor prognosis in patients with COVID-19. However, it seemed noteworthy to mention a few more points that would enhance the quality of this article and enrich its conclusion.

The retrospective sort of study has drawn numerous concerns due to the possibility of recall bias, which could be addressed if authors had included present cases of that time. The author could use a larger population to supplement the results. For instance, a 2020 prospective cohort study describes the characteristics of 5,279 patients with COVID-19 treated at a large quaternary academic health system[2]. Also, conducting a study at a particular location could create bias due to different socio-economic, health, and environmental conditions. As in this study, patients were all from a single geographical region and treated within a single health system; factors associated with poor prognosis might differ elsewhere. In the 2022 cohort study, patients were included in a multicenter international registry that had data from 31 centers in 7 countries[4].

As it is entrenched that patients with a positive diagnosis of COVID-19 were included, laboratory confirmation of the cases could also be addressed. Numerous studies[2,4] confirmed cases of COVID-19 using genetic sequencing or real-time reverse transcriptase polymerase chain reaction (RT-PCR). Additionally, the author should have analyzed further laboratory test findings, including blood urea nitrogen, serum creatinine, low-density lipoprotein levels (LDL), and liver function tests. A 2021 study concluded that elevated levels of aspartate aminotransferase (AST), creatinine, blood urea nitrogen, and bilirubin were significantly associated with unfavorable outcomes, and these parameters can be used to predict disease prognosis[5]. The author should also have briefed about different chronic conditions such as dyslipidemia, heart disease, chronic kidney disease, cancer, cerebrovascular disease, Parkinson's disease, and dementia since these comorbidities can influence mortality[6]. Dyslipidemia plays an important role in the pathological development of COVID-19. LDL levels are inversely correlated with disease severity, which could be a predictor of disease progress and poor prognosis[7].

Although it was stated that pulmonary involvement at the first chest tomography was considered a risk factor for an unfavorable outcome, it can also be mentioned that pleural effusion and a higher CT score on the CT scan at the time of admission are imaging predictors of poor prognosis in COVID-19 patients[3]. Finally, these findings lead to the conclusion that different parameters can be used to predict disease prognosis. Monitoring disease progression from the early stages will help in reducing unfavorable outcomes and allow more targeted lines of treatment, leading to a reduction in the rate of mortality.

ETHICS

The study was conducted in accordance with the Declaration of Helsinki and followed ethical standards.

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[1]Dow University of Health Sciences – Karachi, Pakistan.
*Corresponding author: marium.amjad5746@gmail.com

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Evangely, the subcategorization has been announced in the 2023 Bethesda system for reporting thyroid cytopathology: let bygones be bygones in thyroidology!

Ilker Sengul[1,2], Demet Sengul[3*]

Dear Editor,

The 2010 TBSRTC, first edition, was initially proposed in Bethesda, Maryland, USA, in 2007, providing thyroidologists a standardized, reporting system for thyroid FNA, in Volume 19, Thyroid[1]. Wielding TBSRTC has also been endorsed by the 2015 American Thyroid Association management guidelines[2] through the delicate papillon gland[3-7]. A special 2½-h symposium was moderated by Ali and Vielh at ICC in Pacifico Yokohama, Japan, on 28 May–01 June 2016[8-10]. The 2017 TBSRTC, second edition, was then published in Volume 27, Thyroid, by amendment of indeterminate cytology[11]. However, (re)appraisal for atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), category III, is still one of the most challenging issues in thyroidology as well as for Endocrine and Head & Neck Radiologists[12-17]. To this end, we emphasized whether it is essential to maintain category III as a unique one in February 2021, Volume 67, Rev Assoc Med Bras[18]. In October 2021, we declared blurred lines for managing thyroid nodules in the era of category III in a possible forthcoming TBSRTC, third edition. Of note, we postulated the so-called subdivision in category III as (i) IIIA: AUS/FLUS without nuclear atypia (AUS/FLUS w/o NA) and (ii) IIIB: AUS/FLUS with nuclear atypia (AUS/FLUS w/ NA) in Volume 67, Rev Assoc Med Bras[19]. Finally, we have currently recommended working with subsets to resolve the ongoing debate on "indeterminate cytology," similar to "intermediate suspicion" in Radiology, in Ultrasonography with a submission date of June 08, 2023[20].

Evangely, just 1 month later, the third edition of this lexicon, the 2023 TBSRTC, has been announced after two former successful editions by Ali et al., on July 08, 2023, in Thyroid. They have stated that the 2023 TBSRTC discontinues the term "FLUS" to avoid confusion with reporting terminology; henceforth, only "AUS" will be used. Of note, the up-to-date third edition declared the subcategorization of category III as (i) AUS-NA and (ii) AUS-other. Today, this two-tiered subclassification[21] has confirmed our previous recommendation for subdivision: (i) AUS w/ NA and (ii) AUS w/o NA in Volume 67, Rev Assoc Med Bras[19].

Hic et ubique terrarum, NAs have non-negligible clues in these nodules with indeterminate cytology. E fructu arbor cognoscitur. Eventually, the subcategorization has been announced after a long expectancy. Evangely, let bygones be bygones![21] We are deeply grateful to Cibas and Ali, founders of this crucial thyroid lexicon stating "just keep study" instead of "just keep stu(ea)dy", particularly for the indeterminate era in thyroidology. Novi sub sole, subdivision is no more debatable, in thyroidology, as we kindly have advocated in Rev Assoc Med Bras[22].

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IS: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **DS:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

[1]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
[2]Giresun University, Faculty of Medicine, Department of General Surgery – Giresun, Turkey.
[3]Giresun University, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
*Corresponding author: demet.sengul.52@gmail.com

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Gram-stained smear in the diagnosis of acute urethritis: is it coming to an end?

Mehmet Sarier[1,2*], Esin Kasap[3]

Acute urethritis is the most common infection of the male genital tract. Approximately 89 million new cases of non-gonococcal urethritis (NGU) and 62 million new cases of gonococcal urethritis (GU) are reported globally every year, and these numbers continue to increase[1]. Acute urethritis is most commonly caused by sexually transmitted pathogens. The three cardinal symptoms are urethral discharge, dysuria, and itching. The traditional diagnostic method for acute urethritis is a Gram-stained smear (GSS) of urethral discharge. GSS is widely used because it is of low cost and is easy to perform. Not only does GSS diagnose acute urethritis but it also allows the dichotomization of cases as GU caused by *Neisseria gonorrhoeae* with the detection of gram-negative diplococci or NGU in their absence[2]. However, GSS is a test susceptible to inter- and intra-observer errors.

In the classical approach, the treatment of acute urethritis is managed through GSS. GSS inevitably leads the clinician to empirical treatment, especially in cases of NGU, as the specific identification of NGU pathogens by conventional methods is a long process. However, treatment failure occurs in up to 20% of NGU patients who receive empirical treatment based on the results of GSS[3]. Moreover, empirical treatment practices also contribute to the development of resistant strains. Antibiotic resistance in *N. gonorrhoeae* and *Mycoplasma genitalium* is a serious public health problem[4], and *M. genitalium* alone is responsible for 41% of recurrent urethritis cases[5].

The widespread use of nucleic acid amplification tests such as polymerase chain reaction (PCR) has brought about significant advances in the management of acute urethritis[6]. PCR enables the rapid identification of multiple pathogens from a single sample with high sensitivity and specificity and has become the gold standard for identifying urethritis pathogens[7]. PCR also allowed inquiry into the effectiveness of GSS. The inability of GSS to detect coinfections of NGU pathogens with GU is an important limitation. GSS is also ineffective in urethritis patients with low inflammation, some of whom may even be asymptomatic. In a recently published study, 68.7% of urethritis cases evaluated by PCR did not have apparent urethral discharge, making it difficult to detect these cases with GSS[8]. This results in misdiagnosis and patients continuing to act as a vector of the contagion.

Traditionally, the threshold for GSS is ≥5 polymorphonuclear leucocytes (PMNL)/high-power field (HPF)[9]. However, a PCR confirmation study demonstrated that GSS had 55.6% sensitivity in the diagnosis of NGU when using a threshold of ≥5 PMNL/HPF in cases of acute urethritis[10]. Current guidelines recommend a GSS threshold of ≥2 PMNL/HPF for the diagnosis of NGU[11]. Considering that the frequency of NGU pathogens in acute urethritis is above 80%[12], GSS may be seriously inadequate in identifying the majority of acute urethritis cases.

In terms of cost, GSS and PCR are not comparable at present. However, the risks of misdiagnosis, antibiotic resistance, and recurrent urethritis associated with GSS warrant a critical scrutiny of its cost-effectiveness. In our view, GSS has fulfilled its role in the diagnosis of acute urethritis. Going forward, PCR assay should be regarded as the first choice for both the diagnosis of acute urethritis and the identification of causative pathogens. In our view, this is, first and foremost, the right approach for public health.

AUTHORS' CONTRIBUTIONS

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

[1]Istinye University, Department of Urology – İstanbul, Turkey.
[2]Medical Park Hospital, Department of Urology – Antalya, Turkey.
[3]University of Health Science, Tepecik Education and Research Hospital, Department of Obstetrics and Gynecology – İzmir, Turkey.
*Corresponding author: drsarier@gmail.com
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SHORT COMMUNICATION

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The cystic adventitial disease of the popliteal artery: from imaging to histopathology

Jan Roman[1,2], František Jalůvka[1,2], Ilker Sengul[3,4*], Demet Sengul[5], Tomáš Jonszta[6], Pavel Hurník[7,8], Anton Pelikán[1,2,9], Václav Procházka[6]

Dear Editor,

Cystic adventitial disease (CAD) is a rare non-atherosclerotic vascular disease, presenting with claudication symptoms usually in young males without any risk factors. Even though many cases have been described, the precise cause of this disease remains unknown. The incidence of CAD is 1:1200 calf claudication cases[1]. The disease causes myxomatous cystic tumor growth in the adventitial layer of a blood vessel, effectively causing external compression of the lumen and slowing down the blood flow. It mostly involves arteries, typically in (but not limited to) the popliteal region. However, it can also affect veins. Even though this disease can only be observed in asymptomatic patients, the only definitive treatment relies on surgical removal or marsupialization of the cyst. The following vignette case describes the clinical factors, treatment, and response of an adult who presented with a CAD of the popliteal artery. A 41-year-old white male, treated only for Klinefelter syndrome with testosterone substitution, presented with 14 days of claudication pain in the right calf. He reported pain, which occurred after a walking distance of 50–100 m and was accompanied by cold and pins-and-needles sensations in the affected area, without any limitation to movement or sensitivity. However, no similar problems had been experienced beforehand, and on examination, a colder right leg below the knee was revealed, with no palpable pulse in the popliteal area or more distally, with a positive Homans sign. The rest of his physical examination was unremarkable, and laboratory findings were recognized in the physiological ranges. A Doppler ultrasonography demonstrated the signs of embolization in the area of popliteal artery trifurcation. Moreover, the proximal blood flow was not compromised with triphasic Doppler waveforms and 100–80 cm/s flow. However, distally from the popliteal artery division, no blood flow was notified. A well-developed collateral flow around the knee was also noticed and pathology of the deep vein system was detected. A computed tomographic (CT) angiography was performed, confirming the findings from the sonography and localized the area of obstruction to the P1 segment of the popliteal artery (Figure 1A). He was indicated for surgical embolectomy with the signs of popliteal artery embolization and, shortly after admission, an indirect embolectomy through the Fogarty catheters was performed. Of note, no embolus was removed. However, the arterial backflow was notified in normal values. Moreover, an intraoperative angiography was performed, exhibiting a residual narrowing of the P1 segment of the popliteal artery to 30–40% due to doubts about the quality of perfusion. Perioperatively, the clinical status of the affected limb was largely improved, therefore the surgical procedure was completed without any further interventions. Postoperatively, due to pathological stenosis in a young male, an investigation continued based on the clinical imaging studies. By using Doppler ultrasonography and antegrade digital subtraction angiography (DSA), the morphology of the stenosis was established as eccentric, gradually rising from the arterial wall, with a well-developed collateral arterial flow (Figure 1B). The flow of the contrast agent was slowed down. However, the morphology of the lower limb

[1]University Hospital Ostrava, Department of Surgery – Ostrava, Czech Republic.
[2]University of Ostrava, Faculty of Medicine, Department of Surgery – Ostrava, Czech Republic.
[3]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
[4]Giresun University, Faculty of Medicine, Department of Surgery – Giresun, Turkey.
[5]Giresun University, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
[6]University Hospital Ostrava, Department of Radiology – Ostrava, Czech Republic.
[7]University Hospital Ostrava, Institute of Molecular and Clinical Pathology and Medical Genetics – Ostrava, Czech Republic.
[8]University of Ostrava, Institute of Molecular and Clinical Pathology and Medical Genetics, Faculty of Medicine – Ostrava, Czech Republic.
[9]Tomas Bata University in Zlin, Faculty of Humanities, Department of Health Care Sciences – Zlín, Czech Republic.
*Corresponding author: ilker.sengul.52@gmail.com
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arterial tree was normal. As such, a diagnosis of CAD was suggested and the patient was scheduled for an early open surgical revision of the popliteal artery, which was performed 4 days later. During the surgery, in the prone position, using an S-shaped incision, a popliteal artery and a vein were identified. The subcutaneous tissue was highly fibrous with plenty of collateral vessels, and the preparation of both critical structures proved challenging. A 9,000 IU of heparin was administered for further embolization prevention and the procedure of vascular clamps was placed to prevent eventual unnecessary bleeding. Afterward, the adventitial layer of the popliteal artery was incised, revealing a small cavity containing gelatinous material similar to coagulated blood, albeit browner (Figure 1C). As there was no apparent communication with the arterial lumen, the applied clamps were released, without any signs of bleeding. In fine, the surgical incision was closed as usual with a placement of 10 French Redon drains after the total duration of the surgery of 160 min. Postoperatively, the 5700 IU of nadroparin was administered subcutaneously twice daily, together with 250 IU sulodexide per os also twice daily. No complications occurred during the postoperative period and his recovery was uneventful. A Doppler sonography was performed before discharge, exhibiting a normal triphasic flow in all parts of the arterial system of his lower extremity. During the follow-up, the patient was completely symptom-free at 1- and 3-month periods. Histopathological examination revealed a CAD consisting of an adventitial fibrous tissue with small blood vessels and discrete chronic inflammatory cellularization. Microscopically, the lumen of the cystic structure was not lined with an epithelium layer but filled with mucoid contents (Figure 2A). Histochemically, the wall of the cystic structure was permeated by the mucosubstances positivity in the Alcian blue staining (Figure 2B). The CAD was originally described in 1947 by Atkins and Key[2]. Even though many cases have been described, the precise pathophysiology remains unknown. Several theories have been proposed. However, none of them has been proven. The original proposition described mucinous degeneration of the adventitial layer[3], but the theory of repeated trauma quickly replaced this theory[4]. As CAD can also affect other locations in the body and is present mostly in young patients with no history of trauma, an alternative theory of synovial involvement was soon proposed, which is based on the fact that vessels located near joints are usually affected. Of note, traumatic events affecting the joint capsule or a spontaneous migration of synovial cells cause the production of mucin along arterial or venous walls resulting in the development of cysts. Some authors also proposed the idea of the embryogenic origin of mucinous cells[5]. The disease mostly affects young people and males are affected approximately five times more often[6], and commonly, less to no known risk factors are present for the aforementioned phenomenon. Affected patients typically present with claudication symptoms of various intensities and speeds of onset. Even though no other

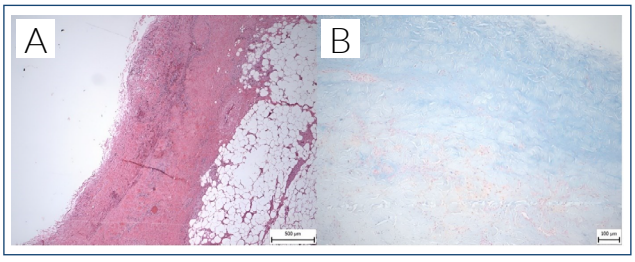

Figure 2. (A) Fibrous wall of a cystic lesion of the adventitia (hematoxylin-eosin; original magnification, 40×). (B) Alcian-positive mucoid masses diffusely permeating the wall (Alcian blue; original magnification, 40×).

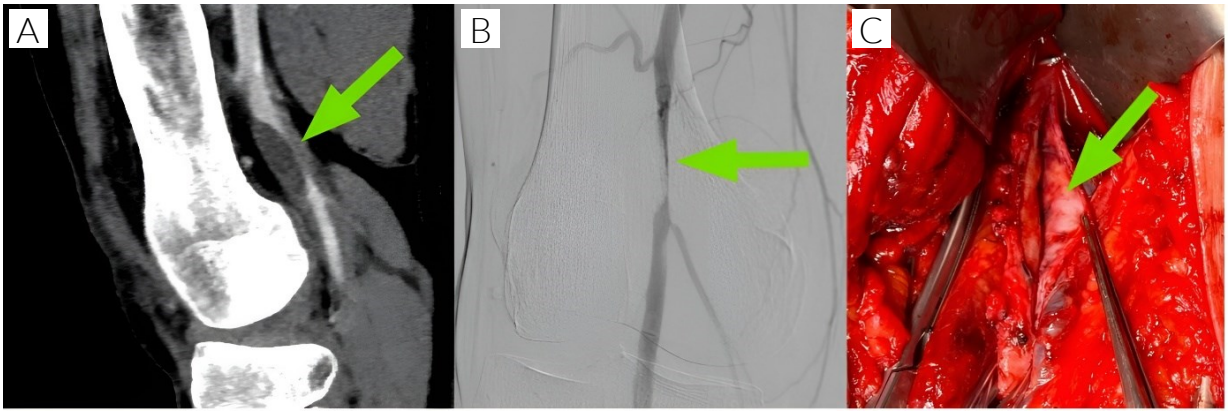

Figure 1. (A) A computed tomographic image showing the location of the stenosis at the P1 segment of the popliteal artery. (B) A digital subtraction angiography showing the eccentric, gradually rising stenosis. (C) An intraoperative image exhibiting the opened adventitial cyst with all gelatinous contents removed.

symptoms may be present, sometimes changes in peripheral pulse quality can be detected. These symptoms mostly result from the presence of arterial cysts (frequently in the popliteal region, in up to 85% of cases[7]). However, veins can also be rarely affected. In case of vein involvement, that condition may result in deep vein thrombosis. The first line diagnostic modality is Doppler ultrasound, which typically proves a reduction of blood flow distally from the affected area. Some sonographic signs are considered specific for CAD, such as cyst wall visualization, sonographic scimitar sign, and no vascularization of the cyst. However, due to the rarity of the disease, these findings usually need to be verified by CT angiography or DSA. To this end, these examinations are able to verify a typical shape of the stenosis (scimitar sign and hourglass shape of the lumen), which is usually gradual, with no post-stenotic dilation (as opposed to other forms of stenosis)[8]. Of note, magnetic resonance imaging is also a viable option, showing classical signs of cystic mass – hypointensity in T1- and hyperintensity in T2-weighted images. While some cases can be managed as "watch-and-wait" with regular check-ups, there were cases of spontaneous resolution of symptoms[9]. However, most are symptomatic and require treatment options. A step-up treatment can be rationally recommended. However, there is no strong evidence in the literature regarding the optimal treatment of CAD and the long-term prognosis of patients. As such, the first-line method is CT-guided aspiration of the cyst, which can be sometimes successful on its own. Notably, cases treated by the percutaneous intervention were also published, with variable outcomes. In case of recurrence, surgery is the method of choice in the management of this phenomenon. No high-quality comparison of various surgical methods is available, and the final treatment method depends on the experience of the surgeon. In English-language literature, the arterial region affected by a cyst is usually completely resected, with the affected region being replaced by a bypass procedure. This is successful in 94% of cases, and the overall long-term patency of grafts is high (80.9 months)[10]. In conclusion, peripheral arterial disorders[11,12] remain the challenging issue of vascular surgery and vascular pathology. Anecdotally, the adventitial cystic disease of the popliteal artery might lead to claudication symptoms and mimicking symptoms of chronic limb Ischemia, mostly in younger, without risk factors. Available literature reveals that the affected arterial structure can easily be preserved. From a surgical perspective, physiological blood flow is usually restored, resulting in a complete resolution of symptoms, after surgical intervention. Postoperatively, the majority of cases remain symptom-free in the long term, and recurrences are typically described following interventional procedures. To this end, we once again emphasize that physicians should be vigilant for this rare entity during their daily routine in both out- and inpatient clinics.

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AUTHORS' CONTRIBUTIONS

JR: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **IS:** Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **DS:** Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TJ:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **PH:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **AP:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **VP:** Investigation, Methodology, Project administration, Resources, Validation, Visualization. **FJ:** Investigation, Methodology, Project administration, Resources, Validation, Visualization.

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ORIGINAL ARTICLE

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Effect of *Laurus nobilis* on bacteria and human transforming growth factor-β1

Okan Sancer[1,2*], Uğur Şahin[2,3], Emel Sesli Çetin[4], Muhammet Yusuf Tepebaşi[5], Yasemin Cezaroğlu[4], Göksel Bilir[4], Sibel Yünlü[2,3], Ahmet Koca[6]

SUMMARY

OBJECTIVE: In this study, we aimed to determine the phenolic compounds, the antibacterial activity of extract from *Laurus nobilis* leaves, and its possible effect on transforming growth factor-β1 expression level in peripheral blood mononuclear cells.

METHODS: The phenolic components of *Laurus nobilis* were identified by the high-performance liquid chromatography method. The antibacterial activity of this extract was determined by disk diffusion and broth microdilution methods. The transforming growth factor-β1 expression was analyzed using the RT-qPCR method.

RESULTS: Epicatechin was found in the highest amount and o-coumaric acid in the lowest amount. The half-maximal inhibitory concentration (IC_{50}) was determined to be 55.17 μg/mL. The zones of inhibition and minimum inhibitory concentration for *Staphylococcus aureus, Enterococcus faecalis*, and *Klebsiella pneumoniae* were 15, 14, and 8 mm and 125, 250, and 1000 μg/mL, respectively. The change in transforming growth factor-β1 expression levels was found to be statistically significant compared with the control groups ($p<0.0001$).

CONCLUSION: *Laurus nobilis* extract was found to be effective against bacteria and altered the expression level of transforming growth factor-β1 in peripheral blood mononuclear cells.

KEYWORDS: Epicatechin. *Laurus nobilis*. MTT. o-Coumaric acid.

INTRODUCTION

Laurus nobilis L. is an aromatic herb that spreads worldwide on the coasts of southern Europe and Asia Minor, where the Mediterranean climate prevails[1]. Laurel is widely used in alternative medicine, food, and cosmetics industries. The main reasons for its use in these areas are due to its antimicrobial, antifungal, antioxidant, anxiolytic, antidepressant, and antistress properties[2].

Fruits, herbs, and other plant-based foods contain sources of compounds such as (poly)phenols and flavonoids that protect against inflammation and chronic disease. T lymphocytes are instrumental in supporting the production of pro-inflammatory and anti-inflammatory cytokines in tissues and circulation. However, less is known about the relative potency of different (poly)phenols in modulating cytokine release by lymphocytes[3].

In the immune system, transforming growth factor-β1 (TGF-β1) is a general regulatory activity that affects many types of immune cells. By regulating T lymphocyte development, differentiation, homeostasis, and tolerance, the evolutionarily highly conserved TGF-β cytokine crucially supports a functional T cell pool. TGF-β1 plays a critical role in maintaining peripheral tolerance to endogenous and harmless antigens such as food and commensal bacteria and in controlling the immune response to pathogens[4].

Laurus nobilis is a natural medicinal plant and a rich source of bioactive compounds. The biological properties of its various extracts and its essential oil are documented, in particular their antimicrobial and antioxidant effects[5] and wound-healing properties in animal models[6].

Our aim in this study was to determine the effect of the phenolic components of *L. nobilis* leaf extract against the bacteria *Staphylococcus aureus, Enterococcus faecalis,* and *Klebsiella pneumoniae* that cause various infectious diseases and the effect of TGF-β1 expression in human peripheral blood mononuclear cells (PBMCs) in the presence of these bacteria.

[1]Suleyman Demirel University, Faculty of Medicine, Department of Medical Biology – Isparta, Turkey.
[2]Suleyman Demirel University, Innovative Technologies Application and Research Center, Genetic Research Unit – Isparta, Turkey.
[3]Suleyman Demirel University, Faculty of Arts and Sciences, Department of Chemistry – Isparta, Turkey.
[4]Suleyman Demirel University, Faculty of Medicine, Department of Medical Microbiology – Isparta, Turkey.
[5]Suleyman Demirel University, Faculty of Medicine, Department of Medical Genetic – Isparta, Turkey.
[6]Isparta University of Applied Sciences, Sütçüler Prof. Dr. Hasan Gürbüz Vocational School – Isparta, Turkey.
*Corresponding author: okansancer@hotmail.com
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METHODS

The flowchart of the overall work is shown in Figure 1.

Plant

Fresh leaves from *L. nobilis* were collected in Isparta/Türkiye in June 2022. The plant material was recorded at the Iğdır University Wildlife Museum (Herbarium Specimen) with reference number INWM00000111. Species verification and taxonomic assessment of *L. nobilis* were completed by Dr. Ahmet KOCA.

Determination of the phenolic compounds

Determination of phenolic compounds in extracts made by the high-performance liquid chromatography (HPLC) method according to our previous study[7]. After weighing 2.7 g of *L. nobilis* leaves, 30 mL of 99% methanol (Merck, Germany) was added and homogenized with a household mixer. They were mixed in an ultrasonic bath for 1 h. The extracts were filtered on Whatman filter paper (no. 4). Then the filtered extracts were evaporated at 40°C. The remainder in the Erlenmeyer flask was taken with 5 mL of methanol. This solution of 20 µL was injected into the HPLC device. The method of Caponio et al., was used for the determination of phenolic compounds by HPLC[8]. Detection of phenolic compounds was carried out at a wavelength of 278 nm and a flow rate of 0.8 mL/min. A reverse-phase column (5 µm) Agilent Eclipse XDB C18 (4.6 × 250 mm) was used. The column temperature was 30°C. The separation was performed with a binary solvent system using a gradient program. Solution A was 3% acetic acid, and solution B was methanol.

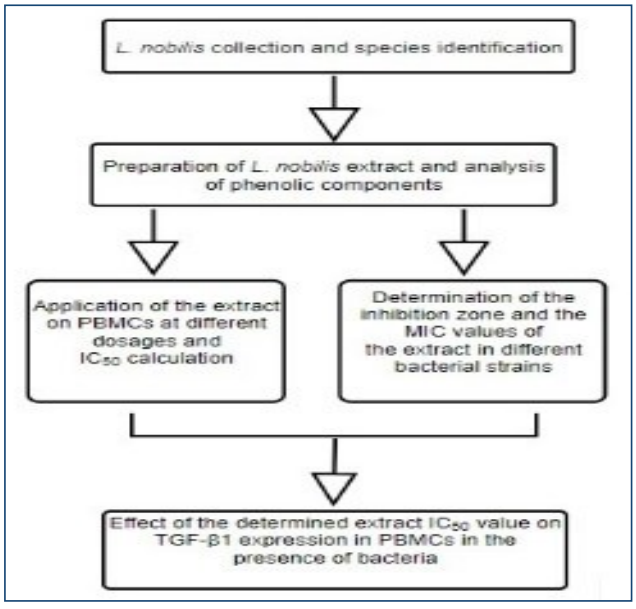

Figure 1. Flowchart of the overall work.

Preparation of *Laurus nobilis* extract for bacterial and peripheral blood mononuclear cells culture

Laurus nobilis extracts were freeze-dried (Labconco FreeZone 6 plus, USA). After the lyophilized samples were dissolved in RPMI 1640 (Biological Industries, Israel) and the extract was filtered with a 0.45-µm filter, the extracts were added to the incubation medium with human peripheral lymphocytes. In parallel, the lyophilized samples were dissolved in dH_2O and used in bacterial disk diffusion and minimum inhibitory concentration (MIC) tests.

Isolation of peripheral blood mononuclear cells

Whole blood was collected from lithium heparin tubes from a healthy 34-year-old volunteer who had not been exposed to radiation or drugs for 6 months and who did not smoke.

The isolation of PBMCs was performed according to the protocol of Panda et al.[9] RPMI 1640 medium and heparinized whole blood samples were mixed 1:1. Then this mixture was slowly added to the tube on Histopaque 1077 (Sigma-Aldrich, Switzerland). The mixture was centrifuged at 2000 rpm for 20 min at 4°C. The "Buffy coat" PBMC layer was removed and transferred to the new tube. After centrifugation, PBMCs and RPMI 1640 medium were resuspended in a 1:1 ratio. Then it was centrifuged at 2500 rpm for 5 min at 4°C. Then the supernatant was taken out. Cell viability was determined to be 98% using trypan blue stain.

In vitro cell culture

The cell culture of PBMCs was performed according to the protocol of Panda et al[10]. To determine the non-toxic dose of *L. nobilis* leaf extract, 1×10^4 cells/well PBMCs were seeded at 96-well flat-bottomed microplates (Sarstedt AG, Germany). Then the plate was incubated at 37°C in a 5% CO_2 and humidified incubator for 24 h. *L. nobilis* extracts of 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, and 0.0 µg/mL were cultured with cells at 37°C in a 5% CO_2 and humidified incubator for 24 h. The medium consisted of the following components: RPMI-1640 (Biological Industries, Israel) medium supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, USA), penicillin (100 IU/mL), and streptomycin (100 µg/mL) (Sigma-Aldrich, USA).

Determination of IC_{50} value

After incubation, cell viability was evaluated using the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide

(MTT) colorimetric assay (Sigma Aldrich, USA) to determine the IC_{50} value of the *L. nobilis* extract. MTT reagent was added to each well. The final concentration was adjusted to 0.5 mg/mL, and the cells were incubated in a humidified atmosphere at 37°C in 5% CO_2 for 4 h. The plate was centrifuged at 800 g for 5 min, and then the supernatants were removed. The formazan crystals were dissolved in 200 µLµL of dimethyl sulfoxide (Thermo Fisher Scientific, USA) and shaken at room temperature for 15 min. Optical densities were recorded at 570 nm using a multiscan plate reader (Synergy HTX BioTek, USA)[11]. A plot of viability versus extract concentration was used to calculate IC_{50} values for PBMCs.

The bacteria strains

Staphylococcus aureus (ATCC: 27853), *E. faecalis* (ATCC: 29212), and *K. pneumoniae* (ATCC: 700603) were used in this study. All the strains were obtained from the Microbiology Laboratory at Suleyman Demirel University Hospital, Isparta.

Antibacterial susceptibility of *Laurus nobilis* leaf extract using the disk diffusion method

The disk diffusion method for antibacterial susceptibility testing was performed using the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol[12]. A 6-mm sterile disk filter paper (Schleicher and Schul, 2668, Dassel, Germany) was applied by impregnating 50 µL of *L. nobilis* leaf extract. The bacterial cultures were inoculated on Nutrient Broth (Becton Dickinson and Company, USA) and incubated at 37°C for 24 h. Adequate quantities of Mueller Hinton Agar (GBL, Türkiye) were dispensed into sterile plates and subjected to solidification under aseptic conditions. Bacterial crop counts were adjusted to yield 1×10^6 using the McFarland standard count method. The test microorganisms (0.1 mL) were inoculated with a sterile swab on the surface of the suitable plate solid medium. The agar plates inoculated with the test microorganisms were incubated for 1 h before placing the paper disk impregnated with the extract onto the plates. The bacterial plates were incubated at 37°C for 24 h. Following incubation, the parameters of the growth inhibition zones of all plates were measured in millimeters. Gentamicin (10 µg/disk) (Becton Dickinson and Company, USA) and meropenem (10 µg/disk) (Becton Dickinson and Company, USA) for *K. pneumoniae*, penicillin (1 µg/disk) (Becton Dickinson and Company, USA) and cefoxide for *S. aureus* (30 µg/disk) (Becton Dickinson and Company, USA), and ampicillin (2 µg/disk) (Becton Dickinson and Company, USA) and vancomycin (5 µg/disk) (Becton Dickinson and Company, USA) for *E. faecalis* were used as positive control.

Determination of the minimum inhibitory concentration

The MICs of the raw extracts were achieved by broth micro-dilution using the 96 multiwell microtitration plates[13]. *L. nobilis* extracts (1000 µg/mL) and Mueller Hinton broth (GBL, Türkiye) were applied in the first row of the plate. Mueller Hinton broth was added to other wells. Then, serial dilutions were applied at the rate of 1/2 from the first well to the last well. Standard bacterial strains were adjusted to 0.5 McFarland (10^8 CFU/mL) turbidity standard and diluted 1/100 with Mueller Hinton Broth (GBL, Türkiye) to 10^6 CFU/mL. Finally, 10 µL of bacterial suspension was added to each well. The plates were incubated for 24 h at 37°C. The lowest concentration of plant extract that inhibited bacterial growth was considered the MIC.

Transforming growth factor-β1 expression

Peripheral blood mononuclear cells (1×10^4 cells/well) were incubated at 37°C in a 5% CO_2 and humidified incubator for 24 h. Then PBMCs and bacterial strains (10^6 CFU/mL) were cultured with a determined IC_{50} value, 37°C in 5% CO_2, and humidified atmosphere for 24 h. Total RNA from PBMCs was extracted with the Hibrigen total RNA isolation kit (Hibrigen, Türkiye). RNA purity and concentration were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., DE); 1 µg RNA was reverse transcribed with the 5× i-Script RT supermix and nuclease-free water (BioRad, USA). The total volume was adjusted to 20 µL. Primer designs were performed by detecting specific mRNA sequences. Possible primer sequences were tested using the NCBI website. TGF-β1 (Forward 5′-CAATTCCTGGCGATACCTCAG-3′ and Reverse 5′-GCACAACTCCGGTGACATCAA-3′), primers were designed to amplify. The β-actin gene was used as a housekeeping gene and the CT values of this gene were used for normalization. Notably, 0.1 mL PCR tubes were used in the instrument, and the final reaction volume was 20 µL. The reaction mixture was prepared according to the manufacturer's protocol (A.B.T., Turkey). The resulting reaction mixture was loaded into a real-time qPCR instrument with a thermal cycle determined by the kit manufacturer's protocol.

The CT values of the target genes were determined, and formula $2^{-\Delta\Delta Ct}$ (Livak method) was used to determine their relative expression levels[14].

Statistical analysis

The results of the TGF-β1 expression study were evaluated using the SPSS 18.0 statistical analysis software (SPSS Inc., USA). Comparisons between groups were performed in the present study by one-way analysis of variance and Tukey analysis. PBMC culture, MTT assay, and TGF-β1 expression were performed in triplicate. Concentration-response curves and IC_{50} values were generated with GraphPad Prism 5.

RESULTS

Phenolic compound analysis

In our study, protocatechic acid, p-hydroxybenzoic acid, catechin, luteolin, caffeic acid, camperol, epicatechin, o-coumaric acid, vanillin, ferulic acid, rutin, p-coumaric acid, and cinnamic acid were detected. The analyses of the phenolic compounds of the *L. nobilis* extract are presented in Table 1.

In vitro viability assay

The IC_{50} of *L. nobilis* extract was determined to be 55.17 µg/mL on PBMCs. The results show that increasing concentrations of *L. nobilis* extract leads to a reduction in the survival rate of PBMCs.

Antibacterial activity

Zones of inhibition of *L. nobilis* extract and standard antibiotics are presented in Table 2. The inhibition zone diameter detected with the extract was 15 mm for *S. aureus*, followed by 14 mm for *E. faecalis* and 8 mm for *K. pneumoniae,* respectively. The antibacterial activity of *L. nobilis* extract was tested at concentrations from 7.8 to 1000 µg/mL, and MIC values were determined to be 125, 250, and 1000 µg/mL for *S. aureus*, *E. faecalis,* and *K. pneumoniae*, respectively.

Transforming growth factor-β1 expression

Positive, negative control and bacteria/extract groups were compared, and the expression results were found to be statistically significant ($p<0.0001$). The results are shown in Figure 2. Values were presented as means±SD.

DISCUSSION

One of the global problems is the development of bacterial resistance to antibiotics. People have been using herbal medicine for centuries for its safety, effectiveness, cultural acceptance,

Table 1. Analysis result of the phenolic compounds.

Phenolic compounds	Laurus nobilis (µg/g)
Gallic acid	*
Protocatechic acid	85.2
Catechin	173.2
p-Hydroxybenzoic acid	123.6
Chlorogenic acid	*
Caffeic acid	78.2
Epicatechin	2113.7
Syringic acid	*
Vanilin	48.9
p-Coumaric acid	16.7
Ferulic acid	64.2
Sinapinic acid	*
Benzoic acid	*
o-Coumaric acid	2.7
Rutin	246.2
Hesperidin	*
Rosmarinic acid	*
Eriodictiol	*
Cinnamic acid	5.5
Quercetin	*
Luteolin	14.7
Kamferol	34.3

*Could not be detected.

Table 2. Detection of inhibition zones in bacteria.

Microorganisms	Inhibition zones (mm)						
	Positive controls						
	50 µL	GEN	MER	P	CFX	AMP	VAN
Staphylococcus aureus	15	NT	NT	18	22	NT	NT
Enterococcus faecalis	14	NT	NT	NT	NT	17	13
Klebsiella pneumoniae	8	23	31	NT	NT	NT	NT

50 µL: *Laurus nobilis* extract; NT: not tested; GEN: gentamicin 10 µg; MER: meropenem 10 µg; P: penicillin 10 µg; CFX: cefoxitin 30 µg; AMP: ampicillin 2 µg; VAN: vancomycin 5 µg.

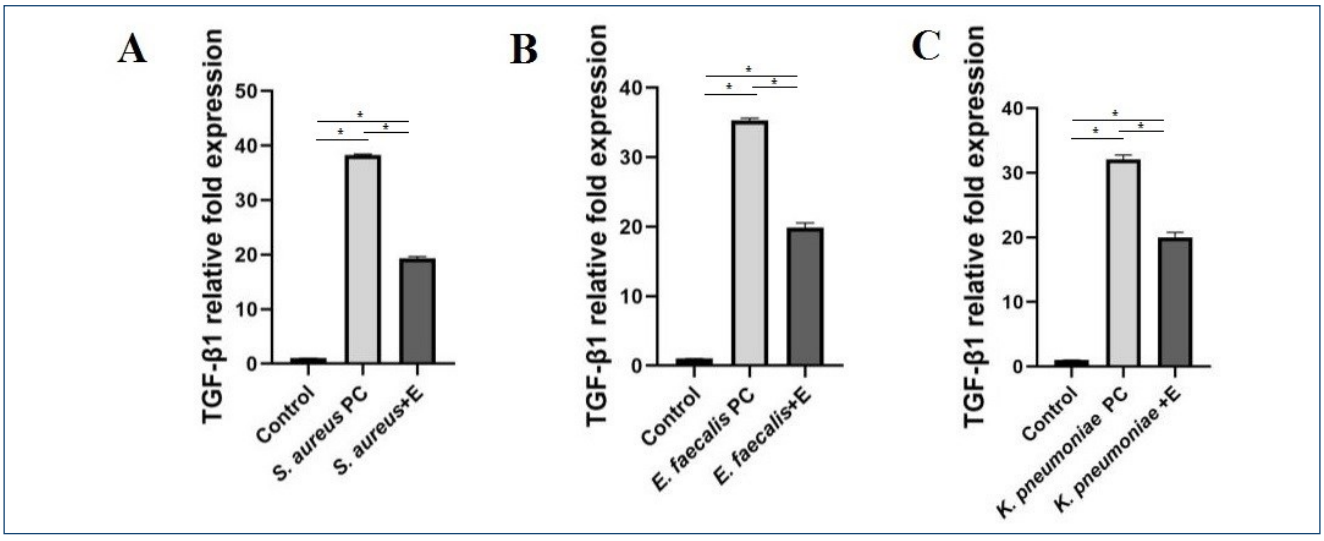

Figure 2. Transforming growth factor-β1expression fold change. (A) *Staphylococcus aureus* TGF-β1 relative fold expression. (B) *Enterococcus faecalis aureus* TGF-β1 relative fold expression. (C) *Klebsiella pneumoniae* TGF-β1 relative fold expression. **p*<0.0001.

and fewer side effects[15]. *L. nobilis* leaves have been used as a medicinal herb and have pharmacological activity that includes antibacterial and anti-inflammatory effect[16].

The antibacterial effect of laurel essential oil on human pathogenic bacteria was tested by the disk diffusion method against *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus faecalis*, *Staphylococcus flexneri*, *Pseudomonas aeroginosa, Serratia marcescens, Salmonella Typhi, K. pneuomoniae*, and *Escherichia coli*. The results showed that *L. nobilis* essential oil has potent antibacterial effects[17]. The antibacterial activity of *L. nobilis* essential oil on some bacterial species was determined as follows: *E. coli* (27.1 mm), *E. faecalis* (28.0 mm), and *Salmonella pullorum* (25.2 mm)[18].

In our study, the inhibition zone diameter detected with the extract was 15 mm for *S. aureus*, followed by 14 mm for *E. faecalis* and 8 mm for *K. pneumoniae*, respectively. These results show that the antibacterial effect of *L. nobilis* essential oil is much stronger than that of the extract. However, the use of extracts for antimicrobial purposes is also effective. In addition, the diameter of the zone of inhibition of the Gram-positive bacteria *S. aureus* and *E. faecalis* is approximately two times larger than that of the Gram-negative bacteria *K. pneuomoniae*. We suggested that this may be a difference due to the uptake of the active ingredients in *L. nobilis* extract into the bacteria depending on the cell walls or membranes of Gram-positive and Gram-negative bacteria. Recent literature information shows that the antimicrobial activity of phenolic compounds is more sensitive in Gram-positive bacteria than in Gram-negative bacteria[19,20]. The differences in the mechanisms of action in Gram-positive and Gram-negative bacteria are also not entirely clear[21]. However, it has been reported that the antimicrobial potential of phenolic compound molecules with hydroxyl groups can result in weaker interactions due to the strong outer membrane electronegativity in the cell wall of Gram-negative bacteria[22].

In our study, we found that *L. nobilis* extract has an antibacterial effect on *S. aureus*> *E. faecalis*> *K. pneumoniae*. Furthermore, we found that epicatechin was present in the highest amount (2113.7 µg/g) in the extract.

In Hep-G2 cells, the IC_{50} values of *L. nobilis* extract were found as follows: ethyl acetate: 3.80 µg/mL, petroleum ether: 10.60 µg/mL, and methanol extract: 23.20 µg/mL[23].

In this study, we determined the IC_{50} value of *L. nobilis* methanol extract to be 55.17 µg/mL in PBMCs. The changes in the IC_{50} determined in the various studies may be due to the application to different cell cultures. In addition, the environmental conditions under which *L. nobilis* grows can also have an impact.

Traditionally, TGF-β has been suggested to have potent anti-inflammatory effects on the immune system. However, TGF-β can have pro-inflammatory and anti-inflammatory effects depending on the context in which it acts[24]. Our study showed that *L. nobilis* leaf extract altered TGF-β1 expression on PBMCs compared with positive and negative controls. In this study, the results of relative TGF-β1 expression showed a very similar profile for *S. aureus*, *E. faecalis*, and *K. pneumonia.*

The fact that *L. nobilis* leaf extract applied according to the IC_{50} value calculated at a lower dose than the MIC value

showed a similar profile in all bacterial/extract groups compared with the controls indicates that the anti-inflammatory effect is largely achieved through inhibition of the TGF-β1 signaling pathway. This demonstrated that *L. nobilis* leaf extract is a candidate for suppressing the inflammatory pathway caused by pathogens due to its anti-inflammatory effect in addition to its antimicrobial activity.

Inflammation is a natural response of the innate and adaptive immune system to infection. However, when inflammation is left uncontrolled, it can lead to autoimmune or autoinflammatory diseases, neurodegenerative diseases, or cancer[25]. *L. nobilis* leaf extract controls inflammation by suppressing activation of the NLRP3 inflammasome[26].

CONCLUSION

The leaves of *L. nobilis* contain many phenolic compounds that contribute to the antimicrobial properties of this plant. The extract was also shown to alter TGF-β1 expression on PBMCs. The phenolic compounds are believed to be responsible for these activities. There is a need to expand research by performing more detailed studies and different cell types and in vivo studies.

INFORM CONSENT

The consent form was obtained from all patients participating in the study.

ETHICS APPROVAL

The study was prepared in agreement with the protocols of the Helsinki Declaration. This study was approved by the Isparta Süleyman Demirel University Medical Faculty Ethics Committee (No. 18/244).

AUTHORS' CONTRIBUTIONS

OS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. **UŞ:** Formal Analysis, Investigation, Methodology, Validation, Writing – review & editing. **ESÇ:** Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **MYT:** Formal Analysis, Validation, Writing – review & editing. **YC:** Formal Analysis, Investigation, Methodology, Writing – review & editing. **GB:** Formal Analysis, Investigation. **SY:** Resources, Validation, Writing – review & editing. **AK:** Investigation, Methodology.

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ORIGINAL ARTICLE

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Can supplementation of tryptophan in parenteral nutrition increase melatonin and alleviate inflammatory response?

Necdet Fatih Yaşar[1*], Bartu Badak[1], Mustafa Salış[2], Fatih Kar[3], Setenay Öner[4]

SUMMARY

OBJECTIVE: Endogenous melatonin is produced from tryptophan which is an essential amino acid. Besides its role in the regulation of sleep patterns, melatonin has anti-inflammatory effects. In this case-control study, we aimed to compare tryptophan and melatonin levels and their relationship with the inflammatory response, specifically serum interleukin-1, interleukin-6, and c-reactive protein levels following major abdominal surgery in patients with food restriction and who receive parenteral nutritional therapy.

METHODS: We enrolled 40 patients between the ages of 18 and 65 years in the study. We collected blood and urine samples 48 h before the operation and on postoperative days 1, 3, and 5.

RESULTS AND CONCLUSION: The tryptophan levels in the experimental group were higher than in the control group but failed to reach any statistical difference. Melatonin levels were increased in both groups following the surgery compared with preoperative levels. The increase in the experimental group was statistically different 3 days after the surgery. The difference in the level of interleukin-1 between the control and the experimental groups was greatest on postoperative day 3. On postoperative day 3, the interleukin-6 level in the treatment group was slightly higher than in the control group. We did not find any difference in the levels of c-reactive protein between the groups. As a result, the levels of tryptophan and melatonin were increased in the parenteral nutrition group, irrespective of the postoperative inflammatory response.

KEYWORDS: Melatonin. Tryptophan. Inflammatory response. Surgery. Nutrition. Parenteral nutrition.

INTRODUCTION

Endogenous melatonin is produced from tryptophan which is an essential amino acid[1]. Besides its role in the sleep patterns, melatonin has anti-inflammatory effects. Studies have shown that melatonin may counteract proinflammatory response[2-5]. Thus, the interaction between inflammation and melatonin secretion seems to be essential for hemostasis after surgery. In this regard, our previous study has shown that improving sleep quality may ameliorate inflammatory response after major abdominal surgery by increasing melatonin secretion[6].

It has been shown that acute tryptophan depletion in healthy volunteers decreased plasma tryptophan levels as well as melatonin levels[7,8]. On the contrary, we have observed that postoperative melatonin levels increase in our previous study regardless of sleep quality[6]. However, there is no study about the effects of postoperative food restriction on tryptophan levels and melatonin secretion. In this case-control study, we compared tryptophan and melatonin levels and their possible relationship with the inflammatory response following abdominal operations in patients with food restriction and in patients who receive parenteral nutritional therapy.

METHODS

This study has been approved by the Ethics Committee of Eskisehir Osmangazi University and conducted in accordance with the principles of the Declaration of Helsinki (No. E-25403353-050.99-93363). We enrolled 40 patients between the ages of 18 and 65 years who underwent abdominal operations in the study. Informed consents were taken from all patients. Patients with inflammatory diseases and hormone-related conditions, such as medication or neoplasms, and patients with malnourishment were excluded. Patients were divided into two groups, the dietary restriction group and the parenteral nutritional therapy group [Oliclinomel N4-550E, (Baxter, Turkey) which had 0.040 g/L of tryptophan], depending on the anticipation if the patient would be able to start eating after 5 days postoperatively.

[1]Osmangazi University, Faculty of Medicine, Department of General Surgery – Eskişehir, Turkey.
[2]Eskişehir City Hospital, Department of General Surgery – Eskişehir, Turkey.
[3]Kütahya Health Sciences University, Faculty of Engineering and Natural Sciences, Department of Biochemistry – Kütahya, Turkey.
[4]Osmangazi University, Faculty of Medicine, Department of Biostatistics – Eskişehir, Turkey.
*Corresponding author: nfyasar@gmail.com

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Preoperative blood and urine samples were collected 48 h before the operation. The demographics of the patients were recorded. Postoperative samples were collected on days 1, 3, and 5. We monitored melatonin production by the determination of urine 6-sulfatoxymelatonin (aMT6s). A 24-h urine sample was collected. Besides urine aMT6, serum tryptophan levels were measured. We monitored inflammatory response by measuring parameters, including interleukin-1 (IL-1), interleukin-6 (IL-6), and c-reactive protein (CRP).

We assayed the urinary aMT6 levels using a commercial enzyme-linked immunosorbent assay (ELISA) kit [IBL International GmbH ELISA kit (Hamburg, Germany)]. We analyzed serum levels of IL-1, IL-6, and tryptophan using the Bioassay Technology Laboratory ELISA kits (Zhejiang, China) and CRP levels using an automated analytical system (SiemensDimension Vista^{O} 1500, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Statistics

As the Shapiro-Wilk test showed that the variables were not distributed normally, we compared the experimental and control groups using the Mann-Whitney U test. We evaluated the difference between preoperative, postoperative days (PODs) 1, 3, and 5 variables in both groups using the Tukey's HSD test.

RESULTS

There was no difference between the control and the experimental groups concerning demographics.

There was no difference between the preoperative and postoperative plasma tryptophan levels in the control group. The tryptophan levels in the experimental group were increased beginning on the third day after the surgery and reached the highest level on POD 5 but failed to reach any statistical difference (Table 1). Melatonin levels were higher in both the control and experimental groups following the surgery compared with preoperative levels, but the increase in the experimental group reached a statistical difference on POD 3 and the peak level on POD 5. Similar to the tryptophan levels, POD 3 and POD 5 melatonin levels were greater in the experimental group than in the control group (Table 1 and Figure 1).

The level of IL-1 was increased in both groups on POD 1, and the difference between the control and the experimental group was greatest on POD 3. However, the difference was diminished on POD 5 (Table 1 and Figure 2). On the contrary, the level of IL-6 on POD 3 in the treatment group was slightly greater than in the control group (Table 1).

The levels of CRP were less in the treatment group, and the difference was greatest on POD 3 but statistically not different (Table 1).

DISCUSSION

In this study, we have observed that the levels of tryptophan and melatonin were increased in the experimental group irrespective of the inflammatory response.

Previous studies by Zimmermann et al., have shown that acute tryptophan depletion reduces plasma tryptophan and melatonin levels in healthy subjects at night[7]. They also showed that urinary 6-SM can be used as a valid and reliable indicator of melatonin production[8]. On the contrary, Ploder et al., did not observe any significant changes in the tryptophan concentrations but increased kynurenine concentrations after tryptophan depletion in trauma patients[9]. Similarly, our results have shown no difference between the preoperative and postoperative plasma tryptophan levels in the control group. However, the tryptophan levels in the experimental group were increased and reached the highest level on POD 5, but the difference was not statistically important. Melatonin levels were higher in both the control and experimental groups, which was consistent with the findings in our previous study in which we investigated how sleep quality affects melatonin levels following surgery[6]. This finding was also demonstrated in the study of Ram et al[10]. However, the rise in melatonin levels in the experimental group was more conspicuous.

In this study, consistent with the previous studies, the response of IL-1 secretion was not different in both control and treatment groups, whereas the increase in the IL-6 levels was greater in the treatment group[6,11]. Similar to previous studies, which showed the anti-inflammatory effects of increased levels of melatonin, we showed a significant decrease in the levels of POD 3 IL-1[2-6]. This might be related to higher levels of melatonin in the experimental group. However, the levels of POD 3 IL-6 were greater in the experimental group. We claim that this might be caused by the administration of tryptophan within parenteral nutrition. In these previous studies, melatonin was either administered or its plasma level was manipulated by sleep quality. However, in this study, plasma levels of melatonin were increased parallel to the increase in the level of tryptophan. It is well known that indoleamine 2,3-dioxygenase 1 (IDO 1) is the most essential enzyme that catalyzes the degradation of tryptophan to kynurenine[12]. IL-6 may upregulate IDO 1 expression and is closely associated with the tryptophan metabolism[13,14]. Therefore, the increased level of plasma tryptophan might have triggered an increase in IL-6 levels to

Table 1. Basic patient demographics and preoperative and postoperative plasma tryptophan, 6-sulfatoxymelatonin, interleukin-1, interleukin-6, and c-reactive protein levels.

	Control group Median (25–75%)	Experimental group Median (25–75%)	p
Age Gender (number of males)	61.00 (51.50–64.50) 12	61.00 (55.00–64.00) 13	0.860
Preoperative			
Tryptophan (µg/mL)	24.95 (22.14–27.18)	25.70 (20.94–27.34)	0.957
aMT6 (µg/day)	68.03 (45.03–91.42)	71.64 (59.72–117.21)	0.500
IL-1 (pg/mL)	34.61 (29.56–68.61)	31.99 (29.08–35.93)	0.062
IL-6 (pg/mL)	43.08 (30.61–55.79)	41.57 (31.32–53.53)	0.482
CRP (mg/L)	2.70 (2.25–4.15)	2.25 (1.00–3.45)	0.285
POD 1			
Tryptophan (µg/mL)	24.95 (23.63–28.13)	25.39 (20.50–45.75)	0.818
aMT6 (µg/day)	101.03 (71.17–139.18)	106.12 (78.47–138.87)	0.844
IL-1 (pg/mL)	39.99 (30.60–64.51)	36.06 (30.32–40.44)	0.168
IL-6 (pg/mL)	63.84 (54.20–71.22)	65.44 (50.62–112.84)	0.490
CRP (mg/L)	86.30 (41.35–119.65)	73.25 (54.30–124.50)	0.957
POD 3			
Tryptophan (µg/mL)	24.75 (24.89–31.97)	28.77 (23.30–57.64)	0.665
aMT6 (µg/day)	93.27 (65.93–128.37)	126.46 (104.13–189.94)	**0.046**
IL-1 (pg/mL)	45.65 (35.28–72.89)	32.87 (29.14–40.71)	**0.006**
IL-6 (pg/mL)	67.54 (58.23–68.95)	75.80 (58.03–100.88)	0.525
CRP (mg/L)	167.25 (69.50–207.65)	144.50 (107.15–214.80)	0.892
POD 5			
Tryptophan (µg/mL)	23.89 (23.53–32.47)	29.27 (23.34–53.52)	0.285
aMT6 (µg/day)	108.51 (67.46–127.49)	144.32 (94.48–179.29)	**0.038**
IL-1 (pg/mL)	36.60 (34.40–40.42)	35.08 (28.54–40.68)	0.185
IL-6 (pg/mL)	65.04 (61.46–79.73)	64.64 (57.18–110.04)	0.957
CRP (mg/L)	67.05 (40.10–109.05)	60.30 (35.45–93.95)	0.534

Statistically significant values are indicated in bold.

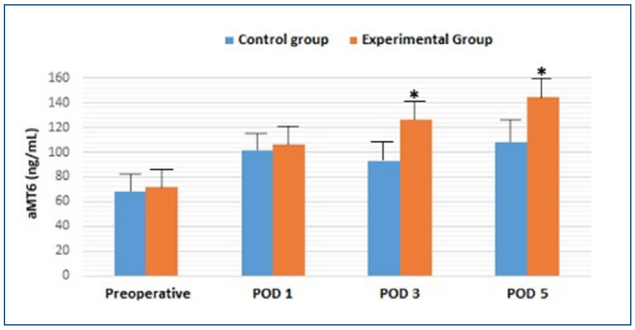

Figure 1. The effects of parenteral nutrition on plasma 6-sulfatoxymelatonin levels after major abdominal surgery. *p<0.05 versus experimental group.

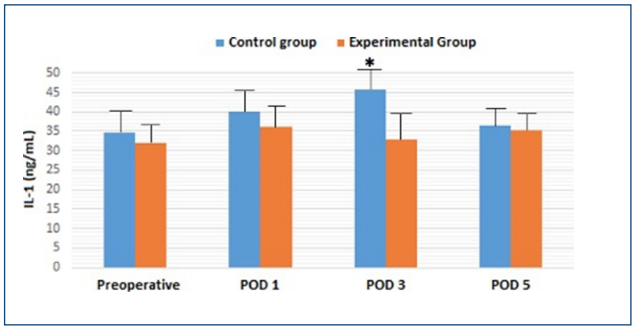

Figure 2. The effects of parenteral nutrition on plasma interleukin-1 levels after major abdominal surgery. *p<0.05 versus experimental group.

induce the degradation of tryptophan in the treatment group. The CRP levels were less in the treatment group, and the difference was apparent 3 days after the surgery but statistically not different. This could be explained by the complex balance between the secretions of melatonin and IL-6. Probably, even if the difference between groups in the melatonin levels reached the highest on PODs 3 and 5, the level of IL-6 in the treatment group was greater than in the control group on POD 3 as well. Thus, probably, the net effect led to an insignificant difference in the levels of CRP.

The study has several limitations which should be reviewed. Our small sample size limited the power of the statistical analysis. Another limitation was the complexity of the relationship between tryptophan, melatonin, and immune response. Supplementation of peripheral protein, glucose, and lipids has effects on immune response and might have interfered with our results. Therefore, treatment with tryptophan after surgery would be more informative about the relationship between tryptophan, melatonin, and postoperative immune response. Furthermore, we believe that still there might be some unknown interactions. Thus, more investigations should be conducted to clarify these complex interactions.

AUTHORS' CONTRIBUTIONS

NFY: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **MS:** Conceptualization, Data curation, Investigation, Resources, Validation, Writing – original draft, Writing – review & editing. **FK:** Data curation, Methodology, Software, Visualization, Writing – review & editing. BB: Formal Analysis, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing. **SÖ:** Formal Analysis, Methodology, Software, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationships between internet addiction, smartphone addiction, sleep quality, and academic performance among high-school students

Yasin Guclu[1], Ozge Aydin Guclu[2*], Hakan Demirci[3]

SUMMARY

OBJECTIVE: In this study, we aimed to evaluate the relationships between Internet addiction, smartphone addiction, sleep quality, and academic success.
METHODS: In this cross-sectional study, high-school students were surveyed to evaluate sleep quality, Internet addiction, and smartphone addiction. Students were queried about their demographics, and grade averages from the previous term were taken as an indicator of academic success.
RESULTS: A total of 1,959 students were enrolled in this study, with 1,034 (52.8%) girls and 925 (47.2%) boys, and the median age of the participants was 16 (13–21) years. Multivariate analyses found that poor sleep quality in students who did not have breakfast before going to school was 1.58 times higher than those who did ($p<0.001$). Students who stayed in a dormitory had 1.79 times more poor sleep quality than those who stayed with their family, and a one-unit increase in the total score of the Young's Internet Addiction Test short form resulted in a 1.08-fold increase (both, $p<0.001$).
CONCLUSION: Our study has shown that students' sleep quality was predicted to be lower if they stayed in a dormitory and skipped breakfast. In addition, Internet and smartphone addictions have a negative effect on sleep quality and academic performance.
KEYWORDS: Academic performance. Cross-sectional study. Students. Internet addiction. Sleep quality. Smartphone addiction.

INTRODUCTION

Having entered people's lives in the mid-1980s, the Internet gained rapid popularity and became a focus of attention among all age groups. In the past few years, there has been a remarkable expansion in the number of Internet users. Internet, which has become an important source of information, particularly for university students and adolescents, may lead to addiction in the said populations[1]. The Global Digital Report 2023 states that, of the world's more than 8.01 billion people, 5.16 billion (64.4%) use the Internet and 5.44 billion (68%) use mobile phones[2]. According to the household information technologies usage survey results, it was determined that 95.5% of households in Turkey have access to the Internet from home in 2023[3]. In broad terms, Internet addiction can be characterized by the inability to overcome the urge toward excessive usage; the time spent offline losing its significance; irritability and aggressiveness in its absence; and the gradual impairment of one's occupational, social, and family lives[1,4]. School is a place of learning for adolescents. It not only plays an important role in education but also creates an environment for health promotion among young people.

Smartphones enhance daily lives by providing diverse social statuses and identities for younger people, compensating for shortcomings in social life. Adolescence is a critical period with biological, social, and psychological changes, leading to potential problems, making assessing smartphone addiction urgent. According to the Information Technologies Usage Research in Children 2021, 64.4% of children aged 6–15 years in Turkey used smartphones in 2021, with boys accounting for 65.7% and girls accounting for 63.0%[5].

Sleep is a crucial daily activity affecting individuals' quality of life and health, with physiological, psychological, and social dimensions. Adolescents need adequate sleep to regulate developmental functions and develop new skills, insights, and expectations. Sleep deprivation can increase daytime sleepiness and carelessness.

In this study, we aimed to evaluate the relationship between Internet and smartphone addictions, sleep quality, and academic success.

METHODS

In line with the permission received from the Boyabat District Directorate of National Education and with the board of ethics approval given by the Clinical Research Ethics Board of

[1]Soğanlı Family Health Center, Department of Family Medicine – Bursa, Turkey.
[2]Bursa Uludağ University, Faculty of Medicine, Department of Pulmonary Disease – Bursa, Turkey.
[3]Bursa Yuksek Ihtisas Training and Research Hospital, Department of Family Medicine – Bursa, Turkey.
*Corresponding author: drozgeaydinguclu@gmail.com
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Sinop University, a cross-sectional survey study was planned for February and March 2019. The survey comprised students from eight high schools in Boyabat, a rural region of Sinop province. Totally answering all questions in the surveys was regarded as satisfactorily completed.

Participants

High-school students were surveyed to evaluate sleep quality, Internet addiction, and smartphone addiction. Students were queried about their demographics. Grade averages from the previous term were taken as an indicator of academic success. All of the questionnaires were filled out anonymously and on a voluntary basis, after the aim and scope of the study were explained to the students and were received from school administrations upon completion.

Procedure

Pittsburgh Sleep Quality Index (PSQI)

The PSQI uses Likert and open-ended responses to assess sleep quality during the previous month. The PSQI measures subjective sleep quality, sleep latency, sleep length, habitual sleep efficiency, sleep disruptions, sleep medication, and daytime dysfunction. A total score of 0–21 is calculated by adding component values of 0–3. A global PSQI score over 5 indicates poor sleep and higher scores indicate poorer sleep quality. The reliability and validity of the Turkish version of PSQI were shown[6].

Young's Internet Addiction Test short form (YIAT-SF)

Developed by Young[1] and shortened by Pawlikowski and friends[7], YIAT-SF is a 5-point Likert (1=Never, 5=Very Frequent) type measure that consists of 12 questions. High scores on this measure show high levels of Internet addiction. The reliability and validity of the Turkish version were shown[8].

Smart-phone Addiction Survey—Short Version (SAS-SV)

Consisting of 10 questions assessed by a 6-point Likert scale, SAS-SV was developed by Kwon et al., to measure the risk of smartphone addiction among adolescents[9]. Each item is rated on a scale ranging from 1 to 6. The overall score on the survey ranges from 10 to 60, with higher scores suggesting a greater risk of addiction. It is a single-factor survey without subscales. Cutoff scores are determined to be 31 for men and 33 for women. SAS-SV's Turkish version was found to be reliable and valid[10].

Data analysis

The Shapiro-Wilk test was used to evaluate the normality of variable distribution. When continuous variables are normally distributed, they are expressed as mean and standard deviation. When they are not normally distributed, they are presented as median (min–max). Categorical variables were shown as n (%). According to normality test results, the Mann-Whitney U test was used for comparisons made between two groups and the Kruskal-Wallis test was used if more than two groups existed. To compare categorical variables, the Pearson chi-square test was performed. Correlation analysis was applied to investigate the relationships between continuous variables, and Spearman's correlation coefficient was calculated. A backward stepwise conditional logistic regression was performed to determine independent predictors of students' poor sleep quality. To measure model fit, Hosmer-Lemeshow goodness-of-fit statistics were used. The SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) software was used for statistical analysis, with $p<0.05$ taken as statistically significant.

RESULTS

In accordance with the number of students enrolled at the high schools identified by the Boyabat District Directorate of National Education, 2,464 surveys were distributed to schools. A total of 1,959 students from eight schools in the district of Boyabat with 1,034 (52.8%) girls and 925 (47.2%) boys were enrolled in this study. Of the surveys distributed to schools, 79.5% were satisfactorily completed. The median age of the participants was 16 (13–21) years. The demographics of the students are presented in Table 1. Of the students, 9.08% (n=178) did not own smartphones.

Assessment of factors related to grade averages showed that female students had higher grade averages than male students (78.0 [30.0–98.5] vs 70.0 [25.0–98.6], $p<0.001$); there was no difference between grade averages of students whose parents were together and of those whose parents were separated (74.0 [25.0–98.9] vs 72 [37–97], $p<0.107$); students who had breakfast in the mornings had higher averages than those who had not (75.0 [32.0–98.9] vs 72.0 [25.0–98.0], $p<0.001$); and students who had a room of their own had higher grade averages than those who had not (73.0 [35.0–98.0] vs 75.0 [25.0–98.9], $p<0.001$), while students who stayed in a dormitory had higher grade averages than students who stayed with their families (80.0 [35.0–98.9] vs 72.0 [25.0–98.5], $p<0.001$). Significant differences were found when the students' grade averages were compared with their parents' education levels, occupations, income levels, and study years ($p<0.001$ for each).

In terms of gender, no significant difference was found in YIAT-SF total scores (girls 26 [12–60]; boys 26 [12–55]; $p<0.618$).

Table 1. Socio-demographic characteristics of high-school students.

Age (years)	n=1959 16 [13–21]
Sex (girl)	1034 (52.8%)
Year of study	
Year 9	515 (26.3%)
Year 10	549 (28%)
Year 11	490 (25%)
Year 12	405 (20.7%)
Mother's level of education	
Illiterate	66 (3.4%)
Primary	1182 (60.3%)
Secondary	419 (29.4%)
High school	227(11.6%)
University	65(3.3%)
Father's level of education	
Illiterate	12 (0.6%)
Primary	823 (42%)
Secondary	508 (25.9%)
High school	61 (34.9%)
University	29 (16.6%)
Mother's occupation	
Housewife	1643 (83.9%)
Worker	221 (11.3%)
Farmer	9 (0.5%)
Retired	8 (0.4%)
Tradesman	16 (0.8%)
Civil servant	62 (3.2%)
Father's occupation	
Unemployed	64 (3.3%)
Worker	870 (44.4%)
Farmer	268 (13.7%)
Retired	123 (6.3%)
Tradesman	377 (19.3%)
Civil servant	257 (13.1%)
Marital status of parents	
Married	1849 (94.4%)
Separated	110 (5.6%)
Income level	
<2000	160 (8.2%)
2000–3000	780 (39.8%)
3000–4000	757 (38.6%)
>4000	261 (13.3%)
BMI (kg/m^2)	21.05 ± 3.19
Breakfast (yes)	1311 (66.9%)
Own room (yes)	1230 (62.8%)
Commute to school	
By a vehicle	803 (41%)
Walking	1156 (59%)
Resides	
With family	1460 (74.5%)
In a dormitory	499 (25.5%)
Previous term grade average	72.8 ± 14.11
PSQI total score	6 [0–19]
YIAT-SF total score	26 [12–60]
SAS-SV total score	23 [10–60]

Continuous variables with normal distribution were presented as mean ± SD; non-normal variables were reported as median [min.: max.]. Categorical variables were shown as n (%). BMI: body mass index, PSQI: Pittsburgh Sleep Quality Index, YIAT-SF: Young's Internet Addiction Test Short Form, SAS-SV: Smart-phone Addiction Survey—Short Version.

With the SAS-SV cutoff score taken to be 31 for men and 33 for women, 27.2% (n=281) of the girls, 28% (n=258) of the boys, and 27.5% (n=539) of all students were found to have smartphone addiction. In terms of smartphone addiction, there was no significant difference between the two sexes (p=0.685).

The end-of-term grade averages of the students were found to be positively correlated with age and BMI values (r=0.200, p<0.001 and r=0.050, p=0.029, respectively) and negatively correlated with PSQI, YIAT-SF, and SAS-SV total scores (r=0.062, p=0.006; r=0.083, p<0.001; and r=0.072, p<0.002, respectively). Assessing the relationship between quality of sleep and Internet and smartphone addiction, it was found that the PSQI total scores were positively correlated with the YIAT-SF and the SAS-SV total scores (r=0.362, p<0.001 and r=0.291, p<0.001, respectively). A positive correlation was found between the total scores of YIAT-SF and SAS-SV (r=0.707, p<0.001) (Table 2).

With a PSQI score of 5 or higher indicating poor sleep quality, it was found that 920 (47%) students had good and 1,039 (53%) students had bad sleep quality. Evaluating the factors that interact with poor sleep quality revealed that students with poor quality of sleep had lower grade averages (p=0.004) along with higher YIAT-SF total score and SAS-SV total score (p<0.001, for each) (Table 3).

Multivariate analyses evaluated independent factors contributing to an increased risk of poor sleep quality. Poor sleep quality was 1.58 times higher in students who skipped breakfast (OR: 1.58, CI: 1.28–1.95, p<0.001). Being a year 10 student significantly increased poor sleep quality risk by 1.48-fold compared with being a year nine student (OR: 1.48, CI: 1.14–1.93, p=0.003), while year 11 students led to a 1.88-fold increase, and year 12 students led to a 1.98-fold increase (p<0.001 for each). Students who live in a dorm had 1.79 times the poor sleep quality of students who live with their families (OR: 1.79, CI: 1.42–2.26, p<0.001), and finally a one-unit increase in the total score of the YIAT-SF results in a 1.08-fold increase in poor sleep quality (OR: 1.08, CI: 1.06–1.09, p<0.001) (Table 4).

Table 2. Correlation analysis of the factors associated with grade point averages.

Factor	r	p
Age, years	0.200	<0.001
BMI (kg/m^2)	0.050	0.029
PSQI	-0.062	0.006
YIAT-SF total score	-0.083	<0.001
SAS-SV total score	-0.072	0.002

BMI: body mass index; PSQI: Pittsburgh Sleep Quality Index; YIAT-SF: Young's Internet Addiction Test Short Form; SAS-SV: Smart-phone Addiction Survey—Short Version.

Table 3. Factors related to sleep quality.

	Sleep quality		p
	Poor (n=1039)	Good (n=920)	
Age (years)	16 [13-20]	16 [13-21]	<0.001[a]
Sex (girl)	563 (54.2%)	471 (51.2%)	0.189[c]
Year of study			
Year 9	221 (21.3%)	294 (32%)	<0.001[c]
Year 10	305 (29.4%)	224 (26.5%)	
Year 11	286 (27.5%)	204 (22.2%)	
Year 12	227 (21.8%)	178 (19.3%)	
Marital status of parents			
Married	977 (94%)	872 (94.8%)	0.472[c]
Separated	62 (6%)	48 (5.2%)	
BMI (kg/m^2)	20.6 [14.5–36.2]	20.5 [14.4–36.2]	0.677[a]
Breakfast (yes)	636 (61.2%)	675 (73.4%)	<0.001[c]
Own room (yes)	654 (62.9%)	576 (62.6%)	0.888[c]
Resides			
With family	740 (71.2%)	720 (78.3%)	<0.001[c]
In a dormitory	299 (28.8%)	200 (21.7%)	
Previous term grade average	74 [25–98.9]	75 [32–98.5]	0.004[a]
YIAT-SF total score	29 [12–56]	23 [12–56]	<0.001[a]
SAS-SV total score	27 [10–60]	20 [10–58]	<0.001[a]

Continuous variables with non-normal variables were reported as median [min.: max.]. Categorical variables were shown as n (%). [a]Mann-Whitney U test. [c]Chi-square test. BMI: body mass index; YIAT-SF: Young's Internet Addiction Test Short Form; SAS-SV: Smart-phone Addiction Survey—Short Version.

Table 4. Factors predicting poor sleep quality.

	Univariate			Multivariate		
	OR	95%CI	p	OR	95%CI	p
Age, years	1.20	1.11–1.29	<0.001	–	–	–
Sex (girl)	1.12	0.94–1.35	0.186	–	–	–
Year of study (Ref. year 9)				–	–	–
Year 10	1.66	1.30–2.11	<0.001	1.48	1.14–1.93	0.003
Year 11	1.70	1.30–2.21	<0.001	1.88	1.43–2.48	<0.001
Year 12	1.86	1.45–2.39	<0.001	1.98	1.47–2.68	<0.001
Breakfast (Ref. yes)	1.75	1.44–2.11	<0.001	1.58	1.28–1.95	<0.001
Resides (Ref. with family)	1.45	1.18–1.78	<0.001	1.79	1.42–2.26	<0.001
Previous term grade average	0.99	0.98–0.99	<0.001	–	–	–
YIAT-SF total score	1.08	1.06–1.09	<0.001	1.08	1.06–1.09	<0.001
SAS-SV total score	1.04	1.03–1.05	<0.001	–	–	–

YIAT-SF: Young's Internet Addiction Test Short Form; SAS-SV: Smart-phone Addiction Survey—Short Version.

DISCUSSION

Aiming to evaluate the relationship between sleep quality and academic success to Internet and smartphone addiction among high-school students, our study found that the risk of poor sleep quality went up 1.6 times for students who did not have breakfast and 1.8 times for students who lived in a dormitory. Compared with year 9 students, year 10 students were found to increase the risk of poor sleep quality by 1.48 times, year 11 students by 1.88 times, and year 12 students by 1.98 times.

There was no difference between the sexes in terms of sleep quality. Arbinaga et al., have shown that poor sleep quality is more common by a factor of 1.52 among females[11]. In our study, it was determined that the average Internet and smartphone addiction survey scores of students with poor sleep quality were higher and their grade point averages were lower. Barahona et al., have found that, in general, students with poor quality of sleep perform poorly in academics[12]. In their study, Maheshwari et al., have shown 64.2% of participating students to be poor sleepers and that poor sleep negatively influenced academic performance[13]. Given that students in rural locations have limited access to the Internet and health information, guidance from health professionals can assist them in developing healthy habits[14]. When the relationship between quality of sleep and Internet and smartphone addictions was assessed, PSQI total scores were found to be positively correlated with YIAT-SF and SAS-SV total scores. In a study conducted by Chen YL et al., it was found that adolescents who had difficulties falling asleep or maintaining sleep were more susceptible to Internet addiction[15]. Our study has found a strong correlation between YIAT-SF and SAS-SV total scores. Similarly, in the study conducted by Tateno et al., a positive correlation was observed between YIAT-SF and SAS-SV[16].

Significant differences in sleep quality and grade averages were observed between years of study. Female and senior-year students had higher grade averages. No difference was found between students with married or separated parents, as Brand et al., found no impact on children's education[17].

Our study has found that the students who have breakfast as opposed to those who do not and the students who have a room of their own as opposed to those who do not have higher grade averages. Meanwhile, in comparison with students staying with their own families, those students who stayed in a dormitory had higher grade averages. In a study by Araujo et al., the proximity of the dormitory to the school campus was suggested to have a positive impact on the academic success of the students[18]. While Soheilipour et al.[19] found no significant relationship between having breakfast and academic success, Burns et al.[20] have reported that the academic success of students who regularly had breakfast improved by a factor of 1.72.

Our study found that higher-income students perform better academically, with differences in grade averages influenced by parents' education, occupations, and study year. Benner et al., have shown that children from families with higher socio-economic status perform better academic performance[21]. Similarly, Duan et al., have established that parents' low socio-economic status has a negative impact on the child's academic performance and socialization[22]. End-of-term grade averages of students were found to be positively correlated with their age and BMI while negatively correlated with PSQI, YIAT-SF, and SAS-SV total scores. A study of adolescents conducted in Korea has shown that students who perform well academically are less inclined toward Internet addiction[23].

The study found no significant difference in smartphone addiction between male and female students. Students with addiction had higher YIAT-SF total scores and lower grade averages. Tateno et al., found smartphone addiction more prevalent among female students, with rates of 28% for girls and 22.8% for boys[16]. In another study, Kwon et al., have also shown smartphone addiction to be more prevalent among female students[9].

In terms of the degree of Internet addiction, our study found no significant difference between the two sexes. In their study, Shek et al., found that Internet addiction negatively impacts academic performance[24], while Haroon et al., found it more prevalent among girls[25]. Other studies on the subject have revealed male dominance in Internet addiction[26,27]. Young et al., suggested that men use the Internet more frequently, which may explain the higher prevalence of Internet addiction among men[1].

Our study has certain limitations. The participants consisted of Turkish adolescents; hence, the results cannot be generalized to other populations. As our study has made use of subjective tests, it might have led to response bias.

CONCLUSION

Our study has shown that students' sleep quality was predicted to be lower if they stayed in a dormitory and did not have breakfast. In addition, Internet and smartphone addictions had a negative effect on sleep quality and academic performance. In this respect, it is important to raise the awareness of parents and teachers who regularly interact with teenagers so that they can take the necessary steps to address increased Internet and smartphone usage during adolescence and the addiction that comes with it.

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AUTHORS' CONTRIBUTIONS

YG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **OAG:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **HD:** 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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Is polycystic ovary syndrome a risk factor for depression and anxiety?: a cross-sectional study

Osman Samet Gunkaya[1*], Arzu Bilge Tekin[1], Ayşegül Bestel[2], Oguz Arslan[1], Fatih Şahin[3], Bilge Dogan Taymur[1], Niyazi Tuğ[1]

SUMMARY

OBJECTIVE: The objective of this study was to learn more about the prevalence and pathophysiology of depression and anxiety that may be caused by polycystic ovary syndrome and to make plans for taking necessary precautions for this vulnerable group.

METHODS: This case-control study was conducted between January 2022 and October 2022. A total of 120 women with polycystic ovary syndrome and 143 controls were included in the study. All healthy volunteers and women with polycystic ovary syndrome were evaluated using self-administered questionnaires and physical examination. Anthropometric data such as weight and height and laboratory value were documented.

RESULTS: There was no significant difference between the groups in terms of demographic characteristics. When the Hospital Anxiety and Depression Scale scores of both groups were compared, both depression and anxiety scores were found to be significantly higher in women with polycystic ovary syndrome compared with the control group (OR: 3.319, 95%CI, 1.563–7.047, p<0.001 and OR: 3.238, 95%CI, 1.659–6.315, p<0.001). In the Hospital Anxiety and Depression Scale questionnaire, the rate of irregular menstruation and Ferriman-Gallwey score were statistically significant in women with polycystic ovary syndrome with high depression and anxiety scores. While serum LH levels and LH/FSH ratios were significantly different in women with polycystic ovary syndrome with high depression scores, serum LH, LH:FSH ratios, and serum total testosterone levels were found significant in women with polycystic ovary syndrome with high anxiety scores.

CONCLUSION: It is clear that depression and anxiety are more common in patients with polycystic ovary syndrome than in healthy women. Our findings support previous recommendations regarding routine screening for depression and anxiety in this population.

KEYWORDS: Depression. Anxiety. Polycystic ovary syndrome. Hyperandrogenism.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a chronic gynecological disease with endocrine and metabolic components that affects approximately 5–20% of women of childbearing age[1]. The characteristic clinical features are menstrual irregularities, hirsutism, polycystic ovaries on ultrasound, and clinical or laboratory findings of androgen increase[1,2]. Women with PCOS are at risk for metabolic, cardiovascular, and many systemic diseases (diabetes, dyslipidemia, hypertension, metabolic syndrome, obstructive sleep apnea, pregnancy complications, and endometrial cancer), especially obesity[3]. Although these endocrine and metabolic results increase the incidence of psychiatric disorders such as depression and anxiety in women with PCOS, the underlying mechanisms are not fully understood[4,5]. Chronic illness is a known risk factor for depression, but less is known about the interaction between chronic illness and anxiety[6]. Additionally, although a significant link between PCOS and sexual dysfunction has not been proven, some studies have reported that most women with PCOS experience more stress than healthy women due to infertility concerns and dissatisfaction with their body image[7,8]. Furthermore, whether treated or not, these women's phenotype and clinical course are of great concern[9].

Androgen excess is found in almost 85% of women with PCOS, indicating that this syndrome is the main cause of hirsutism[10,11]. It has been observed that women with PCOS who experience emotional distress exhibit clinical symptoms of alopecia, acne, hirsutism, and infertility[12]. Moreover, the neuroendocrine feature of PCOS is sustained rapid LH (GnRH) pulsatility; this increases the pituitary synthesis of LH over that of FSH, resulting in increased LH concentrations and increased PCOS-specific LH:FSH ratios. Low levels of FSH contribute to ovulatory dysfunction, while high levels of LH increase ovarian androgen production[13]. Increased blood testosterone levels

[1]University of Health Sciences Turkey, Sehit Prof. Dr. Ilhan Varank Sancaktepe Training and Research Hospital, Department of Obstetrics and Gynecology – İstanbul, Turkey.
[2]University of Health Sciences Turkey, Kanuni Sultan Suleyman Training and Research Hospital, Clinic of Obstetrics and Gynecology – İstanbul, Turkey.
[3]University of Health Sciences Turkey, Istanbul Prof.Dr. Cemil Taşcioğlu City Hospital, Department of Obstetrics and Gynecology – İstanbul, Turkey.
*Corresponding author: dr.sametgunkaya@hotmail.com
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have also been associated as a biochemical factor in increased depression and anxiety in PCOS[13].

The aim of this study was to learn more about the prevalence of depression and anxiety that can be caused by PCOS and the impact of physical and biochemical changes on depression and anxiety in PCOS women and to make plans to take the necessary precautions for this sensitive group.

METHODS

We conducted this case-control study on volunteers who applied to the gynecology department at a tertiary medical center between January 2022 and October 2022. The study was conducted with the institutional ethics committee (E-46059653-020). It complies with the Declaration of Helsinki. All patients confirmed written informed consent before they were enrolled in the study. The diagnosis of PCOS was made using the Rotterdam criteria[2]. Briefly, the diagnosis of PCOS required hyperandrogenic symptoms (hirsutism, acne, and androgenic alopecia) and oligomenorrhea (menstrual cycle more than 35 days apart)/amenorrhea (absence of menstrual cycle for at least 6 months), as well as biochemical hyperandrogenemia (serum total testosterone level.0.77 ng/mL) and polycystic ovaries (12 follicles [2–9 mm in size] per ovary by transvaginal ultrasonography or an ovarian volume >10 mL per ovary by transabdominal ultrasound with distended bladder for virgin women)[2]. The Ferriman-Gallwey grading system was used to assess the presence of hirsutism[10]. None of the women have been prescribed medication for symptoms prior to enrollment. As a control group, the healthy volunteers, not diagnosed with PCOS, who came to the same facility for routine health check-ups, were invited to participate in this study. Exclusion criteria were treatment of depression and anxiety, other endocrine, systemic abnormalities, and use of oral contraceptives and drugs known to affect the hypothalamic-pituitary-ovarian system. All healthy volunteers and women with PCOS were evaluated using self-administered questionnaires (Hospital Anxiety and Depression Scale (HADS)), face-to-face interviews with a gynecologist, and physical examination. All participants in the control group had regular ovulatory cycles (mean cycle length 25–32 days) and showed no clinical or biochemical signs of hyperandrogenism. Anthropometric data such as weight and height were measured by a qualified nurse. To evaluate laboratory findings, blood samples were taken from women with PCOS on the third day of the spontaneous or induced menstrual cycle, in the early follicular phase.

The HADS questionnaire was used to measure depression and anxiety. The HADS is a validated questionnaire consisting of 14 questions, with 7 questions to assess anxiety and 7 questions to assess depressive symptoms. The HADS score of 11 or higher was considered abnormal[14]. The women with high HADS scores were referred to the psychiatry department.

Statistical analysis was performed using SPSS, version 21. The conformity of the variables to the normal distribution was evaluated with the Shapiro-Wilk test, Q-Q plot, and histogram graphics. Continuous variables were presented as mean±standard deviation, median (quartiles 25–75), or (minimum–maximum). Categorical data were presented as frequency (percentage). For analyzing continuous data, the Mann-Whitney U test was used. Analyzing categorical variables, the chi-square test was employed. The significance level was defined as a p-value less than 0.05.

RESULTS

A total of 120 women with PCOS and 143 controls were included in the research. The flow diagram is shown in Figure 1. In this study, the prevalence of anxiety and depression behaviors in women with PCOS was 27.5 and 21.7%, respectively. When women with PCOS were evaluated within themselves, although there was no difference between the groups in terms of BMI, menstrual irregularity and high Ferimann-Gallwey scores were related to high depression and anxiety scores. In addition, serum LH levels, LH:FSH ratios, and serum total testosterone levels were found to be statistically significant in women with PCOS diagnosis with high anxiety scores.

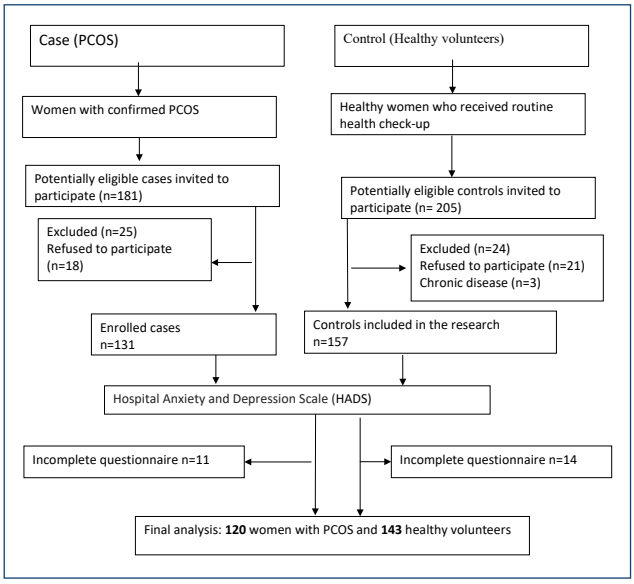

Figure 1. Flow diagram.

There was no significant difference between women with PCOS and the control group in terms of demographic characteristics (Table 1). When the HADS scores of both groups were compared, both depression and anxiety scores were found to be significantly higher in women with PCOS compared with the control group (Table 1) OR: 3.319, 95%CI, 1.563–7.047, p<0.001 and OR: 3.238, 95%CI, 1.659–6.315, p<0.001, respectively. Women with PCOS who had a high depression score (HADS≥11) in the HADS questionnaire had a statistically significant difference in the rate of irregular menstruation (76.9%, n=20, p< 0.001) and Ferriman-Gallwey scores (9.5±6.0 median±IQR, p=0.014) when compared with those with low depression scores in terms of clinical features. Other clinical parameters are detailed in Table 2. The statistical comparison of women with PCOS with a high depression score (HADS≥11) and women with PCOS with a low depression score in terms of laboratory results is shown in Table 2. Women with PCOS who had higher anxiety scores on the HADS questionnaire (HADS≥11) had statistically significant differences in menstrual irregularity rate (84.8%, n=28, p<0.001) and Ferriman-Gallwey score 9.0 (6.0; median (IQR), p<0.001). There was a difference compared with those with low scores for clinical features of anxiety. Other clinical parameters are detailed in Table 3. When women with PCOS with high anxiety scores (HADS≥11) and women with PCOS with low anxiety scores were compared statistically in terms of laboratory results, serum LH, LH:FSH ratio, and serum total testosterone levels were found to be significant (Table 3).

DISCUSSION

This study showed that the prevalence of anxiety and depression behaviors was more common in women diagnosed with PCOS than in women without PCOS. When women with PCOS were evaluated within themselves, although there was no difference between the groups in terms of BMI, it was observed that menstrual irregularity and high Ferimann-Gallwey scores were associated with high depression and anxiety scores. Furthermore, blood LH levels, LH:FSH ratios, and total testosterone levels were higher in PCOS-diagnosed women with high anxiety scores.

Anxiety and depression are among the most common mental disorders[15,16]. Anxiety disorders are a group of mental disorders characterized by anxiety and fear, while depressive disorders are characterized by sadness, loss of interest and happiness, feelings of guilt and low self-esteem, and difficulty sleeping and concentrating[15]. In women, the global

Table 1. Demographic characteristics and Hospital Anxiety and Depression Scale of women with polycystic ovary syndrome and controls.

	PCOS (n=120)	Controls (n=143)	p		
Age (years) mean±SD	26.1±5.8 (16–44)	25.1±5.8 (16–44)	0.173[1]		
BMI (kg/m^2) mean±SD	26.4±4.9 (17.4–45.8)	26.0±4.3 (17.3–33.8)	0.435[1]		
Gravity median (IQR)	0 (1) (0–6)	0 (1) (0–6)	0.989[2]		
Parity median (IQR)	0 (1) (0–4)	0 (1) (0–4)			
Type of delivery			0.871[2]		
Nulliparty, n (%)	78 (65)	91 (63.6)			
Vaginal birth, n (%)	23 (19.2)	31 (21.7)			
Cesarean, n (%)	19 (15.8)	21 (14.7)			
HADS scale	**PCOS (n=120)**	**Controls (n=143)**	**95%CI**	**OR**	**p**
Depression Scale (HADS≥11)(%)	26 (21.7)	11 (7.7)	1.563 to 7.047	3.319	**0.001[2]**
Anxiety Scale (HADS≥11)(%)	33 (27.5)	15 (10.5)	1.659 to 6.315	3.238	**0.001[2]**

[1]Independent-samples t-test. [2]Chi-square tests. Categorical variables were shown as n% and continuous variables were shown as mean±SD or median (IQR) with regard to distribution characteristics. OR: odds ratio; BMI: body mass index; PCOS: polycystic ovary syndrome; HADS: Hospital Anxiety and Depression Scale. Bold indicates statistically significant p-values.

Table 2. Clinical and laboratory characteristics of women with polycystic ovary syndrome with depression (HADS≥11) and without depression (HADS<11).

	Depression (HADS≥11) (n=26)	Without depression (HADS<11) (n=94)	p
Age (years) median±IQR	27.0±9.0 (18–39)	25.0±6.0 (16–44)	0.745[1]
BMI (kg/m^2) median±IQR	25.2±9.2 (17.4–38.9)	26.1± 7.3(17.9–45.8)	0.863[1]
Oligo/amenore (%)	19 (73.1)	64 (68.1)	0.626[2]
Irregular menstruation (%)	20 (76.9)	47 (50.0)	**0.001[2]**
Ferriman-Gallwey score median±IQR	9.5±6.0 (5–18)	9.0 ± 5.0 (0–16)	**<0.014[1]**
FSH median±IQR	5.6±(2.1) (3.4–9.8)	5.6±(1.7) (1.2–9.9)	0.977[1]
LH median±IQR	11.3±(7.5) (6.7–21.0)	7.2±(4.3) (1.4–21.0)	**<0.001[1]**
LH:FSH median±IQR	2.3±(1.1) (0.7–5.5)	1.3±(0.8) (0.4–4.9)	**<0.001[1]**
DHEA-SO$_4$ median±IQR	209±(64.3) (70.4–357)	229.5±(99.3) (55.8–412)	0.424[1]
Prolactin median±IQR	15.2±(8.6) (7.6–45)	15.4±(11.8) (1.0–55.9)	0.990[1]
Total testosterone median±IQR	0.3±(0.2) (0.2–0.7)	0.3±(0.2) (0.1–0.9)	0.433[1]
TSH median±IQR	1.7±(1.2) (0.7–6.5)	1.8±(1.5) (0.3–5.5)	0.541[1]
Estradiol (E$_2$) median±IQR	45.7±(20.5) (26–68)	45.9±(25.5) (11–170)	0.967[1]

[1]Mann-Whitney U test. [2]Chi-square test. BMI: body mass index; FSH: follicle-stimulating hormone; LH: follicle-stimulating hormone; LH/FSH: the ratio of luteinizing hormone to follicle-stimulating hormone; DHEA-SO$_4$: dehydroepiandrosterone sulfate; TSH: thyroid-stimulating hormone; HADS: Hospital Anxiety and Depression Scale. Bold indicates statistically significant p-values.

Table 3. Clinical characteristics of women with polycystic ovary syndrome with anxiety (HADS≥11) and without anxiety (HADS<11).

	(Anxiety HADS≥11) (n=33)	Without anxiety (HADS<11) (n=87)	p
Age (years) median±IQR	27.0±9.0 (18–40)	25.0±5.0 (16–44)	0.128[1]
BMI (kg/m^2) median±IQR	24.9±8.6 (17.4–35.9)	26.2±7.3 (17.9–45.8)	0.472[1]
Oligo/amenore (%)	25 (75.8)	58 (66.7)	0.336[2]
Irregular menstruation (%)	28 (84.8)	39 (44.8)	**<0.001[2]**
Ferriman-Gallwey score median±IQR	9.0±6.0 (5–18)	7.0±5.0 (0–17)	**<0.001[1]**
FSH median±IQR	5.7±(1.8) (2.2–9.9)	5.6±(1.8) (1.2–10)	0.892[1]
LH median±IQR	10.8±(7.3) (4.7–21)	7.3±(4.0) (1.4–17.0)	**0.001[1]**
LH:FSH median±IQR	2.1±(1.0) (0.7–5.5)	1.3±(0.6) (0.4–3.5)	**0.003[1]**
DHEA-SO4 median±IQR	230±(87.5) (70.4–412)	220±(87.0) (55.8–405)	0.711[1]
Prolactin median±IQR	18.6±(13.7) (7.6–55.6)	14.9±(10.8) (1.0–56.9)	0.109[1]
Total testosterone median±IQR	0.4±(0.3) (0.2–0.9)	0.3±(0.2)(0.1–0.7)	**0.040[1]**
TSH median±IQR	1.8±(1.2) (0.7–4.7)	1.8±(1.5) (0.3–6.5)	0.981[1]
Estradiol (E$_2$) median±IQR	44.0±(19.5) (26–106)	48.0±(25.3) (11–170)	0.486[1]

[1]Mann-Whitney U test. [2]Chi-square test. BMI: body mass index; FSH: follicle-stimulating hormone; LH: follicle-stimulating hormone; LH/FSH: the ratio of luteinizing hormone to follicle-stimulating hormone; DHEA-SO$_4$: dehydroepiandrosterone sulfate; TSH: thyroid-stimulating hormone; HADS: Hospital Anxiety and Depression Scale. Bold indicates statistically significant p-values.

incidence of depression and anxiety is 5.1 and 4.6%, respectively. In addition, WHO data indicated that the prevalence of anxiety and depression in women aged 20–40 years was 5–6% and 6–7%, respectively[15]. Since the prevalence of anxiety and depression symptoms is high and the patients are benefitted from treatments very likely, routine screening was recommended[4]. In our study, the rates of high scores of anxiety and depression in all women with PCOS were 21.7

and 27.5%, respectively, and this result was similar to various studies that have reported a higher prevalence of anxiety and depression symptoms in women with PCOS than in healthy women[4]. Cooney et al., reported increased odds for depression and anxiety symptoms when the BMI was matched for PCOS and control groups[4]. Hollinrake et al., stated that obesity alone cannot explain the high risk of depression in PCOS[15]. Similarly, in this study, there was no difference in BMI values for the two study groups, and high depression and anxiety scores were recorded for the PCOS group. Irregular menstruation and hirsutism are common in women with PCOS, and some studies suggest that these features are associated with more severe anxiety and depression symptoms in women with PCOS[17,18]. Negative feelings about one's appearance, in particular, can contribute to poor mental health conditions such as anxiety and depression among women with PCOS[4]. Moreover, a meta-analysis by Cooney et al., reported that clinical and biochemical hyperandrogenism was associated with the prevalence of anxiety/depression-like behaviors in women with PCOS, suggesting that hirsutism, the clinical manifestation of hyperandrogenism, is associated with an increased prevalence of depression and anxiety[4]. In addition, Ekbäck et al., used the Ferriman-Gallwey score to evaluate hirsutism in their study and found that hirsutism was associated with anxiety and depression symptoms in women with PCOS[17]. In this study, Ferriman-Gallwey scores were significantly higher in the population with high scores of depression and anxiety. Despite numerous studies on this topic, it is still unclear which factors influence levels of anxiety and depression in women with PCOS. Therefore, the aim of our study was not only to determine the frequency of these disorders but also to evaluate the relationship of depression and anxiety with the hormone profile. Although serum LH and LH:FSH ratio were found to be statistically significant in women with PCOS with high anxiety and depression scale scores in our study, this finding was supported by many studies while others could not show this relationship[15,19,20]. In support of these studies, no difference was found in serum testosterone levels between women with PCOS with and without depression in our study. PCOS is a disease with hyperandrogenism, but depression and anxiety seem to be caused not by circulating androgen dominance, but by changes in body image like hirsutism. However, some studies show that exogenous DHEA-SO_4 supplementation has beneficial effects on depression[20].

Previous studies have also shown a relationship between high testosterone levels and anxiety[21]. In our study, although testosterone levels were similar in women with high depression scores, it was observed that testosterone levels were statistically significantly higher in women with PCOS with high anxiety scores. This result shows that the high anxiety score in women with PCOS may have an effect on biochemical hyperandrogenism as well as body image disorder.

One of the limitations of this study was its cross-sectional design, which limits our ability to assess the potential impact of PCOS on the future risk of depression and anxiety. Another limitation was a lack of data on a family history of mood disorders and potential confounding factors such as diet and physical activity.

Our work has several strengths. We confirmed the use of the Rotterdam criteria to diagnose PCOS in all participants and performed a preliminary assessment to confirm that the control group did not have undiagnosed PCOS.

In conclusion, it is clear that depression and anxiety are more common in patients with PCOS than in healthy women, and particularly, menstrual irregularities and hirsutism were related to depression and anxiety symptoms. Hyperandrogenism, which is one of the biochemical markers, appears to be associated with high anxiety scores and also causes hirsutism. The reason for this appears to be a cause–effect relationship between hirsutism and hyperandrogenism. Our findings support the previous recommendations about routine screening for depression and anxiety in this population. For managing PCOS, collaboration with nutritionists, endocrinologists, and behavioral medicine specialists might provide comprehensive care.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

OSG: Conceptualization, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **ABT:** Formal Analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **OA:** Data curation, Formal Analysis, Project administration, Resources. **AB:** Data curation, Formal Analysis, Project administration, Resources, Software. **FS:** Data curation, Formal Analysis. **BDT:** Data curation, Project administration, Resources, Software. **NT:** Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Modified thoracoabdominal nerves block through perichondrial approach for laparoscopic cholecystectomy

Ela Erten[1*], Umut Kara[1], Fatih Şimşek[1], Muharrem Öztaş[2], Mehmet Anıl Süzer[3], Hasan Kamburoğlu[4], Mehmet Burak Eşkin[1], Serkan Şenkal[1], Ahmet Çoşar[1]

SUMMARY

OBJECTIVE: A new block, namely, modified thoracoabdominal nerves block through perichondrial approach, is administered below the costal cartilage. We sought to compare the analgesic efficacy of the modified thoracoabdominal nerves block through perichondrial approach block with local anesthetic infiltration at the port sites in an adult population who underwent laparoscopic cholecystectomy.

METHODS: Patients who will undergo laparoscopic cholecystectomy were randomized to receive bilateral ultrasound-guided modified thoracoabdominal nerves block through perichondrial approach blocks or local anesthetic infiltration at the port insertion sites. The primary outcome was the total amount of tramadol used in the first 12 h postoperatively. The secondary outcomes were total IV tramadol consumption for the first postoperative 24 h and visual analog scale scores.

RESULTS: The modified thoracoabdominal nerves block through perichondrial approach group had significantly less tramadol use in the first 12 h postoperatively ($p<0.001$). The modified thoracoabdominal nerves block through perichondrial approach group's visual analog scale scores at rest (static) and with movement (dynamic) were significantly lower compared with the port infiltration group ($p<0.05$).

CONCLUSION: Patients who received modified thoracoabdominal nerves block through perichondrial approach block had significantly less analgesic consumption and better pain scores than those who received port-site injections after laparoscopic cholecystectomy.

KEYWORDS: Laparoscopic cholecystectomy. Nerve block. Postoperative pain. Anesthetics, local.

INTRODUCTION

Laparoscopic cholecystectomy (LC) is less invasive and offers benefits compared with open surgery. However, postoperative pain remains a significant predictor of recovery[1,2]. Regional blocks such as TAP, erector spinae plane, rectus sheath, quadratus lumborum, and paravertebral blocks are commonly used in LC[3-5]. However, these blocks do not adequately block abdominal walls' anterior and lateral parts and may cause sensory blockade of the surgical field[6,7].

The thoracoabdominal nerves block through perichondrial approach (TAPA) is a new block that affects the thoracoabdominal nerves' branches up to T5 in the cephalic direction and T1-L1 in the caudal direction[4,8]. In the TAPA block, LA is injected twice, at the costochondral corner, on the lower and upper surfaces of the chondrium. In contrast, modified TAPA (M-TAPA) only requires a single injection immediately below the costal cartilage[9].

We sought to compare the analgesic efficacy of the M-TAPA block with that of LA infiltrations at the laparoscopic access sites in patients who underwent LC. The primary aim was to evaluate the total tramadol consumption via patient-controlled analgesia (PCA). The secondary aims were to compare postoperative visual analog scale (VAS) scores and frequency of rescue analgesic administration. We hypothesized that the M-TAPA block would offer improved pain management compared with port-site infiltration.

METHODS

This study was carried out at a training hospital in Ankara, Turkey. The study was approved by the ethics committee of the Gülhane Training and Research Hospital (No. 2023/4). Our study was carried out in accordance with the provisions of the Consolidated Reporting Studies statement and the Declaration of Helsinki[10].

[1]University of Health Sciences, Gulhane Training and Research Hospital, Department of Anesthesiology and Reanimation – Ankara, Turkey.
[2]Institute of Health Sciences, Gülhane Training and Research Hospital, Department of General Surgery – Ankara, Turkey.
[3]Private Çankaya Hospital, Department of Anesthesiology and Reanimation – Ankara, Turkey.
[4]Private Acıbadem Ataşehir Hospital, Department of Anesthesiology and Reanimation – Ankara, Turkey.
*Corresponding author: drelacaliskan@hotmail.com
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According to the guidelines of American Society of Anesthesiology (ASA), patients with physical status I–II aged 18–65 years who will have elective LC were included. Before the study, patients were informed, and written consent was obtained. Patients with known allergies to any of the medications used in the study, local or systemic infection, a history of alcohol or drug abuse, inability to cooperate and understand the Turkish language, and patients with pregnancy were excluded. After obtaining a computer-generated randomization list consisting of eight blocks of 10 with an intergroup ratio of 1:1 and equally distributed in the two groups, 80 opaque envelopes with numbers 1–80 were sealed. The patients were randomly assigned M-TAPA group or port infiltration group.

All patients received general anesthesia with sevoflurane and remifentanil infusion. The surgery was performed using the standard four-trocar method. All patients received 1 g of paracetamol and 50 mg of dexketoprofen intravenously. Thirty minutes prior to emergence, all patients received 4 mg of ondansetron intravenously. At the end of the surgery, 2 mg/kg of sugammadex was used to reverse neuromuscular blockade.

Modified thoracoabdominal nerves block through perichondrial approach block

Ultrasound-guided M-TAPA block was administered bilaterally prior to emergence from general anesthesia after the surgical procedure by the same anesthesiologist. A linear high-frequency probe was used to identify the anatomy (Figure 1). At the level of the 10th rib in the midclavicular line, 20 mL of 0.25% bupivacaine was injected (arcus costarum) between the transversus abdominis muscle's upper fascia and the lower fascia of costochondral tissue. The same technique was followed on the other side.

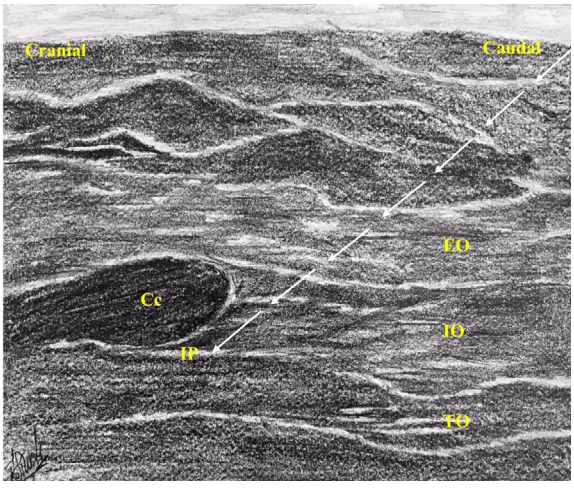

Figure 1. Modified thoracoabdominal nerves block through perichondrial approach block; reverse ultrasound anatomy. Cc: costal cartilage; EO: external oblique muscle; IO: internal oblique muscle; TO: transversus abdominis muscle; IP: injection point.

Port infiltration

The surgeon infiltrated 10 mL of 0.25% bupivacaine at each of the four laparoscopic port areas at the end of the surgery.

After the block and waking up from anesthesia, the patients were transported to the post-anesthesia care unit (PACU). Data collection was continued for 24 h in the ward. Rescue analgesia in the form of 75 mg of diclofenac was administered intramuscularly when the VAS score was >4. In case of nausea and vomiting in the postoperative period, 4 mg of ondansetron was administered as a rescue antiemetic.

Pain was measured using the VAS score at rest (static) and during movement (dynamic). It was rated from 0 to 10, from no pain to unbearable pain. The VAS scores were recorded postoperatively at PACU, 1, 2, 4, 8, 12, and 24 h. Postoperative complications related to the surgery such as nausea/vomiting, shoulder tip pain, or dizziness were evaluated. The Turkish version of the QoR-15 was used to evaluate the quality of recovery of the patients 24 h after surgery[11].

The primary outcome of the study was the total amount of tramadol used in the first 12 h postoperatively. The secondary outcomes included the total IV tramadol consumption for the first postoperative 24 h (mg), the VAS scores at rest (in supine position) and with mobilization (described as transition from supine to sitting position), the QoR-15 score at 24 postoperative hours, rescue analgesic requirement (yes/no), incidence of postoperative complications (yes/no), incidence of nausea and vomiting (yes/no), length of stay (LOS) in PACU (min), and LOS at the hospital (days).

The retrospective data collected from patients undergoing LC showed that the tramadol consumption during the first 12 postoperative hours in the port infiltration group was 139±66 mg. We aimed to detect a reduction of at least 35% in tramadol consumption with a threshold of significance alpha=0.05 and a power of 90% as a result of the M-TAPA block. Therefore, the necessary sample size was 30 patients in each group. Sample size calculation was done using G-power[12]. After a dropout rate of 20%, 72 patients were enrolled in the study. Continuous variables are described as means±SD or median (Q1–Q3). We used the Shapiro-Wilk test to determine the normality of the data distribution. Normally distributed data were analyzed using an independent t-test, and non-normally distributed data were analyzed using the Mann-Whitney U test. Corrected confidence intervals and p-value were calculated by

Bonferroni's method for multiple testing of repeated measurements. The effect size was calculated by dividing the absolute standardized test z-statistic by the square root of the number of pairs (n=60). A $p<0.05$ was considered statistically significant. Categorical variables were expressed as numbers (percentages), and their evaluations were made with Pearson's chi-square test. IBM SPSS Statistics (version 25.0, IBM Corp., Armonk, NY) was used for statistical analyses.

RESULTS

A total of 73 patients were screened for eligibility between February and May 2023. We excluded six patients from the study because they did not meet the criteria, and four patients did not want to participate in the study. Notably, 63 patients were included in the study and randomized. One patient withdrew consent prior to anesthesia, and the surgical approach was changed from laparoscopic to open cholecystectomy in two patients. Thus, the study was completed by 60 patients without any adverse events or complications.

Data from 60 patients, including 31 patients from the M-TAPA group and 29 patients from the port infiltration group, were included in the analysis. The mean age of the patients was 49.26±12.16 years, and 44 (73.3%) patients were women. The mean surgical time was 95.08±26.83 min, and the mean total anesthesia time was 109.25±27.92 min. The median PACU-LOS was 30 min, and the median hospital LOS was 1 day in both groups. When the groups were compared in terms of these basic characteristics, they were found to be similar ($p>0.05$).

The following scores were significantly lower in the M-TAPA group than those in the port infiltration group: the postoperative pain VAS score at rest in the PACU and 1, 2, 12, and 24 postoperative hours and the postoperative pain VAS score during movement at all observation times ($p<0.05$) (Table 1). Figure 2 shows the comparisons of pain intensities between the two groups. The M-TAPA block reduced the mean tramadol requirements by 52.7% during the first 24 h compared with port-site infiltration.

DISCUSSION

We compared the analgesic efficacy of LA infiltration at the port-insertion sites with that of ultrasound-guided M-TAPA block in patients undergoing LC. We observed that patients who received M-TAPA block had significantly lower tramadol consumption and better VAS scores than patients who received port-site infiltrations.

The sources of pain after LC are visceral and somatic pain. Somatic pain is caused by the port-site incisions, whereas visceral pain is caused by the peritoneal stretch and manipulation of the abdominal tissues[1]. Ultrasound-guided interfascial plane blocks offer varying effective analgesia for abdominal surgeries compared with neuraxial blocks[5]. Serratus intercostal plane (SIP) and oblique subcostal transversus abdominis plane (OSTAP) blocks affect anterior dermatomes, and quadratus lumborum and erector spinae plane blocks affect the posterior dermatomes.

The TAPA block has analgesic effects by blocking the anterior and lateral parts of the thoracoabdominal wall. The anterolateral abdominal wall innervation is done by T7-L1 nerves anterior branches[13]. Intercostal nerves pass under the chondrium and connect the origin of the transversus abdominis muscle to the cartilage[14]. In the M-TAPA block, LA is injected under the costal cartilage at the junction of the transversus abdominis muscle with the 10th rib and blocks the anterolateral cutaneous branches of the T5–T12/L1 thoracoabdominal nerves[15]. Ohgoshi et al., demonstrated that the M-TAPA block affects anterior branches of T6–T12 thoracoabdominal nerves[16], whereas Bahadır et al., reported an effect in a wide dermatome area extending from T4 to T11–T12[17].

Several previous studies have evaluated the effects of interfacial plane blocks on pain after LC. Saxena et al., compared ultrasound-guided abdominal field block with port-site infiltrations and reported that abdominal field block provided superior analgesia after LC[18]. Molfino et al., compared TAP block with port-site infiltrations after LC and reported that both groups had similar analgesic effectiveness[19]. In contrast to the results obtained in our study, port-site infiltration and TAP block may have similar analgesic effects since the TAP block can be effective in the T7–12 dermatome region. The procedures on the supraumbilical area may involve the use of the interfacial plane or SIP blocks[6,20]. The M-TAPA block should be preferred over these blocks in LC because, to reduce the pain at the incision sites in LC, the anterior and lateral branches of the intercostal nerves must be blocked same time. However, the anterior and lateral cutaneous branches cannot be blocked with the SIP and OSTAP blocks, respectively[6,7]. The literature review revealed that three studies have evaluated the effects of the M-TAPA block on post-LC pain. Gungor et al., compared the M-TAPA block with LA infiltration after LC and reported that, in the M-TAPA group, postoperative pain and the need for rescue analgesia were significantly lower[21]. Bilge et al., showed that the M-TAPA block decreased pain and consumption of tramadol compared with the control group[22]. Erturk et al., compared the TAPA block with the M-TAPA block after LC and showed that

Table 1. Comparison of pain scores, tramadol consumptions, and postoperative datas.

Variable	M-TAPA group (n=31)	Port infiltration group (n=29)	Effect size[a] (95%[d] or 99%[b] CI)	Corrected[b] p-value
PACU VAS-R*	2.45±1.72	4.27±2.46	-1.82[c] (-2.91 to -0.73)[d]	0.001[b]
1st hour VAS-R	3.00 (2.00, 4.00)	4.00 (3.00, 6.00)	0.48 (0.000 to 0.000)[b]	<0.001[b]
2nd hour VAS-R	2.00 (1.00, 3.00)	3.00 (2.00, 4.00)	0.34 (0.007 to -0.012)[b]	0.009[b]
4th hour VAS-R	2.00 (1.00, 3.00)	2.00 (1.00, 4.00)	0.16 (0.195 to 0.216)[b]	0.206[b]
8th hour VAS-R	2.00 (0.00, 2.00)	2.00 (1.00, 3.00)	0.24 (0.051 to -0.063)[b]	0.057[b]
12th hour VAS-R	1.00 (0.00, 2.00)	2.00 (1.00, 3.50)	0.36 (0.003 to 0.006)[b]	0.004[b]
24th hour VAS-R	1.00 (0.00, 1.00)	1.00 (1.00, 2.00)	0.44 (0.000 to -0.001)[b]	0.001[b]
PACU VAS-M*	3.00 (2.00, 5.00)	5.00 (3.00, 6.50)	0.30 (0.013 to 0.020)[b]	0.017[b]
1st hour VAS-M	3.00 (2.00, 4.00)	5.00 (4.00, 6.00)	0.48 (0.000 to 0.000)[b]	<0.001[b]
2nd hour VAS-M	2.83±1.50	4.34±1.79	-1.50[c] (-2.361 to 0.650)[d]	0.001
4th hour VAS-M	2.00 (1.00, 3.00)	3.00 (2.00, 5.00)	0.38 (0.002 to 0.005)[b]	0.004[b]
8th hour VAS-M	2.00 (1.00, 3.00)	3.00 (2.00, 4.00)	0.34 (0.004 to 0.008)[b]	0.006[b]
12th hour VAS-M	1.00 (0.00, 2.00)	3.00 (2.00, 5.00)	0.53 (0.000 to 0.000)[b]	<0.001[b]
24th hour VAS-M	1.00 (1.00, 2.00)	2.00 (1.00, 3.00)	0.39 (0.001 to 0.004)[b]	0.003[b]
2nd hour tramadol consumptions (mg)	24.61±17.25	40.00±18.65	-15.38 (-25.59 to -5.17)[e]	0.004[a]
4th hour tramadol consumptions (mg)	42.30±22.85	84.16±31.74	-41.85 (-57.49 to -26.21)[e]	<0.001[a]
8th hour tramadol consumptions (mg)	56.92±30.82	125.83±54.20	-68.91 (-93.73 to -44.08)[e]	<0.001[a]
12th hour tramadol consumptions (mg)	73.84±42.99	169.16±73.42	-95.32 (-129.21 to -61.42)[e]	<0.001[a]
24th hour tramadol consumptions (mg)	86.15±59.53	200.00±85.26	-113.84 (-155.39 to -72.29)[e]	<0.001[a]
24th hour QoR-15 score	110.77±19.13	98.48±18.09	—	0.013
Rescue analgesic requirement (Yes:No)	1:30 (3.2%:96.8%)	12:17 (41.4%:58.6%)	—	<0.001
Postoperative complications (Yes:No)	12:19 (38.7%:61.3%)	19:10 (65.5%:34.5%)	—	0.038
Nausea/vomiting (Yes:No)	10:21 (32.3%:67.7%)	18:11 (62.1%:37.8%)	—	0.021

Values are presented as mean±standart deviation, median (Q1, Q3) or frequency (%). VAS-R: visual analog scale score at rest; M-TAPA: modified thoracoabdominal nerves block through perichondrial approach; PACU: post-anesthesia care unit; VAS-M: visual analogue scale score at movement; QoR: quality of recovery. [a]Effect size is calculated by dividing the absolute standardized test statistic z by the square root of the number of pairs (n=60). [b]99%CI and corrected p-value were calculated by Bonferroni's correction for multiple testing of repeated measurement. [c]The mean difference was used instead of the effect size. [d]95%CI was calculated. [e]95%CI and corrected p-value were calculated by Bonferroni's correction for multiple testing of repeated measurement. *Measured immediately after PACU arrival. p<0.05 is considered statistically significant.

NRS scores, tramadol consumption, and rescue analgesic use were similar in the postoperative period[4]. Our results are consistent with the results of these three studies that the M-TAPA block prevents post-LC pain.

Another issue is the evaluation of the quality of postoperative recovery from the patient's perspective. Gungor et al., evaluated patient satisfaction during the postoperative period and reported that the M-TAPA group's scores were better[21]. Bilge et al., also found better QoR scores in the M-TAPA group[22].

The study has a few limitations. First, the postoperative pain was evaluated for 24 h only. The analgesic effect of a single-shot interfascial plane block persists for 24–48 h[5]. Second, the dermatome field affected by the M-TAPA block or port-site infiltration was not examined to evaluate the actual effects of the blocks. Finally, a fixed volume of LA was used for the block.

CONCLUSION

Compared with port-site infiltrations, the M-TAPA block has resulted in a significant decrease in the systemic analgesic demand following LC. Furthermore, considering the dermatome

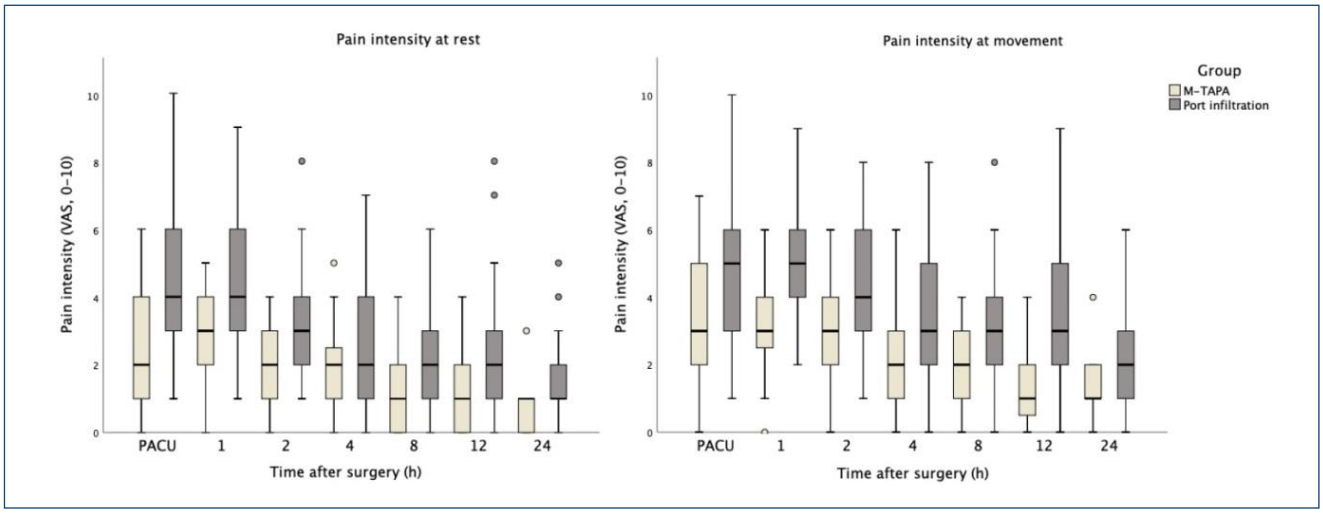

Figure 2. Comparisons of pain intensity at rest between the two groups. The box plot represents the median and interquartile range of the NRS in the modified thoracoabdominal nerve block through the perichondral approach and port infiltration groups during the study period. The upper lines represent the maximum value, whereas the lower lines represent the minimum value, excluding outliers. M-TAPA: modified thoracoabdominal nerve block through the perichondral approach; VAS: visual analog scale.

areas impacted by the M-TAPA block, further research should be conducted on its usefulness in open surgical procedures.

INFORMED CONSENT

Written informed consent was obtained from all individual participants included in this study. This retrospective study was approved by the Ethics Committee of Gülhane Training and Research Hospital, Turkey (No. 2023/4).

AUTHORS' CONTRIBUTIONS

EE: Conceptualization, Data curation, Investigation, Project administration, Writing – original draft, Writing – review & editing. **UK**: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **FŞ**: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **MÖ**: Investigation, Project administration. **MAS**: Methodology. **HK**: Software. **MBE**: Formal Analysis. **SŞ**: Formal Analysis. **AÇ**: Supervision, Validation.

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ORIGINAL ARTICLE

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Relationship between skeletal muscle mass loss and metabolic dysfunction-associated fatty liver disease among Chinese patients with metabolic dysregulation

Miao Xu[1,2], Yi Lin[3], Naibin Yang[4], Jialin Li[2], Li Li[2], Huiqing Ding[5], Chengfu Xu[1*]

SUMMARY

OBJECTIVE: The aim of this study was to explore the correlation between skeletal muscle content and the presence and severity of metabolic dysfunction-associated fatty liver disease in patients with metabolic dysregulation in China.

METHODS: A cross-sectional study was conducted among patients from the endocrinology outpatient department at Ningbo First Hospital, in Ningbo, China, in April 2021. Adult patients with metabolic dysregulation who accepted FibroScan ultrasound were included in the study. However, those without clinical data on skeletal muscle mass were excluded. FibroScan ultrasound was used to noninvasively evaluate metabolic dysfunction-associated fatty liver disease. The controlled attenuation parameter was used as an evaluation index for the severity of liver steatosis. Bioelectrical impedance analysis was used to measure the skeletal muscle index.

RESULTS: A total of 153 eligible patients with complete data were included in the final analysis. As the grading of liver steatosis intensifies, skeletal muscle index decreases (men: P_{trend}<0.001, women: P_{trend}=0.001), while body mass index, blood pressure, blood lipid, uric acid, aminotransferase, and homeostatic model assessment of insulin resistance increase (P_{trend}<0.01). After adjusting for confounding factors, a negative association between skeletal muscle index and the presence of metabolic dysfunction-associated fatty liver disease was observed in men (OR=0.691, p=0.027) and women (OR=0.614, p=0.022). According to the receiver operating characteristic curve, the best cutoff values of skeletal muscle index for predicting the metabolic dysfunction-associated fatty liver disease presence were 40.37% for men (sensitivity, 87.5%; specificity, 61.5%) and 33.95% for women (sensitivity, 78.6%; specificity, 63.8%).

CONCLUSION: Skeletal muscle mass loss among patients with metabolic dysregulation was positively associated with metabolic dysfunction-associated fatty liver disease severity in both sexes. The skeletal muscle index cutoff value could be used to predict metabolic dysfunction-associated fatty liver disease.

KEYWORDS: Metabolic syndrome. Skeletal muscle. Fatty liver. Sarcopenia. China.

INTRODUCTION

With the improvement of living standards worldwide, metabolic dysfunction-associated fatty liver disease (MAFLD) is increasingly common, with a global prevalence of 25% among the healthy population[1]. In China, the prevalence of MAFLD among healthy people in Gansu Province in 2015, Henan Province in 2017, and northeast China in 2018 was 21.03–35.28%[2-4]. The prevalence of overweight/obesity, dyslipidemia, hypertension, and hyperglycemia is much higher in China[2]. Sarcopenia is defined as the progressive loss of skeletal muscle mass, strength, and function[5]. Studies have found that sarcopenia increases the risk of diabetes, dyslipidemia, and cardiometabolic diseases[6,7].

Obesity is an independent risk factor for MAFLD[8]. Weight management may be an effective method to maintain body weight and weight loss[9,10] for the prevention of MAFLD[11]. Besides obesity, sarcopenia has been reported to have strong correlations with non-alcoholic fatty liver disease (NAFLD) and progressive cirrhosis[12]. The prevalence of sarcopenia was 46.0% in the older Chinese generation (men: 71.3 years and

[1]Zhejiang University School of Medicine, The First Affiliated Hospital, Zhejiang Provincial Clinical Research Center for Digestive Diseases, Department of Gastroenterology – Hangzhou, China.
[2]The First Affiliated Hospital of Ningbo University, Department of Endocrinology and Metabolism – Ningbo, China.
[3]University of Nottingham, Faculty of Humanities and Social Sciences, Center for Health Economics – Ningbo, China.
[4]The First Affiliated Hospital of Ningbo University, Metabolic (Dysfunction)-Associated Fatty Liver Disease Research Center, Department of Hepatology – Ningbo, China.
[5]The First Affiliated Hospital of Ningbo University, Department of Obstetrics and Gynecology – Ningbo, China.
*Corresponding author: xiaofu@zju.edu.cn
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women: 69.9 years)[13]. Aging, a sedentary lifestyle, and low body mass index (BMI) are directly associated with sarcopenia[14]. Evidence of sarcopenia among young people with metabolic dysregulation is unknown.

Most studies have focused on fat mass accumulation in obese individuals. However, muscle mass loss has not received sufficient attention from researchers and clinicians. To the best of our knowledge, no study has been conducted on the association between muscle mass level and the presence and severity of steatosis with MAFLD in China, especially among patients with metabolic dysregulation. Therefore, this study aimed to investigate the association between muscle mass loss and MAFLD in patients with metabolic dysregulation in Ningbo, China.

METHODS

Ethics

Ethical approval was obtained from the Research Ethics Committee of Ningbo First Hospital (2019-R057). Written informed consent was obtained from all patients.

Study design and patients

A cross-sectional study was conducted among patients from the endocrinology outpatient department at Ningbo First Hospital in Ningbo, China, in April 2021. Patients suspected of metabolic dysregulation and willing to undergo FibroScan ultrasound (PRO, Echosens, France) examinations were recruited. The data for anthropometric measurements, liver steatosis levels, skeletal muscle mass, biochemical tests, and questionnaires were all obtained during this visit. The inclusion criteria were as follows: 1. patients aged 18–75 years; 2. visiting the endocrinology outpatient department at Ningbo First Hospital for the first time; and 3. being diagnosed with metabolic dysregulation and underwent FibroScan ultrasound examination. Those patients without data on SMI were excluded. The diagnostic criterion for metabolic dysregulation satisfied at least one of the following conditions[15]: (1) overweight/obesity (BMI≥23 kg/m^2)[16]; (2) type 2 diabetes mellitus (T2DM, according to the American Diabetes Association criteria); and (3) the presence of metabolic syndrome[17].

Data collection and study variables

All patients with metabolic dysregulation, willing to participate in this study, were asked to complete questionnaires, including demographic information (e.g., age and sex), medical history [T2DM, hypertension, hypertriglyceridemia, and low level of high-density lipoprotein (HDL) cholesterol], and medication records. Using the standardized protocol, anthropometry was measured by well-trained nurses, and biochemical parameters were analyzed by the laboratory staff.

Anthropometric measurements

The BMI (kg/m^2) was calculated as weight (kg)/height^2 (m^2). Bodyweight was measured to the nearest 0.1 kg with light clothes using a calibrated automatic digital weight. Height was measured to the nearest 0.5 cm without shoes in the standing position using a height scale (HNH-318, Omron, Japan). Waist circumference (in cm) was measured to the nearest 0.5 cm at the mid-point between the lower rib and iliac crest using a 150-cm medical tape. Blood pressure was measured on the right or left arm using an electronic sphygmomanometer (HBP-1100U, Omron, Japan) in a seated position after a 10-min rest.

Biomarker measurements

Glycosylated hemoglobin (HbA1c) (%) was analyzed using high-performance liquid chromatography (D-10 Hemoglobin Analyzer, Bio-Rad, USA). Fast plasma glucose (FPG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), HDL cholesterol, low-density lipoprotein cholesterol (LDL-C), and uric acid (UA) were assessed by enzymatic assays (AU5400, Beckman Coulter, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was estimated using the following formula: fasting insulin (U/mL)×fasting glucose (mmol/L)/22.5. All anthropometric and biochemical parameters were obtained on the same day after overnight fasting for 8–10 h.

Liver steatosis examination

FibroScan was designed to perform liver stiffness measurement using vibration-controlled transient elastography (VCTE) as a noninvasive medical device. The controlled attenuation parameter (CAP, dB/m), which is the ultrasonic attenuation coefficient of the ultrasonic signals used during VCTE examination, is correlated with hepatic steatosis. The CAP assessment was performed by an experienced sonologist, according to the FibroScan instructions[18]. Liver steatosis grade was determined by the cutoff values of CAP according to previous reports as follows: a. CAP<233.5 dB/m denoted no steatosis (S0), b. 233.5≤CAP<268.5 dB/m denoted mild steatosis (S1), c. 268.5≤CAP<301.2 dB/m denoted moderate (S2), and d. CAP>301.2 dB/m denoted severe steatosis (S3)[19].

Skeletal muscle mass measurement

Bioelectrical impedance analysis (BIA) was used to examine impedance for each segment, including the four limbs and trunk,

using the InBody 770 body composition analyzer (InBody, Seoul, Korea). Multi-frequency measurements were performed to estimate appendicular skeletal muscle mass (ASM). In this study, skeletal muscle index (SMI, %) was calculated using the following equation: ASM/body weight (kg).

Statistical analysis

Continuous variables with normal and skewed distributions are presented as the mean (standard deviation) and median (interquartile range), respectively. Continuous variables were analyzed using one-way analysis of variance and the Kruskal–Wallis test. Categorical variables are presented as numbers (percentages) and compared using the chi-square test according to the degree of liver steatosis. Multivariate logistic regression models were used to examine the associations between SMI and the presence of MAFLD using four models: (1) unadjusted model; (2) adjusted for age; (3) adjusted for age, diastolic blood pressure (DBP), and FPG; and (4) adjusted for age, DBP, FPG, TG, TC, UA, and ALT. Receiver operating characteristic (ROC) curve analysis of SMI was used to calculate the cutoff value of SMI for predicting the development of MAFLD. The results were considered statistically significant at a two-tailed level of 0.05. Statistical analyses were performed using IBM SPSS Statistics version 26.0 for Windows.

RESULTS

A total of 188 patients diagnosed with metabolic dysregulation who underwent FibroScan ultrasound examinations were included, while 35 patients without clinical data on SMI were excluded. Finally, 153 patients (52.9% men and 47.1% women) with a mean age of 41.9 years were included in this study.

Table 1 shows the clinical characteristics and laboratory test results stratified by liver steatosis level. Approximately 26.8%, 17.6%, and 35.9% of patients were diagnosed with mild, moderate, and severe MAFLD, respectively. Significantly increasing trends were found in BMI, SBP, DBP, TG, TC, LDL-C, UA, ALT, AST, and HOMA-IR across groups of liver steatosis levels. In contrast, only SMI declined in both sexes.

Multivariate logistic regression was used to investigate the relationship between SMI and the presence of MAFLD stratified by sex (Table 2). Positive associations were found in model 1 for both men [odds ratio (OR)=0.713, p=0.002] and women (OR=0.764, p=0.009). After controlling for age,

Table 1. Characteristics of the study participants stratified by liver steatosis grades.

	The grade of liver steatosis					
	S0<5% (n=30)	S1 5–33% (n=41)	S2 34–66% (n=27)	S3≥67% (n=55)	F/H/χ^2 values	P_{trend}
Male, n (%)	16 (53.3)	24 (58.8)	13 (48.1)	28 (50.9)	0.857[b]	0.848
Age, years	48.5 (34.5, 58.0)	46.0 (32.0, 55.0)	40.0 (34.0, 57.0)	34.0 (26.0, 45.0)*	9.155[a]	**0.027**
BMI, kg/m^2	25.75 (23.43, 27.25)	27.1 (23.8, 29.65)	27.7 (26.4, 31.2)*	30.2 (27.5, 37.0)**	37.8[a]	**<0.001**
DBP, mmHg	71.17 (9.26)	80.53 (11.39)**	75.52 (9.40)	83.80 (12.17)**	17.004	**<0.001**
FPG, mmol/L	5.49 (4.92, 7.44)	6.59 (5.07, 8.99)	5.68 (4.98, 7.18)	6.06 (5.30, 8.49)	5.033[a]	0.169
HbA1c, %	6.2 (5.4, 7.0)	6.2 (5.5, 7.2)	5.85 (5.28, 7.55)	6.5 (5.5, 8.4)	3.949[a]	0.267
TG, mmol/L	0.98 (0.73, 1.21)	1.52 (1.13, 2.73)**	1.54 (0.99, 1.88)	1.99 (1.32, 2.77)**	23.275[a]	**<0.001**
TC, mmol/L	4.47 (1.10)	5.13 (1.31)	4.66 (1.20)	5.48 (1.25)**	7.559	**0.007**
UA, μmol/L	319.49 (80.74)	355.06 (75.35)	344.00 (122.90)	389.02 (97.45)*	7.674	**0.006**
ALT, U/L	19.0 (11.0, 22.0)	23.0 (16.0, 32.0)	25.5 (19.5, 33.25)	36.0 (24.5, 85.0)**	31.902[a]	**<0.001**
HOMA-IR	2.61 (2.21, 3.94)	5.33 (3.09, 7.51)*	5.06 (4.13, 6.52)	7.57 (5.43, 15.00)**	31.943[a]	**<0.001**
CAP, dB/m	209.5 (200.5, 224.0)	254.0 (243.0, 263.0)**	285.0 (276.0, 296.0)**	334.0 (318.5, 357.0)**	140.062[a]	**<0.001**
SMI, %						
Male	42.37 (2.63)	40.14 (3.27)	40.10 (3.05)	36.74 (4.04)**	24.355	**<0.001**
Female	35.86 (2.97)	34.31 (3.36)	32.46 (3.30)*	33.55 (3.45)*	12.607	**0.001**

Statistically significant values are denoted in bold. *compared with S0 group, p<0.05; **compared with S0 group, p<0.01; [a]H values; [b]χ^2 value. Liver steatosis grade was decided by the cutoff values of CAP; CAP<233.5 dB/m denoted no steatosis (S0), 233.5≤CAP≤268.5 dB/m denoted mild (S1), 268.5≤CAP≤301.2 dB/m denoted moderate (S2), and CAP>301.2 dB/m denoted severe steatosis (S3). BMI: body mass index; DBP: diastolic blood pressure; FPG: fast plasma glucose; HbA1c: glycosylated hemoglobin; TG: triglyceride; TC: total cholesterol; UA: uric acid; ALT: alanine aminotransferase; HOMA-IR: homeostatic model assessment of insulin resistance; CAP: controlled attenuation parameter; SMI: skeletal muscle index.

DBP, FPG, TG, TC, UA, and ALT, SMI was still maintained in model 4 in both men (OR=0.691, p=0.027) and women (OR=0.614, p=0.022).

The cutoff value of SMI for predicting MAFLD was analyzed using ROC curve analysis (Figure 1). The areas under the ROC curve were 0.772 [95% confidence interval (CI) 0.665–0.879, p=0.001] in men and 0.743 (95%CI 0.613–0.873, p=0.005) in women. The optimal cutoff values to predict MAFLD were 40.37% (with a sensitivity of 87.5% and a specificity of 61.5%) and 33.95% (with a sensitivity of 78.6% and a specificity of 63.8%) in men and women, respectively.

Table 2. Odds ratio and 95% confidence intervals of skeletal muscle index for the presence of metabolic dysfunction-associated fatty liver disease by sex.

Male			
	OR	95% CI	p-value
Model 1	0.713	0.576–0.884	**0.002**
Model 2	0.711	0.570–0.887	**0.003**
Model 3	0.713	0.538–0.947	**0.019**
Model 4	0.691	0.498–0.959	**0.027**
Female			
	OR	95% CI	p-value
Model 1	0.764	0.625–0.934	**0.009**
Model 2	0.749	0.592–0.946	**0.015**
Model 3	0.64	0.46–0.891	**0.008**
Model 4	0.614	0.404–0.934	**0.022**

Statistically significant values are denoted in bold. Model 1: unadjusted; Model 2: adjusted for age; Model 3: adjusted for age, DBP, and FPG; and Model 4: adjusted for age, DBP, FPG, TG, TC, UA, and ALT. SMI: skeletal muscle index; DBP: diastolic blood pressure; FPG: fast plasma glucose; TG: triglyceride; TC: total cholesterol; UA: uric acid; ALT: alanine aminotransferase.

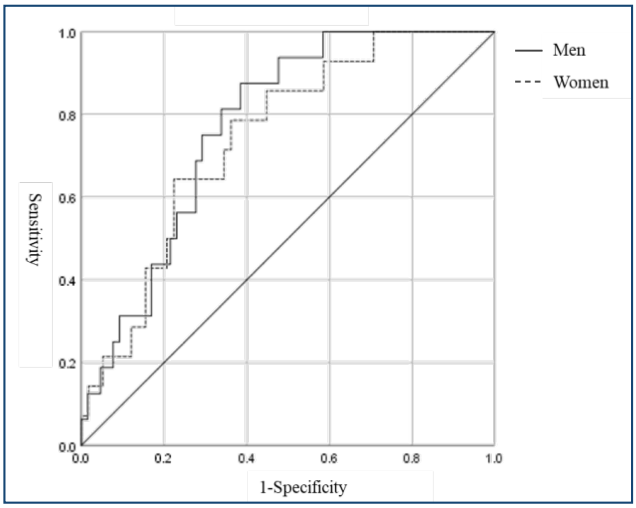

Figure 1. Receiver operating characteristic curves of skeletal muscle index to predict the presence of metabolic dysfunction-associated fatty liver disease by gender. Men (AUC=0.772, p=0.001), the optimal cutoff value was 40.37% with a sensitivity of 87.5% and a specificity of 61.5%; women (AUC=0.743, p=0.005), the optimal cutoff value was 33.95% with a sensitivity of 78.6% and a specificity of 63.8%.

DISCUSSION

This is the first cross-sectional study to examine the relationship between BIA-assessed skeletal muscle mass and liver steatosis in Chinese patients with metabolic dysregulation. We found that the loss of skeletal muscle mass was associated with the presence and severity of MAFLD in both men and women. The optimal cutoff values used to predict MAFLD were 40.37 and 33.95% in men and women, respectively.

There is a lack of information from research studies on the mechanism by which skeletal muscle works on abnormal fat accumulation in internal organs, especially MAFLD in patients with metabolic dysregulation. A previous meta-analysis, including 19 studies[20], in line with our findings, indicated that the SMI level in patients with NAFLD was lower than that in healthy individuals. We found that MAFLD severity was positively associated with BMI, blood pressure, UA, HOMA-IR, TG, and TC. Insulin resistance might be the common pathogenesis of these metabolic disorders and MAFLD[21] and promote the "first hit" of liver steatosis, characterized by hepatic TG accumulation[22]. Sarcopenic obesity has an increased risk of developing physical dysfunction compared to sarcopenia or obesity alone[23]. We hypothesized that skeletal muscle loss plays an important role in MAFLD occurrence and development. Skeletal muscle loss and intramuscular fat accumulation cause insulin resistance, oxidative stress, inflammatory cytokines, and mitochondrial dysfunction[24]. All of these factors may promote the "second hit" of MAFLD[22]. One myostatin secreted by skeletal muscle as an endocrine organ plays a role not only in regulating skeletal muscle mass and metabolism but also in liver steatosis[25]. In our study, after adjusting for confounders, including age, blood pressure, blood glucose, lipids, UA, and liver enzymes, we found that SMI was an independent protective factor for MAFLD in both men and women. Furthermore, the SMI cutoff value was estimated to predict MAFLD in approximately 40.37% of men and 33.95% of women.

Limitations and strength

This is the first study to investigate the association between muscle mass and the presence and severity of steatosis with MAFLD among patients with metabolic dysregulation in China. However, this study had some limitations. First, the causal

relationship could not be determined due to the cross-sectional study design. Second, the small sample size may have influenced the accuracy of this association. BIA and FibroScan are not the best methods for measuring body composition and liver steatosis, and golden standards should be used in the future.

CONCLUSION

Skeletal muscle mass loss among people with metabolic dysregulation was positively associated with MAFLD severity in both sexes. The SMI cutoff value could be used to predict MAFLD. Furthermore, a prospective study with sufficient samples and intervention studies should be conducted to gain a deeper insight into the effect of skeletal muscle mass loss on MAFLD among patients with metabolic dysregulation.

DATA SHARING

The dataset will be available upon request, unless there are legal or ethical reasons for not doing so.

AUTHORS' CONTRIBUTIONS

MX: Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. **YL:** Writing – original draft, Writing – review & editing. **NY:** Writing – review & editing. **JL:** Data curation, Methodology, Supervision. **LL:** Conceptualization, Funding acquisition, Resources. **HD:** Funding acquisition, Writing – review & editing. **CX:** Writing – conceptualization, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Serum adiponectin and peroxisome proliferator-activated receptors-γ levels in obese patients with and without prediabetes

Mehmet Ali Gul[1]*, Duygu Tozcu[2], Akın Tekcan[3], Mustafa Capraz[4], Hatice Dortok Demir[1]

SUMMARY

OBJECTIVE: Obesity is an increasingly prevalent global health problem, which is generally caused by the increase in body fat mass above normal and observed in all societies. If the blood glucose level is higher than normal but not high enough to diagnose diabetes, this condition is defined as prediabetes. Adiponectin increases fatty acid oxidation and insulin sensitivity and is closely associated with obesity. One of the nuclear receptor superfamily member peroxisome proliferator-activated receptors is shown to have an important role in various metabolic reactions. This study aimed to investigate the serum levels of adiponectin and peroxisome proliferator-activated receptors-gamma parameters, which are closely related to adipose tissue, energy metabolism, and insulin sensitivity, in obese patients with and without prediabetes.

METHODS: For this purpose, 52 obese patients with prediabetes, 48 obese patients with non-prediabetes, and 76 healthy individuals were included in this study. Serum adiponectin and peroxisome proliferator-activated receptors-γ levels were analyzed by ELISA.

RESULTS: Serum adiponectin levels were significantly higher in obese patients with prediabetes (18.15±15.99) compared with the control group (15.17±15.67; p=0.42). No significant difference was observed in both adiponectin and peroxisome proliferator-activated receptors-γ levels in the obese patients with the non-prediabetes group compared with the control group. However, no significant difference was observed in the obese patients with prediabetes group and obese patients with non-prediabetes group.

CONCLUSION: Our results suggest that adiponectin may serve as an indicator of prediabetes. This implies that examining adiponectin levels in individuals diagnosed with prediabetes may enhance our understanding of the metabolic processes closely linked to prediabetes and related conditions.

KEYWORDS: Prediabetes. Obesity. Adiponectin. PPAR-gamma.

INTRODUCTION

Obesity is a disease characterized by an increase in the ratio of body fat to the whole body, defined by a body mass index of 30 kg/m² or greater (weight divided by the square of height). The prevalence of obesity is increasing day by day and its prevalence has reached a pandemic level worldwide[1].

Although it is inevitable for patients with prediabetes to be type 2 diabetes mellitus (T2DM), some prediabetes do not develop diabetes and it is estimated that the annual conversion rate of prediabetes to diabetes is 5–10%[2,3]. Therefore, it is important to investigate whether prediabetes, which is one of the T2DM developmental stages of obesity, is a causal risk factor[4].

Metabolic disorders that start with insulin resistance in obesity can cause prediabetes. Prediabetes clearly increases the risk of T2DM. Although it is an important health problem, most prediabetics are not aware of their prediabetes[5]. Adipokines are secreted from adipose tissue and play a role in many physiological events, such as lipid metabolism, glucose metabolism, and inflammation, which are closely related to obesity and prediabetes[6,7]. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a regulatory nuclear protein found mainly in adipose tissue, has anti-inflammatory properties, increases insulin sensitivity, and has important effects on adipocyte proliferation and cell cycle control[8,9]. Adiponectin and PPAR-γ associated with obesity and diabetes have a direct effect on lipid metabolism, insulin sensitivity, and glucose-energy metabolism. In light of this information, this study aimed to investigate the serum levels of adiponectin, which is an important member of the adipokine family, and PPAR-γ, which is an important member of the nuclear receptor family, on prediabetes and obesity.

METHODS

A total of 52 obese patients with prediabetes, 48 obese patients with non-prediabetes, and 76 healthy individuals who applied to Amasya University Sabuncuoğlu Şerefeddin Training and

[1]Amasya University, Faculty of Medicine, Department of Medical Biochemistry – Amasya, Turkey.
[2]Amasya University, Faculty of Medicine, Department of Physiology – Amasya, Turkey.
[3]Amasya University, Faculty of Medicine, Department of Medical Biology – Amasya, Turkey.
[4]Amasya University, Faculty of Medicine, Department of Internal Diseases – Amasya, Turkey.
*Corresponding author: mehmetali.gul@amasya.edu.tr

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Research Hospital Internal Diseases Department were included in the study. Anthropometric measurements of the individuals were included in the study during their application to the outpatient clinic. At the same time, the patients' age, gender, different drug use, diet, smoking, alcohol, and other disease anamnesis were taken. The blood samples taken from the patients who applied after at least 8–12 h of fasting were routinely examined. Blood samples were taken for routinely requested serum samples, and 5 mL of blood was collected in yellow-capped gel tubes. It was slowly turned upside down 5–6 times. After waiting for at least 30 min, it was centrifuged at 1500–2000×g for 10 min with a centrifuge device. The serum part, which was separated at the top of the tube, was aliquoted and transferred to the Eppendorf tubes. The transferred samples were stored in a deep freezer at -80°C until the working day. Adiponectin and PPAR-γ levels were determined from the stored serum samples by using the ELISA method. ELISA analyses were performed according to the kit procedure instructions. The study was approved by the Ethics Committee of Amasya University (03/2021 Decision Number:6/100) and performed in accordance with the ethical standards specified in the Declaration of Helsinki.

Serum adiponectin and PPAR-γ levels were determined using the Human Adiponectin and Human PPAR-γ ELISA kit (Bioassay Technology Laboratory, Birmingham, UK) according to the manufacturer's instructions. Serum adiponectin and PPAR-γ measurements were performed using the Chromate 4300 Elisa reader (Awareness Technology, Inc., Martin Hwy., Palm City, USA).

Statistical analysis of the data was carried out using Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp., Armonk, NY, USA). Whether the data were normally distributed was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Also, the skewness and kurtosis values were analyzed. The Mann-Whitney U test, which is one of the nonparametric tests, was used for the data that did not comply with the normal distribution. The results were given as mean±standard deviation (mean±SD), p-value below 0.001 were contemplated very significant, and p-value below 0.05 were contemplated statistically significant.

RESULTS

There was no statistical difference between the control, obese patients with non-prediabetes, and obese patients with prediabetes group in terms of age (years) (50.13±9.49 vs. 46.34±10.50 vs. 50.88±9.58), height (cm) (160.29±11.68 vs. 161.25±10.73 vs. 159.72±10.32), smoking, alcohol use, and other diseases. A statistical difference was determined between control, obese patients with non-prediabetes, and obese patients with prediabetes group considering the presence of weight (kg) (80.53±14.81 vs. 94.52±17.70 vs. 86.65±12.89; p<0.001), BMI (31.69±6.92 vs. 36.27±5.40 vs. 34.11±5.41; p<0.001), and waist circumference (cm) (98.14±10.91 vs. 106.19±11.14 vs. 103.43±8.24; p=0.001), which were found to be statistically high in the obese patients with non-prediabetes group (Table 1).

Table 1. Baseline clinical and demographic features of the patients and controls.

Characteristics	Controls (n=76)	Obese patients with non-prediabetes (n=48)	Obese patients with prediabetes (n=52)	p-value
Gender, F/M, n (%)	56/20 (73.7/26.3)	36/12 (75.0/25.0)	33/19 (63.5/36.5)	
Age, mean±SD, years	50.13±9.49	46.34±10.50	50.88±9.58	
Adiponectin (ng/mL), mean±SD	15.17±15.67	16.63±15.64	18.15±15.99	**0.023***
PPAR-γ (ng/mL), mean±SD	3108.33±2932.72	3244.16±3324.42	2950.45±2490.87	0.885
PPAR-γ/adiponectin ratio	259.32±208.38	206.44±114.71	206.57±229.66	**0.047****
Height (cm), mean±SD	160.29±11.68	161.25±10.73	159.72±10.32	0.786
Weight (kg), mean±SD	80.53±14.81	94.52±17.70	86.65±12.89	**0.000****
BMI, mean±SD	31.69±6.92	36.27±5.40	34.11±5.41	**0.000****
Waist circumference, mean±SD	98.14±10.91	106.19±11.14	103.43±8.24	**0.001****
Smoking, yes/no, n (%)	12/64 (15.8/84.2)	7/41 (14.6/85.4)	6/46 (11.5/88.5)	
Alcohol, yes/no, n (%)	0/76 (0.0/100.0)	0/48 (0.0/100.0)	2/50 (3.8/96.2)	
Other diseases, yes/no, n (%)	21/39 (35.0/65.0)	27/14 (65.9/34.1)	25/22 (53.2/46.8)	

F: female; M: male; SD: standard deviation; DM: diabetes mellitus; BMI: body mass index. *Between the control group and obese patients with prediabetes group. **Between all groups. Statistically significant p-value are denoted in bold.

There was no statistical difference between the obese patients with prediabetes (2950.45±2490.87) and obese patients with non-prediabetes (3244.16±3324.42). Also, there was no statistical difference between the obese patients with non-prediabetes and control groups (3108.33±2932.72) in terms of PPAR-γ levels (ng/mL). However, there was no significant difference between serum adiponectin levels in the obese patients with non-prediabetes and control groups. Our remarkable finding was that serum adiponectin levels (ng/mL) of the obese patients with prediabetes group (18.15±15.99) were higher than the control group (15.17±15.67; p=0.42).

In addition, PPAR-γ to adiponectin ratio was statistically higher in the control group (259.32±208.38), obese patients with prediabetes group (206.57±229.66), and obese patients without prediabetes group (206.44±114.71).

Receiver operating characteristic (ROC) analysis was applied for adiponectin in obese patients with prediabetes compared with controls. According to our results, the analysis of adiponectin shows low diagnostic accuracy in obese patients with prediabetes compared with healthy controls (Figure 1).

DISCUSSION

In this study, we have shown that circulation of serum adiponectin and PPAR-γ concentrations differed based on the degree of prediabetes in obese and non-obese patients. We demonstrated that lower serum concentrations of adiponectin were present in control groups than in obese patients with prediabetes. Also, when obese patients with non-prediabetes compared with healthy non-obese patients, there were no significant differences between serum adiponectin and PPAR-γ levels. When obese patients without prediabetes were compared with healthy non-obese patients, no difference was found between serum PPAR-γ levels.

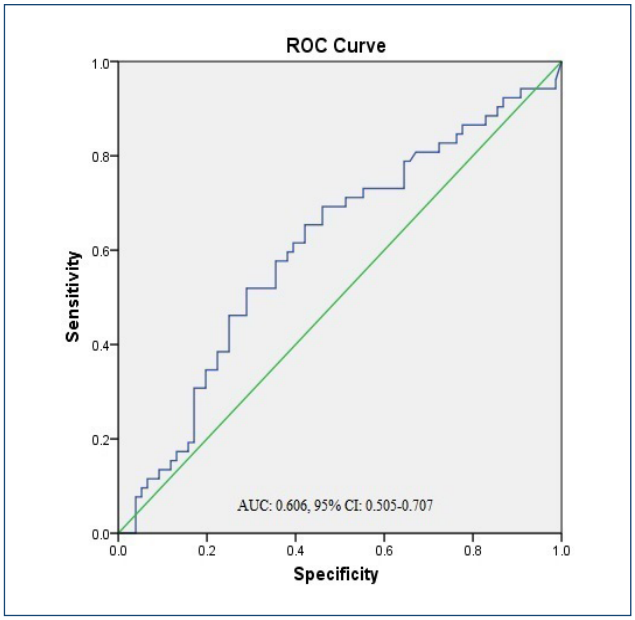

Figure 1. The receiver operating characteristic analysis results of adiponectin in obese patients with prediabetes compared to controls.

Adiponectin is a member of the adipokine family, which is secreted from adipose tissue, which is considered an endocrine organ, has antidiabetic and anti-inflammatory properties, and has functions such as insulin sensitivity, atherosclerosis, cell proliferation, and regulation of energy metabolism[10-12]. Obesity is an important public health problem and increases the risk of serious diseases such as T2DM and cancer. It is known that altered expression of adipokines affects fat accumulation in obesity. It is important to investigate this issue as adiponectin can act as a protective and safe factor to prevent obesity progression[13].

Gateva et al., showed lower adiponectin levels in patients with prediabetes compared with those without prediabetes, and Stojanović et al., indicated that decreasing the level of adiponectin was strongly implicated in the development of insulin resistance and may be a useful marker for coronary artery disease, metabolic syndrome, and prediabetes[14,15].

In this study, levels of serum adiponectin in obese patients with prediabetes were found statistically significantly higher than controls ($p<0.42$). However, there was no difference between obese patients with prediabetes and obese patients with non-prediabetes ($p>0.05$). Also, there was no difference between obese patients with non-prediabetes and control groups ($p>0.05$).

To the best of our knowledge, there are not many studies on serum PPAR-γ and adiponectin levels in obese and non-obese prediabetes. Contrary to our results, although this study is not specific to obese patients, in their meta-analysis study, Lai et al., showed that prediabetes patients had lower adiponectin levels than healthy controls, based on the lower circulating adiponectin levels before the onset of diabetes[16]. In their study among healthy adults whose parents had a history of T2DM, Jiang et al., showed adiponectin level as a strong risk marker for prediabetes and explained this by the fact that adiponectin is evident during the transition from normoglycemia to prediabetes at a much earlier stage of pathogenesis, due to its well-known association with diabetes risk[17].

It has been shown that prediabetic people have low serum adiponectin levels[16]. The low adiponectin levels observed in obese and T2DM individuals can be explained by the fact that adiponectin increases insulin sensitivity in target tissues[18,19].

In this study, no statistically significant difference was found in serum PPAR-γ levels between all groups (between

obese patients with prediabetes, obese patients with non-prediabetes, and control groups).

In their study on obese and non-obese patients with newly diagnosed T2DM, Liu et al., showed that there was a significantly reduced serum adiponectin level in the obese T2DM group compared with the T2DM group with normal BMI[20].

Studies on obesity have shown that PPAR-γ is an important regulator of fat cell formation and their normal functions[21]. The relationship between adiponectin and PPAR-γ is also present at the gene level, and adiponectin gene expression can be stimulated by PPAR-γ agonists[22]. Jones et al., showed that by ablating PPAR-γ from adipose tissue using a tissue-specific gene ablation approach, adiponectin gene expression was significantly reduced in adipocytes and also resulted in decreased circulating levels[23]. Treatment using a PPAR-γ agonist has been shown to increase adiponectin secretion and improve insulin resistance in rats and humans[24]. The clinical benefits of administering these agonists in preventing the progression of prediabetes to T2DM are not yet fully known. Although the irregularity of serum adiponectin levels in prediabetes may play a role in the pathogenesis of the disease, it may be better to prefer lifestyle changes to pharmacological treatment that will increase serum adiponectin levels because the results of using agonists are not known exactly[25].

CONCLUSION

We aimed to investigate serum adiponectin and PPAR-γ levels of both prediabetic obese and non-prediabetic obese patients in the design of our study as obesity is known to be a risk factor for the development of T2DM and prediabetes is a risk factor for the development of T2DM. As it is known, adiponectin increases insulin sensitivity in adipose tissue. Obesity and prediabetes also cause the development of T2DM as a result of metabolic disorders that begin with insulin resistance. In this context, we think that the findings of this study will contribute to the literature, as we have not encountered a study similar to the patient grouping design of our study in the literature.

Among the findings of our study, the fact that serum adiponectin levels were significantly higher in obese patients with prediabetes compared with the control group supports both our hypothesis and the literature. However, the fact that the PPAR-γ/adiponectin ratio was statistically higher in the control group compared with the prediabetic and non-prediabetic obese patient groups also supports our hypothesis. High adiponectin levels in obese patients with prediabetes may be an important marker for investigating the changes in lipid and glucose metabolism associated with both prediabetes and obesity, and even inflammatory and cardiovascular risks. If similar studies with a larger number of patients are supported, our results suggest that adiponectin may be an indicator of prediabetes, may help to understand metabolic disease processes closely related to prediabetes, and should be investigated in patients diagnosed with prediabetes.

ETHICS INFORMATION

The study was approved by the Ethics Committee of Amasya University (03/2021 Decision Number:6/100) and performed in accordance with the ethical standards specified in the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

MAG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **DT:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **AT:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MC:** Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **HDD:** Data curation, Formal Analysis, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Could signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 be a therapeutic target in the pathogenesis of preeclampsia?

Kenan Toprak[1*], Zafer Yıldız[2], Selim Akdemir[2], Kamil Esen[2], Rahime Kada Düken[3]

SUMMARY

OBJECTIVE: Determination of biomolecules that play a role in the etiopathogenesis of preeclampsia and their application as therapeutic targets may increase surveillance in this patient group. The aim of this study was to investigate the relationship between signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1, a marker of endothelial dysfunction and platelet activation, and the development of preeclampsia.

METHODS: In this observational cross-sectional study conducted between April 2021 and December 2022, 73 consecutive pregnant women with preeclampsia and 73 healthy pregnant women were included. Blood samples were taken from all patients with preeclampsia to measure signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 levels at the time of hospitalization. Excluded from the study were pregnant women with certain medical conditions or treatments, and the signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 levels of the groups were compared according to the development of preeclampsia.

RESULTS: Signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 levels were significantly higher in the preeclampsia group than in the controls ($p<0.001$). In multivariate analysis, signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 was determined as an independent predictor for preeclampsia (OR: 1.678, 95%CI 1.424–1.979, $p<0.001$). Receiver operating characteristic curve analysis showed that the best cutoff value of signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 at 3.25 ng/mL predicted the development of preeclampsia with 71% sensitivity and 68% specificity (area under the curve, 0.739; 95% confidence ınterval (95%CI), 0.681–0.798, $p<0.001$).

CONCLUSION: Signal peptide complement C1r/C1s, Uegf, and Bmp1, and epidermal growth factor-containing protein 1 is significantly elevated in pregnant women with preeclampsia compared with healthy controls.

KEYWORDS: Preeclampsia. SCUBE1. Biomarkers.

INTRODUCTION

Preeclampsia is one of the most important causes of maternal and perinatal mortality and morbidity all over the world[1]. Preeclampsia is a serious pregnancy-specific hypertensive disease that presents with various organ failures, especially dysfunction of the kidneys, liver, and lungs[2]. Currently, the only known definitive treatment for preeclampsia is to terminate the pregnancy and deliver the newborn[3,4]. Preeclampsia occurs in approximately 3–6% of all pregnancies, with an incidence 1.5–2 times higher in first pregnancies[5]. Generalized vasospasm, endothelial dysfunction, and secondary decreased organ perfusion with the activation of the coagulation cascade have been implicated in the pathogenesis of preeclampsia[6]. Maternal comorbidities closely associated with endothelial dysfunction and thrombotic complications such as chronic kidney disease, hypertension, and obesity play an important role in the etiopathogenesis of preeclampsia, and there is a lot of evidence that endothelial disease, the underlying mechanism of preeclampsia, is not limited to pregnancy but increases cardiovascular risk in later life[7].

Although many comprehensive studies have been carried out in recent years to understand the pathogenesis of preeclampsia, the underlying pathogenesis still remains unclear[4,8,9]. It is claimed that these mechanisms play a role in the basic etiopathogenesis of preeclampsia, mainly related to endothelial dysfunction[10]. Due to the release of placental factors into the systemic circulation at the end of poor placental perfusion, triggering systemic inflammation, vascular endothelial dysfunction, oxidative stress, and platelet activation, signs of increased blood

[1]Harran University, Faculty of Medicine, Department of Cardiology – Şanlıurfa, Turkey.
[2]Harran University, Faculty of Medicine, Department of Obstetrics and Gynecology – Şanlıurfa, Turkey.
[3]Siverek State Hospital, Department of Obstetrics and Gynecology – Şanlıurfa, Turkey.
*Corresponding author: kentoprak@hotmail.com
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pressure, proteinuria, and hypercoagulation develop, and the clinical picture of preeclampsia emerges[11].

Signal peptide complement C1r/C1s, Uegf, and Bmp1 and epidermal growth factor-containing protein 1 (SCUBE1) are recently identified cell surface proteins that can be expressed and secreted during early embryogenesis[12]. SCUBE1 is predominantly stored in the alpha granules of inactive platelets and endothelial cells[13]. After platelet activation, its expression increases and migrates toward the cell surface and is released into the circulation as small soluble particles. These circulating particles are considered a platelet activation marker because they increase platelet-platelet adhesion and agglutination in thrombotic conditions[13]. In addition, it is accepted as a marker of endothelial damage and vascular biology because its levels increase in the circulation in conditions associated with acute endothelial damages such as acute coronary syndrome, ischemic stroke, and hypertensive crisis[14-16]. We thought that there may be a causal relationship between SCUBE1 and preeclampsia, as the main presentations of preeclampsia, such as endothelial damage, hypertensive state, and prothrombotic environment, are pathophysiological conditions in which SCUBE1 also plays an active role, as mentioned before. In addition, the fact that aspirin, which is an antithrombotic agent, is the only proven treatment method in preeclampsia prophylaxis today strongly suggests the active role of SCUBE1, which is a platelet activation marker, in preeclampsia[17]. Thus, this biomarker may be a potential diagnostic marker and therapeutic target for preeclampsia.

As there are no enough data in the literature, our aim in this study was to compare the levels of SCUBE1, which is a marker of endothelial dysfunction and platelet activation, between healthy pregnant women and pregnant women with preeclampsia, and also to investigate the relationship between SCUBE1 levels and the severity of preeclampsia.

METHODS

Study setting and population

This observational cross-sectional study included 73 consecutive pregnant women hospitalized for preeclampsia between April 2021 and December 2022 and 73 healthy normotensive pregnant women matched by gestational age. Pregnant women with diabetes, history of chronic hypertension, liver disease, chronic kidney failure, history of thromboembolic event or thrombophilic disease, active infection, multiple pregnancies, having had preeclampsia before, HELLP syndrome, pregnant women using antiaggregant or anticoagulant, and those whose written consent could not be obtained for the study were excluded. Blood samples were taken from all patients with preeclampsia to measure SCUBE1 levels at the time of hospitalization. SCUBE1 levels were compared between the gestational age-matched healthy control group and the preeclampsia group. The sample size for this study was estimated based on common assumptions for a two-sample t-test with a significance level (alpha) of 0.05 and a power (1 - beta) of 0.80. We assumed a moderate effect size (Cohen's d=0.5) for the difference in SCUBE1 levels between the preeclampsia group and the healthy control group. An estimated sample size of approximately 67 participants in each group (preeclampsia and healthy control) would be required to detect a significant difference in SCUBE1 levels. Therefore, the estimated sample size was found to be adequate to detect significant differences in SCUBE1 levels between the two groups, considering the stated assumptions.

This study was conducted in line with the principles of the Declaration of Helsinki. The study was approved by the local ethics committee (Date: 21.03.2021, No. 21.02.01). Informed consent was obtained from all participants.

Clinical definitions

Preeclampsia was defined as the presence of one or more of the following new-onset conditions in pregnant women diagnosed with hypertension after 20 weeks of gestation: (1) proteinuria; (2) maternal organ dysfunction, including (a) renal failure (creatinine>90 μmol/L; 1 mg/dL), (b) liver involvement (elevated transaminases with or without right upper quadrant or epigastric abdominal pain), (c) neurological complications (including eclampsia, altered mental status, blindness, stroke, hyperreflexia with clonus, severe headaches with hyperreflexia, and persistent visual scotomata), and (d) hematological complications (thrombocytopenia with a platelet count below 150,000/dL, disseminated intravascular coagulation, and hemolysis); and (3) uteroplacental dysfunction (such as fetal growth retardation and abnormal umbilical artery Doppler wave)[18].

Laboratory analysis

Venous blood samples were obtained from pregnant patients with preeclampsia shortly after hospitalization and during routine polyclinic examinations from the control group matched for gestational age. Plasma and serum samples were obtained after centrifugation at 2750×g for 10 min. Routine biochemical analyses were performed on blood samples. Serum samples for SCUBE1 analysis were frozen and stored at -20°C until assayed. SCUBE1 levels were measured using the commercial

enzyme-linked immunosorbent assay (ELISA) kits (Human SCUBE1 ELISA kit: Aviva Systems Biology, San Diego, USA). The ELISA kit range was at a concentration of 0.156–10 ng/mL. The results are presented in ng/mL for SCUBE1. The mean coefficients of variation (CV) ranged from intra-assay: CV<6.5% to inter-assay: CV<9.5%.

Statistical analysis

Statistical Program for Social Sciences 26 (IBM SPSS, Chicago, IL, USA) was used for statistical calculations. The Kolmogorov-Smirnov test was used to determine whether the data fit the normal distribution. Continuous variables that fit the normal distribution were expressed as means±standard deviation (SD), and those that did not fit the normal distribution were expressed as median with interquartile range (IQR). Comparisons between subjects with preeclampsia and the control group were analyzed using the Mann-Whitney U test and independent-sample t-test where appropriate. The chi-square test was applied to categorical variables. Multivariate regression analyses were performed to determine the independent predictors of preeclampsia. The selection of independent variables for logistic regression analysis was guided by a stepwise variable selection approach. The "forward selection" method was used, in which variables were added to the model one by one according to their statistical significance. At each step, the variable with the lowest p-value was included and the goodness of fit of the model was evaluated. Variables that did not contribute significantly to the model or improve its fit were not included. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimum cutoff value of SCUBE1 levels, and we employed the ROC curve analysis to explore the potential utility of SCUBE1 as a biomarker for distinguishing between preeclampsia and healthy pregnancies. Two-tailed p<0.05 were considered statistically significant.

RESULTS

A comparison of demographic, clinical, and laboratory parameters of pregnant women with preeclampsia and the control group is given in Table 1. The mean age of the preeclampsia group was significantly higher than the control group (33.5 [4.9] vs 32.1 [5.9], p=0.039). While hematocrit levels were significantly lower in the preeclampsia group than in the control group (p=0.012), creatinine, white blood cell (WBC), and C-reactive protein (CRP) were higher (p=0.016, p=0.039, and p=0.016, respectively). SCUBE1 levels were significantly higher in the preeclampsia group than in the control group (4.63 [1.90] vs 3.09 [1.70]; p<0.001) (Table 1). In multivariate analysis, age (odds ratio [OR]: 1.056, 95% confidence interval (CI): 1.004–1.110, p=0.035), creatinine (OR: 1.280, 95%CI 1.059–1.569, p=0.041), WBC (OR: 1.152, 95%CI

Table 1. Comparison of demographic characteristics and hematological and biochemical parameters of the study groups.

Variables	Control group (n=73)	Preeclampsia group (n=73)	p-value
Age (years)	32.1±5.9	33.5±4.9	0.039
Body mass index (kg/m^2)	29.1±1.9	29.3±3.4	0.486
Gravidity (n)	4.8±3.4	5.0±2.5	0.550
Parity (n)	3.3±2.6	3.3±2.8	0.994
Gestational age at blood sampling (weeks)	35.04±3.61	35.08±3.61	0.216
Hemoglobin (g/dL)	10.77±0.55	10.75±0.41	0.735
Hematocrit (%)	35.5 (33.0–37.0)	34.5 (32.9–36.0)	0.012
Blood urea nitrogen (mg/dL)	38.0 (32.0–44.9)	36.0 (27.0–44.9)	0.101
Uric acid (mg/dL)	5.2 (4.4–6.1)	5.3 (4.3–6.1)	0.605
Creatinine (mg/dL)	0.8 (0.7–0.9)	0.8 (0.7–1.0)	0.016
White blood cell (×1000/mm^3)	6.52 (5.29–8.20)	7.80 (4.48–10.45)	0.039
C-reactive protein (mg/dL)	0.50 (0.18–1.00)	0.64 (0.32–1.50)	0.016
SCUBE1 (ng/mL)	3.09±1.70	4.63±1.90	<0.001
Newborn's birth weight (g)	2976±413	2672±452	0.093
Gestational age at delivery	38 (37–40)	37 (35–38)	0.109

Values are mean±SD, n (%), or median (interquartile range) unless otherwise stated.

1.043–1.273, p=0.005), and SCUBE1 (OR: 1.678, 95%CI 1.424–1.979, p<0.001) were determined as independent predictors for preeclampsia (Table 2). ROC curve analysis showed that the best cutoff value of SCUBE1 at 3.25 ng/mL detected the development of preeclampsia with 71% sensitivity and 68% specificity (area under the curve (AUC), 0.739; 95%CI 0.681–0.798, p<0.001). Also, when SCUBE1 and other predictors (i.e., WBC, creatinine, and age) were compared pairwise, the predictive power of SCUBE1 to predict preeclampsia was stronger than the other predictors (p<0.001 for all) (Figure 1).

DISCUSSION

In this study, we found that SCUBE1 was significantly higher in preeclamptic pregnant women than in the healthy control group. In addition, we found SCUBE1 significantly higher in the severe preeclampsia group than in the mild preeclampsia group, indicating that SCUBE1 may also be closely related to the severity of preeclampsia. Furthermore, we demonstrated that SCUBE1 may be an independent predictor of preeclampsia.

Preeclampsia is a pregnancy-specific disease that significantly increases maternal and perinatal mortality and morbidity. This disease causes the fetus to be premature and the risk of cardiovascular disease increases significantly in the long term in the mother[19]. Preeclampsia is manifested by new-onset maternal hypertension and often proteinuria after the 20th week of pregnancy and may result in hepatic, renal, and cerebral end-organ damage, and nowadays almost the only treatment is delivery of the placenta and fetus[4,5]. Although many pathophysiological mechanisms and risk factors have been identified in the etiopathogenesis of preeclampsia, its pathophysiology is still not fully elucidated[5]. It is suggested that unsuccessful transformation of uterine spiral arteries by trophoblasts in early pregnancy leads to poor placentation, causing hypoperfusion in the placental bed and fetal tissues, and as a result, placental factors released from the placenta into the maternal circulation lead to endothelial dysfunction, which is the main pathophysiology of preeclampsia[20]. This endothelial dysfunction creates a prothrombogenic predisposition and also increases the risk of thrombotic complications in these patients[21].

Many endothelial functions are mediated by proteins selectively expressed on the endothelial surface, such as SCUBE1[22]. SCUBE1 shares homology with proteins involved in thrombotic processes such as fibrillin, thrombomodulin, and protein C[22]. In addition to endothelial cells, it is stored in the alpha granules of platelets and actively participates in platelet adhesion and aggregation; therefore, it is considered a marker of vascular biology and platelet activation[14]. Studies have shown that SCUBE1 plays an active role in diseases such as acute coronary

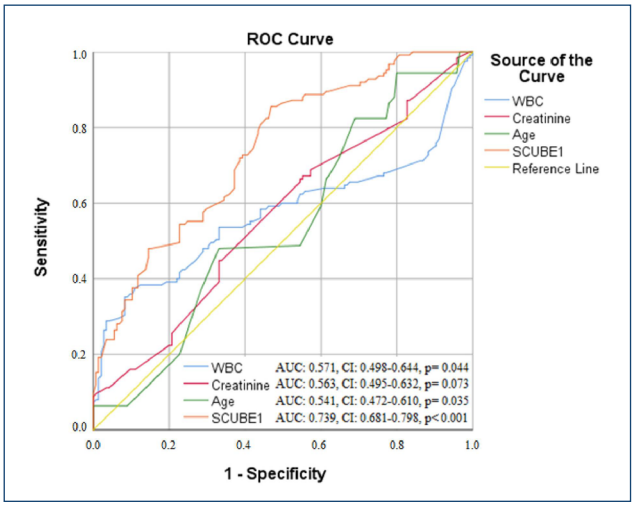

Figure 1. Receiver operating characteristic curve analysis showed that the best cutoff value of signal peptide complement C1r/C1s, Uegf, and Bmp1 and epidermal growth factor-containing protein 1 at 3.25 ng/mL predicted the development of preeclampsia with 71% sensitivity and 68% specificity (area under the curve, 0.739; 95% confidence interval, 0.681–0.798, p<0.001). Also, when signal peptide complement C1r/C1s, Uegf, and Bmp1 and epidermal growth factor-containing protein 1 and other predictors (i.e., white blood cell, creatinine, and age) were compared pairwise, the predictive power of signal peptide complement C1r/C1s, Uegf, and Bmp1 and epidermal growth factor-containing protein 1 to predict preeclampsia was stronger than the other predictors (p<0.001 for all).

Table 2. Univariate and multivariate regression analysis to identify independent predictors of preeclampsia.

	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.046 (1.002–1.091)	0.040	1.056 (1.004–1.110)	0.035
Hematocrit	0.996 (0.980–1.012)	0.612	0.988 (0.967–1.009)	0.266
Creatinine	1.976 (1.098–2.496)	0.006	1.280 (1.059–1.569)	0.041
White blood cell	1.155 (1.061–1.256)	0.001	1.152 (1.043–1.273)	0.005
SCUBE1	1.604 (1.383–1.859)	<0.001	1.678 (1.424–1.979)	<0.001

syndrome, ischemic stroke, acute mesenteric Ischemia, and hypoxic renal damage that present with endothelial dysfunction and thrombotic processes[15]. Studies have found a causal relationship between SCUBE1 and hypertension, which is one of the major risk factors for the development of preeclampsia[23]. In another study, SCUBE1 levels were found to be significantly higher in case of hypertensive crisis, and preeclampsia is actually a hypertensive crisis specific to pregnant women[17]. The fact that endothelial dysfunction and prothrombotic processes that trigger these comorbidities are also involved in the basic pathophysiological processes that trigger preeclampsia supports the causal relationship between preeclampsia and SCUBE1, an endothelial dysfunction, vascular damage, and thrombosis marker. In another study, it was shown that SCUBE1 could be a marker that could indicate placental dysfunction in patients with gestational diabetes[24]. Previous studies have shown a close relationship between preeclampsia and gestational diabetes and its associated placental dysfunction, and these results support the association between SCUBE1 and preeclampsia[25].

In conclusion, our findings suggest that SCUBE1 may serve as a valuable biomarker and predictor for the development and severity of preeclampsia. Identifying elevated SCUBE1 levels in pregnant women could potentially enhance the surveillance and early intervention in this patient group, ultimately contributing to improved maternal and perinatal outcomes. Furthermore, the role of SCUBE1 in the pathophysiology of preeclampsia highlights its potential as a therapeutic target. Future research, including larger randomized controlled studies, should aim to confirm these results and explore the clinical implications and therapeutic applications of SCUBE1 in the prediction and management of preeclampsia.

Limitations

Our study has some limitations. First of all, our study population was relatively small and it was an observational study. Second, only a single third-trimester measurement was taken for SCUBE1 in our study, and serial measurements were not taken before and after pregnancy. Third, long-term follow-up was not performed for thrombotic complications and cardiovascular diseases. The addition of serial measurements and long-term follow-up would have made the study more valuable. Fourth, markers such as the sFlt-1/PLGF ratio that were previously proven in the development of preeclampsia were not evaluated in our study. Fifth, our study is a cross-sectional study and therefore does not reveal a definitive relationship between SCUBE1 and preeclampsia. Finally, the ethnic diversity of the sample may have affected the results, although we included consecutive patients in the study. Larger randomized controlled studies are needed to confirm our results.

CONCLUSION

In our study, we detected higher levels of SCUBE1 in pregnant women with preeclampsia compared with healthy controls. Furthermore, we showed that SCUBE1 may be an independent predictor for the development of preeclampsia. These results indicate that the development of preeclampsia, in which endothelial dysfunction is a hallmark in etiopathogenesis, may be mediated by SCUBE1, an endothelial dysfunction and vascular biology marker. In this context, SCUBE1 may be a marker that can help explain the pathogenesis of preeclampsia.

AUTHORS' CONTRIBUTIONS

KT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **ZY:** Data curation, Formal Analysis, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **SA:** Investigation, Methodology, Resources, Writing – original draft. **KE:** Investigation, Methodology, Resources, Writing – original draft. **RKD:** Investigation, Methodology, Resources, Writing – original draft.

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ORIGINAL ARTICLE

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Comparison of rocuronium priming vs. standard rapid sequence intubation technique in emergency department patients requiring intubation

Nurullah İshak Işık[1*], Ayhan Özhasenekler[2], Çağdaş Yıldırım[2], Alp Şener[2], Fatih Ahmet Kahraman[2], Gül Pamukçu Günaydın[2]

SUMMARY

OBJECTIVE: In our study, we aimed to compare the effect of standard rapid sequence intubation protocol and the application of rocuronium priming technique on the procedure time and hemodynamic profile.

METHODS: Patients who applied to the emergency department and needed rapid sequence intubation were included in our study, which we conducted with a randomized controlled design. Randomization in the study was made according to the order of arrival of the cases. Rapid sequence intubation was performed in the standard group. In the priming group, 10% of the rocuronium dose was administered approximately 3 min before the induction agent. Intubation time, amount of drug used, vital signs, and end-tidal CO_2 level before and after intubation used to confirm intubation were recorded.

RESULTS: A total of 52 patients were included in the study, of which 26 patients were included in the standard group and 26 patients in the priming group. While intubation time was 121.2±21.9 s in the standard group, it was calculated as 68.4±11.6 s in the priming group (p<0.001). While the mean arterial pressure was 58.3±26.6 mmHg in the standard group after intubation, it was 80.6±21.1 mmHg in the priming group (p=0.002).

CONCLUSION: It was observed that priming with rocuronium shortened the intubation time and preserved the hemodynamic profile better.

Clinical Trial Registration Number: NCT05343702.

KEYWORDS: Airway management. Rapid sequence induction and intubation. Emergency medicine. Rocuronium.

INTRODUCTION

Rapid sequence intubation (RSI) is a commonly used technique for elective intubation, aiming to provide optimal physiological conditions while carrying some inherent risks such as hypotension, hypoxia, and aspiration of gastric content[1,2]. The primary goal of RSI is to achieve the intubation procedure as quickly as possible, minimizing complications in the subsequent process[1]. Emergency department patients often undergo this procedure without prior evaluation, which may increase the likelihood of complications. Although there is procedural consensus on measures to prevent gastric aspiration and hypoxia, clear recommendations for managing hypotension are currently lacking[1-4].

The advent of rocuronium, a non-depolarizing neuromuscular agent (NMBA), has increased its popularity, making it one of the most widely accepted agents for RSI[5]. The priming technique, first proposed by Foldes, has been shown in numerous studies to expedite the onset of NMBA action during intubation[6,7]. Theoretically, a small preceding dose of NMBA administered before induction leads to faster receptor stimulation and quicker onset of action.

Although the priming technique is generally considered hemodynamically neutral, the existing literature mainly comprises pre-evaluated patients in operating rooms and intensive care units. Unfortunately, there is a lack of studies focusing on emergency department patients[8].

In our study, we aimed to investigate the impact of rocuronium priming technique compared with the standard RSI protocol on intubation times and hemodynamic responses in patients requiring intubation upon presentation to the emergency department.

METHODS

Our study was conducted between July 15, 2021, and December 01, 2021, at the Ankara City Hospital Emergency Department, which is a tertiary care center with approximately 450,000 annual patient admissions. The study design was prospective and

[1]Ankara Etlik City Hospital, Department of Emergency Medicine – Ankara, Turkey.
[2]Ankara Bilkent City Hospital, Department of Emergency Medicine – Ankara, Turkey.
*Corresponding author: nurullahishak01@gmail.com

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randomized, and it received ethical approval from the Ankara City Hospital Ethics Committee 2 (Ethical Approval No. E2-21-631). Written consent was obtained from all patients, and the study was registered on clinicaltrial.gov (No. NCT05343702).

The inclusion criteria for our study were as follows:

- Age 18 years and above;
- Requirement for advanced airway management;
- Written consent from the patient or consent from the patient's relatives if the patient was unable to provide consent.

The exclusion criteria were as follows:

- Presence of crush (rescue) airway indication;
- Meeting any of the LEON criteria, indicating a difficult airway;
- Ineligibility for ketamine administration due to clinic-related reasons or allergy.

Data were collected using a data collection form, which included information on patients' age, gender, presence of diabetes mellitus, indication for intubation, vital signs (i.e., blood pressure, pulse rate, and oxygen saturation) before and 10 min after intubation, intubation duration, drug dosages used, end-tidal CO_2 level for intubation confirmation, number of successful attempts for intubation, and the need for alternative airway management. We only recorded diabetes mellitus as a chronic disease because diabetes patients have a higher likelihood of prolonged neuromuscular blockade[9]. The selection of the initial and 10th-min vital signs was based on the assumption that the physiological response to intubation (resulting from intense sympathetic and parasympathetic stimulation in the upper airway) would almost return to normal by the 5th min, while the effect of rocuronium would still be present, leading to more accurate results for the primary outcomes of our study[10]. For randomization, patients included in the study were assigned to the standard technique group if their admission number was odd and to the priming technique group if their admission number was even.

Our study's power analysis followed Şen et al.'s approach, which utilized onset time of action data for "Depolarizing and non-depolarizing neuromuscular agents." To achieve 99% power and a 5% type-1 error rate, each group required a minimum of 21 patients[11].

A common induction agent and a neuromuscular blocking agent (NMBA) were used for both groups. Ketamine was used as the induction agent with a dosage of 1 mg/kg, while rocuronium was used as the NMBA with a total dosage of 1 mg/kg. Chin relaxation was considered the indication of NMBA effectiveness in both groups. The start of the stopwatch was considered the moment when ketamine administration began for both groups. The end of the stopwatch was considered when the tube passed through the vocal cords, and the operator declared "I have passed." The onset of action of ketamine was considered 30 s for both groups. Capnometry was used to measure end-tidal carbon dioxide levels for verification after the intubation procedure. Vital signs were measured again at the 10th minute after the procedure. The standard technique followed the RSI protocol, while the priming technique involved administering 10% of the total planned rocuronium dose before induction and the remaining dose after the onset of action of the induction agent.

Our primary outcomes were intubation duration and hemodynamic response. Statistical analyses utilized SPSS for Windows 22.0. Normality was assessed with the Shapiro-Wilk test. Normally distributed data were presented as mean, standard deviation, and 95% confidence interval, and non-normally distributed data were shown as median, interquartile range, minimum, and maximum. Categorical variables were compared using Pearson chi-square and Fisher's exact tests. The independent-samples t-test assessed normally distributed continuous data for independent group comparisons and the paired-samples t-test for dependent group comparisons. The Mann-Whitney U test analyzed non-normally distributed continuous data for independent groups and the Wilcoxon signed-rank test for dependent groups. Significance was set at $p<0.05$.

RESULTS

After applying the exclusion criteria, a total of 52 patients were initially included in the study. However, data from one patient were excluded due to missing information. Consequently, the study was completed with 26 patients in the priming group and 25 patients in the standard group, and their data were analyzed (Figure 1).

There were no statistically significant differences between the groups in terms of mean age, gender distribution, and presence of diabetes among the included patients. Analysis of the pre-intubation hemodynamic profiles of the patients also revealed similar characteristics and no statistically significant differences between the two groups (Table 1).

When looking at the primary outcome, which is the intubation duration, it was found that the priming group had a significantly shorter intubation duration (Table 2). Another primary outcome, hemodynamic stability, was examined, and it was observed that the priming group exhibited a more stable hemodynamic profile at the 10th-min post-intubation.

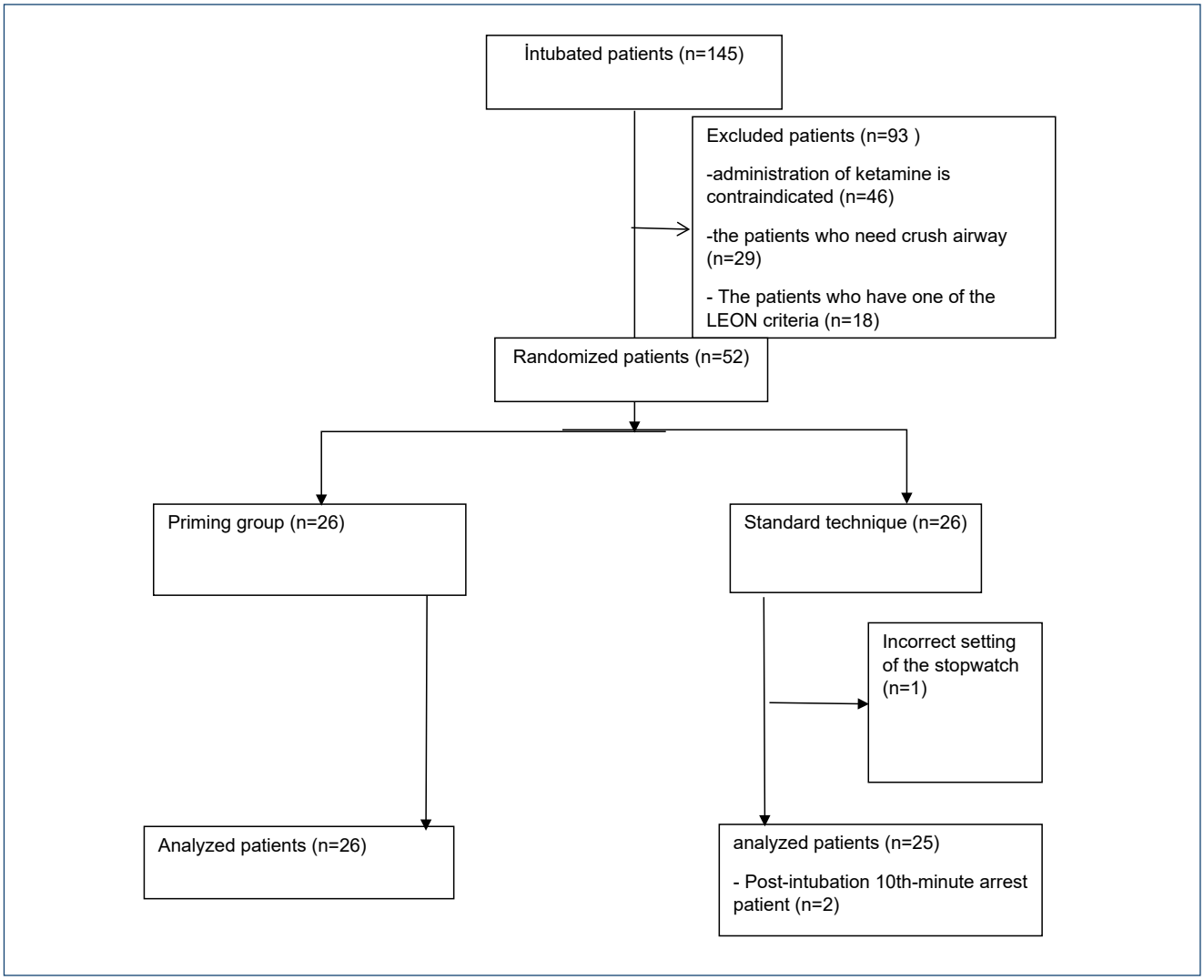

Figure 1. Flow diagram.

Table 1. Demographic characteristics and vital signs.

Variables	Standard group (n=25)	Priming group (n=26)	p-value
Age median (IQR)	80.0 (70.0–84.0)	79.0 (69.0–88.0)	0.806
Sex			
Male (%)	13 (52.0)	15 (57.7)	0.683
Female (%)	12 (48.0)	11 (42.3)	0.683
Diabetes mellitus (%)	9 (36.0%)	8 (30.8%)	0.692
SBP median (IQR) mmHg	110 (96–131)	110 (101–135)	0.434*
DBP median (IQR) mmHg	62 (53–77)	64.5 (56–75)	0.699†
MAP median (IQR) mmHg	84.3 (66.0–92.0)	79.7 (71.3–96.7)	0.378†
Heart rate (IQR) pulse/min	115 (98–139)	106 (94–118)	0.511*
SO_2 median (IQR) %	86 (78–92)	81,5 (71–89)	0.163*

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; IQR: interquartile range. *Independent-samples t-test. †Mann-Whitney U test.

Table 2. Post-intubation hemodynamic parameters and intubation times.

	Standard median (IQR)	Priming median (IQR)	p-value
Intubation time	117.0 (107.0–132.0)	67.5 (59.0–76.0)	<0.001†
Post SBP (mmHg)	80 (60–105)	104.5 (85–137)	0.007*
Post DBP (mmHg)	50 (32–60)	61 (53–75)	0.001*
Post MAP (mmHg)	59.7 (41.7–73.7)	72.7 (64.7–89.0)	0.002†
Heart rate (pulse/min)	124 (95–135)	106.5 (97–135)	0.659*
Post SO_2 (%)	95 (87–97)	93.5 (90–97)	0.762†

*Independent-samples t-test. †Mann-Whitney U test.

When analyzing the data at the 10th-min post-intubation, the priming group had higher values for systolic blood pressure, diastolic blood pressure, and mean arterial pressure compared with the standard group, and there was a statistically significant difference between the groups. However, there was no statistically significant difference between the groups regarding pulse rate and oxygen saturation (Table 2).

When examining the success of intubation based on the groups, there was no statistically significant difference between the groups. The median number of attempts in the priming group was 1.0 (mean value), while in the standard group, it was also 1.0 (p=0.362).

Regarding the verification of intubation placement, we analyzed the post-intubation end-tidal CO_2 values. The median value in the priming group was 26.0 mmHg (interquartile range: 20.0–29.0), while in the standard group, it was 29.0 mmHg (interquartile range: 24.0–59.0). There was a statistically significant difference between the groups (p=0.029).

DISCUSSION

When examining the results of our study, it was found that the priming technique resulted in a significantly shorter intubation duration and a more stable hemodynamic profile compared with the standard RSI.

As known, certain complications during intubation can lead to various undesired outcomes for patients in the short and long term. For instance, hypotension is a feared occurrence during intubation in both emergency services and urgent surgical cases, particularly for patients with no fasting period. Hypotension develops in almost one-fourth of patients, and this subgroup is associated with a higher mortality rate[12]. Our study designated these two issues as the primary outcomes and demonstrated that the priming technique was more beneficial.

When reviewing the literature on priming dose, it is evident that most studies have been conducted by anesthesiologists. Our study stands out as the first one conducted in the emergency department setting. Notably, the distribution of characteristics, including age, gender, and additional features, was similar between the groups. Our study includes older patients, with an average age of 79 years, which is not commonly seen in the literature[13,14]. Some studies suggest that the priming technique should not be applied to elderly patients or those with poor lung reserves[8,15]. However, we could not find any specific recommendations in the literature pertaining to our patient population.

It is well-known that the priming technique reduces intubation duration, and several studies have provided evidence supporting this finding[7]. The results of our study align with the literature in this regard. Moreover, being the first study conducted specifically in the emergency department setting and involving a higher proportion of elderly patients adds to its distinctiveness. Regarding hemodynamic stability, rocuronium is among the best nondepolarizing neuromuscular agents, and it is generally accepted that priming application does not impact the hemodynamic response[16]. There is a wealth of data on intubation-related hypotension, which can be influenced by various factors such as the physiology of intubation, drugs used, and the patient's clinical condition (e.g., hypovolemia and shock). Additionally, there are articles in the literature indirectly investigating the relationship between rocuronium use and hypotension[17]. When examining the literature, we notice a common feature of planned RSI performed in low-risk, young patients. In contrast, our study involves patients with relatively unstable conditions in the emergency department, including older patients. The age-related differences in rocuronium pharmacokinetics and the fragility of these patients might have contributed to the emergence of a significant difference. This suggests that priming dosage might be more beneficial for this critical patient group. Furthermore, the higher end-tidal CO_2 values in the priming group could confirm better perfusion in these patients.

In conclusion, our study's findings indicate that the priming technique results in a significantly shorter intubation duration and a more stable hemodynamic profile compared with the standard technique. These results hold potential importance for critical patients in the emergency department. However, further studies with larger patient populations and diverse settings are needed to validate these findings.

LIMITATIONS

One of the main limitations of our study is that it was single-blinded. We attribute this to the fact that the study was conducted in a large hospital with intubations performed by various personnel in multiple areas. To overcome this limitation, we ensured that the data collection process was carried out by an independent individual.

CONCLUSION

Our study demonstrated that the priming technique significantly reduced intubation duration and resulted in a more stable and consistent hemodynamic profile in terms of systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure.

AUTHORS' CONTRIBUTIONS

NİI: Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **FAK:** Data curation, Visualization. **AŞ:** Formal Analysis, Methodology. **AÖ:** Funding acquisition, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **ÇY:** Resources, Writing – original draft, Writing – review & editing. **GPG:** Validation.

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ORIGINAL ARTICLE

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Effect of obesity on mood regulation and eating attitudes in mental disorders

Hayriye Baykan[1], Sinan Altunöz[1*], Merve Şahin Can[1]

SUMMARY

OBJECTIVE: The precise relationship between obesity and eating habits, attitudes, and emotion regulation is still ambiguous. The purpose of this study was to investigate possible correlations among body mass index, challenges related to managing emotions, and attitudes toward eating among adult participants with known psychiatric diagnoses.

METHODS: The body mass indices of participants were calculated, and data on eating styles were collected using the Dutch Eating Behavior Questionnaire. The level of difficulty in managing emotions was evaluated using the Difficulties in Emotion Regulation Scale.

RESULTS: The research findings indicated a meaningful positive association. An observation was made between body mass index and results from the Eating Attitude Test-40, as well as the restrained eating subdimension of the Dutch Eating Behavior Questionnaire. Conversely, a meaningful reverse relationship was identified between the scores of the "strategies" subdimension of the Difficulties in Emotion Regulation Scale. No meaningful differences in eating attitudes and emotion regulation were found between non-obese and obese patients.

CONCLUSION: While a partial and meaningful correlation was observed among body mass index, eating attitudes, and emotion regulation difficulties, it is suggested that factors such as patients' age, disease duration, current body mass index, and the simultaneous presence of depression and anxiety should be considered.

KEYWORDS: BMI. Eating behaviors. Emotion regulation. Obesity. Mood.

INTRODUCTION

Body mass index (BMI)≥30 kg/m² is known as obesity and has a multifactorial etiopathogenesis, involving genetic, environmental, metabolic, lifestyle, and behavioral factors[1]. The existence of overweight or obesity elevates the likelihood of numerous diseases, making it a serious global epidemic linked to stroke, heart disease, colorectal cancer, high cholesterol, high blood pressure, gallbladder disease, diabetes mellitus, and increased mortality[2].

Research indicates a strong bidirectional association among diverse psychiatric disorders and obesity. These conditions can be impacted by factors such as age, gender, and socioeconomic status[3]. Anxiety and mood disorders are widespread among patients looking bariatric surgery for obesity, and binge eating disorder is commonly associated with severe obesity[4]. During life, people are continually unprotected from internal and environmental stimuli that can cause emotional reactions, and these feelings manifest themselves with some cognitive, behavioral, and physiological responses[5]. The moment emotion is experienced, and the presence of emotion regulation comes into question[6].

Emotion regulation is essential for processing and managing emotions effectively. It involves skills to identify, comprehend, and accept emotions, using adaptive regulation strategies based on individual goals and values[7].

People may turn to eating as a coping mechanism when faced with negative emotions. Fernandes et al., found varied emotional processing and regulation challenges in obese individuals with and without binge eating disorder. Shriver et al., suggested that in adolescents, lack of emotion regulation predicts future obesity through emotional eating[8,9].

Our research explored the relationship between BMI, emotion regulation challenges, and eating attitudes in adults with psychiatric diagnoses at a psychiatry clinic. We also investigated the relationship between psychiatric diagnoses, emotion regulation difficulties, and eating attitudes. Our hypothesis is that higher BMI would be linked to more eating attitude disorders and emotion regulation difficulties, which are associated with underlying psychiatric disorders. Addressing these goals will provide information for interventions for individuals with psychiatric disorders and obesity.

[1]Balikesir University, Faculty of Medicine, Department of Mental Health and Diseases – Balıkesir, Turkey.
*Corresponding author: sinanaltunoz03@gmail.com
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METHODS

Before starting the study, ethical approval (No. 2022/123) was obtained from Balikesir University Health Sciences Non-Interventional Research Ethics Committee on 03.08.2022. Participants were selected from individuals seeking treatment at the Psychiatry Clinic. Inclusion criteria were adults aged 18–68 years with known psychiatric diagnoses. Exclusion criteria were severe cognitive impairment or inability to provide informed consent.

Procedure

Participants provided information on purpose, procedures, risks, and benefits. Verbal and written consent was obtained, emphasizing voluntary participation and the right to withdraw without repercussions. The size of the study group was determined using the G-Power 3.1 software (α=0.05 and power=80%), resulting in 140 participants. The final sample was 123 adults seeking treatment at the Psychiatry Clinic, with 78.4% females (n=96) and 21.96% males (n=27). The age range was 18–68 years, with an average age of 37.94 (SD=12.09) years.

Data collection tools

General Information Form

Researchers utilized a sociodemographic data form they developed to assess participants' socio-demographic participant characteristics, including age, gender, and other relevant information.

The Difficulties in Emotion Regulation Scale

The Difficulties in Emotion Regulation Scale (DERS) by Gratz and Roemer assessed participants' emotion regulation challenges. It is a 5-point Likert-type scale (1=almost never to 5=almost always) without a specified cut-off score. Higher scores indicate more severe challenges. DERS has six subdimensions: Awareness (components 2, 6, 8, 10, 17, 34, and 34), Clarity (components 1, 4, 5, 7, and 9), Rejection (components 11, 12, 21, 23, 25, and 29), Strategies (components 15, 16, 22, 28, 30, 31, 35, and 36), Impulse (components 3, 14, 19, 24, 27, and 32), and Goal (components 13, 18, 20, 26, and 33). The internal consistency coefficient of the scale is 0.93, and the subdimensions range from 0.80 to 0.89[10].

Eating Attitude Test

The Eating Attitude Test-40 (EAT-40) is a 6-point Likert-type self-report measure (ranging from "Always" to "Never") with 40 items. Developed by Garner and Garfinkel, it identifies individuals at risk for eating disorders and assesses symptoms. Applicable to ages 11 years and above, specific items have different scoring criteria. Scores≥30 on the EAT-40 indicated "prone to eating behavior disorder" in this study. Savaşır and Erol examined the validity of EAT-40 in Turkey, yielding a reliability coefficient of 0.65, and Cronbach's alpha coefficient was 0.70 for internal consistency[11].

The Dutch Eating Behavior Questionnaire

Van Strien et al., developed a 33-item scale assessing emotional, restrained, and external eating styles which includes 10 items for restrained eating, 13 for affective eating, and 10 for extrinsic eating. Responses range from "1: Never" to "5: Very often," with item 31 scored reversely. Psychometric characteristics were studied in a Turkish university sample. Cronbach's alpha was 0.94 for the entire scale, 0.90 for external eating, 0.91 for restrained eating, and 0.97 for emotional eating subdimensions. The test–retest reliability was 0.72, indicating DEQB's reliability in Turkey[12].

Body Mass Index Measurement

BMI is a common noninvasive measure for obesity assessment. Height was accurately measured after removing shoes and excess clothing. BMI was calculated using the formula BMI=Weight (kg) / Height (m)^2. Participants were categorized based on BMI values: underweight (<18.50 kg/m^2), normal weight (18.50–24.99 kg/m^2), overweight (25.0–29.99 kg/m^2), and obese (≥30.0 kg/m^2)[13].

Statistical analysis

Study data were analyzed using the SPSS 25.0 software. Descriptive statistics (N, %, $\bar{x}$, SD, M) summarized data. Normality was assessed with the Kolmogorov-Smirnov test and histograms. The independent-samples t-test compared normally distributed quantitative data. The Mann-Whitney U test analyzed non-normally distributed data. The Spearman correlation coefficient was used for non-normally distributed quantitative variables, and the Pearson correlation coefficient was used for normally distributed variables. The chi-square test compared categorical data. Test selection was based on the nature of the data and research hypotheses. The significance level of $p<0.05$ and a 95% confidence interval were used for the assessment of results.

RESULTS

Table 1 shows the sociodemographic data and psychiatric diagnoses of participants [n=123, with 78.04% females (n=96) and 21.96% males (n=27)]. The average age of the participants was 37.94±12.09 years (range: 18–68 years). The average BMI of

the participants was 28.98±6.83 kg/m² (range: 15.00–45.50 kg/m²). The diagnoses were depressive disorder (51.21%, n=63), anxiety disorder (39.02%, n=48), and obsessive-compulsive disorder (9.75%, n=12).

There were 50.40% (n=62) obese (BMI≥30 kg/m²) and 49.59% (n=61) nonobese participants. The average ages of the obese and nonobese groups were 44.27±11.11 and 31.51±9.40 years, respectively. A statistically significant relationship was found between EAT-40 scale scores and BMI (r=0.233; p=0.01). Particularly in depressive disorder individuals, EAT-40 scores significantly correlated with BMI (r=0.249; p=0.049).

A noteworthy negative correlation was observed between DERS2 (Strategies) results and BMI (r=-0.184; p=0.041). A statistically significant negative correlation was observed in anxiety disorder patients (r=-0.296; p=0.022), but not in depressive disorder patients (r=-0.043; p=0.740).

A statistically meaningful correlation was found between DEBQ1 (restrained eating) results and BMI (r=0.212; p=0.019). In depressive disorder volunteers, a significant correlation between DEBQ1 (restrained eating) results and BMI (r=0.282, p=0.025) was observed, but not in anxiety disorder patients (r=0.132; p=0.315).

No statistically substantial correlations were found in paired comparisons between obese and nonobese groups using the study scales (Table 2). The average scores obtained from the scales used in the study are presented in Table 3.

DISCUSSION

Previous studies in the literature have documented that those people with eating disorders have at least one psychiatric comorbidity before or concurrently with the establishment regarding the identification of an eating disorder and persisted after the eating disorder has been cured[14]. Depression is more prone to occur after the progress of an eating disorder, while anxiety tends to manifest before the progress of an eating disorder[15]. According to this, the hypothesis was formulated that anxiety may be linked to more severe symptoms of the eating disorder[16]. Based on the current literature data, our research found that the average EAT-40 result was below the

Table 1. Descriptive statistics.

	Female (n=96)	Male (n=27)	Total (n=123)
Gender	78.04%	21.96%	100%
Age (mean±SD) (years)	37.38±11.92	39.96±12.73	37.94±12.09
Body mass index (mean±SD) (kg/m²)	28.24±7.33	29.37±4.64	28.49±6.83
Depressive disorder % (n)	36.58 (45)	14.63 (18)	51.21 (63)
Anxiety disorder % (n)	33.33 (41)	5.69 (7)	39.02 (48)
Obsessive-compulsive disorder % (n)	8.13 (10)	1.62 (2)	9.75 (12)

Table 2. Correlation between the scales utilized in the study and body mass index.

p<0.05*	Body mass index					
	All participants		Depression		Anxiety disorder	
	r	p	r	p	r	p
EAT-40	0.233	0.010	0.249	0.049	0.230	0.078
DEBQ1 (Restrained eating)	0.212	0.019	0.282	0.025	0.132	0.315
DEBQ2 (Emotional eating)	0.059**	0.515	0.063	0.625	0.037	0.776
DEBQ3 (External eating)	0.017	0.849	0.009	0.945	0.095	0.472
DERS1 (Goal)	0.105	0.250	0.210	0.099	-0.021	0.873
DERS2 (Strategies)	-0.184	0.041	-0.043	0.740	-0.296	0.022
DERS3 (Impulse)	0.087	0.341	0.169	0.185	-0.082	0.536
DERS4 (Awareness)	0.026	0.779	-0.059	0.645	0.008	0.954
DERS5 (Clarity)	0.131	0.148	0.153	0.233	0.052	0.694
DERS6 (Nonacceptance)	-0.042	0.641	-0.022	0.863	-0.124	0.344

*Level of statistical significance. **Spearman correlation coefficient.

Table 3. Mean (±SD) scores obtained from the scales used.

Scales	Mean±SD
EAT-40	20.87±10.68
DEBQ1 (Restrained eating)	24.24±8.91
DEBQ3 (External eating)	28.00±8.44
DERS1 (Goal)	14.80±6.18
DERS2 (Strategies)	16.98±6.02
DERS 3 (Impulse)	14.92±6.02
DERS4 (Awareness)	16.42±4.50
DERS5 (Clarity)	20.85±8.41
DERS6 (Nonacceptance)	12.94±4.52
Scale	Med. (min.–max.)
DEBQ2 (Emotional eating)	20 (13–65)

cutoff point (20.87±10.68). This could explain the absence of significant correlations between BMI, EAT-40, and restrained eating subdimension scores from the DEBQ1 in patients with anxiety disorder.

Anxious mood showed a selective association with eating psychopathology in anorexia nervosa participants compared with other affective temperaments. However, the significant effect of depressive mood on anorexia nervosa psychopathology was also emphasized[17].

Our research detected a significant association among BMI, eating attitudes, and challenges in emotion regulation. However, unlike the existing literature, no notable distinction was detected between the obese (BMI≥30 kg/m^2) and non-obese patient groups regarding eating attitudes and emotion regulation difficulties. We acknowledge that our study's clinical sample and cross-sectional approach may introduce confounding factors. Important limitations include environmental influences, disease duration, current BMI, and levels of anxiety and depressive symptoms between the non-obese and obese groups.

Previous research emphasizes the effect of energy intake variations and individual eating behavior differences on obesity. Morbidly obese patients often exhibit problematic eating behaviors. Categorizing eating behaviors into emotional, external, and restrained styles is common and relevant in obesity etiological models[18,19]. Despite variations in the data from different studies, a notable link has been observed between restrained eating behaviors and obesity as well as BMI[20-22]. Our study uncovered a statistically meaningful association among the scores of the restrained eating subdimension in the DEBQ and BMI, which aligns with the findings from previous research.

Difficulties in emotion regulation are significant in individuals with eating disorders, often leading to unhealthy eating habits as a means of coping with negative emotions[23,24]. Many studies have suggested that unhealthy eating habits could be due to efforts to regulate negative emotions. Studies comparing healthy and obese volunteers have shown that obese individuals more frequently face emotion regulation challenges. However, investigations on high BMI individuals without eating disorders are limited[25]. In our study, a clear negative correlation was found between the "Strategies" subdimension results of the DERS2 scale and BMI.

The absence of statistically meaningful distinction between obese (BMI≥30 kg/m^2) and non-obese groups in eating attitudes and emotion regulation challenges suggests the role of environmental factors, disease duration, age, BMI, and comorbidities (depression and anxiety) in influencing outcomes.

Significant implications for clinical practice

Clinicians should conduct a thorough assessment of eating attitudes and emotion regulation in patients with psychiatric diagnoses, as these factors significantly relate to BMI. Special attention should be given to restrained eating behavior, which shows a positive correlation with BMI. Comorbidities such as depression and anxiety should be considered when evaluating these relationships. Individualized treatment plans should be developed, considering factors such as age, disease duration, current BMI, and comorbidities. Further research, including healthy volunteer studies, is necessary to better understand the complexities of these correlations. A multidisciplinary approach involving mental health professionals and nutritionists may be beneficial in providing comprehensive care to patients.

Limitations

Cross-sectional design hampers causal relationships. Longitudinal studies better explore BMI, emotion regulation, and eating attitudes over time. Focusing on clinical sample limits generalizability. Healthy controls would offer a comparative perspective. Relying on self-reports may introduce response bias and miss variable complexity. Future studies need objective assessments and diverse, larger samples to address these limitations.

CONCLUSION

The research found significant correlations between BMI, eating attitudes, and emotion regulation challenges in adult patients with psychiatric diagnoses at a psychiatry clinic. Higher BMI correlated positively with restrained eating and negatively with emotion regulation strategies. Interventions targeting emotion

regulation and maladaptive eating attitudes could benefit individuals with psychiatric diagnoses and high BMI. Future research should explore longitudinal associations, use larger, diverse samples, and include healthy volunteers to understand BMI, emotion regulation, eating attitudes, and psychiatric diagnoses better. The research supports targeted interventions for individuals with psychiatric disorders and obesity.

ETHICAL APPROVAL

Before starting the study, ethical approval (No. 2022/123) was obtained from Balikesir University Health Sciences Non-Interventional Research Ethics Committee on 03.08.2022. During the implementation phase, the participants were briefly informed about the purpose of the study, and verbal and written consent was obtained from those who agreed to participate in the study.

AUTHORS' CONTRIBUTIONS

HB: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **SA**: Conceptualization, Writing – original draft, Writing – review & editing. **MŞC**: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Mobile app for pelvic floor muscle training for urinary incontinence during the coronavirus disease 2019 pandemic: clinical trial

Camila Carvalho de Araujo[1], Luiz Gustavo Oliveira Brito[1], Andrea Marques[1], Marcela Bardin[1], Cássia Raquel Teatin Juliato[1*]

SUMMARY

OBJECTIVE: The objective of this study was to evaluate the effects of home-based pelvic floor muscle training in women with urinary incontinence, addressing the difficulties arising from social isolation due to the coronavirus disease 2019 pandemic by utilizing a specialized mobile app.

METHODS: This randomized, single-group clinical trial aimed to assess the efficacy of pelvic floor muscle training guided by a mobile app (Diario Saúde) in women with stress urinary incontinence. Participants were instructed via telephone to engage in pelvic floor muscle training exercises twice a day for 30 days. Pre- and post-treatment, participants completed validated questionnaires regarding urinary symptoms and quality of life through telephone interviews. Additionally, treatment adherence was evaluated.

RESULTS: A total of 156 women were enrolled in the study, with a mean age of 49.3±14.2 years. Significant improvements in urinary incontinence symptoms and quality of life were observed following pelvic floor muscle training guided by the mobile app ($p<0.001$). Notably, 74.3% of the participants reported performing the exercises with appropriate frequency. Of the participants, 62% reported either complete or substantial improvement in urinary symptoms post-treatment.

CONCLUSION: This study revealed notable enhancements in stress urinary incontinence, urinary storage, and overall quality of life subsequent to pelvic floor muscle training guided by a mobile app, particularly during the coronavirus disease 2019 pandemic. The mobile app demonstrated robust acceptance and adherence among women experiencing urinary incontinence.

KEYWORDS: Adherence. COVID-19. Mobile app. Pelvic floor. Urinary incontinence.

BACKGROUND

Urinary incontinence (UI) represents a substantial global health concern for women, exerting adverse effects on their quality of life and contributing to increased morbidity and mortality rates[1]. Pelvic floor muscle training (PFMT) stands as the primary conservative approach for managing UI in women[2], yielding favorable outcomes. Nevertheless, adhering to PFMT presents complexities, requiring modifications in behavior and active patient engagement[3].

The onset of the coronavirus disease (COVID-19) pandemic demanded both patients and healthcare providers adapt to new norms, emphasizing social isolation and rigorous sanitation protocols[4]. As a result, addressing non-urgent conditions like UI became challenging due to pandemic-induced restrictions[4].

Telemedicine emerged as an innovative solution during this period, gaining widespread acceptance[4,5]. Although mobile apps for PFMT had been explored previously, the effectiveness, acceptance, and adherence of women with UI to a structured app-based PFMT regimen took on heightened significance within the context of the pandemic.

Adherence to PFMT exercises remains crucial for sustaining long-term effectiveness and preventing UI recurrence[6]. A recent systematic review confirmed the safety and efficacy of home device apps[7]. This study aimed to evaluate the effects of home-based PFMT in women with UI, addressing the difficulties arising from social isolation due to the COVID-19 pandemic by utilizing a specialized mobile app Diário Saúde®[8].

DESIGN AND SETTING

This randomized, single-group, clinical trial study was conducted from December 2020 to March 2022 at the University of Campinas, Brazil. The study was approved by the local research ethics board (CAAE: 36515520.0.0000.5404) and registered at the Brazilian Clinical Trials Registry (RBR-7k2hpj4). Informed consent was obtained from all participants through

[1]Universidade Estadual de Campinas, School of Medical Sciences, Department of Obstetrics and Gynecology – Campinas (SP), Brazil.
*Corresponding author: cjuliato@unicamp.br, cassia.raquel@gmail.com
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a recorded telephone call after the agreement of the participant, who also provided a digital signature through the consent term sent by email.

Participants

We included women aged 18 years or older, with complaints of stress urinary incontinence (SUI) or mixed UI with stress predominance according to the International Continence Society criteria[9] lasting for at least 4 weeks, who had an Android mobile phone, were able to read, agreed to answer the questionnaires through telephone contact, and performed PFMT using the mobile app Diário Saúde®[10]. We excluded women who were currently pregnant or using medication for UI, had any prior UI treatment, had recurrent urinary tract infection, had a history of neurological diseases, and were <1 year postpartum.

Procedure and intervention

Recruitment of participants for the study was initiated through outreach on social networks, inviting interested women to connect with the principal researcher (a qualified physiotherapist) for an initial screening interview aimed at confirming eligibility criteria. Subsequently, comprehensive interviews were conducted with all participants, during which the exercise program was introduced and explained via telephone communication.

Participants underwent a baseline assessment encompassing sociodemographic and gynecological information, details about COVID-19 testing and outcomes, and any changes in urinary symptoms when COVID-19 was confirmed and answered the validated Portuguese versions of the following questionnaires: Questionnaire for Urinary Incontinence Diagnosis (QUID), International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF), International Consultation on Incontinence Questionnaire-Overactive Bladder (ICIQ-OAB), and Incontinence Quality of Life Questionnaire (I-QOL)[10-13]. Participants received thorough education regarding the role of pelvic floor muscles, accurate perception of their activation, and the correct techniques for contraction and relaxation. Subsequently, participants were guided to install the dedicated mobile app.

The home-based training protocol was based on the regimen outlined by Araujo et al.[9] involving exercises lasting around 5 min each, to be performed twice daily (in sitting, lying, or standing position) over a span of 30 days.

A post-treatment assessment was conducted by the researcher 30–45 days after the exercise period's conclusion, utilizing the same baseline assessment tools. Additionally, participants were asked about their satisfaction with the treatment intervention and to rate their satisfaction with the treatment. Finally, adherence to the treatment regimen was measured through the question, "How often did you adhere to the prescribed exercises?"

Diário Saúde® app

The Diário Saúde® app uses a visual stimulus similar to electromyography as a guide for PFMT contraction, which shows when the participant is supposed to contract and sustain or contract and relax, along with the duration of contraction and the number of repetitions. The music rhythm is synchronized with the duration of the contractions, and the volume changes during the contraction or relaxation of pelvic floor muscle visual stimuli[8]. Importantly, the app also offers twice-a-day reminders to encourage practicing PFMT.

Outcomes

The main objective of this study was to assess the enhancement in urinary symptoms, urinary storage, and quality of life. The secondary objective was to encompass the assessment of both adherence to and satisfaction with the intervention among women experiencing UI.

Statistical analysis

The sample's characteristics were depicted by presenting frequency tables for categorical variables with absolute frequency (n) and percentage (%) values. Descriptive statistics, including mean values and standard deviations, were employed to portray the numerical variables. To compare categorical variables before and after the intervention, the McNemar test was applied for related samples involving two categories. In cases where numerical variables lacked a normal distribution, the Wilcoxon test was employed to compare related samples before and after the intervention. The threshold for statistical significance was set at 5%, denoted as $p<0.05$[14].

RESULTS

A total of 156 women with complaints of SUI or mixed UI with stress predominance were included in the study (Figure 1). The mean age of the women was 49.3±14.2 years. Among the women affected by UI, 23.7% had tested positive for COVID-19, and of those, half noted a deterioration in UI symptoms subsequent to the infection (Table 1).

The majority of participants in our study exhibited severe to very severe SUI, as indicated by ICIQ-SF scores ranging from 5 to 21, with a median of 14.4 (±4) and a mean of 15, accompanied by interquartile 25 and interquartile 75 values of 12 and 18, respectively. Significant enhancements were

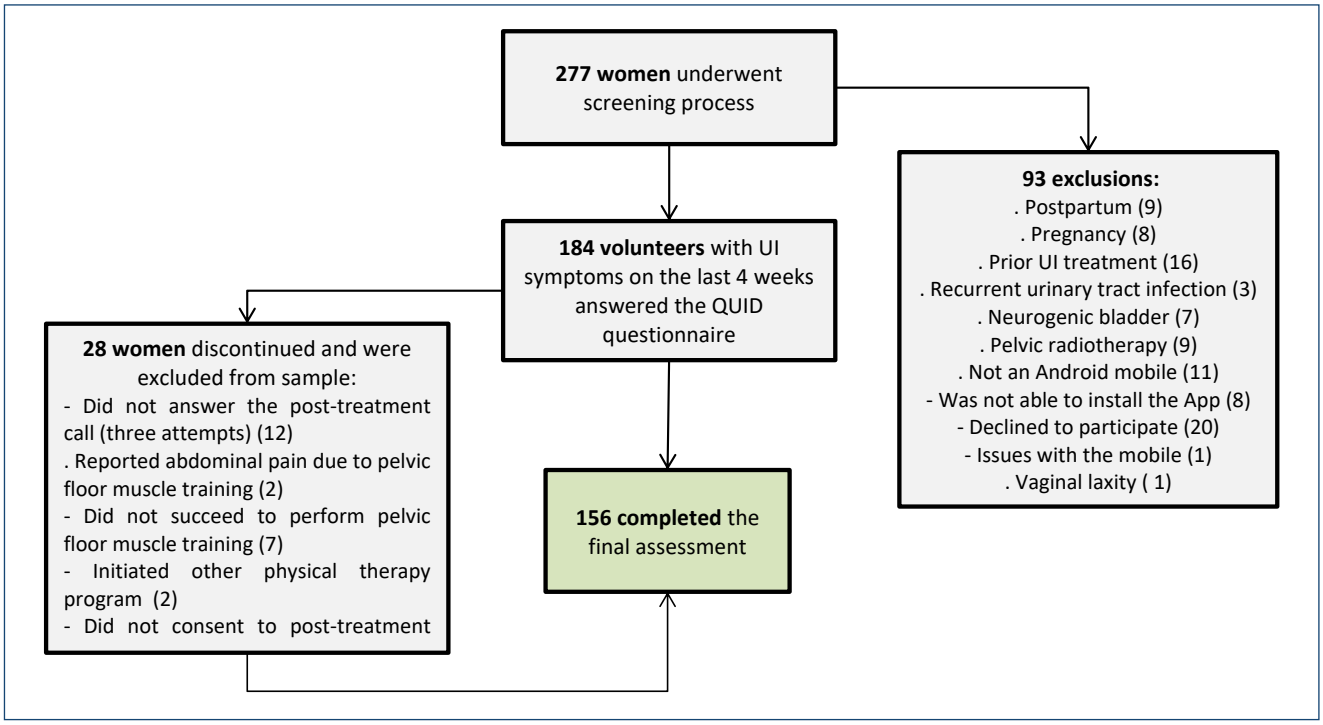

Figure 1. Participant flowchart and drop-out. UI: Urinary incontinence; PFMT: pelvic floor muscle training.

observed in UI symptoms assessed by QUID and ICIQ-SF following the mobile app-based home treatment (p<0.001). Furthermore, improvements were evident in urinary storage symptoms post-treatment (7.3 vs. 6.3, p<0.001). Evaluation of quality of life indicated overall improvement post-treatment, encompassing aspects such as general well-being, avoidance or limitation of behaviors, psychological impact, and social discomfort (p<0.001, Table 2).

In terms of exercise adherence, women reported engaging in the prescribed exercises with satisfactory frequency ("always," "almost always," and "many times") 74.3% of the time. Remarkably, the PFMT regimen guided by the Diário Saúde® app demonstrated robust acceptance, with only 2% of participants reporting noncompliance with the exercises. Additionally, over 62% of women reported either complete alleviation or a notable improvement in symptoms post-treatment, with an average satisfaction rating of 7.4 on a scale of 0–10.

A post-hoc power analysis was conducted, comparing score values (QUID, ICIQ-SF, and I-QOL) before and after the intervention, based on data from 156 women with UI and utilizing mean and standard deviation values from both assessment points. The analysis revealed a high level of statistical power, ranging from 98.3 to 99.9%, for the comparison of questionnaire scores pre- and post-intervention.

DISCUSSION

Our findings showed improvements in SUI symptoms, urinary storage symptoms (such as urinary frequency, nocturia, urinary urgency, and urge incontinence), and quality of life. The utilization of the Diário Saúde® app exhibited high levels of adherence, complemented by significantly positive feedback from the participants.

Among various physiotherapeutic modalities, PFMT requires consistent execution and sustained efforts by the patient within a home setting. Notably, our study's outcomes align with the findings of Barret et al.[15] indicating a preference for app-based guidance over traditional verbal or written instructions for PFMT.

Employing app-guided PFMT holds distinct advantages, primarily stemming from cost reduction compared to in-person sessions due to the elimination of transportation expenses and material resources. Moreover, it facilitates heightened adherence by accommodating flexible timing and location for therapy, further reinforced by reminders and alarms to minimize forgetfulness. Our study revealed an encouraging 74.3% adherence rate among participants who reported regularly performing exercises through the app, reflecting substantial acceptance and compliance with the app's guidance. This level of adherence surpassed that reported in previous studies, indicating the app's efficacy in promoting consistency[15,16].

During the COVID-19 pandemic, the utilization of the Diário Saúde® app for PFMT exhibited efficacy comparable to in-person programs. Notably, a Cochrane review demonstrated that women undergoing PFMT reported significant improvement, with a success rate of 55% in symptom relief or improvement, compared to 3.2% among untreated individuals[17]. Our study demonstrated a similar success rate of 62.2%, consistent with the outcomes from other mobile app-based interventions[18].

Table 1. Characteristics of UI women included the study.

Variables	Total (n=156)
Age, mean (SD)	49.3 (14.2)
Marital status, n (%)	
Single	28 (18)
Married/living with a partner	86 (55.1)
separated/divorced/widowed	42 (26.9)
Ethnicity, n (%)	
White	108 (69.2)
Others	48 (30.8)
BMI, mean (SD)	29.1 (6.4)
Number of pregnancies, mean (SD); n=140	
Vaginal delivery	1.5 (1.2)
Cesarean	0.8 (0.8)
Abortion	0.5 (0.9)
Pregnancies, mean (SD); n=140	
Vaginal	106 (75.7)
Cesarean	78 (55.7)
Abortion	44 (31.4)
Weight birth (grams), mean (SD); n=136	3385 (699)
Education (years), mean (SD)	12.3 (3.8)
Comorbidities, n (%)	
Arterial hypertension	50 (32)
Diabetes mellitus	29 (18.6)
Chronic obstructive pulmonary disease	7 (4.5)
Smoking, n (%)	18 (11.5)
Menopause, n (%)	77 (49.4)
Physical activity, n (%)	58 (37.2)
Intestinal constipation, n (%)	65 (41.7)
Onset of UI symptoms, n (%)	
During pregnancy	71 (45.5)
After menopause	28 (18)
Don't know	57 (36.5)
Positive COVID test, n (%)	37 (23.7)
Worsening of IU symptoms after COVID, n (%); n=37	19 (51.3)

UI: urinary incontinence; SD: standard deviation; N: absolute frequency; BMI: body mass index.

Our findings emphasized marked improvement in UI symptoms, as indicated by QUID and ICIQ-SF scores, along with enhanced condition-specific quality of life. These outcomes mirror those observed in supervised or unsupervised PFMT interventions[19,20] and align with a systematic review involving unsupervised behavioral and PFMT programs[21]. A previous randomized controlled trial focusing on a UI app similarly demonstrated enhanced ICIQ-SF scores and quality of life after 3 months of treatment[22].

Notably, our study documented a significant amelioration of storage symptoms, consistent with the potential of PFMT to mitigate such symptoms[23]. Pelvic floor contractions play a pivotal role in reducing detrusor pressure, elevating urethral pressure, and suppressing the micturition reflex. Our results substantiate the effectiveness of the home-based app-guided exercise regimen in enhancing storage symptoms.

Remarkably, a quarter of our study population tested positive for COVID-19, with half of these individuals reporting exacerbation of UI symptoms. While respiratory symptoms have been the primary focus of COVID-19, it is worth noting that the noticeable increase in abdominal pressure resulting from heightened respiratory efforts, coughing, sneezing, and overall weakness could potentially lead to added strain on the pelvic floor that contributes to the worsening of UI.

Strengths of our study include its pioneering approach in evaluating UI treatment through an app during the COVID-19 pandemic, ensuring continued care and access to health services within the prevailing limitations. The app's incorporation of reminder notifications substantially contributed to participant adherence, a pivotal factor in achieving positive outcomes. Despite the relatively short study duration, the rate of loss to follow-up remained negligible.

The limitation of the study was the absence of physical assessment for pelvic floor muscles, necessitated by pandemic restrictions. Furthermore, the lack of an extended follow-up hinders our understanding of the long-term effects of the treatment. Additionally, the absence of a control group limits the scope of comparison. Future studies should consider control groups, extended follow-up, and face-to-face assessments for a comprehensive evaluation of UI treatment. Despite these limitations, our study demonstrates the feasibility and efficacy of treating UI using the Diário Saúde® app, particularly in the face of pandemic-induced challenges. This approach holds promise for enhancing access to conservative treatment and fostering adherence to PFMT.

Table 2. Urinary symptoms and quality of life in women with urinary incontinence before and after treatment with an app-guided pelvic floor muscle training.

Questionnaires	Baseline assessment (n=156)	Post-treatment assessment (n=156)	p-value
QUID TOTAL score (0–15), mean (SD)	3.3 (1.3)	2.5 (1.3)	<0.001
SUI	7.6 (3.7)	5.8 (3.4)	<0.001
OAB	7 (4)	5.9 (3.7)	<0.001
ICIQ-SF score (0–21), mean (SD)	14.4 (4)	12.1 (4.7)	<0.001
ICIQ-OAB score (0–16), mean (SD)	7.3 (3.7)	6.3 (3.7)	<0.001
I-QOL TOTAL score (22–110), mean (SD)	51.8 (23.7)	58.4 (23.5)	<0.001
Avoidance or limiting behaviors	50.1 (23.1)	57.8 (22.8)	<0.001
Psychological impacts	61.7 (27.3)	66.5 (25.9)	<0.001
Social embarrassment	36.9 (24.9)	42.2 (25.6)	<0.001

QUID: Questionnaire for Urinary Incontinence Diagnosis; SUI: Stress Urinary Incontinence; OAB: overactive bladder; SD: standard deviation; ICIQ-SF: International Consultation on Incontinence Questionnaire-Short Form; ICIQ-OAB: International Consultation on Incontinence Questionnaire-Overactive Bladder; I-QOL: Incontinence Quality of life Questionnaire; Wilcoxon test.

Table 3. Adherence, subjective improvement and satisfaction of women (n=156) with urinary incontinence treatment.

Adherence treatment, n (%)	Total (n=156)
Always or almost always	57 (36.5)
Many times	59 (37.8)
Same times	29 (18.6)
Few times	8 (5)
Never or almost never	3 (1.9)
Subjective improvement	n (%)
Cured	16 (10.2)
Almost cured	76 (52)
Same symptoms as before treatment	49 (31.4)
A little worse	15 (9.6)
A lot worse	0
Self-reported* satisfaction (0–10)	Mean (SD)
	7.5 (2.5)

UI: urinary incontinence; app: mobile application; PFMT: pelvic floor muscle training; N: absolute frequency; SD: standard deviation; *scale from 0 to 10.

CONCLUSION

In light of the challenges posed by the COVID-19 pandemic, the implementation of a home-based mobile app program has proven to be successful in reducing symptoms of SUI. This innovative strategy not only resulted in significant enhancements in both quality of life and storage symptoms but also exhibited remarkable levels of adherence and participant satisfaction with the treatment.

AUTHORS' CONTRIBUTIONS

CCA: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **LGOB**: Formal Analysis, Writing – original draft, Writing – review & editing. **AM**: Conceptualization, Data curation, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. **MB**: Writing – original draft, Writing – review & editing. **CRTJ**: Conceptualization, 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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Diagnostic value of transthoracic needle biopsy in lung tumors

Ozlem Sogukpinar[1*], Ulku Aka Akturk[1], Makbule Ozlem Akbay[1], Erdal Tatlidil[2], Dilek Ernam[1]

SUMMARY

OBJECTIVE: Thoracic ultrasonography is widely used in imaging peripheral lesions and invasive interventional procedures. The aim of this study was to assess the diagnostic value of thoracic ultrasonography-guided transthoracic needle aspiration biopsy and the factors affecting the diagnosis of peripheral tumoral lung lesions.

METHODS: The lesion size, biopsy needle type, number of blocks, complications, and pathology results were compared in 83 patients between January 2015 and July 2018. The cases with pathological non-diagnosis and definite pathological diagnosis were determined. For the assessment of the factors affecting diagnosis, the size of the lesions and the biopsy needle type were evaluated. Biopsy preparations containing non-diagnostic atypical cells were referred to a cytopathologist. The effect of the cytopathological examination on the diagnosis was also evaluated.

RESULTS: Pathological diagnosis was made in 66.3% of the cases; cell type could not be determined in 22.9% of the cases, and they were referred to a cytopathologist. After the cytopathologist's examination, the diagnosis rate increased to 80.7%. Diagnosis rates were higher when using tru-cut than Chiba and higher in cases with tumor size >2 cm than smaller.

CONCLUSION: Thoracic ultrasonography-guided transthoracic needle aspiration biopsy is a preferred approach to the diagnosis of peripheral tumoral lung lesions, given its high diagnostic rate, in addition to being cheap, highly suitable for bedside use, and safe, and the lack of radiation exposure.

KEYWORDS: Biopsy, fine-needle aspiration. Thoracic neoplasms. Interventional ultrasonography. Cytopathology.

INTRODUCTION

The most non-invasive and safest method should be preferred for the tissue diagnosis of lung lesions, considering the lesion localization, the patient's pulmonary function capacity, and the availability of diagnostic resources. Based on the method of sample collection, biopsies can be planned as bronchoscopic, percutaneous, thoracoscopic, or surgical. An imaging modality guides percutaneous biopsies.

Transthoracic needle aspiration biopsy (TTNAB) procedures have improved over time in line with advances in imaging technologies[1], the improvement of punction needles, and the improvement of cytological examination methods[2]. TTNAB can be performed under the guidance of ultrasonography (USG) or computerized tomography (CT)[3,4], fluoroscopy, or magnetic resonance imaging (MRI), with CT-guided TTNAB being the most commonly used approach[3,5]. USG-guided TTNAB is preferred for lesions attached to the chest wall that are of sufficient size[6].

The advantages of thoracic USG over other imaging methods include its low expense, portability, repeatability, bedside applicability, and absence of radiation exposure during the procedure[7,8]. Thoracic USG guidance is used by pulmonologists during various pulmonary procedures, including thoracentesis, chest tube placement, transthoracic aspiration, and biopsies[9], in that it allows the visualization of needle placement and movement during biopsies[5], while other advantages include the opportunities presented for real-time biopsy, the multi-dimensional images, which allow the lesion to be approached from different angles, and the ability to make a dynamic evaluation of proximity to vascular structures[10].

Thoracic USG eases access to peripheral lesions attached to the chest wall, and USG-guided TTNAB provides similar diagnostic accuracy and safety to CT-guided TTNAB, in addition to reducing the time needed for the biopsy[11]. Complication rates are also lower than with CT-guided TTNAB[12]. USG-guided TTNAB is a minimally invasive procedure that is safe[12,13] and fast[14] and offers high diagnostic accuracy[8,15-17].

Based on these advantages, USG-guided biopsy should be the first choice of clinicians for eligible patients to diagnose peripheral lung lesions.

In this study, we investigate the diagnostic value of thoracic USG-guided TTNAB in patients with peripheral tumoral lesions and the factors affecting it.

[1]University of Health Sciences, Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital, Department of Chest Diseases – İstanbul, Turkey.

[2]Denizli State Hospital, Department of Chest Diseases – Denizli, Turkey.

*Corresponding author: ozlemsogukpinar@yahoo.com

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METHODS

We carried out a retrospective review of the patients who underwent thoracic USG-guided TTNAB in the pulmonology clinics between January 2015 and July 2018. Excluded from the study were patients with missing information. In the review of the medical files of the patients who underwent biopsy, information including demographics, the size of the peripheral lesion, the biopsy needle type, the number of blocks, and the pathological diagnosis were all recorded. All biopsies were guided by the same USG device (Mindray, North America) using a 3.5–5 MHz convex probe, a 22-gauge Chiba needle, and a tru-cut biopsy needle. For the assessment of the factors affecting diagnosis, the lesions were classified into two groups: those larger and those smaller than 2 cm, considering the lesion size and the biopsy needle type.

Cases in which no diagnosis could be made after a pathological examination were considered non-diagnostic, while those with a definitive pathological diagnosis were considered diagnostic. Preparations including atypical cells but without type confirmation were referred to a cytopathologist, and the impact of the cytopathological examination on the diagnosis was recorded. We also recorded any complications reported in the medical records of the patients who were followed up after undergoing biopsy. Pneumothorax was evaluated with chest radiographs taken immediately in symptomatic patients and 2 h after the procedure in asymptomatic patients.

The study was designed by the International Helsinki Declaration, and institutional ethics committee approval was granted for the continuation of the present study (116.2017.R-250).

Statistical analysis

The Statistical Package for the Social Sciences 22.0 version (IBM Corporation, Armonk, New York, USA) package program was used for the analysis of the data. Descriptive statistics were used to present baseline characteristics. Continuous variables were expressed as mean±standard deviation and median (range). Qualitative data were calculated as percentages. Chi-square test (^{2}) was used to compare the differences between groups. Student's t-test was used in case of normal distribution of variables. Mann–Whitney U test was used to compare parameters that did not show normal distribution. A $p<0.05$ was considered statistically significant.

RESULTS

The files of 110 patients who underwent thoracic USG-guided biopsy on the specified dates were examined, and 83 patients with complete data were identified. The mean age of the patients was 61.2±9.8 years, and 62.7% (n=52) were male.

While a definitive pathological diagnosis was made in 66.3% (n=55) of the patients, the type could not be confirmed in 22.9% (n=19) despite the detection of atypical cells. Preparations containing atypical cells but not a diagnostic type were referred to a cytopathologist and examined. Among the 19 patients evaluated by a cytopathologist, 12 (68%) were given a definitive diagnosis, while the subtype could not be identified in 7 patients (32%). The diagnosis rate increased from 66.3 to 80.7% in patients with atypical cells referred to a cytopathologist. The diagnostic rate of USG-guided biopsy was thus found to be 80.7%.

The rate of diagnosis based on biopsies performed using a tru-cut needle was higher than that of those performed using a Chiba needle (p=0.04). In contrast, the rate of diagnosis did not differ depending on the number of blocks. The diagnosis rate was higher for tumors larger than 2 cm (p=0.03). Table 1 presents the distribution of the pathological findings after a diagnostic examination, while a comparative analysis of the diagnosis rates associated with biopsy needle, tumor size, and number of blocks is presented in Table 2.

Table 1. Distribution of the pathological findings after a diagnostic examination.

Distribution of diagnoses (n=83)	
Adenocarcinoma	27 (32.6%)
Squamous cell carcinoma	27 (32.6%)
Non-small cell carcinoma	14 (16.8%)
Small-cell carcinoma	7 (8.4%)
Adenocarcinoma with lepidic growth	3 (3.6%)
Neuroendocrine tumor	3 (3.6%)
Organized pneumonia	1 (1.2%)
Sarcoidosis	1 (1.2%)

Table 2. A comparative analysis of the diagnosis rates associated with biopsy needles, tumor sizes, and number of blocks.

	Rate of diagnosis (%)
Biopsy needle	
Tru-cut	88.4
Chiba	78.2
Tumor size	
<2 cm	65.3
≥2 cm	86.6
Number of blocks	
1	77.6
≥2	84.3

None of the pathological examinations led to a diagnostic outcome in 10.8% (n=9) of the patients, for whom more invasive procedures were needed.

Among the major complications, one patient developed pneumothorax and required chest tube placement, and another experienced bleeding in the lesion with consolidation around the mass.

DISCUSSION

It is found in the present study that thoracic USG-guided TTNAB is associated with high diagnostic and low complication rates in patients with peripheral lung lesions. The rate of diagnosis using thoracic USG-guided TTNAB was higher for tumors with diameters larger than 2 cm than for smaller tumors. Moreover, biopsies performed using tru-cut needles were associated with a higher diagnosis rate than those conducted with a Chiba needle. A cytopathologist was consulted for the cases with atypical cells identified in the pathological examination but in which no subtype classification could be made, which led to a definitive diagnosis in some of the cases, increasing the overall diagnosis rate.

The diagnosis rate with USG-guided TTNAB varies between 71.8 and 88.7% in the literature, depending on the malignancy potential of the lesion, the originating tissue, the size of the lesion, the presence of necrosis, and the biopsy needle used[8,15-17]. Consistent with the literature, the rate of diagnosis with thoracic USG was found to be 80.7% in this study.

Another important issue is the selection of the biopsy needle and the gauge. Conflicting results have been reported regarding the diagnostic effect of larger-diameter needles[18,19]. Diacon et al., reported diagnostic accuracy rates of 82 and 76% for USG-guided TTNAB and cutting-needle biopsy, respectively, and that the combination of them increases diagnostic accuracy to 89%[13]. In another study, Dogan et al., reported diagnostic accuracy rates of 71.8% with USG-guided TTNAB and 81.2% with tru-cut biopsy, and that the diagnostic accuracy rate increased to 93.7% when combined[15]. In this study, the diagnostic rate with TTNAB using a Chiba needle was 78.2 and 88.4% when the tru-cut-needle biopsy method was used, representing a significant difference (p=0.04). There are also studies in which no difference was found between cutting needles and TTNAB. A systematic review of 11 studies found no significant difference between biopsy needles[20], and there is still no standard approach to needle selection in thoracic USG-guided TTNAB.

Small lesions can be challenging in thoracic USG-guided TTNAB as they are dynamic during respiration, and the biopsy needle will have a smaller range of motion. Guo et al., reported lower diagnostic rates in lesions smaller than 2 cm when compared to larger lesions and lower rates in lesions larger than 5 cm due to necrosis[18]. Huang et al., reported thoracic USG-guided biopsies to be appropriate and safe for lesions smaller than 2 cm and that biopsies using cut-needle methods were 3.4 times more diagnostic than thin-needle biopsy approaches[21]. In our study, diagnostic accuracy was higher for lesions larger than 2 cm than for smaller lesions (p=0.03). As lesions get smaller, inserting the needle into the lesion becomes more challenging, and the amount of collected biopsy material is limited. While small lesion size is not an obstacle for thoracic USG-guided TTNAB, it is essential that the needle be placed inside the lesion and sufficient biopsy material be collected. Thoracic USG is associated with a higher diagnostic accuracy rate in larger lesions, as it allows multidimensional access, but it is also a safe and convenient biopsy guide with a diagnostic accuracy rate that cannot be underestimated in lesions smaller than 2 cm. As USG allows real-time biopsy, the respiration-associated movements of smaller lesions can be easily managed.

The number of transitions in biopsies is not defined; it is determined by factors like lesion accessibility, complication risk, sample quality, needs for real-time pathology examination, and sample sufficiency[3]. Lee et al., reported that fewer transitions would be needed during thoracic USG-guided biopsies than with CT-guided biopsies, which they attributed to the efficacy of needle placement and the collection of sufficient samples that real-time imaging USG allows[5]. In this study, we found that the number of blocks collected during biopsy made no significant difference for pathological diagnosis, with high rates of diagnosis reported with both single and double blocks of 77.6 and 84.3%, respectively.

The data pooled from 10 studies before 2015 investigating thoracic USG-guided TTNAB showed that the most common complication was pneumothorax, occurring in 4.4% of cases, while the same was much higher (20.5%) when BT-guided TTNAB was used[17]. Another study reported the overall complication rate to be lower when using USG-guided TTNAB (7%) when compared to CT-guided TTNAB (24%)[5]. A lower complication rate can be expected with thoracic USG-guided TTNAB than with CT-guided TTNAB since there is no lung tissue between the probe and the lesion when using USG, and therefore no lung tissue is invaded during the procedure. Visualizing the lesion would otherwise not be possible with USG. Huang et al., reported bleeding and pneumothorax complication rates of 6.7 and 2.1%, respectively[21]. In this study, only one patient developed pneumothorax requiring chest tube placement, while one patient developed bleeding

into the lesion with consolidation around the mass, and both were successfully managed. The findings of the present study support the use of thoracic USG-guided TTNAB as a safe procedure for the diagnosis of peripheral lung lesions and its association with a low rate of complications. Another advantage of USG-guided biopsy is that it allows the detection of such complications as pneumothorax immediately after the procedure. The sliding sign in thoracic USG, meaning the sliding of visceral pleura and parietal pleura over each other, can successfully exclude pneumothorax formation[22]. In this study, sonographic controls were made to check for pneumothorax after each biopsy.

This study was limited by its single-center, retrospective design. Furthermore, the number of patients was limited, and the biopsy needle and method were selected in the light of the available radiological findings by the interventional pulmonologist performing the procedure, preventing randomization.

CONCLUSION

Based on the findings of the present study, thoracic USG-guided TTNAB can be considered a safe, fast, and effective diagnostic method for peripheral lung lesions that can be visualized by thoracic USG. Due to the advantages described, experienced centers should make more frequent use of thoracic USG for the diagnosis of peripheral lung lesions.

ETHICAL STANDARDS DISCLOSURE

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving research study participants were approved by the S.BU. Süreyyapaşa Chest Diseases and Thoracic Surgery Non-Interventional Ethics Committee Approval/116.2017.R-250 [S.BÜ. Süreyyapaşa Göğüs Hastalıkları ve Göğüs Cerrahisi Girişimsel Olmayan Etik Kurul onayı/116.2017.R-250].

AUTHORS' CONTRIBUTIONS

OS: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **UAA:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation. **MOA:** Data curation, Investigation, Methodology, Resources. **ET:** Data curation, Investigation, Methodology, Resources. **DE:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation.

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ORIGINAL ARTICLE

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Quality of sleep and excessive daytime sleepiness among medical students in a Brazilian private university

Anna Karolinna Ribeiro Souza[1], Rhaissa Siqueira Sandes[1], Raquel Fernandes Vanderlei Vasco[1], Edoarda Vasco de Albuquerque Albuquerque[1*]

SUMMARY

OBJECTIVE: The aim of this study was to assess medical students' quality of sleep and excessive daytime sleepiness in different graduation cycles.
METHODS: A cross-sectional study was carried out at a private university in Maceió, Brazil, from August 2021 to March 2022. The sample was composed of medical students aged 18 years and above from years 1–2 (basic cycle), 3–4 (clinical cycle), and 5–6 (internship) of Medical School who were invited to answer two validated questionnaires: the Pittsburgh Sleeping Quality Index and the Epworth Sleepiness Scale.
RESULTS: A total of 179 students participated; most of them were female (78.2%), aged 19–25 years (73.7%), and with a body mass index<25 kg/m² (73.7%), with smaller participation from students from the basic cycle (21.2%). Analyzing the Pittsburgh Sleeping Quality Index, 55.9% of the students were classified as having poor sleep quality, with no difference in sleep category between gender, age, body mass index, and graduation cycle. Students with a body mass index of ≥25 kg/m² had longer sleep latency (p=0.016) and shorter sleep duration (p=0.027). The Epworth Sleepiness Scale assessment showed that 44.1% of the students exhibit daytime sleepiness. Women had more daytime sleepiness than men (p=0.017), with no difference between age, body mass index, and graduation cycle.
CONCLUSION: About half of the medical students experience poor sleep quality and daytime sleepiness, regardless of the graduation cycle. This should trigger a targeted institutional intervention to promote better mental and physical health, as well as sleep hygiene, to reduce future health issues.
KEYWORDS: Sleep hygiene. Students, medical. Sleepiness.

INTRODUCTION

Sleep disorders, mainly insomnia and excessive daytime sleepiness (EDS), are a public health issue[1]. Prolonged sleep deprivation can be deleterious to physical and cognitive performance since higher functions, such as learning and memory retention, and also metabolic health, depend on sleep quality[2-4].

Medical students are often subjected to an extensive curriculum, exhausting extracurricular activities, and internships with night shifts, and all of that combined can modify sleep architecture[5-9]. Evidence shows that poor sleep quality can lead to lower academic performance, compromising students' abilities to reason, acquire, and process information, correctly interpret and solve clinical cases, and make students more prone to depression and anxiety[6,8,9]. In addition, sleep deprivation also increases reaction time and systolic blood pressure post-exercise, and the first could magnify the risk of accidents, both work-related and not work-related[6,10].

All of these could generate a vicious cycle where students deprive even more of their sleep time to study for exams and increase substance abuse (caffeine and/or medications)[5,11]. This creates a chronic problem and is often overlooked by professors and other medical professionals, labeling this situation as "normal" and, in a certain way, promoting sleep deprivation[11].

In this setting, considering the population studied as a risk factor for developing sleep disorders and knowing that there is an interference of these disorders over academic performance[8], this study proposed to assess sleep quality and EDS among medical students from a private university in the Brazilian Northeast, comparing these aspects in different graduation cycles.

METHODS

Study design and participants

An observational, descriptive cross-sectional study was conducted at Centro Universitário Tiradentes (UNIT-AL), a private university in Maceió, Alagoas, from August 2021 to March 2022. The study population included medical students from years 1 to 6, allocated into three groups: basic cycle (BC; years 1–2), clinical cycle (CC; years 3–4), and internship (IN; years 5–6).

[1]Centro Universitário de Maceió (UNIMA/Afya) – Maceió (AL), Brazil.
*Corresponding author: edoarda.vasco@gmail.com, edoarda.vasco@souunit.com.br, edoarda@alumni.usp.br
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During this study, the university had 864 registered medical students, distributed into 302 students in BC, 302 students in CC, and 260 in IN. The questionnaires were answered online, using the tool Google Forms to collect the data. Students from all years were recruited by online invitation during the pandemic and also by personal approach when the flexibilization of social distance measures was possible, providing a quick response code to the questionnaires for the participants.

The inclusion criteria were students aged ≥18 years registered in the institution's medical school. Those who filled out the forms incorrectly or incompletely were excluded. This study was approved by the local Ethics Committee (CAAE 48301921.3.0000.5641).

Instruments

The selected instruments were the Pittsburgh Sleep Quality Index (PSQI), in its translated and validated version to Brazilian Portuguese[12], and the Epworth Sleepiness Scale (ESS), also validated for usage in Brazil[13]. Demographic data regarding gender, age, body mass index (BMI), and previous sleep or chronic disease diagnoses were also collected.

Pittsburgh Sleeping Quality Index assesses sleep quality and disturbances in the last month, and it is composed of 19 items—and five more for individuals who share the bedroom or bed with another individual. These items are arranged in seven components: (1) subjective sleep quality, (2) sleep latency, (3) sleep duration, (4) habitual sleep efficiency, (5) sleep disturbances, (6) use of sleeping medication, and (7) daytime dysfunction. The global score achieved ranges from 0 to 21 points, and scores from 0 to 5 indicate good sleep quality, scores from 6 to 10 indicate poor sleep quality, and >10 points may indicate a sleep disorder.

ESS is a tool to identify excessive daytime sleepiness, including eight items that grade the probability of the individual dozing in inappropriate situations. The scoring system is from 0 to 3, in which 0 means "would never doze," 1 means "slight chance of dozing," 2 means "moderate chance of dozing," and 3 means "high chance of dozing." The global score ranges from 0 to 24. Scores >10 points suggest EDS, and scores >15 indicate severe daytime sleepiness.

Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics Client version 22.0 Multilingual, with a confidence level of 95% ($p<0.05$). Sample calculation was performed in G*Power version 3.1.7 using goodness-to-fit multiple proportions with an α-error of 0.05, power of 0.8, and effect size ω of 0.3, resulting in a total of 108 subjects, with 36 in each group. Individuals were allocated into groups related primarily to graduation cycle (BC, CC, and IN), as well as to gender (male and female), age (19–25 and 26 years or older), and BMI (<25 kg/m² and ≥25 kg/m²). We used the Shapiro–Wilke test to assess the variable distribution and the Levene test to evaluate homogeneity. We used the t-test to compare two parametric variables and the analysis of variance to compare three variables. For comparison of non-parametric variables, we used the Mann–Whitney test when two non-paired variables were analyzed and the Kruskal–Wallis test when three or more non-paired and non-homogeneous variables were analyzed.

RESULTS

A total of 179 students answered both questionnaires (20.7% of the total) from all graduation cycles (BC=38, 21.2%; CC=72, 40.2%; IN=69, 38.5%). Most participants were female (n=140, 78.2%), aged 19–25 years (n=132, 73.7%), with a BMI<25 kg/m² (n=132, 73.7%). Around 23.7% of women and 44.7% of men were overweight or obese (p=0.018), and there was no significant difference between age and gender, or BMI and gender. Twenty-four students (13.4%) reported chronic diseases such as hypothyroidism (n=5) and asthma (n=4). Nine participants (5.0%) reported previous sleep disorder diagnoses, notably insomnia (n=4).

Regarding sleep disturbances in the last month, at least once a week, 56.9% (n=102) of the participants could not sleep within 30 min, 58.1% (n=104) woke up in the middle of the night, 50.2% (n=90) had to get up to use the bathroom, 16.2% could not breathe comfortably, 17.3% (n=31) coughed or snored loudly, 39.6% had bad dreams, 15.1% (n=27) had pain, and 16.7% (n=30) used prescribed or "over the counter" medicine to help sleep. Moreover, 39.1% (n=70) had trouble staying awake while driving, eating meals, or during social activities at least once a week, and 83.4% (n=149) reported not having enough enthusiasm to perform daily activities.

About 29.6% (n=53) of the participants shared a bed or bedroom with another individual. Their partners reported that at least once a week, 13.2% (n=7) of them snored loudly, 33.9% (n=18) twitched their legs or jerked, and 13.2% had episodes of disorientation or confusion during sleep.

Concerning the reported sleep/waking times, students go to sleep around 11:35 p.m., with a median sleep latency of 20 min, and wake up around 06:38 a.m., with a median sleep duration of 6.5 h (Table 1). Sleep duration among individuals aged 19–25 years was longer than among individuals aged ≥26 years, and sleep duration was also longer and sleep latency was shorter in individuals whose BMI was <25 kg/m² than whose BMI was ≥25 kg/m² (Table 1).

The median PSQI score was 7 points [interquartile range (IQR): 5–10 points]. In a categorical analysis, 55.9% of the participants obtained 5–10 points, therefore being classified as having poor sleep quality (Figure 1). There was no significant difference in the PSQI among the graduation cycles (p=0.09, Figure 1) or scores among various PSQI components. Nevertheless, in component 1, which assesses the subjective quality of sleep, 34.2% of participants from BC, 20.8% from CC, and 14.5% from IN classified their sleep quality as poor in the last month (Figure 1).

Regarding gender, there was no difference in the assortment of categories among men and women (p=0.186, Figure 1). In a subanalysis of the various components, women had higher scores in components 5, sleep disturbances (2 versus 1 point, p=0.01), and 7, daytime dysfunction (2 versus 1 point, p=0.024). There was no difference between age groups in category assortment or individual scale components by PSQI (Figure 1, Table 1). Concerning BMI, there was no difference among category assortments (p=0.128, Figure 1).

Analyzing ESS, 44.1% (n=79) of the sample showed some degree of daytime sleepiness. The mean score was 9.8±4.2, and women had a worse score (female 10.4±4.2; male 7.5±3.3; p<0.001) and were more assorted as having EDS (Table 2). Younger individuals had higher scores (19–25 years 10.3±4.0; ≥26 years 8.3±4.5; p=0.004), but they did not differ in daytime sleepiness classification (Table 2).

Table 1. Sleep latency and duration according to graduation cycle, gender, age, and body mass index.

	Sleep latency (IQR)	p	Sleep duration (IQR)	p-value
Total	20 (10–30)		6.5 (5.5–7.0)	
Gender				
Male	20 (10–30)	0.789	6.5 (5.5–7.0)	0.656
Female	20 (10–30)		6.0 (6.0–7.0)	
Age (years)				
19–25	20 (10–30)	0.054	7.0 (6.0–7.0)	**0.025**
>25	30 (15–40)		6.0 (5.5–7.0)	
BMI (kg/m²)				
<25	20 (10–30)	**0.016**	7.0 (6.0–7.3)	**0.027**
≥25	30 (15–40)		6.0 (5.5–7.0)	
Graduation cycles				
Basic	20 (10–41.2)	0.824	6.0 (5.5–7.0)	0.208
Clinical	20 (10–30)		7.0 (6.0–7.0)	
Internship	25 (10–32.5)		6.5 (5.5–7.5)	

Statistically significant values are denoted in bold.

DISCUSSION

In this study, medical students reported a median sleep duration of 6.5 h. Although total sleep time may vary according to age, the mean value is around 7–8 h per night[14]. Therefore, it is noticeable that our sample has less sleep time than recommended, and individuals with less than 7 h of sleep are more likely to develop health issues, regardless of gender[14].

Likewise, 55.9% of medical students had poor sleep quality, and 16.2% had a probable sleep disturbance. In a study performed on the adult population also from Maceió, poor sleep quality was found in 48% of participants[15], reinforcing the idea that medical students are at risk of sleep disturbances[7]. In a study performed in Botucatu, 87.1% of medical students obtained >5 points in PSQI, a rate higher than our study (72.1%) and the 61.5% were found in Paraiba, Brazil[16,17]. In parallel, a study assessing bruxism and smartphone usage with the performance of PSQI in Brazil showed a median score of 7 points, similar to our findings[18]. Regarding daytime sleepiness, about 11.6–36% of the general population exhibited EDS[19], while in our study this rate was higher at 44.1%. Daytime sleepiness directly impacts performance, and 83.4% of the students felt they lacked enthusiasm for daily activities. This could lead to impaired learning and memory, thus increasing psychological distress in this population[20]. Curiously, women showed more daytime sleepiness than men, which could be a selection bias since male participation was low.

The participation of students from BC was 12.5% of the registered students in this cycle, lower than the other two assessed periods (CC 23.8% and IN 26.5%). A possible explanation is the selected age of 18 years or older for participation in this study since first-year students have a higher proportion of underaged individuals. Nevertheless, the participation in the BC group was enough to achieve the minimum number of subjects necessary to maintain this study's strength.

Although about half of the students were classified as poor sleepers, analyzing component 1 of PSQI, only 34.2% of the participants from BC, 20.8% from CC, and 14.5% from IN agreed with this classification, showing a different perception of the students from what was measured by PSQI. This emphasizes that sleep quality and self-consciousness about lifestyle need debating during graduation. Also, 16.7% of the population used hypnotic medications in the last month, a higher rate compared to similar studies in other universities around Brazil, where the mean rate of medication usage was 8.6%[16,19].

Regarding weight, individuals with a BMI≥25 kg/m² had higher PSQI scores, shorter sleep duration, and longer sleep latency. There is a correlation between obesity and poor sleep quality, often related to sleep apnea. However, a study in Kuwait with a cohort of

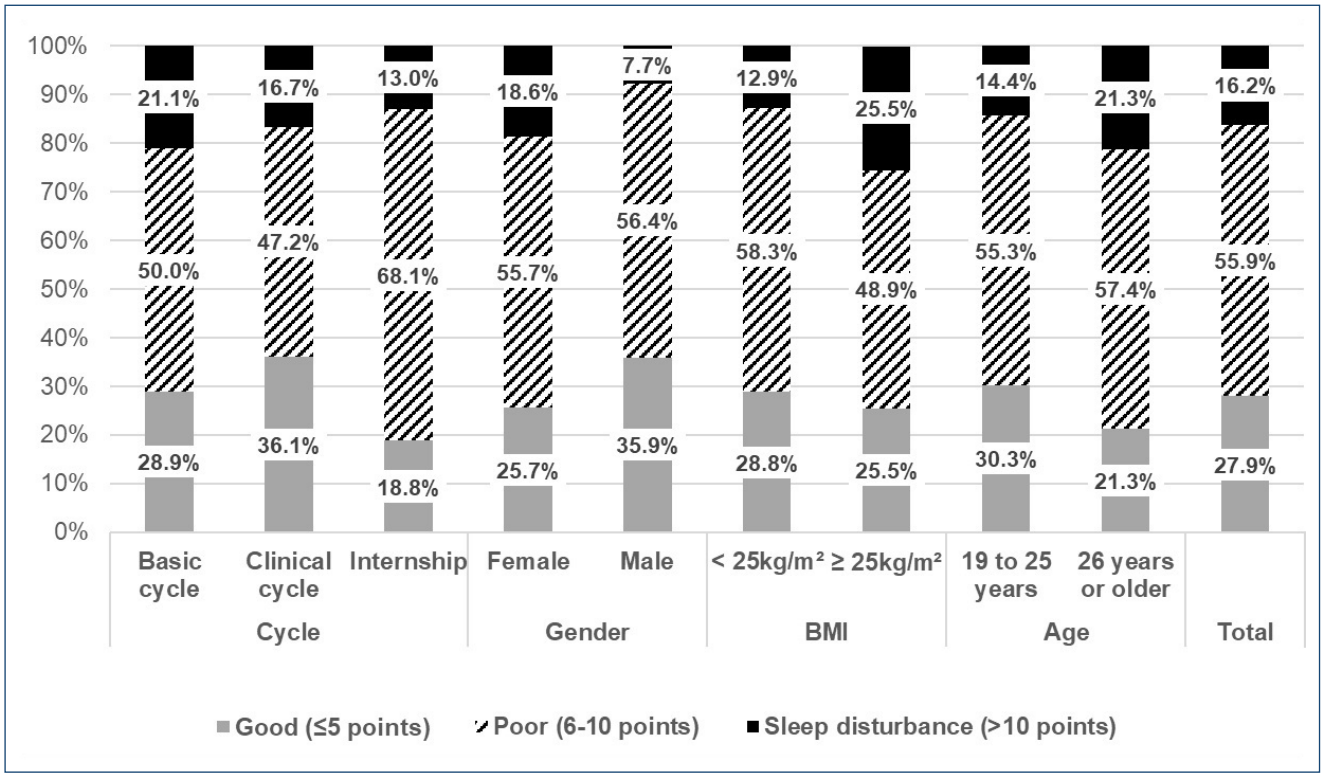

Figure 1. Distribution according to sleep quality classification by Pittsburgh Sleep Quality Index among graduation cycles, gender, body mass index, and age. Note: there was no statistically significant difference among any of the groups.

Table 2. Daytime sleepiness classification according to Epworth Sleepiness Scale distributed by graduation cycle, gender, age, and body mass index.

	No EDS (<10 points)	EDS (10–15 points)	Severe EDS (>15 points)	p-value
Total	55.9%	33.0%	11.2%	
Gender				
Male	71.8%	28.2%	0.0%	**0.017**
Female	51.4%	34.3%	14.3%	
Age (years)				
19–25	51.5%	36.4%	12.1%	0.144
>25	68.1%	23.4%	8.5%	
BMI (kg/m²)				
<25	53.0%	34.8%	12.1%	0.434
≥25	63.8%	27.7%	8.5%	
Graduation cycles				
Basic	44.7%	44.7%	10.5%	0.149
Clinical	66.7%	23.6%	9.7%	
Internship	50.7%	36.2%	13.0%	

Statistically significant values are denoted in bold. EDS: excessive daytime sleepiness.

individuals without sleep apnea observed an independent effect of weight on sleep quality, and BMI values were directly proportional to worse PSQI scores and shorter sleep duration and efficiency[21].

Concerning the graduation cycles, despite no statistical significance, internship students exhibit a worse quality of sleep, followed by the basic and then clinical cycles, without difference in daytime sleepiness, a similar pattern found in the countryside of Paraíba, Brazil[17]. This corroborates that there is a reduction in available time for adequate sleep over the graduation years, probably due to workload and night shifts. The finding of worse quality sleep in basic compared with the clinical cycle can be justified by the transition from high school to university[14,16].

This study has several limitations. Overall participation of students from BC was low, probably due to the cutoff age of 18 years, which could attenuate differences among graduation cycles due to selection bias. Furthermore, male participation and individuals with a BMI≥25 kg/m² were also low, and the findings may not be generalized to these populations.

CONCLUSION

In this study, we identified a high prevalence of poor sleep quality and EDS among medical students from a private university. These findings should trigger an intervention to promote

better mental and physical health, along with sleep hygiene, to reduce future health issues.

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AUTHORS' CONTRIBUTIONS

AKRS: Data curation, Investigation, Resources, Visualization, Writing – original draft. **RSS:** Data curation, Investigation, Resources, Visualization, Writing – original draft. **RFVV:** Data curation, Formal Analysis, Methodology, Software, Validation, Writing – review & editing. **EVAA:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing review & editing.

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ORIGINAL ARTICLE

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Janus kinase-signal transducer and activator of transcription signaling pathway in the ocular cells of rat fetuses exposed to maternal saturated fat ingestion

Lorrany da Silva Avanci[1], Thiago Guedes Pinto[1], Daniel Vitor de Souza[1], Lais Vales Mennitti[1], Daniel Araki Ribeiro[1*], Luciana Pisani[1]

SUMMARY

OBJECTIVE: The aim of this study was to analyze possible alterations (morphological and inflammatory) in the ocular cells of fetuses from mothers with insulin resistance exposed to saturated fatty acids through the period of pregnancy.

METHODS: Wistar female rats were induced to develop insulin resistance before pregnancy. Fetuses' skulls were collected on the 20th day of intrauterine life. The rats were separated on the first day of management into two groups according to the diet applied: control group (C): diet containing soybean oil as a source of fat; and saturated fatty acid group (S): diet containing butter as a source of fat.

RESULTS: Histological and immunohistochemical analyses have been conducted. The immunohistochemical analyses of interleukin 6, suppressor of cytokine signaling, 3 and signal transducer and activator of transcription 3 did not demonstrate alterations in the expression of proteins in the fetuses of mothers fed with a saturated fatty diet. Moreover, no histopathological changes were noticed between groups.

CONCLUSION: The saturated fatty diet does not induce tissue changes or activate the Janus kinase/signal transducer and activator of transcription signaling pathway during eye development in the fetuses of mothers with insulin resistance.

KEYWORDS: JAK/STAT pathway. Eye. Fatty acids.

INTRODUCTION

Several studies have indicated that dietary behavior and health conditions impact not only the health of mothers but also fetus, therefore affecting its development[1]. Even though fetal development is basically driven by the program encoded in its genome, the epigenetic regulation of its growth is directly influenced by the intrauterine environment[2]. In this context, the fetus' health depends on the supply of nutrients and oxygen from the mother, and a malfunction of this system in the gestational period may lead to metabolic effects throughout the fetus' life, encompassing childhood and adolescence[3].

According to some authors, a significant imbalance in macronutrient intake may lead to health harm. Saturated fatty acids (such as lauric acid, myristic acid, palmitic acid, and stearic acid), for instance, may induce transcription of factor κB (nuclear factor-kappa B) and culminate in the activation of proinflammatory genes (such as cyclooxygenase-2)[4]. In this regard, this cascade of events could represent an additional factor in increasing cholesterol levels and triggering cardiovascular diseases[5].

Although in past decades dietary guidance has almost universally advocated reducing the intake of total and saturated fat, these recommendations, as well as the link between fat consumption and the risk of human disease, have been among the most vexed questions in public health in order to fully understand whether dietary fats are harmful[6].

The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway (one of the main signaling pathways) in eukaryotic cells might be useful as it is activated in several growth and developmental processes in multiple tissues in order to control cell proliferation, differentiation, survival, and apoptosis[7]. It is activated when certain cytokines bind to the receptor complex, JAK1 and JAK3, allowing the recruitment and phosphorylation of STAT3, which dimerizes and enters the nucleus, activating a transcription program of the target genes, and stabilizing the receptor complex[8]. Also, such binding may activate the signaling pathway of mitogen-activated protein kinase and phosphoinositide 3-kinase signaling, which may induce the transcription of certain cytokine signaling suppressors (SOCS), therefore inhibiting the JAK/STAT pathway[9]. Besides, the proinflammatory cytokine interleukin 6 (IL-6) demonstrates JAK-STAT signaling dependence, which justifies our choice to study such biomarkers in the current study[10].

[1]Universidade Federal de São Paulo, Department of Biosciences – Santos (SP), Brazil.
*Corresponding author: daribeiro@unifesp.br
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Of particular importance, *Drosophila melanogaster*'s eyes have been extensively used as a platform to understand signaling pathways, and several reports have demonstrated that the JAK/STAT signaling pathway plays pleiotropic roles in *Drosophila* eye development. The biological mechanism involves activation (dependent on the ligand Upd) of the mentioned pathway in eye tissues during development[8]. On the contrary, retinal degenerative diseases are a major cause of severe visual impairment or blindness in humans, and genes and proteins of the JAK/STAT signaling pathway have been shown to play an important role in models of retinal degeneration, which justifies the conduct of studies within the field[9].

The purpose of this study was to evaluate the histopathological changes as well as the expression of the JAK/STAT pathway in the eyes of rat fetuses from dams with insulin resistance treated with a saturated-fat normolipidic diet during pregnancy.

METHODS

Animals and experimental design

Every procedure of the current study was carried out following the International Standards for Research involving Animals. Regarding animals' features, a total of 13 2-month-old virgin Wistar female rats were obtained from Centro de Desenvolvimento de Modelos Experimentais para Medicina e Biologia (CEDEME). Concerning environmental conditions, the rats were kept under light-cycle conditions (12 h light and 12 h dark) at 24°C±1°C with food and water *ad libitum*. This study was approved by the Animal Ethics Committee under Protocol # 8298100720. Furthermore, female rats were induced to develop insulin resistance through high-fat (HF) diet preconception and mated when they were 4 months old, and on the following day, the presence of spermatozoa in the vaginal lumen was verified with the aid of a microscope (through the introduction of a small amount of 0.9% saline solution into the vagina with posterior aspiration with a dropper). After likely conception confirmation, the females were kept in individual plastic cages and distributed sequentially into two different groups. During pregnancy, each group received a different experimental diet as follows: control group (C): diet containing soybean oil as a source of fat (n=5) and saturated fatty diet group (S): diet containing butter as a source of fat (n=5). The recommendations of the American Institute of Nutrition (AIN-93G) were followed in the composition of the diets[11]. The experimental design was established in a previous study[12]. Also, body weight and food intake were recorded weekly, considering both animal groups.

On the 20th day of gestation (from a total of 21–22 days), the pregnant rats were euthanized to obtain the fetuses. The rats were anesthetized with isoflurane (Isoflurane®; BioChimico Ltda, Itatiaia, RJ, Brazil) by inhalation and then decapitated after 4 h of fasting. Fetuses were collected (regardless of sex) by caesarean section and euthanized by decapitation. Fetal skulls were collected for histological analysis of ocular tissue.

Histopathological analysis

After collecting the skulls, the tissue was embedded in paraffin, and then histological sections of 3 µm thickness were performed. The following parameters were evaluated: cell morphology, evaluation of cell nuclei, presence of inflammatory infiltrate, presence of hemorrhage, and atypical cells.

Immunohistochemical analysis

Histological sections were serially cut in the microtome sections with a 3-µm thickness, and then the slides were deparaffinized in xylene, rehydrated in ethanol (99.5%), and pretreated with citric acid buffer (10 nM, pH 6, 0.1 M citric acid, Synth®, São Paulo, Brazil; 0.1 M sodium citrate—Synth®, São Paulo, Brazil), and antigenic retrieval was performed in microwaves (for 3 cycles of 5 min). Primary antibodies (Santa Cruz Biotechnology, Inc., USA) were used as follows: IL-6 dilution 1:150; STAT 3 diluted 1:100; and SOCS 3 diluted 1:200, applied to slides and incubated overnight at 4°C. Following day, the slides were washed twice with phosphate-buffered saline (PBS) and incubated with biotinylated secondary antibody (Starr Trek Universal HRP Detection Kit, Biocare Medical®, USA) for 30 min, washed with PBS and incubated with streptoavidin conjugated with hydrogen peroxide for 30 min, and then stained with 3,3-diaminobenzidine, (DAB, 0.05%—DAKO North America Inc.®, California, USA). Finally, counterstaining with hematoxylin was performed, and the slides were mounted with resin (Entellan® new, Merck, Germany).

RESULTS

Maternal oral glucose tolerance test and area under the curve

Figure 1 illustrates the increased area under the curve (AUC) (Figure 1B) and blood glucose levels after 45 min of oral glucose administration (Figure 1A) in dams that received the HF diet (43% lipids) during the pregestational period. In contrast to the dams in the control group, baseline glucose levels (time 0) were not recovered in the HF group after 120 min from the

start of the test. These findings have shown that consuming the HF diet for 8 weeks before pregnancy impaired the glycemic response in the female rats, reducing oral glucose tolerance and influencing the development of insulin resistance.

Histopathological analysis

The analysis of the ocular tissue of Wistar rat fetuses, as shown in Figure 2, occurred through observation of the cornea, retina, vitreous humor, and crystalline lens in both the control group (A) and the group treated with saturated fatty acid (B). The groups were mutually compared, and it was not possible to observe changes in all analyzed tissues in this setting. In the lens, the integrity of the fibers arranged in layers, capsules, and epithelium was observed. Also, the vitreous humor (composed of an aqueous substance containing collagen fibers, hyaluronic acid, and the hyaloid vascular system) presented preserved structure with no changes after the treatment period. Retinal ganglion cells also showed preserved cellular morphology and structure after the experimental treatment (Figures 2C and 2D).

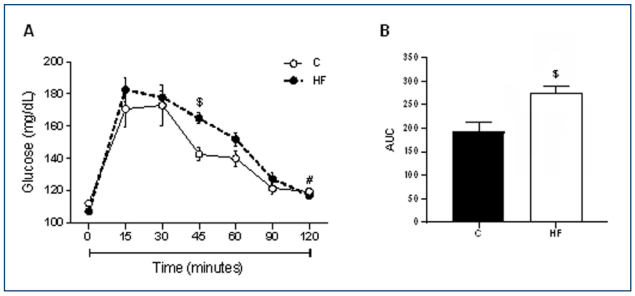

Figure 1. Oral glucose tolerance test and area under the curve in the female rats after 8 weeks of dietary treatment. **(A)** Glycemia at baseline, 15, 30, 45, 60, 90, and 120 min after 2 g of glucose/kg of body weight, and **(B)** area under the curve in the female rats. C: female rats fed a control diet; and HF: female rats fed a high-fat diet. $p≤0.05 versus C and zero (controls) and #p≤0.05 HF basal versus HF after 120 min.

Immunohistochemical analysis

Immunohistochemical analyses were performed for the detection of IL-6, STAT-3, and SOCS-3. In Figure 3, the experimental groups and the control group did not show significant relevance between them, since the expressions of pro-inflammatory cytokine IL-6, STAT-3 protein, and SOCS-3 protein were observed in the retinal and corneal tissues of both groups. In the other tissues studied in this research, expression was not observed for the tests performed.

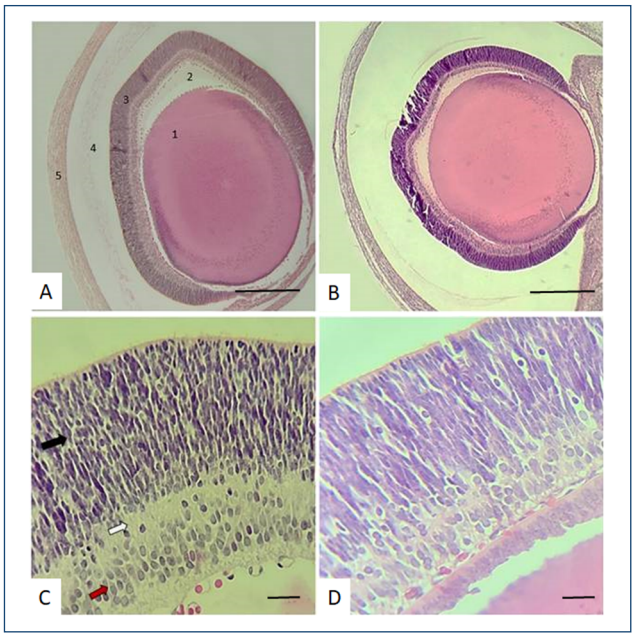

Figure 2. Ocular tissue in the control group **(A):** 1 crystalline; 2 vitreous humor; 3 retina; 4 posterior chamber; 5 cornea; and treated group **(B):** no remarkable changes. **(C)** High magnification of the control group and the treated group **(D):** the black arrow points to the photoreceptor cone cell layer; the white arrow points to the interneuron layer; and the red arrow points to the location of the retinal ganglion cells. No change was observed in cell morphology between the two analyzed groups. Hematoxylin and eosin analysis. Scale bar=34 μm.

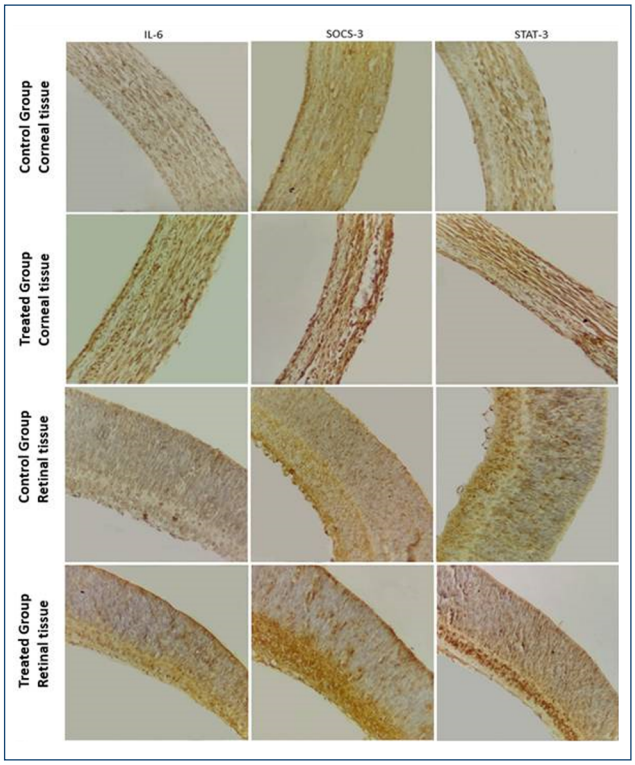

Figure 3. Corneal and retinal tissue immunohistochemistry for interleukin, suppressor of cytokine signaling, and signal transducer and activator of transcription 3. Immunohistochemical stain.

DISCUSSION

Some review papers have suggested that saturated fatty diets have no effect on cardiovascular disease, cardiovascular mortality, or even total mortality[5]. Despite the fact that medical literature is still full of articles arguing opposing positions, the recommendation to limit dietary saturated fatty acid intake has persisted, even though most recent meta-analyses of randomized trials and observational studies found no beneficial effects of reducing saturated fatty diet intake on human diseases. Some studies even suggest that saturated fatty acid intake may lead to protective effects against stroke[6].

It is known that the development of ocular tissue in rats is similar to the development of ocular tissue in humans and, in both species, development, biochemical, and metabolic processes are widely involved[13]. Regarding the human eye structure, it is important to highlight that it is produced from the coordinated development of multiple tissues (neuroectodermal, ectodermal, and mesodermal). Also, three layers can be distinguished in this complex organ: the outer region, composed of the cornea and the sclera; the middle one, composed of the iris, ciliary body, and choroid; and finally, the inner layer, which is composed of the retina with two different cell types (rods and cones).

According to a great number of studies, harmful effects of maternal insulin resistance and consumption of high amount of saturated fatty acids, especially during pregnancy or lactation, have been observed[14,15]. Therefore, an increased consumption of saturated fatty acids may lead to increased concentrations of glucose, insulin, leptin, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1, IL-6, NF-κB, and Toll-like receptor 4 activation[16,17]. In this sense, it has also been demonstrated that the increased production of TNF-α in the vascular endothelium is probably related to the risk of cardiovascular disease development, which involves inflammatory processes that may ultimately affect the skeletal muscle and the liver negatively[18]. Furthermore, high concentrations of TNF-α demonstrated a positive association with newborns from mothers with pre-eclampsia[19].

Currently, less information is available on whether and to what exent a saturated fatty diet is able to induce changes following ocular development. In our study, no histopathological changes were observed in all tissues analyzed in the experimental and control groups in this setting. In addition, no remarkable changes were noticed in the IL-6, STAT-3, and SOCS-3 expressions. By comparison, some authors have postulated that a high content of saturated fat, cholesterol, and sugar significantly increased retinal leukocyte accumulation and endothelial injury in streptozotocin-diabetic rats[20]. The consumption of diets containing higher palmitic acid concentrations modulates rats' mucus granule surfaces in their goblet cells[21]. Taken as a whole, we assume that the maternal diet during gestation with a normolipidic diet based on saturated fatty acids did not appear to trigger significant changes in the ocular tissue of fetuses (such as other cell morphology changes, cell atypicity, or the presence of degenerations), even in the presence of maternal insulin resistance.

CONCLUSION

The saturated fatty diet does not induce tissue changes that activate the JAK/STAT signaling pathway during eye development in the fetuses of mothers fed a saturated-fat normolipidic diet. Given the absence of similar studies for possible comparisons, it can be concluded that, despite the previously related high inflammatory potential of fatty acids and their direct exposure to fetus health, the normolipid maternal diet, regardless of the type of fatty acid intake, does not induce eye damage in the fetuses, emphasizing the importance of diet quality during pregnancy. Further studies involving other cellular signaling pathways within the field are necessary to elucidate the issue.

AUTHORS' CONTRIBUTIONS

LSA: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **TGP:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **DVS:** Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **LVM:** Formal Analysis, Writing – original draft, Writing – review & editing. **DAR:** Data curation, Formal Analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **LP:** Formal Analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Analysis of Down syndrome newborn outcomes in three neonatal intensive care units in Rio de Janeiro, Brazil

Hanna Gabriela da Cruz Alfaro[1], Saint Clair Gomes Junior[1], Renato Augusto Moreira de Sá[1,2], Edward Araujo Júnior[3,4]*

SUMMARY

OBJECTIVE: The aim of this study was to analyze the outcomes of newborns with Down syndrome admitted to three neonatal intensive care units in the city of Rio de Janeiro, Brazil.

METHODS: A retrospective cohort study was conducted by analyzing the medical records between 2014 and 2018 of newborns with Down syndrome admitted to three neonatal intensive care units. The following variables were analyzed: maternal and perinatal data, neonatal malformations, neonatal intensive care unit intercurrences, and outcomes.

RESULTS: A total of 119 newborns with Down syndrome were recruited, and 112 were selected for analysis. The most common maternal age group was >35 years (72.07%), the most common type of delivery was cesarean section (83.93%), and the majority of cases were male (53.57%). The most common reasons for neonatal intensive care unit hospitalization were congenital heart disease (57.66%) and prematurity (23.21%). The most common form of feeding was a combination of human milk and formula (83.93%). The second most common malformation was duodenal atresia (9.82%). The most common complications during neonatal intensive care unit hospitalization were transient tachypnea of the newborn (63.39%), hypoglycemia (18.75%), pulmonary hypertension (7.14%), and sepsis (7.14%). The mean length of stay in the neonatal intensive care unit was 27 days. The most common outcome was discharge (82.14%). Furthermore, 12.50% of newborns were transferred to an external neonatal intensive care unit, and 6% died.

CONCLUSION: Newborns with Down syndrome are more likely to be admitted to the neonatal intensive care unit, and the length of hospital stay is longer due to complications related to congenital malformations common to this syndrome and prematurity.

KEYWORDS: Down syndrome. Newborn. Intensive care units. Congenital anomalies.

INTRODUCTION

Occasionally, chromosomes can undergo some changes during the mitotic and meiotic processes, resulting in aneuploidies[1]. Down syndrome (DS) is a genetic disorder caused by an imbalance in the chromosomal constitution during the fetal stage. It was described in 1866 by John Longdon Down[1], and its etiology is related to an excess of genetic material resulting from an extra chromosome in pair 21[2]. Its estimated prevalence is 1/700 births, occurring worldwide, regardless of social or ethnic class, and it is considered the most common chromosomal abnormality in newborns[3,4].

Some of the complications associated with DS include generalized hypotonia, neuropsychomotor development, thyroid disorders, hematological disorders, hearing/vision disorders, and orthopedic disorders[5,6]. Gastrointestinal malformations such as duodenal atresia and esophageal atresia are very common[5]. However, the major congenital malformation associated with the clinical course of these newborns is congenital heart disease (CHD), which is present in approximately 40–50%[7] of newborns with DS, most commonly a complete or incomplete atrioventricular septal defect (AVSD)[8].

The presence of congenital anomalies associated with DS, mainly CHD, increases the risk of admission to the neonatal intensive care unit (NICU) after birth and may directly affect the length of stay and outcomes[9]. Risk factors independently associated with primary NICU admission included the antenatal diagnosis of DS, the presence of CHD, pulmonary hypertension (PH), and the need for ventilation[9]. According to Mann et al.[10] neonates with DS are five times more likely to be admitted to the NICU than those born with a normal karyotype (46% vs. 9.2%).

The objective of this study was to evaluate the neonatal outcomes of DS in three NICUs in Rio de Janeiro, Brazil.

METHODS

A retrospective cohort study was conducted, reviewing the medical records of newborns with DS in three NICUs in the city of

[1]Fernandes Figueira Institute, Oswaldo Cruz Foundation, Department of Obstetrics and Fetal Medicine – Rio de Janeiro (RJ), Brazil.
[2]Universidade Federal Fluminense, Department of Obstetrics – Niterói (RJ), Brazil.
[3]Universidade Federal de São Paulo, Paulista School of Medicine, Department of Obstetrics – São Paulo (SP), Brazil.
[4]Universidade Municipal de São Caetano do Sul, Discipline of Woman Health – São Paulo (SP), Brazil.
*Corresponding author: araujojred@terra.com.br
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Rio de Janeiro, Brazil, between January 2014 and December 2018. Inclusion criteria were newborns admitted to the NICU within the first 24 h of life. Exclusion criteria were medical records of newborns transferred to external units, twin pregnancies, incomplete medical records, and medical records that were not available at the time of data collection. This study was approved by the Ethics Committee of the National Institute of Women's Health Child and Adolescent Health Fernandes Figueira/Oswaldo Cruz Foundation under CAAE number 24734719.7.0000.5269.

The variables studied were maternal data (maternal age and ethnicity), birth data (type of delivery, Apgar score, gender, birth weight, need of cardiopulmonary resuscitation, gestational age at delivery, previous diagnosis of DS, presence of CHD, and extra-cardiac malformations), hospitalization data (NICU admission, main route of feeding, use of nutritional therapy, hypoglycemia, hypothermia, transient tachypnea of the newborn, and NICU intercurrences), and outcomes (discharge, transfer, or death, length of hospital stay, and discharge weight). Hypoglycemia was defined as blood glucose<40 mg/dL in the presence of symptoms such as tachypnea, hypothermia, tachycardia, nervousness, hypotonia, or lethargy. Hypothermia was defined as a body temperature<36.0°C. Transient tachypnea in the newborn was defined as a respiratory rate greater than 60 breaths per minute for more than 4 h.

Data were tabulated in the Epi Info program (Centers for Disease Control and Prevention, Atlanta, GA, USA) and analyzed descriptively. Categorical variables were described using absolute and percentage frequencies. Numerical variables were described by median, minimum, and maximum values. The multivariate two-step cluster method was used to classify newborns with similar profiles in terms of birth characteristics, NICU intercurrences, and outcomes. This technique makes it possible to work with categorical and numerical variables simultaneously. The number of clusters was based on the silhouette coefficient. The latter assesses the cohesion and discrimination of the groups formed and ranges from -1 to +1, where positive values greater than 0.5 indicate a reasonable partitioning of the data and values less than 0.2 indicate that the data do not exhibit a cluster structure.

The clustering method is a multivariate statistical analysis approach. This technique makes it possible to identify groups with homogeneous characteristics, which can be used when there are at least three variables. One of the most commonly used clustering techniques is k-means, which consists of dividing a set of objects into smaller subsets according to their characteristics (variables). After mathematical distance calculations, it is possible to assign a measure of proximity (similarity) to all pairs of objects and between each object and the subsets. Subsequently, in an interactive process, i.e., by repeating the previous steps, the subgroups are formed in such a way that the distances between the members of a subgroup are minimal and the distances between the subgroups are maximal[11].

RESULTS

A total of 119 newborns with DS were initially identified. After applying the exclusion criteria, the final sample consisted of 112 newborns. The maternal age group with the highest number of DS births was>35 years [80 cases of 112, 95% confidence interval (CI) 62.7–80.1]. The lowest maternal age was 16 years, and the highest was 42 years. The predominant maternal ethnicity was white (54.46%), followed by mixed (41.07%). DS was diagnosed during the prenatal period only in 20.53% of cases by invasive procedures, being 17.85% by amniocentesis, 1.79% by cordocentesis, and 0.89% by chorionic villus sampling.

The most common type of delivery was cesarean section (94 of 112 cases, 95%CI 75.79–90.19), and males had a slightly higher number of cases of DS (60 of 112 cases). The gestational age at delivery was distributed as follows: 53.57% term (37–42 weeks), 44.64% preterm (from 29 to 36+6 weeks), and 1.79% extremely preterm (<28 weeks). Cardiopulmonary resuscitation was required in 11.61% of newborns, and the mean birth weight was 2,580 g.

The main reason for NICU admission during the study was CHD in 57.66% of the cases studied (53 out of 112), being 30.63% of AVSD, 16.22% of ventricular septal defect, 5.41% of atrial septal defect, 3.60% of tetralogy of Fallot, and 1.80% of ventricular and atrial septal defects. The extra-cardiac malformations leading to NICU admission were duodenal atresia [9.82% (95%CI 4.94–14.7)], followed by esophageal atresia [3.57% (95%CI 0.98–8.89%)]. Table 1 shows all congenital malformations observed in DS newborns.

The most common intercurrence during NICU hospitalization was transient tachypnea of the newborn, which occurred in 63.39% of cases, followed by hypoglycemia with 18.75%. During the NICU hospitalizations, 64.29% of newborns received some type of nutritional therapy, including 56.25% with enteral probing, 7.14% with cup, 11.61% with parenteral nutrition, and 0.89% with translactation (95%CI 75.79–90.19%). The NICU intercurrences in DS newborns are shown in Table 2.

The mean NICU length of stay was 27 days, and the most common outcome was discharge [82.14% 95%CI (73.78–88.14)]. Notably, 12.50% of newborns were transferred to an external NICU, and only 6% died. Causes of death included

Table 1. Distribution of congenital malformations in newborns with Down syndrome.

Congenital malformations	Frequency	
	n	%
Congenital heart disease	53	57.66
Duodenal atresia	11	9.82
Esophageal atresia	4	3.57
Pyelectasis	4	3.57
Clubfeet	3	2.68
Anal imperforation	3	2.68
Hydrocele	3	2.68
Hepatomegaly	3	2.68
Hydronephrosis	2	1.79
Cryptorchidism	2	1.79
Single umbilical artery	2	1.79
Tracheoesophageal fistula	1	0.89
Hypospadias	1	0.89
Pulmonary hypoplasia	1	0.89
Inguinal hernia	1	0.89
Syndactyly	1	0.89
Choroid plexus cyst	1	0.89
Hemivertebra	1	0.89

Table 2. Neonatal intensive care unit intercurrences in newborns with Down syndrome.

Intercurrence	Frequency	
	n	%
Transient tachypnea of the newborn	71	63.39
Hypoglycemia	21	18.75
Pulmonary hypertension	8	7.14
Sepsis	8	7.14
Orotracheal intubation	7	6.25
Gastrostomy	7	6.25
Hypothermia	5	4.46
Necrotizing enterocolitis	6	5.36
Urinary tract infection	6	5.36
Metabolic acidosis	3	2.68
Colostomy	3	2.68
Seizures	1	0.89
Cardiorespiratory arrest	1	0.89
Pulmonary atelectasis	1	0.89

sepsis (4 out of 6 cases, with only 1 being an isolated cause) and metabolic acidosis (2 out of 6 cases, both associated with PH). The other death cases were multiple congenital malformations associated with CHD and extreme prematurity. Of the six recorded deaths, four were male and two were female.

Cluster 01 (n=28) had 75% male newborns, the highest percentage of DS diagnoses in the prenatal period (35.7%), and the highest number of preterm newborns (64.3%). Cluster 01 also had the highest number of NICU intercurrences, the highest rate of orotracheal intubation and necrotizing enterocolitis, and the longest NIUC length of stay (55 days). Cluster 01 had a number of newborns with CHD similar to other groups and was the second group with the highest number of deaths.

Cluster 02 (n=11) was almost evenly split in terms of gender (45.5% female and 54.5% male) and had the highest number of cardiorespiratory resuscitations, the highest need for parenteral nutrition (36.4%), the highest number of CHD (81.8%), and no deaths.

Cluster 03 (n=40), all being female, had a higher percentage of stillbirths (70%), a higher rate of hypoglycemia during NICU hospitalization (22.5%), and a higher rate of transient tachypnea in the newborn (35.0%).

Cluster 04 (n=33), all being male, had the highest number of congenital malformations (66.7%), the highest number of deaths (9.1%), 6.1% of cases of metabolic acidosis, the lowest rate of CHD (9.4%), and the shortest NICU length of stay (12 days). Table 3 shows the variables stratified according to cluster analysis.

DISCUSSION

In this study, we evaluated the incidence of associated congenital malformations, NICU intercurrences, and outcomes of newborns diagnosed with DS in three NICUs in the city of Rio de Janeiro, Brazil. According to Moreira and Gusmão[12], DS occurs as a free trisomy (not a chromosomal disjunction) in 95% of cases. In this study, 98.2% of the cases had this type of trisomy, while 1.78% had a translocation. There was no difference in severity between the newborns who had a translocation in the karyotype.

Screening and prenatal diagnosis programs for the diagnosis of DS in different populations have proven to be very important so that these fetuses can be referred to referral health units in a timely manner[13,14]. In this study, 27.68% of the newborns were diagnosed with DS before birth.

Congenital heart disease was the most common congenital malformation in DS newborns, with a rate of 57.66% of our cases, with AVSD being the most prevalent (30.66%). These

Table 3. Variables stratified according to cluster analysis.

Variables	Clusters			
	01 (n=28)	02 (n=11)	03 (n=40)	04 (n=33)
Gender				
Female	25.0% (7)	45.5% (5)	100% (40)	0% (0)
Male	75.0% (21)	54.5% (6)	0% (0)	100% (33)
Prenatal diagnosis of Down syndrome	35.7% (10)	27.3% (3)	25% (10)	24% (8)
Yes				
Need for cardiopulmonary resuscitation				
Yes	3.6% (1)	27.3% (3)	5% (2)	21.2% (7)
Gestational age				
Preterm[1]	39.9% (19)	63.6% (7)	30% (12)	22.4% (14)
Term[2]	32.1% (1)	36.4% (4)	70% (28)	57.6% (19)
Presence of congenital malformation				
Yes	10.7% (3)	0% (0)	25% (10)	66.7% (22)
Nutrition				
Formula	75% (21)	54.5% (6)	92.5% (37)	90.9% (30)
Breastfeeding	10.7% (3)	9.1% (1)	0% (0)	3% (1)
Parenteral	14.3% (4)	36.4% (4)	7.5% (3)	6.1% (2)
Nutritional therapy				
Translactation	0% (0)	0% (0)	0% (0)	3% (1)
Cup	0% (0)	0% (0)	12% (4)	12.1% (8)
Enteral probing	67.9% (19)	54.5% (6)	55% (22)	48.5% (16)
None	32.1% (9)	45.5% (5)	35% (14)	36.4% (12)
Hypothermia[3]				
Sim	7.1% (2)	0% (0)	2.5% (1)	6% (2)
Hypoglicemia[4]				
Sim	17.9% (5)	9.1% (1)	22.5% (9)	18.2% (6)
Transit tachypnea of the newborn[5]	85.7% (24)	63.6% (7)	55% (22)	54.5% (18)
Yes				
Congenital heart disease				
Yes	67.9% (19)	81.8% (9)	67.5% (27)	28.1% (10)
Respiratory distress				
Yes	14.3% (4)	9.1% (1)	35% (14)	25% (28)
NICU intercurrence				
Yes	60.7% (17)	27.3% (3)	17.5% (7)	27.3% (9)
Duodenal atresia				
Yes	0% (0)	100% (11)	0% (0)	0% (0)
Sepsis				
Yes	28.6% (8)	0% (0)	0% (0)	0% (0)
Orotracheal intubation				
Sim	14.3% (4)	0% (0)	2.5% (1)	6.1% (2)

Continue...

Table 3. Continuation.

Variables	Clusters			
	01 (n=28)	02 (n=11)	03 (n=40)	04 (n=33)
Hypoactivity				
Yes	0% (0)	0% (0)	5% (2)	3% (1)
Necrotizing enterocolitis				
Yes	10.7% (3)	9.1% (1)	2.5% (1)	3% (1)
Cardiorespiratory arrest				
Yes	0% (0)	0% (0)	0% (0)	3% (1)
Metabolic acidosis				
Yes	3.6% (1)	0% (0)	0% (0)	6.1% (2)
Pulmonary hypertension				
Yes	10.7% (3)	9.1% (1)	7.5% (3)	3% (1)
Outcome				
Discharge	67.9% (19)	81.8% (9)	87.5% (35)	87.9% (29)
Transferring	25% (7)	18.2% (2)	10% (4)	3% (1)
Death	7.1% (2)	0% (0)	2.5% (1)	9.1% (3)

[1]Premature: <36+6 weeks; [2]Term>37 weeks; [3]Body temperature<36.0°C; [4]Capillary blood glucose<40 mg/dL; [5]Respiratory rate>60 incursions per minute. NICU: neonatal intensive care unit. Values in relative (%) and absolute frequency.

findings are in agreement with Fudge et al.[15] who retrospectively analyzed 4,350 patients with DS and observed that AVSD was the most common CHD. Taura et al.[16] assessed the prevalence of CHD among 42 patients with DS in southwestern Saudi Arabia. They observed a prevalence of 81% of CHD, with atrial septal defect (28.5%) being the most common, followed by ventricular septal defect (25%), patent ductus arteriosus (16%), and AVSD (14.3%).

Compared to other congenital malformations in newborns with DS, we observed a higher incidence of duodenal atresia (9.82%), followed by esophageal atresia (3.57%). Bermudez et al.[6] retrospectively reviewed data from 1,207 patients with DS and observed a rate of 5% of gastrointestinal malformations, including 13 cases of duodenal atresia, 8 of imperforate anus, 4 annular pancreases, 2 congenital megacolon, 2 esophageal atresias, 2 esophageal compression by an anomalous subclavian, and 1 case of duodenal membrane. Buchin et al.[17] retrospectively reviewed data from 187 patients with DS, and gastrointestinal malformations were observed in 27 (14.4%), similar to our results.

The most common NICU intercurrence in DS newborns was the transient tachypnea of the newborn. MacAndrew et al.[18] in a retrospective cohort analyzed full-term infants with (4,623) and without (606,770) DS. Infants with DS had more respiratory distress, thrombocytopenia, feeding problems, and PH. They received respiratory support for a longer period of time and had a longer length of stay. Infants with DS have a high risk of oral motor difficulties and pharyngeal dysphagia with aspiration, both of which require systematic attention. Clinical interventions should promote swallowing safety and the development of feeding abilities[19]. In our study, 64.29% of DS newborns received some type of nutritional therapy, including 56.25% with enteral probing.

Cluster 02 included all cases of duodenal atresia, which is directly related to the higher number of parenteral nutrition cases (36.4%). In terms of gender, this cluster had the smallest difference between female and male cases (45.5 and 54.5%, respectively). The higher number of premature newborns found in this group may be related to the finding of a greater need for cardiopulmonary resuscitation at birth.

Clusters 03 and 04 differed significantly in terms of gender, but the most striking differences were the higher number of full-term births, cases of CHD, and respiratory distress in females. The cluster formed by males, on the contrary, had a higher number of cases of prematurity, congenital malformations, cardiorespiratory arrest during NICU hospitalization, and metabolic acidosis. No data on significant gender differences in morbidity or mortality in DS were found in the literature.

Congenital heart disease was present in a higher proportion in cluster 02, which was not reflected in the length of hospital stay or the outcome. Therefore, we can assume that the outcome of death was not directly related to CHD, since the

group with the highest number of this outcome was the cluster with the lowest number of newborns with CHD. This suggests that CHD was diagnosed and corrected early and that surgical correction can be considered a protective factor against death.

CONCLUSION

Down syndrome newborns are at higher risk of NICU admission and longer hospital stays due to common congenital abnormalities and prematurity. There is a need for further studies targeting those born with DS in order to improve the quality of neonatal care for them.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Predictors of recurrence in breast cancer patients with pathological partial response

Fadime Didem Can Trabulus[1*], Mehmet Ali Nazli[2], Esra Arslan[3], Ozlem Mermut[4], Fatih Dal[5], Bulent Akce[5], Riza Umar Gursu[6], Esra Canan Kelten Talu[7], Jacqueline Nur Adira Couteau[8]

SUMMARY

OBJECTIVE: Patients with residual disease after neoadjuvant chemotherapy have a relative risk of developing recurrence. This study investigates the risk factors for recurrence in locally advanced breast cancer patients with residual disease and evaluates survival analysis.

METHODS: This is a retrospective, single-center study. Breast cancer patients who failed to achieve a pathological complete response after neoadjuvant chemotherapy were included. Demographic, clinicopathological, and treatment characteristics were evaluated to identify predictive factors of recurrence and survival analysis.

RESULTS: We included 205 patients in this study. After a median of 31 months of follow-up, 10 patients died, and 20 developed distant metastasis. Disease-free survival and disease-specific survival were 73.8% and 83.1%, respectively. Lymphovascular invasion and non-luminal subtype were independent predictors of locoregional recurrence. In situ carcinoma, lymphovascular invasion, ypTIII stage, and non-luminal molecular subtypes were independent predictors of disease-free survival. The only independent factor affecting disease-specific survival was cNII–III. The number of involved lymph nodes was an independent predictor of disease-free survival in patients without complete axillary response.

CONCLUSION: Factors affecting disease-specific survival and disease-free survival were cNII–III and the number of involved lymph nodes, respectively. Patients with non-luminal, large residual tumors with in situ carcinoma, lymphovascular invasion, clinically positive axilla, and residual nodal involvement have a high relative risk for recurrence and may benefit from additional treatments.

KEYWORDS: Breast cancer. Pathologic complete response. Neoadjuvant chemotherapy. Disease free survival. Locoregional neoplasm recurrences.

INTRODUCTION

Neoadjuvant chemotherapy (NAC) is the standard approach for managing locally advanced breast cancer (LABC) (stages IIB and III)[1,2]. NAC downstages tumors by allowing breast-conserving surgery (BCS) instead of mastectomy and avoids axillary lymph node dissection (ALND)-associated lymphedema by allowing sentinel lymph node biopsy (SLNB)[1-3]. NAC is recommended for triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive cancer because these subtypes have higher rates of pathological complete response (pCR)[1-5].

In LABC patients, pCR following NAC can serve as a surrogate marker of treatment efficacy[1,3]. However, not all patients achieve pCR and may develop locoregional recurrence (LRR)[6]. Patients with a partial response may avoid recurrence by the addition of regimens that will sensitize their tumors to ongoing chemotherapy[7]. Therefore, identifying patients at high risk of recurrence is critical for early intervention. This study aimed to identify factors that predict recurrence in LABC patients who failed to achieve pCR after NAC and to conduct a survival analysis.

METHODS

Study design and patient selection

This retrospective study was conducted in a tertiary care hospital. Patients diagnosed and treated for LABC (>18 years) between January 2011 and June 2019 were selected and those who received NAC and failed to achieve pCR were identified.

[1]Bahçeşehir University, School of Medicine, Goztepe Medical Park Hospital, Department of General Surgery – İstanbul, Turkey.
[2]University of Health Sciences, Istanbul Training and Education Hospital, Department of Radiology – İstanbul, Turkey.
[3]University of Health Sciences, Istanbul Training and Education Hospital, Department of Nuclear Medicine – İstanbul, Turkey.
[4]University of Health Sciences, Istanbul Training and Education Hospital, Department of Radiation Oncology – İstanbul, Turkey.
[5]University of Health Sciences, Istanbul Training and Education Hospital, Department of General Surgery – İstanbul, Turkey.
[6]University of Health Sciences, Istanbul Training and Education Hospital, Department of Medical Oncology – İstanbul, Turkey.
[7]University of Health Sciences, İzmir Faculty of Medicine, Department of Pathology – İzmir, Turkey.
[8]Bahçeşehir University, School of Medicine – İstanbul, Turkey.
*Corresponding author: didemcan73@gmail.com
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Patients with incomplete axillary response according to tumor features were also evaluated.

LABC was defined as stage III and stage IIB (T3N0) disease[8].

Notable exclusions were male patients, patients with metastatic disease at presentation, those who received only neoadjuvant hormonotherapy, and patients with pCR.

Demographic, clinicopathological, and treatment characteristics were recorded. The source of medical records was our hospital's electronic database.

Pathology

Diagnosis of invasive breast cancer in the breast and axilla was histopathologically confirmed and defined according to WHO classification[9]. ER, PR, and HER2 statuses were evaluated by immunohistochemistry or fluorescence in situ hybridization before NAC administration. Tumor subtypes were defined as follows [12]: luminal A; ER(+) or PR(+), HER2-neu (–), Ki67 <20%; luminal B, ER(+) or PR(+), and/or HER2-neu (+), Ki67 ≥20%; non-luminal HER2-neu(+), ER(–) PR(–) HER2-neu (+); and triple-negative, ER(–) PR (–) HER2-neu (–).

Treatment

The multidisciplinary team decided on NAC and the type of surgery post-NAC. Most patients received anthracycline-based regimens, followed by a taxane (four cycles of doxorubicin and cyclophosphamide every 3 weeks and 12 cycles of paclitaxel or docetaxel weekly). Patients with HER2-positive tumors received trastuzumab simultaneously with NAC for 1 year[5,10]. No patients received adjuvant chemotherapy. After NAC, patients underwent either BCS or mastectomy. Intraoperative pathological examination was performed to analyze SLNB and surgical margins[11]. Patients with clinically, radiologically, and pathologically positive lymph nodes underwent ALND.

All patients underwent adjuvant radiotherapy. Radiotherapy was applied at 50 Gy in 25 fractions over 4 weeks and 10 Gy boost to the tumor bed for patients who underwent BCS, and peripheral lymph nodes (axillary, supra, and supra infra-clavicular and internal mammary lymph nodes). Patients with ER-positive tumors received adjuvant endocrine therapy with tamoxifen or aromatase inhibitors for at least 5 years[10].

Assessment of response

Pathological response after NAC was assessed using the Miller-Payne classification. pCR (ypT0 and ypN0) was defined as no residual invasive disease in the breast or axilla. Residual ductal carcinoma in situ was included in the pCR category. Partial response was defined as any response besides pCR[12].

Statistical analysis

The endpoint analyses were LRR, distant metastases, and disease-specific survival (DSS). LRR was defined as a recurrent disease in the ipsilateral breast or peripheral lymph nodes. Distant metastasis was considered any recurrence in distant organs or other lymph nodes.

SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The study data were evaluated using descriptive methods (number, percent, and median). Survival calculations were made by the Kaplan-Meier analysis. With the log-rank univariate analysis test, the effects of prognostic factors related to tumor and patient characteristics on disease-free survival (DFS) and DSS were investigated. The effects of prognostic factors on DFS and DSS were investigated using the Cox regression test in multivariate analysis. Proportional differences between the groups were calculated with the chi-square test. Results were evaluated within a 95% confidence interval and a significance level of $p<0.05$.

Ethics

The study protocol was approved by the Istanbul Education and Training Hospital (approval no. 2020-2675). The study was conducted under the 1975 Declaration of Helsinki, as revised in 2013.

RESULTS

A total of 1,247 patients with breast cancer were diagnosed and treated between January 2011 and June 2019. We identified 205 patients with LABC who received NAC. Among them, 54 (26.3%) achieved pCR in the breast, 94 (45.9%) in the axilla, and 43 (21%) in both breast and axilla. Notably, 151 patients with incomplete response in the breast and 111 with incomplete response in the axilla were included in this study.

Demographic and clinicopathological features of patients with partial response are shown in Table 1. The median age at diagnosis was 52 years (range 25–77 years), and 44% (n=67) were younger than 50 years. Notably, 61 (40%) patients underwent BCS and 90 (60%) underwent mastectomy. As for the axillary approach, 92 (61%) patients underwent ALND and 59 (39%) underwent SLNB.

The rate of partial response in the breast was 74%. Evaluation of residual tumors in the breast revealed that 45 (30%) patients had high-grade tumors, 65 (43%) had lymphovascular invasion (LVI), 92 (61%) had coexisting in situ carcinoma, and 100 (66%) had residual tumor in lymph nodes (Table 1).

After a median of 31 months of follow-up (range 12–115 months), 10 patients (6.6%) died and 20 (13.2%) developed distant metastasis. The 5-year DFS and DSS were 73.8

Table 1. Demographic and clinicopathological features of patients who achieved partial response in the breast.

Features	Category	Patients n=151 (%)
Age (years)	<50	67(44.4)
	≥50	84(55.6)
cT stage	I–II	111(73.5)
	III–IV	40(26.5)
cN stage	0–I	130(86.1)
	II–III	21(13.9)
Histological type	Invasive ductal carcinoma	126(83.4)
	Others	25(16.6)
Surgical type	Breast-conserving surgery	61(40.4)
	Mastectomy	90(59.6)
Tumor grade	I–II	106(70.2)
	III	45(29.8)
Lymphovascular invasion	Positive	65(43.0)
In situ carcinoma	Yes	92(60.9)
ypT stage	I	101(66.9)
	II	37(24.5)
	III	13(8.6)
Lymph node invasion	Positive	100(66.2)
Estrogen receptor	Positive	115(76.2)
Progesterone receptor	Positive	96(63.6)
HER2	Positive	33(21.9)
Ki-67	<20%	26(17.2)
	≥20%	125(82.8)
Molecular subtype	Luminal-A	15(9.9)
	Luminal B/HER2(–)	85(56.3)
	Luminal B/HER2(+)	17(11.3)
	Non-luminal B/HER2(+)	16(10.6)
	Triple-negative	18(11.9)
Molecular subtype	Luminal	117(77.5)
	Non-luminal	34(22.5)
Loco-regional recurrence	Breast	0(0.0)
	Thorax wall	6(4.0)
	Axilla	1(0.7)
Systemic recurrence	Yes	20(13.2)

and 83.1%, respectively (Figures 1B and 1C). Seven patients (4.6%) developed LRR, including six chest wall recurrence and one axillary nodal recurrence. The last LRR occurred in the 34th month. The 5-year locoregional free survival was 92.8%. The nodal failure occurred in a patient who underwent ALND. All chest wall recurrences occurred in patients who underwent mastectomy. LRR rate was significantly higher in patients who underwent mastectomy than patients who underwent BCS (8% vs. 0%; p=0.042, respectively).

On univariable analysis, mastectomy, high-grade tumors, LVI, non-luminal tumors, and HER2 overexpression were associated with LRR. On multivariate analysis, independent factors associated with LRR were LVI [HR: 22.35 (1.42–352.62); p=0.027] and non-luminal molecular subtype [HR: 27.34 (2.99–249.14); p=0.003] (Tables 2 and 3).

As for 5-year DFS rates, patients who underwent mastectomy with high-grade tumors, LVI, residual ypTIII, residual in situ carcinoma, and residual tumor in lymph nodes had lower DFS rates than those without these features. Independent factors affecting DFS were LVI [HR: 4.35 (1.18–15.94); p=0.027], residual in situ carcinoma [HR: 7.37 (1.52–35.71); p=0.013], ypTIII stage [HR: 5.42 (1.69–17.35); p=0.004], and non-luminal molecular subtype [HR: 4.41 (1.33–14.58); p=0.015]. We observed that in cases of partial axillary response, higher numbers of metastatic lymph nodes were associated with lower 5-year DFS [(1–3 involved lymph nodes: 81%, 4–9: 70.4% and ≥10: 46.7%) (p=0.025)] (Tables 2–4). However, metastatic tumor size and axillary extranodal extension (ENE) were not associated with survival.

DSS rate of patients with cNII-III stages, LVI, coexisting in situ carcinoma, ypT III stage, and positive lymph nodes were significantly lower than those without these features. We determined that the only independent factor affecting 5-year DSS was cN II–III stage [HR: 5.14 (1.22–21.59; p=0.025] (Tables 2 and 3).

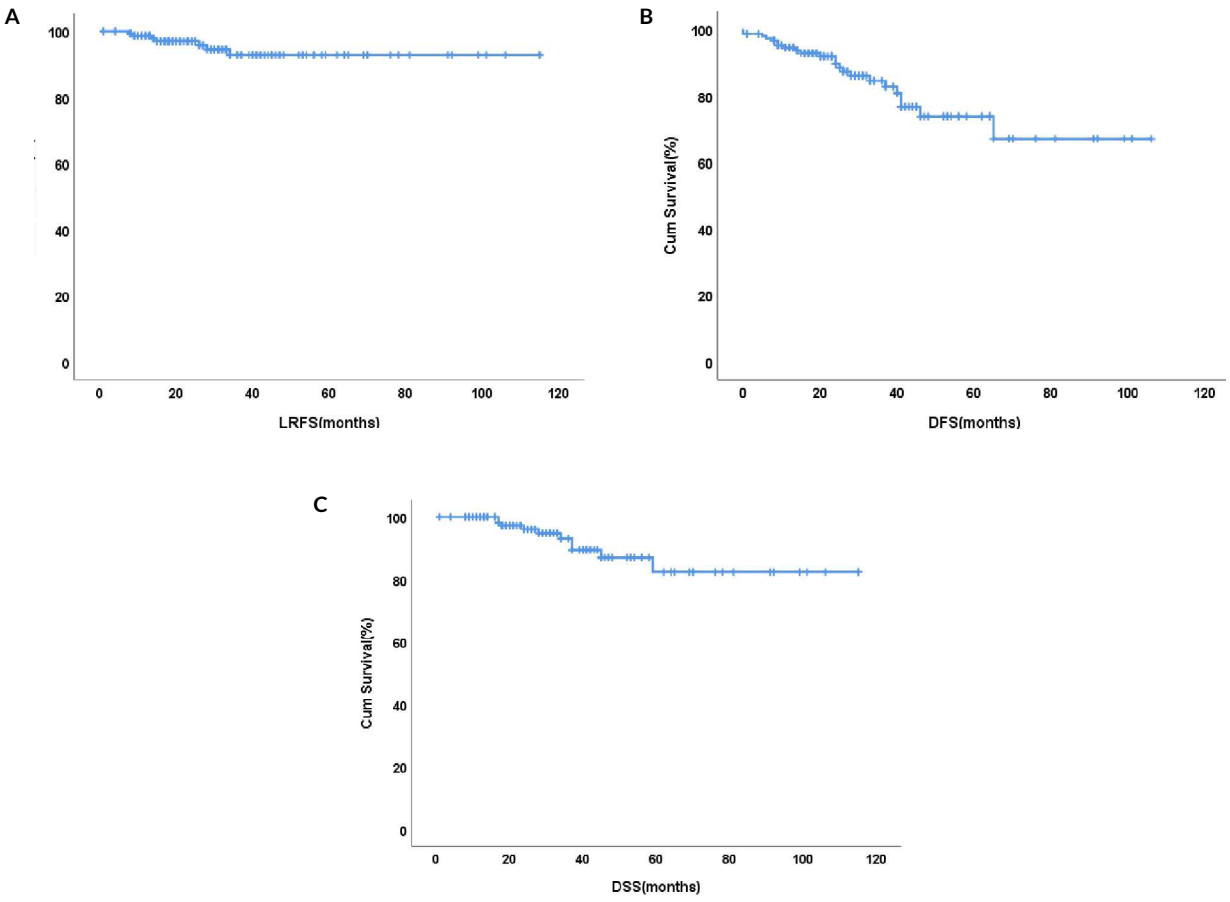
DISCUSSION

This retrospective study evaluates predictive factors for recurrence in LABC patients without pCR after NAC. We found that LVI and non-luminal subtypes were independent predictors of LRR. LVI, in situ carcinoma, ypTIII stages, and non-luminal molecular subtypes were independent predictors affecting DFS. The only independent factor affecting DSS was cN II–III. Our results demonstrate that the number of involved axillary lymph nodes were independent predictors of LRR and DSS for patients without axillary pCR.

Patients with residual disease post-NAC have a higher risk of developing LRR than patients with pCR[3,12,13].

In many studies, the presence of LVI increases recurrence risk in patients without pCR[13,14]. Our results support this by showing that LVI was a predictor of LRR for patients without axillary pCR.

ENE, which is defined as an extension of neoplastic cells through the nodal capsule into perinodal tissue, is associated



DFS: disease-free survival, DSS: disease-specific survival.

Figura 1. The 5-year locoregional free survival. (A) the 5-year disease-free survival, (B) and the 5-year disease-specific survival and (C) of the patients who failed to achieve pathologic complete response.

with recurrence and mortality. Tumors with ENE persisting after NAC may be more likely to recur[15]. Unlike the reported literature, we did not observe any relationship between ENE and survival in patients without axillary pCR.

Although patients with luminal-type, especially hormone-positive, or non-HER2-like tumors are less likely to achieve pCR than patients with non-luminal and HER2-like tumors, they are not necessarily more likely to develop LRR[3]. In fact, studies show that patients with TNBC with residual disease after NAC have a higher recurrence risk[4,13]. Supporting literature, this study shows that hormone-positive tumors, especially luminal B-type tumors, were less likely to achieve pCR than non-luminal tumors. However, the non-luminal subtype was an independent predictor of local or systemic recurrences.

In patients with residual disease post-NAC, lymph node positivity and LVI are predictors of distant metastasis[13,14]. The only nodal failure in this study occurred in the axilla of a patient who underwent ALND. Similarly, all chest wall recurrences occurred among patients who underwent mastectomy, and no in-breast failure occurred among patients who underwent BCS. Based on this, we concluded that more extensive surgeries result in more aggressive tumors. This study also showed that high numbers of involved lymph nodes are predictors of axillary recurrence. Extensive axillary dissections showed no additional benefit in avoiding LRR. As no relapses occurred after SLNB, ALND may be less considered after NAC and SLNB may be preferred even for patients with a partial response.

Our study has several limitations. First, data were collected retrospectively. Second, no formal power analysis was performed to determine the sample size. However, all eligible patients with LABC in our hospital database were recruited. Third, this is a single-center study and does not contain different therapeutic approaches. Therefore, the generalizability of our results may

Table 2. Risk factors associated with 5-year locoregional recurrence-free survival, disease-free survival, and disease-specific survival of patients who achieved partial response in breast (univariate analysis).

Factors	Category	LRRFS (92.8%)	p	DFS (73.8%)	p	DSS (83.1%)	p
Age (years)	<50	91.3	0.485	78.3	0.449	91.2	0.290
	≥50	94.0		70.2		84.6	
cT stage	I–II	93.4	0.932	70.7	0.509	83.7	0.321
	III–IV	90.5		82.0		85.9	
cN stage	0–I	94.3	0.326	75.6	0.421	88.9	0.005*
	II–III	85.5		65.0		69.3	
Histological type	Invasive ductal carcinoma	93.0	0.978	71.8	0.447	81.3	0.219
	Others	91.7		88.0		100.0	
Surgical type	Breast-conserving surgery	100.0	0.040*	86.8	0.044*	96.9	0.108
	Mastectomy	88.9		66.8		77.7	
Tumor grade	I–II	100.0	<0.001*	75.6	0.033*	83.9	0.351
	III	78.4		55.2		79.8	
Lymphovascular invasion	Positive	85.9	0.018*	53.9	<0.001*	62.6	<0.001*
	Negative	97.9		89.1		100.0	
In situ carcinoma	Yes	91.4	0.546	61.5	0.005*	74.3	0.012*
	No	94.9		94.9		100.0	
pT stage	I–II	92.8	0.754	77.0	0.007*	86.8	0.034*
	III	91.7		53.3		77.1	
Lymph node invasion	Yes	89.4	0.058	68.5	0.021*	75.1	0.024*
	No	100.0		82.6		100.0	
Estrogen receptor	Positive	97.3	0.002*	73.5	0.235	83.1	0.325
	Negative	78.8		70.1		81.4	
Progesterone receptor	Positive	98.7	0.005*	74.5	0.169	82.0	0.444
	Negative	82.4		71.1		83.0	
HER2	Positive	81.7	0.003*	77.7	0.590	93.0	0.567
	Negative	96.5		71.7		78.8	
Ki-67	<20%	100.0	0.239	87.5	0.110	100.0	0.659
	≥20%	91.5		71.4		88.2	
Molecular subtype	Luminal-A	100.0	0.002*	83.3	0.507	100.0	0.637
	Luminal B/HER2(–)	98.5		75.2		81.0	
	Luminal B/HER2(+)	91.7		91.7		100.0	
	Non-luminal B/HER2(+)	71.3		63.2		85.7	
	Triple-negative	80.0		72.9		68.6	
Molecular subtype	Luminal	97.3	0.001*	74.3	0.153	83.4	0.259
	Non-luminal	77.4		67.8		80.1	

*p<0.05, p-value given refer to log-rank test. DFS: disease-free survival; DSS: disease specific survival; LRRFS: locoregional recurrence free survival.

be limited due to varying treatment guidelines according to local regulations and resource availability.

In conclusion, our study demonstrated that LVI and non-luminal subtypes are independent predictors of LRR. LVI, in situ carcinoma, ypTIII stage, and non-luminal molecular subtypes are independent predictors of DFS. The only independent factor affecting DSS was cN II-III. Additionally, the number of involved axillary lymph nodes was an independent predictor of LRR and DSS. Residual tumor size and ENE did not demonstrate a significant risk for recurrence in patients without axillary

Table 3. Risk factors associated with 5-year locoregional recurrence free survival, disease free survival and disease specific survival of patients who achieved partial response in breast (multivariate Cox regression analysis).

Factors	Category	Loco-regional recurrence-free survival HR (95%CI)	p-value	Disease-free survival HR (95%CI)	p-value	Disease-specific survival HR (95%CI)	p-value
Age (years)	<50	3.55(0.44–28.94)	0.237	Reference (1)	0.617	Reference (1)	0.125
	≥50	Reference (1)		1.30(0.46–3.65)		3.41(0.71–16.34)	
cT stage	I–II	Reference (1)	0.135	Reference (1)	0.003*	Reference (1)	0.358
	III–IV	5.65(0.58–54.88)		5.66(1.77–18.09)		2.06(0.44–9.62)	
cN stage	0–I	Reference (1)	0.791	Reference (1)	0.803	Reference (1)	0.025*
	II–III	1.34(0.15–11.87)		1.14(0.40–3.27)		5.14(1.22–21.59)	
Histological type	Invasive ductal carcinoma	Reference (1)	0.237	Reference (1)	0.607	Reference (1)	
	Others	5.66(0.39–82.06)		1.49(0.32–6.88)		**	
Surgery	Breast-conserving surgery	Reference (1)		Reference (1)	0.444	Reference (1)	0.200
	Mastectomy	**		1.67(0.45–6.17)		4.23(0.47–38.44)	
Tumor grade	I–II	Reference (1)		Reference (1)	0.180	Reference (1)	0.941
	III	**		2.02(0.72–5.64)		1.06(0.24–4.68)	
Lymphovascular invasion	Positive	22.35(1.42–352.62)	0.027*	4.35(1.18–15.94)	0.027*	**	
	Negative	Reference (1)		Reference (1)		Reference (1)	
In situ carcinoma	Yes	1.04(0.15–6.98)	0.972	7.37(1.52–35.71)	0.013*	**	
	No	Reference (1)		Reference (1)		Reference (1)	
pT stage	I–II	Reference (1)	0.499	Reference (1)	0.004*	Reference (1)	0.089
	III	2.58(0.17–40.34)		5.42(1.69–17.35)		3.87(0.81–18.45)	
Lymph node invasion	Yes	**		3.70(0.77–17.87)	0.103	**	
	No	Reference (1)		Reference (1)		Reference (1)	
Molecular subtype	Luminal	Reference (1)	0.003*	Reference (1)	0.015*	Reference (1)	0.233
	Non-luminal	27.34(2.99–249.14)		4.41(1.33–14.58)		2.75(0.52–14.55)	

*p<0.05; hazard ratio (HR) is presented with their 95% confidence interval (CI) and p-value. **As there is no event, it is not included in Cox regression modeling.

Table 4. Results of 5-year disease-free survival and overall survival related to axillary lymph node status (number of positive lymph nodes, size of the metastasis, and extranodal extension) of patients with axillary partial response.

Factors	Category	DFS (67.8%)	p	DSS (75.8%)	p
Number of metastatic lymph nodes	1–3	81.0	0.025*	92.1	
	4–9	70.4		81.8	
	≥10	46.7		62.5	0.453
Size of metastasis	ITC and micrometastasis	79.1	0.421	90.0	
	Macrometastasis	65.3		73.4	0.603
Extra nodal extension	Yes	62.5	0.385	68.0	
	No	76.5		93.8	0.148

*p<0.05, p-values given refer to log-rank test. DFS: disease-free survival; DSS: disease-specific survival; ITC: isolated tumor cells.

pCR. As patients with residual disease are prone to recurrence, identifying these patients is essential for monitoring and early intervention. Our study also showed that BCS and SLNB are safe after NAC, as no recurrence occurred in patients who underwent these procedures. Patients with non-luminal large residual tumors in the breast and axilla, with in situ carcinoma or LVI, may benefit from adjuvant chemotherapy with agents that sensitize tumors to chemotherapy and radiotherapy to lower recurrence risk. Future prospective, powerful, and genetic-based studies are warranted.

ETHICS APPROVAL STATEMENT

The study protocol was approved by the Clinical Research Ethics Committee of Istanbul Education and Training Hospital in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration (approval no. 2020-2675).

PATIENT CONSENT STATEMENT

The form of concern was not obtained because the data were analyzed anonymously. The ethics committee waived the requirement for informed consent.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Yes.

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AUTHORS' CONTRIBUTIONS

FDCT: Conceptualization, Data curation, Formal Analysis, Methodology Resources, Visualization, Writing – original draft, Writing – review & editing. **MAN:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **EA:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **OM:** Conceptualization, Data curation, Methodology, Writing – review & editing. **FD:** Conceptualization, Formal Analysis, Writing – original draft. **BA:** Conceptualization, Writing – review & editing. **RUG:** Data curation, Methodology, Visualization. **ECKT:** Data curation, Writing – review & editing. **JNAC:** Writing – review & editing.

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ORIGINAL ARTICLE

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Determination of aging anxiety in middle-aged women

Nese Kiskac[1], Mahruk Rashidi[1], Muharrem Kiskac[2*]

SUMMARY

OBJECTIVE: The aim of this study was to determine the state of aging anxiety in middle-aged women.

METHODS: The study was collected from women between the ages of 40 and 59 years by an online survey method. While collecting the data of the participants, the women's personal characteristics diagnostic form and the Aging Anxiety Scale for Middle-Aged Women were used. The data were analyzed with the SPSS 26 statistical software.

RESULTS: The aging anxiety of the women was found to be moderate (53.05±16.26). A significant correlation was found between women's menopausal status, household income, education level, and total score of aging anxiety ($p<0.05$).

CONCLUSION: In addition to working outside the home, women are also burdened with duties inside the home. To improve their quality of life, women need to share many of the tasks imposed on them with other family members. To reduce the anxiety experienced by women during the climacteric period, it is recommended to provide psychosocial support to women and consider this issue in health policies. Healthcare professionals, especially nurses, have important duties to reduce anxiety and stress, which constitute the basis of many chronic diseases. It is recommended that nurses, who are health ambassadors, direct women with anxiety to psychological support services through screenings they will conduct for women during this period.

KEYWORDS: Aging. Anxiety. Female. Gender.

INTRODUCTION

Aging is a process that has bio-psychosocial dimensions and cannot be prevented[1]. It is not enough to consider aging only chronologically. Many factors, such as environmental factors, lifestyle, genetic characteristics, and social and cultural life, affect aging. Therefore, it is necessary to address the concept of aging in all aspects[2].

In recent years, the world's population has been aging rapidly[3]. The World Health Organization (WHO) reported that by 2030, one out of every six people will be over the age of 60 years, and by 2050, the world's population aged 60 years and over will be twofold[4].

Gender plays an important role in determining the frequency of anxiety, onset time, course, and prognosis of diseases. In addition to biological and psychological predispositions, gender-specific social risk factors are also effective in the emergence of anxiety[5]. Factors such as women's position in society, conflicting work, family, and social roles, especially the burden of caring for children and family members, the low status of women, which is more prominent in certain societies, not having equal conditions in working life, and being poorer cause women to be more sensitive to stress. In addition, the fact that women enter the workforce today and carry out all their other roles while working at the same time increases the burden on them even more. Low employment and pension rights, more economic problems in older women, less participation in social environments, and more mental problems increase the fear of aging in middle-aged women[6]. According to the WHO, 45–59 years is defined as middle age[7]. These problems will increase even more with aging[8].

Since the negative conditions of being a woman will make the living conditions more difficult in old age, it will increase the aging anxiety in middle-aged women[9]. According to studies, it has been determined that women between the ages of 40 and 65 years, which is defined as the climacteric period, experience a lot of anxiety[10]. When the literature is examined, it is seen that there are a limited number of studies in which the aging concerns of middle-aged women are measured and the conditions affecting their quality of life are evaluated[11-14]. When the literature studies are examined, it is seen that women in the climacteric period face many situations that will negatively affect their quality of life. The aim of this study was to determine the aging concerns of women aged 40–59 years. It is thought that the results of the research will shed light on the creation of health policies to be created for solutions in the future.

[1]Istanbul Gelisim University, Faculty of Health Sciences, Department of Nursing – İstanbul, Turkey.
[2]Bezmiâlem Vakif University, Faculty of Medicine Hospital, Department of Internal Medicine – İstanbul, Turkey.
*Corresponding author: muharremkiskac@gmail.com

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METHODS

Study design

The research is a descriptive and cross-sectional study.

Sample of the research

The sample consisted of 240 women aged between 40 and 59 who agreed to participate in the study.

Data collection method

The data for the study were collected from the participants in the form of an online survey between May 1 and June 30, 2023.

Data collection

The data for the study were obtained by using the personal data identification form (age, marital status, education level, occupation, child status, with whom they live at home, menopausal status, and household income level) and the Aging Anxiety Scale for Middle-Aged Women (AASMAW).

Aging anxiety scale for middle-aged women

The validity and reliability of the scale developed by Lee et al.[15] was conducted by Daşıkan et al.[16] The scale sample consisted of women aged 40–59 years. While language, content, and construct validity analyses were performed in the validity phase of the scale, internal consistency and stability over time analyses were performed in the reliability phase. Cronbach's α reliability is 0.962 for the overall scale and ranges between 0.836 and 0.949 for the four sub-dimensions. The Cronbach's α value of this study was 0.789. The scale consists of 19 items, and a 5-point Likert scale is used. The scale consists of four sub-dimensions: physical competence (questions 1–4), anxiety about changes in appearance (questions 5–8), social value (questions 9–16), and negative expectations about aging (questions 17–19). Questions 17–19 are reverse-scored. The total value of all sub-dimensions is calculated as a total score. As the total score increases, the level of aging anxiety also increases.

Data analyses

The IBM SPSS 26.0 statistical program was used in the data analysis of this study. In addition to descriptive statistical methods (mean, standard deviation, frequency, and percentage), the Student's t-test was used to compare normally distributed data, and the Mann-Whitney U test was used to compare non-normally distributed data. One-way analysis of variance and Kruskal-Wallis test were used to evaluate more than two normal and non-normally distributed variables, respectively. The results were evaluated at a 95% confidence interval and a $p<0.05$ significance level.

Ethical considerations

Ethics committee approval was obtained from the Istanbul Gelişim University Ethics Committee Presidency with the decision dated April 19, 2023 and numbered 2023-04-141 to conduct the research. The participants to be included in the study were informed before the survey, and a consent form was signed.

RESULTS

The personal data of the participants are shown in Table 1. It was found that 84.2% of the women were married, 60.4% were university graduates, 70.8% were employed, 45.4% had two children, 67.9% were not in menopause, 93.3% lived with their families, 69.2% had medium household income, and the mean age was 46.34±5.24 years (Table 1).

Table 2 shows the mean scores of the participants on the AASMAW and the relationship between them and their personal characteristics. The total score of the AASMAW was found to be 53.05±16.26 at a moderate level. A significant correlation was found between the menopausal status of the women and physical competence, the social value sub-dimension, and the total score of the AASMAW ($p<0.05$) (Table 2).

The relationship between the personal characteristics of the participants and the sub-dimensions of the AASMAW is shown in Table 3. A significant correlation was found between the participants' education level and physical competence, social value, and AASMAW ($p<0.05$). It was observed that the anxiety of illiterates was higher than the others. A significant relationship was found between child status and physical competence ($p<0.05$). A significant correlation was found between the income level of the household and physical competence, social value, and AASMAW ($p<0.05$). Those who found the income level of the household insufficient were found to have higher anxiety than the others.

DISCUSSION

In this study, according to the results of the online questionnaire administered to 240 women aged 40–59 years, women's anxiety about aging was found to be moderate (53.05±16.26) (Table 2). The relationship between the personal characteristics of the participants and the score of the AASMAW was analyzed (Tables 2 and 3). There was a significant correlation between women's menopausal status, household income level, education level, and the AASMAW ($p<0.05$).

Table 1. Personal data of the participants (n=240).

	n	%
Age (years; average)	46.34±5.24	
Marital status		
Single	38	15.8
Married	202	84.2
Educational levels		
Illiterate	3	1.3
Literate	2	0.8
Primary school graduate	18	7.5
Secondary school graduate	13	5.4
High school graduate	59	24.6
University graduate	145	60.4
Profession		
Employee	170	70.8
Pensioner	13	5.4
Housewife	57	23.8
Child status		
None	28	11.7
1 child	58	24.2
2 children	109	45.4
3 children	28	11.7
4 and more children	17	7.1
Menopausal status		
There is	77	32.1
None	163	67.9
Whom she lives with at home		
Alone	14	5.8
With her family	224	93.3
With a friend	2	0.8
Household income level		
Inadequate	23	9.6
Medium	166	69.2
Good	51	21.3

Descriptive statistical methods (mean, standard deviation, frequency, and percentage).

When the literature was analyzed, no study was found to have been conducted using the AASMAW. However, it is seen that there are a limited number of studies in which gender differences, age, occupation, education, socioeconomic factors, and anxiety are studied. Kiely et al.[17] and Kessler et al.[18] stated in their studies that women experience more anxiety about aging than men. Lytle et al.[19] found that anxiety increased with age in a study conducted with 821 participants aged 45–80 years. Calatayud et al.[20] found that anxiety was higher in those with low education. Carrard et al.[21] conducted a study with 331 women aged 45–65 years and found that anxiety increased as women's physical body dissatisfaction increased and they used antiaging products. Woods et al.[22] stated that menopause triggers memory disorders in women, and Castellon et al.[23] stated that psychiatric problems associated with depression increase as memory disorders increase.

Kiely et al.[17] and Kessler et al.[18] stated in their studies that women experience more aging anxiety than men. The study was conducted with only females as a sample. However, the results of the literature reveal the purpose of this study. The fact that women are in a more disadvantaged group than men in factors such as hormonal, work, status, and economic conditions suggests that scientific studies to be conducted in the female gender should be increased.

Lytle et al.[19] found that anxiety increased with increasing age in a study conducted with 821 participants aged 45–80 years. In the present study, the relationship between age and the AASMAW was not significant ($p>0.05$). The results of the literature study and this study are different. In the study, the sample was taken between 40 and 59 years old, while in the study of Lytle et al.[19], the sample was taken between 45 and 80 years old. The reason for the difference in the results can be attributed to the difference between ages.

Calatayud et al.[20] found that anxiety was higher in those with low education. In this study, a significant correlation was found between the level of education of the participants and physical competence, social value, and AASMAW ($p<0.05$). It was observed that the anxiety of illiterates was higher than the others. The result of the study is similar to the result of the literature. The fact that women with higher education level participate in business life and therefore have better social, retirement, and financial opportunities can be considered a factor that reduces anxiety.

Woods et al.[22] stated that menopause triggers memory disorders in women, and Castellon et al.[23] stated that psychiatric problems related to depression increased as memory disorders increased. In the present study, a significant correlation was found between menopausal status in women and the AASMAW ($p<0.05$). Those who had menopause had a higher level of aging anxiety. The result of this study is similar to that of the literature. We can explain the reason for this in two ways. First, in line with the literature, we can explain the decrease in memory functions with menopause and thus the increase in susceptibility to depression. Second, menopause is an inevitable process that usually occurs in women's advanced

Table 2. The relationship between the personal characteristics of the participants and the sub-dimension and total scores of the Aging Anxiety Scale for Middle-Aged Women.

	Physical competence	p-value	Anxiety about changes in appearance	p-value	Social value	p-value	Negative expectations about aging	p-value	AASMAW total score	p-value
Average	12.66±4.28		10.84±4.71		20.98±8.58		8.58±3.33		53.05±16.26	
Menopausal status										
There is	13.57±4.80	**0.02**	11.27±4.57	0.33	22.86±8.74	**0.02**	9.08±2.91	0.**10**	56.77±16.56	**0.01**
None	12.23±3.95		10.64±4.77		20.09±8.39		8.34±3.49		51.29±15.86	
Age (years)										
≥46	12.70±4.55	0.88	11.01±4.61	0.60	21.40±8.89	0.46	8.63±3.10	0.80	53.74±16.59	0.53
<46	12.62±4.04		10.69±4.80		20.60±8.32		8.52±3.53		52.42±15.99	
Marital status										
Married	12.76±4.22	0.41	10.83±4.82	0.91	21.00±8.51	0.94	8.67±3.34	0.31	53.24±16.39	0.67
Single	12.13±4.63		10.92±4.08		20.89±9.10		8.08±3.26		52.02±15.71	

Statistically significant values are denoted in bold. Student's t-test, Mann-Whitney U test.

Table 3. The relationship between the personal characteristics of the participants and the sub-dimension and total scores of the Aging Anxiety Scale for Middle-Aged Women.

	Physical competence p-value	Anxiety about changes in appearance p-value	Social value p-value	Negative expectations about aging p-value	AASMAW total score p-value
Educational level	**0.009**	0.406	**0.002**	0.27	**0.004**
Child status	**0.036**	0.943	0.302	0.052	0.546
Whom she lives with at home	0.844	0.647	0.143	0.644	0.553
Household income level	**0.001**	0.924	**0.042**	0.244	**0.026**
Profession	0.930	0.134	0.240	0.214	0.518

Statistically significant values are denoted in bold. One-way analysis of variance, Kruskal-Wallis test.

age. Women accept the hormonal changes in menopause and try to get used to the idea that they are getting older. We can say that this stage of acceptance affects the woman more emotionally and increases anxiety.

As a result of the review conducted by Aki[24], it was stated that women's economic inadequacies increase depression even more. A significant correlation was found between the income level of the household and physical competence, social value, and AASMAW ($p<0.05$). In the study, those who stated that their household income was inadequate had a higher score than the others. The results of the study are similar to those in the literature. Participation of individuals in working life, being financially independent, and having an economic income in the retirement period are effective in accessing health, protecting health, and maintaining treatment and rehabilitation. In our country, men have more economic independence than women. We can say that women who think that their economic income is insufficient may have high aging anxiety because they think that their health will be negatively affected.

CONCLUSION

In line with the literature data, this study was conducted considering that women's anxiety would be high because they are different from men both biologically and their status in society. As a result of the study, the aging anxiety score of women was found to be moderate. It was determined that women's menopause, low economic income, and low education levels increased the anxiety of aging. In addition to working outside the home, women are also burdened with duties inside the home. To improve their quality of life, women need to share many of the tasks imposed on them with other family members. To reduce the anxiety experienced by women during the climacteric period, it is recommended to provide psychosocial support to women and consider this issue

in health policies. Healthcare professionals, especially nurses, have important duties to reduce anxiety and stress, which constitute the basis of many chronic diseases. It is recommended that nurses, who are health ambassadors, direct women with anxiety to psychological support services through screenings they will conduct for women during this period.

ETHICS COMMITTEE APPROVAL

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AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Can the Glasgow prognostic score predict ischemic stroke in patients with infective endocarditis?

Cihan Aydın[1*], Aykut Demirkıran[1], Hüseyin Aykaç[1], Nurullah Uslu[1], Şeref Alpsoy[1]

SUMMARY

OBJECTIVE: The Glasgow prognosis score is a simple parameter calculated using serum levels of albumin and C-reactive protein. The aim of this study was to examine whether this parameter may predict ischemic stroke in patients with infective endocarditis.

METHODS: A total of 80 patients who were diagnosed with definitive infective endocarditis according to Duke criteria between 2016 and 2023 were included in the study. Glasgow prognosis score was based on serum levels of albumin and C-reactive protein. In imaging methods, patients were divided into two groups according to whether they had a stroke or not. These two groups were compared in terms of biochemical parameters, and infective endocarditis findings on echocardiography and Glasgow prognosis score.

RESULTS: We found that the results were statistically similar except for serum C-reactive protein (Group 1: 54.9±71.1 and Group 2: 39±70.7; p=0.03), neutrophil (Group 1: 19.8±10.8*10^9/L and Group 2: 13.3±7.3*10^9/L; p=0.014), albumin (Group 1: 2.3±0.6 and Group 2: 2.8±0.5; p=0.03), and Glasgow prognosis score (Group 1: median 2, min.–max. (1–2) and Group 2: median 1, min.–max. (0–1); p=0.004). In the receiver operating characteristics analysis, Glasgow prognosis score had 82.4% sensitivity and 58.3% specificity in predicting ischemic stroke if the Glasgow prognosis score cutoff was ≥1. In multivariate logistic regression analysis, chronic renal failure [odds ratio (OR): 1.098; 95% confidence interval: 1.054–1.964; p=0.044], age (OR: 1.050; 95%CI 1.006–1.096; p=0.024), and Glasgow prognosis score (OR: 0.695; 95%CI 0.411–0.949; p=0.035) were independent variables in predicting ischemic stroke.

CONCLUSION: High Glasgow prognosis score is an independent predictor of ischemic stroke in patients with infective endocarditis. Glasgow prognosis score, determined using albumin and C-reactive protein levels, is a simple and practical index for predicting the prognosis of patients hospitalized with infective endocarditis.

KEYWORDS: Prognosis. Transesophageal echocardiography. C-reactive protein. Albumin.

INTRODUCTION

Although infective endocarditis (IE) is uncommon, it is a serious condition with a mortality rate of 12–20% during the initial hospitalization[1]. Mortality rates during hospitalization are around 3%[2]. Several characteristics are frequently reported as indicators of poor prognosis: older age, heart failure, renal failure, staphylococci infection, aortic location, embolisms, IE on the prosthetic valve, and persistent fever despite antibiotic therapy[3]. New prognostic markers are needed to predict high-risk IE patients. Many studies have shown that inflammation plays an important role in the etiopathogenesis of cardiovascular diseases[4].

The Glasgow prognostic score (GPS), calculated from C-reactive protein (CRP) and albumin levels, is a useful tool in predicting prognosis in various cancer types[5]. In addition, GPS has been stated in various studies as an important parameter in predicting survival in heart failure with reduced ejection fraction and preserved ejection fraction and predicting mortality in patients with acute coronary syndrome and IE[5-7]. When accompanied by cerebral embolism, IE, an uncommon illness, has significant morbidity and mortality. We aimed to examine whether this parameter can predict ischemic stroke in patients with IE.

METHODS

Study population

A total of 80 patients who were diagnosed with definite IE according to Duke criteria between 2016 and 2023 were included in the study retrospectively. However, 20 patients with carotid artery disease detected on Doppler ultrasonography and 10 patients with missing laboratory results were excluded from the study. There were no signs of cerebrovascular embolism in the physical examination and imaging methods of the patients with IE included in the study at the time of admission to the hospital. Patients who developed stroke during hospital follow-up were included in Group 1. Patients who did not develop stroke were included in Group 2. Patient data was obtained from the data system of our hospital. This study was carried out at

[1]Namık Kemal University, Faculty of Medicine, Department of Cardiology – Tekirdağ, Turkey.
*Corresponding author: drcihanaydin@hotmail.com
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Tekirdağ Namık Kemal University Hospital, Department of Cardiology. The study protocol was reviewed and approved by the institutional ethics committee (Ethics Committee Number: 2023.150.07.15) and by the principles of the Declaration of Helsinki. Informed consent was obtained from all the patients participating in the study. In exclusion criteria, patients younger than 18 years of age without knowledge of serum albumin and/or CRP levels, patients with other increased inflammatory markers such as malignancy, patients receiving systemic steroid therapy, patients with chronic inflammatory disease, and patients with end-stage liver disease were excluded from the study. Patients with carotid artery disease in Doppler ultrasonography were excluded from the study. Carotid artery stenosis was referred to as a≥50% stenosis of internal carotid artery, with stenosis severity estimated using the North American Symptomatic Carotid Endarterectomy Trial method[8].

Collection of blood samples and laboratory measurement

Basic demographic and clinical variables of the study population, such as medical history, physical examination, age, gender, diabetes mellitus, hypertension, dyslipidemia, and smoking, were recorded from details in the hospital database. Blood tests and GPS were calculated based on the blood values at the time of admission to the hospital. Three sets of blood cultures (six vials in total, three aerobic and three anaerobic) were taken at half-hour intervals, without waiting for the febrile period to occur in the patients. GPS was calculated as follows: patients with both normal CRP (≤1.0 mg/dL) and albumin (≥3.5 mg/dL) levels were given 0 points. One point was given to patients with abnormal CRP or abnormal albumin levels. Patients with both high CRP (>1.0 mg/dL) and hypoalbuminemia (<3.5 mg/dL) were given 2 points[9]. Blood culture positivity and accompanying microorganisms were recorded.

Clinical follow-up

Transthoracic echocardiography and/or transesophageal echocardiography (TEE) were performed with the Epiq 7 device (Koninklijke Philips N.V., Amsterdam, the Netherlands). Intracardiac complications such as vegetation presence, valve type, left ventricular dysfunction, perivalvular abscess, leaflet perforation, paravalvular regurgitation, and new prosthetic valve regurgitation were evaluated, and the results were recorded. Findings other than sinus rhythms were determined from electrographic recordings. Clinical complications such as acute heart failure, acute renal failure, peripheral embolism, the need for surgery, and death were recorded. Newly developed stroke events and septic embolisms were detected using cranial computed tomography (CT) and cranial magnetic resonance imaging (MRI) methods.

Statistical analysis

Statistical analysis was performed using the SPSS 22.0 statistics package (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as percentages. The chi-square test and Fisher's exact tests were used for categorical variables. Normally distributed data were reported as mean±standard deviation after being analyzed with the Kolmogorov–Smirnov test, while non-normally distributed continuous variables were presented as median. The Student's t-test was used to compare normally distributed data, and the Mann–Whitney U test was used to compare non-normally distributed data. Univariate and multivariate logistic regression analyses were used to determine the independent predictors of stroke. Receiver operating characteristic (ROC) analysis was performed to determine the optimal cutoff value of GPS to predict stroke. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 80 patients diagnosed with IE, according to Duke's criteria, were included in our study. These patients were divided into two groups: those who were found to have had a stroke as a result of cranial MRI, CT, and physical examination (Group 1, n=23) and those who did not have a stroke (Group 2, n=57). Out of these 80 patients, 49 (61.3%) were male, and the mean age was 66.7±1.6 years. Tables 1 and 2 describe the main demographic, laboratory, and clinical data of the groups. When we examined the basic laboratory and demographic characteristics of the patients, Group 1 was older (mean age, Group 1: 74.4±11.9 years vs. mean age, Group 2: 63.5±15.3 years; $p=0.03$), and the number of patients with atrial fibrillation (AF) [Group 1: 9 (39.1%) vs. Group 2: 4 (7%); $p<0.001$] was higher in Group 1. When the groups were examined in terms of laboratory parameters, white blood cell and neutrophil, creatinine, and blood urea nitrogen levels were higher in the stroke group. While GPS levels were higher in Group 1, albumin levels were lower in this group. The number of chronic renal failure patients was higher in the stroke group [Group 1: 18 (78.2%) vs. Group 2: 27 (47.3%); $p=0.037$]. The two groups were similar in terms of other demographic and laboratory parameters. While there was vegetation on the native mitral valve in 50 (62.5%) patients, vegetation on the native aortic valve was observed in 32 (40%) patients. In Group 1, the vegetation size of both mitral and aortic valves was larger than in Group 2. The two groups were similar in terms of complications

Table 1. Demographic and laboratory variables of patients.

	All patients (n=80)	Group 1 (n=23)	Group 2 (n=57)	p-value
Age (years)	66.7±1.6	74.4±11.9	63.5±15.3	**0.031**
Gender (male), n (%)	49 (61.3)	11 (47.8)	38 (66.7)	0.117
Height (cm)	167.9±7.4	166.3±7.3	168.6±7.4	0.210
Weight (kg)	75.6±13.6	78.6±14.4	74.4±13.1	0.208
Hospitalization time (days)	26±19	27±20	25±18	0.760
Diabetes mellitus, n (%)	29 (36.3)	10 (43.5)	19 (33.3)	0.393
Hypertension, n (%)	60 (75)	20 (87)	40 (70.2)	0.117
Coronary artery disease, n (%)	34 (43)	10 (45.5)	24 (42.1)	0.788
Congestive heart failure, n (%)	14 (17.5)	5 (21.7)	9 (15.8)	0.860
Atrial fibrillation, n (%)	13 (16.2)	9 (39.1)	4 (7)	**<0.001**
Chronic renal failure, n (%)	45 (56.3)	18 (78.2)	27 (47.3)	**0.037**
Chronic obstructive pulmonary disease, n (%)	7 (8.7)	2 (8.6)	5 (8.7)	0.655
Angina, n (%)	18 (22.5)	5 (21.7)	13 (22.8)	0.918
Dyspnea, n (%)	43 (53.7)	10 (43.4)	33 (57.9)	0.226
Syncope, n (%)	6 (7.5)	2 (8.6)	4 (7)	0.232
Atrioventricular block	4 (5)	1 (4.3)	3 (5.2)	0.113
Hemoglobin (g/dL)	8.9±1.9	8.4±1.6	9.2±2	0.105
White blood cell ($\times10^3/mm^3$)	18.6±9.3	23.7±10.3	16.5±8.1	**0.002**
Neutrophil ($10^9/L$)	15.7±8.8	20.2±10.4	13.9±7.4	**0.003**
Thrombocyte ($\times10^3/mm^3$)	161±10	151.6±7.8	166 ±10.9	0.567
C-reactive protein (mg/L)	40.6±6.3	56.3±6.8	34.3±6.3	0.160
Blood urea nitrogen (mg/dL)	125±34	164±72.9	104.6±71	**0.001**
Creatinine (mg/dL) (min.–max.)	2.7 (0.5–13.5)	4.78 (0.7–6)	1.7 (0.5–3.6)	0.067
Sodium (mmol/L)	134.3±7.43	134.5±9.6	134.41±5.8	0.959
Potassium (mmol/L)	4.7±1.4	4.4±1.1	4.7±0.5	0.937
Alanine aminotransferase (U/L) (min.–max.)	36 (6–580)	28 (6–580)	36 (9–200)	0.430
Aspartate aminotransferase (U/L) (min.–max.)	40 (10–636)	75 (10–721)	32 (12–400)	0.376
Albumin (g/dL)	2.7±0.6	2.3±0.6	2.9±0.5	**<0.001**
Glasgow prognostic score (min.–max.)	2 (0–2)	2 (1–2)	1 (0–2)	**<0.001**

Statistically significant values are denoted in bold. Group 1: patients with stroke; Group 2: patients without stroke. Chronic renal failure was defined as a creatinine value greater than 2 mg/dL.

after IE and surgical needs after complications. However, 34 patients died in their intensive care unit. In the ROC analysis, GPS had 82.4% sensitivity and 58.3% specificity in predicting ischemic stroke if the GPS was ≥1. The area under the curve was 0.625 [95% confidence interval (CI): 0.470–0.780; p=0.002]. In multivariate logistic regression analysis, chronic renal failure [odds ratio (OR): 1.098; 95%CI 1.054–1.964; p=0.044], age (OR: 1.050; 95%CI 1.006–1.096; p=0.024), and GPS (OR: 0.695; 95%CI 0.411–0.949; p=0.035) were independent predictors of stroke (Table 3).

DISCUSSION

Stroke is the third leading cause of death and disability in the world[10,11]. To the best of our knowledge, our study is one of the first studies in the literature to show a relationship between GPS and stroke in patients with IE. Previous studies have shown that long-term survival is significantly reduced in patients with high GPS and that chronic renal failure is an independent predictor of mortality[7]. In our study, we found age, chronic renal failure, and GPS as independent predictors of stroke. Inflammation plays an important role in the etiopathogenesis of cardiovascular diseases.

Table 2. Echocardiographic variables of patients.

	All patients (n=80)	Group 1 (n=23)	Group 2 (n=57)	p-value
Ejection fraction (%)	56.4±7.1	55.9±7.5	56.5±7	0.727
SPAP (mmHg)	33.4±12.2	30±8	34±13.2	0.195
Mitral valve, n (%)				
Native	77 (96.2)	22 (95.6)	55 (96.4)	0.847
Prosthesis	3 (3)	1 (4)	2 (3)	0.113
Aortic valve, n (%)				
Native	71 (88.7)	18 (78.2)	53 (92.9)	0.692
Prosthesis	9 (11.3)	5 (21.7)	4 (7)	0.059
TEE evaluation, n (%)				
Aortic vegetation	32 (40)	6 (26.1)	26 (45.6)	0.107
Mitral vegetation	50 (62.5)	19 (82.6)	31 (54.4)	**0.018**
Tricuspid vegetation	3 (3.7)	1 (4.3)	2 (3.5)	0.192
Abscess	3 (3.7)	1 (4.3)	2 (3.5)	0.192
Fistula	1 (1.25)	1 (4.3)	0 (0)	0.245
Perforation	2 (2.5)	1 (4.3)	1 (1.7)	0.198
Pseudoaneurysm	5 (6.2)	2 (8.6)	3 (5.2)	0.858
Paravalvular leakage	5 (6.2)	2 (8.6)	3 (5.2)	0.125
Prosthetic valve dehiscence	3 (25)	1 (16.6)	2 (33.3)	0.363
Patients requiring surgery	13 (16.2)	4 (17.3)	9 (15.7)	0.287
Vegetation size (cm)				
Aortic valve	0.34±0.19	0.36±0.17	0.17±0.05	**0.009**
Mitral valve	0.73±0.6	0.99±0.77	0.62±0.61	**0.028**

Statistically significant values are denoted in bold. Group 1: patients with stroke; Group 2: patients without stroke. SPAP: systolic pulmonary artery pressure; TEE: transesophageal echocardiography.

Table 3. Univariate and multivariate logistic regression analysis of independent predictors of stroke.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.058 (1.017–1.100)	**0.005**	1.050 (1.006–1.096)	**0.024**
Hypertension	2.833 (0.742–10.816)	0.128	–	–
Chronic renal failure	1.745 (1.307–2.452)	**0.039**	1.098 (1.054–1.964)	**0.044**
Heart failure	1.123 (0.308–4.087)	0.861	–	–
Glasgow prognostic score	2.509 (1.038–6.064)	**0.041**	0.695 (0.411–0.949)	**0.035**
Diabetes mellitus	1.538 (0.571–4.146)	0.394	–	–
Gender (female)	2.182 (0.814–5.850)	0.121	–	–
Atrial fibrillation	0.417 (0.043–4.025)	0.449	–	–

Statistically significant values are denoted in bold.

Inflammatory markers are associated with poor prognosis in cardiovascular diseases[8,9].

Glasgow prognostic score includes two markers: negative acute phase reactant albumin and positive acute phase reactant CRP. In addition to its role in regulating osmotic pressure, albumin is a good indicator of nutritional status and has antioxidant and anti-inflammatory properties[12]. Hypoalbuminemia as a result of increased inflammation is a strong predictor of mortality in cardiovascular disease[13]. Furthermore, hypoalbuminemia is related to coagulation factors and is a prothrombotic condition[14].

The change in the thrombotic state may increase the frequency of stroke and peripheral embolism in IE. The most common complications in IE are embolic events (20.6%), acute renal failure (17.7%), and heart failure (14.1%)[15].

C-reactive protein is an acute-phase reactant and a well-known marker of systemic inflammation. The relationship between high CRP levels and AF has been determined in previous studies. As it is known, one of the most important causes of stroke is AF[16]. Although the frequency of AF was low (16.2%) in our study, this rate was higher in the stroke group (39.1%).

All patients with AF were using oral anticoagulation therapy at the time of admission to the hospital. Their treatments were not changed during hospitalization. Additionally, TEE was performed in all our study patients, and no intracardiac thrombus was detected in any of the patients. Therefore, new cerebrovascular events were not attributed to AF.

Glasgow prognostic score was found to be an independent risk factor for 30-day and 1-year cardiovascular death in individuals with chronic coronary syndrome. Previous studies have revealed that the GPS score can identify a patient who is in poor condition with many diseases[17]. In many studies, GPS has also been reported to be associated with mortality and morbidity in cancer patients and in patients with heart failure with preserved and reduced ejection fraction[18,19]. The role of inflammation in the development of valve diseases is not to be underestimated. Various studies have shown that GPS can predict mortality after transcatheter aortic valve implantation (TAVI) and that low albumin levels prolong intensive care unit stays after TAVI, and high CRP levels increase the need for surgical repair in aortic valve stenosis[20-23].

Infective endocarditis was complicated by a stroke in 20–40% of cases[24]. In our study, this rate was around 28%. Stroke is an independent adverse prognostic factor for survival in IE. Prompt initiation of antibiotic therapy in IE reduces the risk of stroke. Vegetation size (≥10 mm), mitral valve involvement, mobile vegetation, and *Staphylococcus aureus* infection have all been identified as risk factors for embolism. *S. aureus* grew in 25% of the patients in our study, and there was no difference between the two groups in terms of microorganisms grown in blood culture. Vegetation diameters were larger in the aortic and mitral valves in the stroke group.

In the stroke group, 82% of vegetation was on the mitral valve. Surgical requirements were also similar in the two groups. Individuals with chronic renal disease are more susceptible to complications such as malnutrition, cardiovascular events, anemia, and infections.

In our study, 56.3% of the patients had renal failure. This rate was higher in the stroke group (78.2%). Valve diseases are observed more frequently in renal failure due to impaired excretion of calcium and phosphate. When IE develops in the elderly and patients with kidney failure, it is difficult to maintain an effective antibiotic level. In our study, renal failure and age were found to be independent risk factors for stroke. Biomarkers such as GPS can help us determine the risk of stroke in these patients and guide the treatment strategy.

Limitations

Our study was a retrospectively designed single-center study with a small number of patients. The patients were not inquired about using aspirin, statins, or anti-inflammatory medications earlier which would have affected the inflammatory process. GPS was calculated once from the patients' laboratory values at the time of admission. GPS could be calculated from the average of all laboratory values that could be checked during follow-up.

Larger, multi-center prospective studies are needed to validate the findings of this work.

CONCLUSION

High GPS is an independent predictor of ischemic stroke in patients with IE. GPS, determined using albumin and CRP levels, is a simple and practical index for predicting the prognosis of patients hospitalized with IE. These biomarkers may be a guide in determining patient risk before radiological imaging.

AUTHORS' CONTRIBUTIONS

CA: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **AD:** Data curation, Validation. **HA:** Investigation, Software. **ŞA:** Supervision, Visualization. **NU:** Methodology, Resources.

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ORIGINAL ARTICLE

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Are depression and poor sleep quality a major problem in Turkish Women receiving chemotherapy for breast cancer?

Mahmut Buyuksimsek[1*], Ahmet Gulmez[1], Okan Pirinci[2], Mert Tohumcuoglu[1], Mehmet Mutlu Kidi[3]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate depression and sleep quality in Turkish women receiving neoadjuvant or adjuvant chemotherapy for breast cancer and investigate their relationship.

METHODS: This cross-sectional, descriptive, and analytical study included 183 patients who received chemotherapy for non-metastatic breast cancer. Data were collected using the Beck Depression Inventory-II, the Pittsburgh Sleep Quality Index, and a disease-related/sociodemographic information form.

RESULTS: The mean age of the participants was 50.2 years, and 50.3% were in menopause. The mean Beck Depression Inventory-II score was 19.64±10.4. Mild depression was detected in 25.7% (n=47) of the women, and moderate or severe depression in 55.2% (n=101). The mean global score of sleep quality was found to be 8.28±2.62, and the majority of the participants (79.7%, n=146) had poor sleep quality. There was a positive correlation ($p<0.001$, $r=0.43$) between depression and sleep quality scores. While a negative correlation was found between depression scores and age ($p<0.001$, $r=0.26$), the surgical procedure performed did not significantly affect depression scores ($p=0.705$). Additionally, depression scores were positively correlated with sleep duration ($p<0.001$, $r=0.42$) and sleep latency ($p=0.01$, $r=0.48$).

CONCLUSION: Very high rates of depression and poor sleep quality were detected among Turkish women receiving neoadjuvant or adjuvant chemotherapy for breast cancer. The entire healthcare team involved in the treatment process should take this relationship into consideration and use the necessary preventive and therapeutic methods.

KEYWORDS: Breast cancer. Depression. Sleep quality.

INTRODUCTION

Breast cancer is the most common type of cancer affecting women across the world, with one in eight women being diagnosed with this disease during their lifetime[1]. Being diagnosed with breast cancer is a major traumatic experience for women due to its effects on self-image and sexual relationships; therefore, patients with breast cancer experience many psychiatric problems during the treatment process. Issues experienced by survivors include existential concerns, psychological reactions, and physical symptoms that can potentially impair their well-being. The overall prevalence of depression among oncology patients remains unclear, with previous studies reporting a prevalence between 0 and 58%. This wide range may be due to the use of different tools and criteria to define and evaluate depression, as well as differences in the disease stage and treatment modalities of the evaluated patients[2]. The prevalence of depression is high during the first year after breast cancer diagnosis. A review emphasized that although the prevalence of depression varied across studies, the highest risk period for depression was the first year following breast cancer diagnosis[3]. Sleep disorders are reported in up to 50% of cancer patients, and women diagnosed with breast cancer experience sleep disorders at higher rates (67–90%) than other cancer patients[4,5]. Depression and sleep disorders frequently occur together in breast cancer patients, and they both increase the negative impact on patients' quality of life[6]. It is also known that depression and sleep disorders are associated with decreased adherence to treatment and reduced survival among breast cancer patients[7]. Cultural differences in societies may affect depression symptoms, and the relationship between sleep disorders and social and cultural factors is also well established, which can also explain the different results obtained from studies conducted to evaluate depression and sleep quality in cancer patients[8]. In this study, we aimed to evaluate depression and sleep quality in Turkish women receiving adjuvant or neoadjuvant chemotherapy for breast cancer and to investigate the relationship between these two psychological disorders.

[1]University of Health Sciences, Adana Health Practice and Research Center, Department of Medical Oncology – Adana, Turkey.
[2]University of Health Sciences, Adana Health Practice and Research Center, Department of Internal Medicine – Adana, Turkey.
[3]Cukurova University, Department of Medical Oncology – Adana, Turkey.
*Corresponding author: mahmutbuyuksimsek@gmail.com
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METHODS

This cross-sectional, descriptive, and analytical study was conducted from April 2022 to May 2023. The study population consisted of women with breast cancer who received chemotherapy at the outpatient chemotherapy unit of the University of Health Sciences Adana City Training and Research Hospital (Yuregir, Adana). The study included patients aged ≥18 years with non-metastatic breast cancer who had undergone or were scheduled to undergo any type of mastectomy, whose mastectomy type was determined, and who received neoadjuvant or adjuvant chemotherapy. None of the patients had a history of depression or other mental disorders before the diagnosis of breast cancer. In addition, patients with a history of using sleeping pills or other sedative drugs were excluded from the study. Data were collected using the Beck Depression Inventory-II (BDI-II), the Pittsburgh Sleep Quality Index (PSQI), and a disease-related/sociodemographic information form. The disease-related/sociodemographic information form was used to collect data on age, menopausal status, a family history of breast or other cancer, marital status, educational level, number of children, side of the breast involved, mastectomy procedure, household income, number of chemotherapy sessions, type of chemotherapy (neoadjuvant or adjuvant), a history of depression and sleep disorders, and medication use. The depression status of the patients was determined with the BDI-II scale, which is frequently used for this purpose. The BDI-II scale consists of 21 statements, each scored between 0 and 3 according to their severity. The total score that can be obtained from the scale is 63, with higher scores being associated with a higher level of depression. Scores between 0 and 13 indicate minimal depression; those between 14 and 19 indicate mild depression; those between 20 and 28 indicate moderate depression; and those between 29 and 63 indicate severe depression[9]. The PSQI, developed by Buysse et al., is a reliable tool for assessing sleep quality and disorders in the past month. The PSQI evaluates seven sleep components, namely, sleep disorders, sleep medication use, sleep latency, subjective sleep quality, sleep duration, habitual sleep efficiency, and daytime dysfunction. Each component is scored from 0 to 3, and a maximum of 21 points can be achieved in total. A total score of 5 or higher is indicative of poor sleep quality[10]. This study followed the recommendations of the ethics principles published in the Declaration of Helsinki, developed by the World Medical Association, and approved by the Clinical Research Ethics Committee of Adana City Training and Research Hospital (date: March 24, 2022, meeting number: 102, decision number: 1859). The participants were informed about the purposes and method of the study, and their written consent was obtained. The patients were informed that they could withdraw from the study at any time without giving any reason. The resulting data were coded and analyzed using the SPSS v. 22.0 software (SPSS, Inc., Chicago, IL, USA). Analytical tests (Pearson correlation analysis and chi-square test) and descriptive statistics (mean±standard deviation and percentage) were used during statistical analyses.

RESULTS

A total of 183 patients who met the inclusion criteria were included in the study. The mean age of the patients was 50.2±10.75 (range: 25–75) years. Half of the participants (n=92, or 50.3%) stated that they were in menopause, while 48.1% had a family history of cancer. The sociodemographic and disease-related characteristics of the patients are summarized in Table 1. The mean BDI-II score was 19.64±10.4. Mild depression was detected in 25.7% (n=47) of the women, moderate depression was detected in 36.6% (n=67), and severe depression was detected in 18.6% (n=34). Accepting a global PSQI score of 5 or higher as an indicator of poor sleep quality, the majority of the participants (79.7%, n=146) had poor sleep quality. The mean global score of sleep quality was found to be 8.28±2.62. There was a moderately positive correlation between depression scores and sleep quality scores ($p<0.001$, $r=0.43$) (Figure 1). While a negative correlation was found between depression scores and age ($p<0.001$, $r=-0.26$), there was a positive correlation between the number of chemotherapy sessions and depression score ($p<0.001$, $r=0.27$), and these results were statistically significant. We found a negative correlation between depression scores and the number of children ($p=0.887$, $r=-011$) and household income ($p=0.31$, $r=-0.075$), but these results did not reach statistical significance. Also, the group receiving neoadjuvant treatment had a statistically significant tendency toward depression compared to the group receiving adjuvant treatment ($p=0.03$). The depression scores of premenopausal patients were statistically significantly higher than those of menopausal patients ($p<0.001$). Severe depression was detected in 79.4% of premenopausal patients and in 20.6% of menopausal patients. However, no significant relationship was found between educational level and depression scores ($p=0.12$). There was also no statistically significant difference in depression scores according to the presence of a family history of cancer ($p=0.12$) or a family history of breast cancer ($p=0.31$). Marital status and surgical procedures did not significantly affect depression scores ($p=0.143$ and 0.705, respectively). Table 2 presents the mean±standard deviation) scores on the overall PSQI and its subscales. There was a significant

Table 1. Sociodemographic and disease-related characteristics of participants (n=183).

Sociodemographic characteristics		Disease-related characteristics	
Age	**(Years, M±SD)**	**Menopause status**	**n (%)**
	50.19	Yes	92 (50.3)
	(±10.75)	No	91 (49.7)
Number of children			
0	25 (13.8)	**Family history of cancer**	n (%)
1	23 (12.6)		
2	67 (36.6)	Yes	88 (48.1)
3	36 (19.7)	No	95 (51.9)
4	16 (8.7)		
5	5 (2.7)	**Family history of breast cancer**	n (%)
6	3 (1.6)	Yes	60 (32.8)
7	7 (3.8)	No	123 (67.2)
8	1 (0.5)		
Marital status	n (%)		
Single	8 (4.4)	**Chemotherapy type**	n (%)
Married	135 (73.8)	Neoadjuvant	82 (44.8)
Divorced/separated	40 (21.9)	Adjuvant	101 (55.2)
		Involved breast	n (%)
Educational attainment	n (%)	Right	71 (38.8)
İlliterate	32 (17.5)	Left	108 (59)
Elementary school	89 (48.6)	Bilateral	4 (2.2)
High school	44 (24)		
University	18 (9.8)	**Type of mastectomy procedure**	n (%)
Employment	n (%)	Segmental	87 (47.5)
Housewife	142 (77.6)	Total	96 (52.5)
Employee	3 (1.6)		
Nurse (hemşire)	4 (2.2)	**Number of chemotherapy sessions**	n (%)
Teacher	12 (6.6)	<5	41 (22.4)
Secretary	6 (3.3)	>5	142 (77.6)
Retired	5 (2.7)		
Textile exporter	3 (1.6)		
Housekeeper	8 (4.4)		
Household gross income	n (%)		
Good	26 (14.2)		
Moderate	50 (27.3)		
Low	107 (58.5)		

M±SD: mean±standard deviation; n (%): number (percent).

positive correlation between sleep disturbances and habitual sleep efficiency in breast cancer patients receiving chemotherapy ($p<0.001$, $r=0.33$). Additionally, we found a significant positive correlation between the number of chemotherapy sessions and subjective sleep quality ($p=0.046$, $r=0.14$). A negative correlation was observed between subjective sleep quality and daytime dysfunction, but this did not reach statistical significance ($p=0.6$, $r=-0.039$). Finally, depression scores were positively correlated with sleep duration ($p<0.001$, $r=0.42$) and sleep latency ($p=0.01$, $r=0.48$).

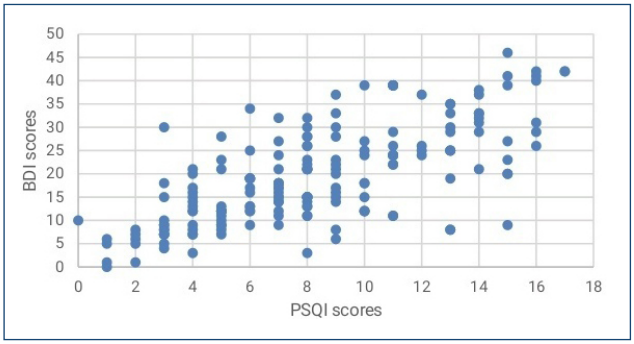

Figure 1. The scatter graph shows a positive, moderate correlation between the Pittsburgh Sleep Quality Index scores and Beck Depression Inventory scores.

Table 2. Mean±standard deviation scores on Global Pittsburgh Sleep Quality Index and Pittsburgh Sleep Quality Index Subscales.

Subscales	Mean (±standard deviation)	Range
Subjective sleep quality	1.32 (±0.99)	0–3
Sleep latency	1.17 (±0.96)	0–3
Sleep duration	1.38 (±0.94)	0–3
Habitual sleep efficiency	1.20 (±0.83)	0–3
Sleep disturbances	1.24 (±0.91)	0–3
Use of sleeping medication	1.06 (±0.92)	0–3
Daytime dysfunction	0.93 (±0.92)	0–3
Global PSQI	8.31 (±3.95)	2–21

PSQI: Pittsburgh Sleep Quality Index.

DISCUSSION

This study aimed to evaluate depression and sleep quality in Turkish women receiving adjuvant or neoadjuvant chemotherapy for breast cancer and investigate their relationship. Approximately 80.9% of the participants reported experiencing mild to severe depression. This rate is significantly higher than the rate (34.7%) reported by Turhal et al., in a healthy Turkish female population[11]. In contrast, in a study conducted in the United States, the prevalence of depression in breast cancer patients was found to be 44%, which is much lower compared to the depression rate in our study[12]. There are numerous factors that can influence the development of depression in individuals diagnosed with breast cancer: age, marital status, educational level, income level, a family history of breast cancer, stage of the disease, menopause status, body image, and the effects of chemotherapy on fertility and sexual life. The presence of several factors influencing depression among patients can explain the observed variations in depression prevalence across different geographies and studies[13]. Our results showed that there was no relationship between the type of mastectomy procedure and depression. Similarly, a meta-analysis and systematic review reported that whether the mastectomy procedure was radical, partial, or reconstructive did not affect depression[14]. However, another additional study indicated that radical mastectomy was correlated with a higher prevalence of depression than partial mastectomy[15]. Undoubtedly, it is difficult to determine whether the association between depression and the type of mastectomy is related to the surgical method or the cancer itself. In this study, we determined that marital status did not affect depression. However, depression decreased as the age of the participants increased. Additionally, depression was more common in premenopausal patients than in menopausal patients. A negative correlation was detected between depression and the number of children, but this did not reach statistical significance. Our findings concerning age, menopausal status, and the number of children supported the literature[16-18]. Young patients' concerns about deterioration in their body image, sexual problems, early menopause, and not being able to have children, as well as the potential recurrence of the disease, may be the reasons for the high prevalence of depression in these patients. The emotional support offered by children to their mothers may be important for stress management. Thus, the higher prevalence of depression in those with a small number of children can be explained by the limited support they receive. In our study, there was a negative correlation between income level and depression, but this did not reach the statistical significance level. Upon reviewing the existing literature, it becomes evident that individuals with a lower income are more prone to experiencing depression[19-21]. We also compared depression and sleep quality between breast cancer patients undergoing neoadjuvant and those undergoing adjuvant chemotherapy and detected a higher prevalence of depression in the former. The results of this study showed that 79.7% of the participants had poor sleep quality. Several factors can potentially influence sleep quality, including the sleep quality scale used, the sociocultural and economic levels of patients, the surgical procedure applied, and the stage of the disease[22-24]. In our study, we also found a moderately positive correlation between depression and poor sleep quality. Disturbances in sleep quality affect the patient's daily mood and create a tendency toward depression. Therefore, evaluating sleep disorders and depression together by taking a holistic approach will greatly facilitate the management of these patients. This study has certain limitations. The single-center design

and the low educational and income levels of the majority of patients make it difficult to generalize the findings to the whole country. Depression was statistically significantly different between those receiving neoadjuvant and adjuvant treatment, but the small number of patients between these two groups can be considered another limitation.

CONCLUSION

Depression and poor sleep quality directly affect breast cancer patients' adherence to treatment, length of hospital stay, and survival. Surgeons, medical oncologists, radiation oncologists, nurses, and psychologists should be alert and cooperate in the management of breast cancer cancers starting from the diagnosis, given the very high rates of depression and sleep disorders in this patient population.

ETHICAL APPROVAL

The study was approved by the Clinical Research Ethics Committee of Adana City Training and Research Hospital (date: March 24, 2022, meeting number: 102, decision number: 1859).

AUTHORS' CONTRIBUTIONS

MB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **AG:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **OP:** Conceptualization, Data curation, Formal Analysis, Methodology. **MT:** Data curation, Formal Analysis, Investigation, Resources, Software. **MMK:** Conceptualization, Resources, Software, Visualization.

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ORIGINAL ARTICLE

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A novel comparison of erector spinae plane block and paravertebral block in laparoscopic cholecystectomy

Elvan Tekir Yılmaz[1*], Duygu Demiriz Gülmez[2], Alparslan Apan[1], Bilge Olgun Keles[1], Mücahit Coşkun[1], Cihan Döger[3], Tugrul Kesicioglu[4], Vedat Ataman Serim[5], Furkan Ali Uygur[4], Ilker Sengul[4,6]

SUMMARY

OBJECTIVE: Erector spinae plane block is an updated method than paravertebral block, possessing a lower risk of complications. This study aimed to compare erector spinae plane and paravertebral blocks to safely reach the most efficacious analgesia procedure in laparoscopic cholecystectomy cases.

METHODS: The study included 90 cases, aged 18–70 years, classified as American Society of Anesthesiologists I–II, who underwent an laparoscopic cholecystectomy procedure. They were randomly separated into three groups, namely, Control, erector spinae plane, and paravertebral block. No block procedure was applied to Control, and a patient-controlled analgesia device was prepared containing tramadol at a 10 mg bolus dose and a 10-min locked period. The pain scores were recorded with a visual analog scale for 24 h postoperatively.

RESULTS: The visual analog scale values at 1, 5, 10, 20, and 60 min at rest and 60 min coughing were found to be significantly higher in Control than in paravertebral block. A significant difference was revealed between Control vs. paravertebral block and paravertebral block vs. erector spinae plane in terms of total tramadol consumption (p=0.006). Total tramadol consumption in the first postoperative 24 h was significantly reduced in the paravertebral block compared with the Control and erector spinae plane groups.

CONCLUSION: Sonography-guided-paravertebral block provides sufficient postoperative analgesia in laparoscopic cholecystectomy surgery. Erector spinae plane seems to attenuate total tramadol consumption.

KEYWORDS: Paravertebral. Block. Laparoscopic cholecystectomy. Postoperative pain.

INTRODUCTION

Laparoscopic procedures have opted for conventional ones for reasons such as causing less surgical trauma, pain, wound site infection, and fewer postoperative respiratory complications, providing better cosmetic results, and allowing early mobilization and discharge from the hospital[1-3]. However, pain remains a challenge for laparoscopic cholecystectomy (LC) cases, although postoperative pain rates are lower than in conventional ones. Given this, opioids and non-steroid anti-inflammatory drugs are frequently selected for the elimination of pain, but they may cause dose-dependent side effects such as nausea, itching, kidney failure, respiratory depression, and addiction, or the analgesic efficacy may remain insufficient. In addition, an increase in the use of interfascial blocks and nerve blocks in postoperative pain has been engaged together with the spread of ultrasonography utilization[4]. Paravertebral block (PVB), per se, has been widely used in postoperative analgesia for many years and has been shown to improve pain scores, attenuate the essentiality for additional analgesia, and improve respiratory function[5-7]. However, the proximity of PVB to the pleura limited its usage due to potential complications, but with the augmented use of sonography, this complication risk has diminished[8]. Although there is some controversy about the effect mechanism of erector spinae plane (ESP) block, its efficacy has been proven by many studies[9,10]. To the best of our knowledge, no study has yet been published that has compared the efficacy and complications of ESP block and PVB in LC operations. The primary aim of this study was to compare ESP and PVB blocks as important postoperative pain management in terms of being able to reliably reach the highest analgesic efficacy in patients who underwent LC, which is a frequently applied surgery.

[1]Giresun University, Faculty of Medicine, Department of Anesthesiology and Reanimation – Giresun, Turkey.
[2]Başakşehir Çam and Sakura City Hospital – İstanbul, Turkey.
[3]University of Health Sciences, Faculty of Medicine, Department of Anesthesiology and Reanimation – Ankara, Turkey.
[4]Giresun University, Faculty of Medicine, Department of General Surgery – Giresun, Turkey.
[5]Giresun University, Faculty of Medicine, Department of Neurology – Giresun, Turkey.
[6]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
*Corresponding author: elvanty28@hotmail.com
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METHODS

Ethical aspects

This study was approved by the Ethics Committee of Clinical Researchers linked to Ordu University, under approval number 0685021.283/2021.

Study design

This prospective randomized, controlled study was conducted between January 2022 and October 2022 using a consolidated standards of reporting trials (CONSORT) flow diagram shown in Figure 1 for the recording of patients (Figure 1). The study incorporated a total of voluntary

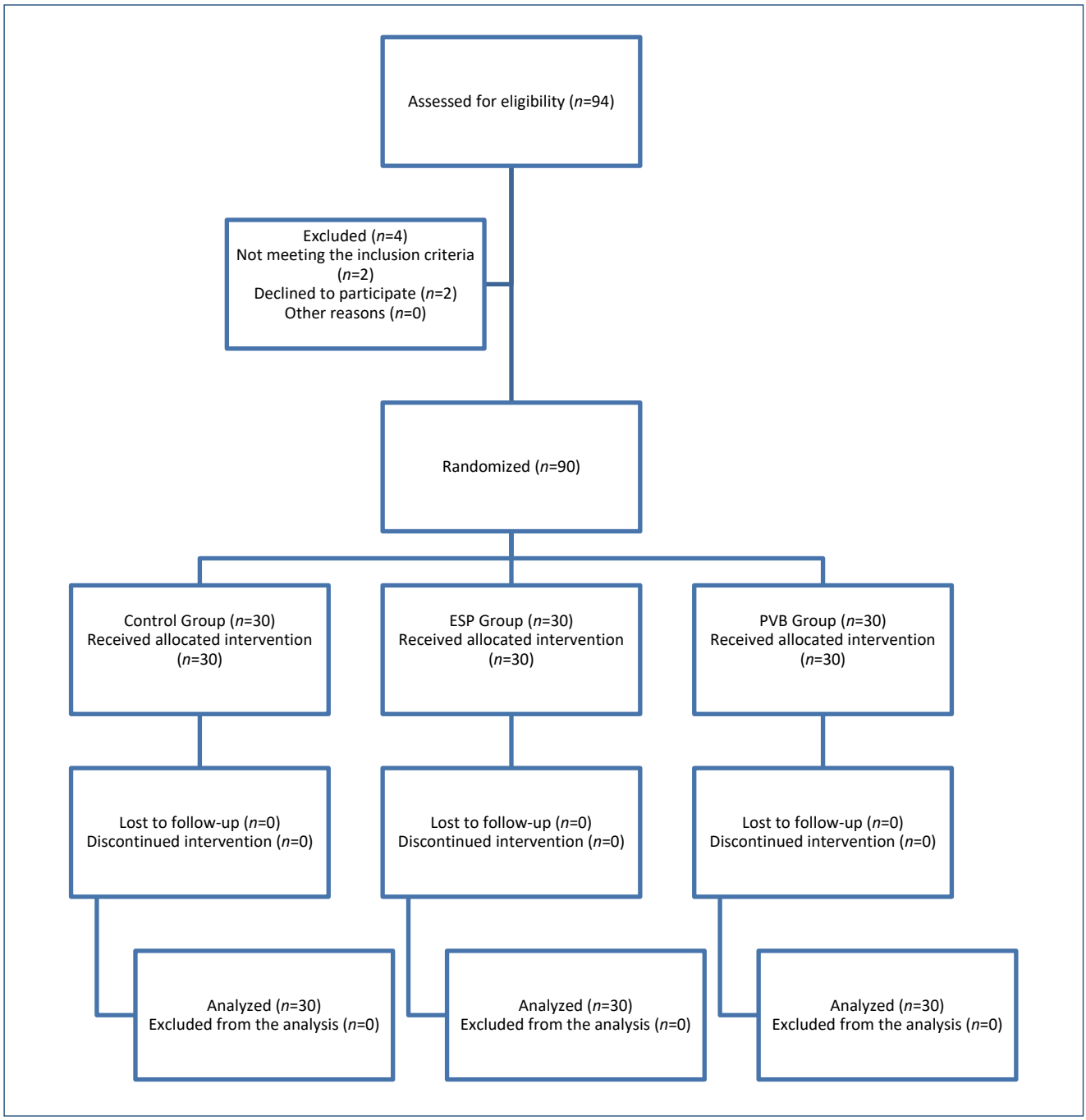

Figure 1. Consolidated standards of reporting trials flow diagram.

basis as those aged >18 years of physical status American Society of Anesthesiologists (ASA) I–II, who were planned to undergo LC. The exclusion criteria were as follows: (i) not providing informed consent, (ii) possessing any psychiatric or mental problem that prevented understanding of the informed consent form, (iii) planning to undergo an emergency cholecystectomy procedure, (iv) having any allergy or hypersensitivity to local anesthetic (LA), (v) possessing an infection in the needle entry area, and (vi) having a history of coagulopathy or the use of anticoagulants. All patients underwent routine general anesthesia protocol in our clinic. Patients were divided into three groups, namely, Control, ESP, and PVB.

Erector spinae plane block technique

After sterilization of the skin with povidone-iodine, the probe covered with a sterile sheath was placed 3 cm lateral of the T8 spinous process. The trapezius, rhomboid major, and erector spinae muscles and the transverse process (TP) of the vertebrae were visualized, and the needle was placed craniocaudally within the fascial plane of the deep surface of the erector spinae muscle above the bone shadow of the TP. The fluid dissemination was confirmed by raising the placement of the needle tip toward the erector spinae muscle and a 20 mL of 0.25% bupivacaine was applied to this region and the spread of LA was observed (Figure 2A).

Paravertebral block technique

The spinous processes of the vertebrae had been marked up to the T8 level. After providing an antisepsis of the skin with 10% povidone-iodine, the ultrasound probe was placed parallel to the vertebral spine at T8. The TP and hyperechoic pleura were observed 2.5 cm right lateral of the spinous process, and the needle was placed in the caudal direction by using the in-plane approach. Furthermore, 20 mL of 0.25% bupivacaine was administered for the block after confirming displacement of the pleura with 0.5–1 mL LA (Figure 2B).

Postoperative pain management

All the cases had been followed up for 30 min in the Postanesthetic Care Unit. Patients with a visual analog scale (VAS) score of ≥4 had been administered 50 mg tramadol. Of note, a patient-controlled analgesia (PCA) device (BodyGuard 595 ColorVision Pump/Belgium) was prepared with tramadol. The infusion dose was not adjusted and was set as 10 mg bolus with a 10-min locked period. In the follow-up of the patients on the ward, 1 g paracetamol had been administered as routine at the sixth hour. A 50-mg dexketoprofen trometamol (Arveles®, 50 mg/2 mL, Menarini Co., Ltd., Istanbul, Turkey) was administrated as rescue analgesia during follow-up, whereas 1 mg morphine (MorfinHidroklorür®, 0.01 g/1 mL Osel Co., Ltd., Istanbul, Turkey) was performed if VAS score did not reduce to <4 within half an hour. At postoperative 1, 4, 12, 18, and 24 h, the VAS scores and tramadol bolus dose were recorded. All the patients were questioned about nausea, vomiting, and shoulder pain.

Sample size determination

The sample size was determined because of power analysis performed using the G*Power 3.1.9.7 software[11]. For the

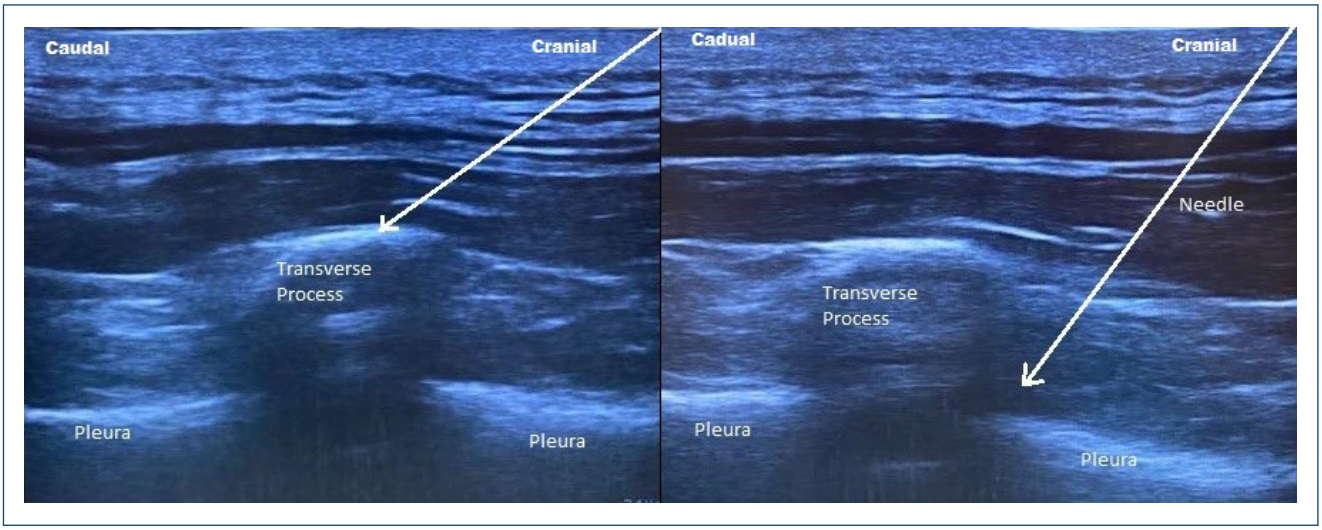

Figure 2. **(A)** A sonographic imaging of thoracal paravertebral block (the arrow indicates the location of the needle, placed craniocaudally within the fascial plane of the deep surface of the erector spinae muscle above the bone shadow of the transverse process). **(B)** A sonographic imaging of erector spinae block (the arrow indicates the location of the needle, placed in the paravertebral space above the pleura). PVP: paravertebral block; ESP: erector spinae block.

analysis of variance (ANOVA) test, it was determined that at least 28 cases were required in each group to give a moderate effect size (f=0.35) as calculated in previous studies[9,12,13] with type 1 error (alpha) of 0.05 and type 2 error (beta) of 0.80. Taking possible losses of 10% into consideration, a sum of 90 patients were included in the study, with 30 in each of the three groups.

Statistical analysis

Data obtained in the study were statistically analyzed using the Statistical Package for Social Sciences (SPSS)25 software (SPSS Inc., Chicago, IL, USA). The conformity of the continuous variables to normal distribution was assessed with the Shapiro-Wilk test, and the subsequent data were stated as mean±standard deviation (SD) values and categorical data as number (n) and percentage (%). The Pearson chi-square test was used to compare categorical data between groups, and the ANOVA test was applied in comparisons of data showing normal distribution. The post hoc Tukey test was applied to determine from which group the difference originated for the parameters where a significant difference was recognized. The subsequent data not exhibiting the normal distribution were compared with the Kruskal-Wallis test. Moreover, the Mann-Whitney U test was applied in paired comparisons to determine the group that was the source of the parameters where a statistically significant difference emerged. Finally, a value of two-tailed p<0.05 was accepted as statistically significant in all the statistical comparisons.

RESULTS

A posteriori, an evaluation was made of a total of 90 cases, comprising 56 (62.2%) females and 34 (37.8%) males with a mean age of 53.2±12.7 years. Herein, all groups were similar with respect to gender distribution, BMI, ASA, duration of anesthesia, surgery, and duration of block application. The HR values at 5, 10, and 20 min after extubation were found to be higher in Control than in ESP (p<0.017). The other hemodynamic parameters were similar in all. The VAS values at 0, 5, 10, 20, and 60 min at rest and at 60 min coughing were found to be significantly higher in Control than in PVB. There is a statistically significant difference in VAS values of only 20 min between the Control and ESP groups. At the other time points, no statistically significant difference between the VAS values of the groups had been recognized. The data of postoperative nausea, gas output, mobilization, shoulder pain, time of first food intake, and time to discharge were similar in the groups. The preference status and satisfaction of the patients in Control were found to be lower than those of the patients in the block groups. The mean tramadol consumption throughout the first 24 h postoperatively was 84.7±98.5 mg in Control, while it was 57.7±83.7 mg in ESP and 21.7±48.6 mg in PVB. A statistically significant difference was determined between Control and PVB or between ESP and PVB. Nevertheless, no statistically significant difference was determined between the ESP and Control groups (p>0.05). No patient required dexketoprofen trometamol or morphine during or after recovery. In the comparisons of the postoperative tramadol consumption of the groups, no significant difference was determined with respect to the first 30-min, 4-h, and 24-h consumptions. The tramadol consumption of Control cases was greater compared with PVB at the first, second, and sixth hours, and PVB was lower than that of the other two at the 12th hour. A sum of 12 patients in Control needed it, which was consistent with the VAS scores, in the first hour (Table 1).

DISCUSSION

This study was aimed to compare postoperative analgesia requirements, side effects, and complication rates in cases undergoing LC surgery with PVB, which has proven efficacy, and ESP block, the efficacy of which has been attempted to be shown in various studies. This study revealed that the total tramadol consumption in the postoperative 24 h was significantly diminished in the PVB compared with the Control and ESP groups. It was determined that less tramadol was consumed by the PVB than by the other groups at 12 h. Tramadol

Table 1. Comparisons of the groups with respect to the postoperative requirement for tramadol.

Time (postoperative)	Control n=30	ESP n=30	PVB n=30	p
30th min	6 (20.0%)	5 (16.7%)	2 (6.7%)	0.311
1st hour	12 (40.0%)[a]	5 (16.7%)	2 (6.7%)	**0.005**
2nd hour	12 (40.0%)[a]	7 (23.3%)	2 (6.7%)	**0.009**
4th hour	8 (26.7%)	7 (23.3%)	4 (13.3%)	0.420
6th hour	14 (46.7%)[a]	7 (23.3%)	4 (13.3%)	**0.013**
12th hour	8 (26.7%)	5 (16.7%)	0 (0.0%)[a,b]	**0.012**
24th hour	4 (13.3%)	2 (6.7%)	1 (3.3%)	0.338
Total	18 (60.0%)	15 (50.0%)	7 (23.3%)[a,b]	0.013

Values are given as number and percentage of cases. The chi-square test was used in the comparisons. A value of p<0.05 was accepted as statistically significant and is mentioned in bold. [a]Difference between the Control and PVB groups p<0.05. [b]Difference between the PVB and ESP groups p<0.05. PVB: paravertebral block; ESP: erector spinae plane block.

consumption of the PVB was 0 at the 12th hour. Some authors reported that PVB was applied preoperatively and postoperatively in LC surgery and compared with Control, and the VAS scores and requirement for additional analgesia in both were significantly low compared with Control[12]. This study supports previous studies that have shown PVB to be a very good option for postoperative analgesia[14,15]. This study revealed significant differences between PVB and ESP, or between PVB and Control with respect to the 24-h total tramadol consumption ($p<0.006$), while the tramadol consumption of Control was greater compared with PVB at the first, second, and sixth hour. PVB has been shown to provide more effective analgesia because of the anatomic proximity to the sympathetic chain that the ESP block did not provide as effective analgesia as PVB, which in this study can be attributed to the surgical areas. Application of a bilateral ESP block might provide better outcomes as abdominal operations such as LC might lead to more widespread pain. Some authors reported that the first 30- and 60-min VAS scores were found to be high when PVB was applied to LC cases, which was attributed to not using any non-opioid agent other than paracetamol[16]. In this study, the VAS scores of the cases were recorded four times in the first 20 min, and the VAS values at 0, 5, 10, 20, and 60 min at rest and at 60 min coughing were higher in Control than PVB. There was no statistically significant difference between the Control and ESP groups. The number of patients in Control requiring analgesia in the first hour was greater and the VAS scores were higher, whereas, at the other time points, the significant difference disappeared and the number of patients requiring additional analgesia decreased which probably occurred secondary to the increased opioid consumption of Control not applied with block and no complications have been developed during or after the procedure. In this study, to better determine the risk–benefit ratio, we purposed to obtain a better result by adding a satisfaction rating that globally evaluates performance (mobilization time) and pain[14]. Patient preference and satisfaction were significantly lower in the control group. We postulate that the lower level of satisfaction in the non-block group in terms of preference and satisfaction makes the results more meaningful.

Limitations

This study has some limitations. As the block applications had been performed without awakening the patients in the postoperative period, sensory tests could not be performed to find dermatomal spread.

CONCLUSION

To the best of our knowledge, this is the first study on the efficacy and complications of ESP and PVB in LC cases. The outcomes of this study indicate that 24-h tramadol consumption is lower in PVB than Control and ESP with a significant superiority in PVB compared with Control and ESP. We postulate that the so-called unilateral ESP block concept might not provide adequate postoperative analgesia in LC surgery. We might point out that the complication rate is lower when PVB is performed under sonographic guidance and PVB can be used for postoperative analgesia in LC surgery.

AUTHORS' CONTRIBUTIONS

ETY: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Validation, Visualization, Writing – original draft. **DDG:** Investigation, Methodology, Software, Visualization, Writing – original draft. **AA:** Investigation, Methodology, Software, Visualization, Writing – original draft. **BOK:** Methodology, Project administration, Validation, Visualization. **MC:** Methodology, Project administration, Validation, Visualization. **CD:** Methodology, Validation, Visualization. **TK:** Investigation, Methodology, Validation, Visualization. **VAS:** Investigation, Supervision, Visualization, Writing – review & editing. **FAU:** Investigation, Supervision, Visualization, Writing – review & editing. **IS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Management of sialorrhea in children: a systematic review

Manuela Leitão de Vasconcelos[1*], Divany Guedes Pereira da Cunha[2], Giorvan Ânderson dos Santos Alves[3], Tatiana Carneiro da Cunha Almeida Santos[2], Luiz Medeiros de Araujo Lima-Filho[4], Karinna Veríssimo Meira Taveira[5], Cristiano Miranda de Araújo[6], Leandro Pernambuco[3]

Systematic Review Registration: PROSPERO website (International prospective register of systematic review – Centre for Reviews and Dissemination University of York – CRD), under number CRD42020220096[1].

BACKGROUND

Sialorrhea is an involuntary loss of saliva through the mouth, considered pathological in children aged 4 years and above, which may be due to increased saliva production or swallowing deficit, the latter being the most frequent condition in children with neurological disorders[2-5].

This non-intentional saliva loss may happen anteriorly or posteriorly[3,4,6]. A child may have both types, with impacts on various dimensions of their and their caregivers' lives. The literature describes health, emotional, and social impacts[2,5,7-9].

The occurrence of sialorrhea ranges from 10 to 83%, with a higher frequency in children with neurological disorders[2,7,8].

The literature points out different intervention methods. It indicates beginning sialorrhea management with less invasive strategies, then progressing to more invasive ones if the patient does not adequately respond to the treatment[8,10].

Less invasive interventions aim to improve swallowing efficiency and frequency, decreasing saliva accumulation in the oral cavity[8,10,11]. Pharmacological therapy administers drugs to decrease saliva production, but it may have side effects such as urine retention and headaches[12]. Botulinum toxin has been used as an alternative to minimize these effects, which is usually applied to the glands that produce the greatest volume of unstimulated saliva. Surgery is the most invasive sialorrhea management strategy, ranging from salivary duct relocation to gland resection[13].

Given the impact this condition may have on children's and caregivers' lives, studies aim to assess the effectiveness of therapies to control sialorrhea[7,8,10,11,13]. Thus, the objective of this review was to analyze the efficacy of interventions to control sialorrhea in children.

METHODS

This systematic review was conducted according to PRISMA 2020[14]. Eligibility criteria were established with PICOS and included research on treatments to control sialorrhea in children. No study was excluded based on language, time of publication, population sex, or ethnicity. Randomized clinical trials approaching sialorrhea control interventions in children aged up to 12 years were included. The exclusion criteria were as follows: 1. studies on therapeutic interventions including children aged above 12 years, without the possibility of distinguishing the specific results of the age group of interest for this review; 2. studies with results of sialorrhea control without a specific sialorrhea control intervention; 3. studies different from clinical trials; and 4. unavailable full-text articles.

Five databases were searched: Excerpta Medica database (EMBASE), Latin-American and Caribbean Health Sciences Literature (LILACS), PubMed/Medline, Scopus, and Web of Science. An additional search was made on the gray literature: Google Scholar, OpenGrey, ProQuest, and the Brazilian

[1]Universidade Federal da Paraíba, Graduate Program in Decision Models and Health – João Pessoa (PB), Brazil.
[2]Universidade Federal da Paraíba, Lauro Wanderley University Hospital – João Pessoa (PB), Brazil.
[3]Universidade Federal da Paraíba, Department of Speech, Language and Hearing Sciences – João Pessoa (PB), Brazil.
[4]Universidade Federal da Paraíba, Department of Statistic – João Pessoa (PB), Brazil.
[5]Universidade Federal do Rio Grande Do Norte, Center for Advanced Studies in Systematic Review and Meta-analysis – Natal (RN), Brazil.
[6]Universidade Tuiuti do Paraná, Center for Advanced Studies in Systematic Review and Meta-analysis – Natal (RN), Brazil.
*Corresponding author: manuela.leitao@gmail.com
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Digital Library of Theses and Dissertations, besides a manual search in the references to the articles included in the review. References were organized, and duplicates were removed using the EndNote® online version[15]. The search took place on March 1, 2021, and was updated on January 16, 2022.

Two independent reviewers conducted the selection steps. All divergences regarding study selection were solved by a third reviewer. The kappa coefficient of agreement between reviewers was 0.7, indicating good agreement[16].

The risk of bias was assessed with the Cochrane Collaboration Risk of Bias Tool[17], and a chart was generated with the RevMan 5.4 software[18]. The difference between before and after the intervention was calculated. The mean difference was calculated for discrete quantitative variables, whereas median variation or percentage frequency was observed for the qualitative variables. The certainty of the evidence was assessed with the Grading of Recommendations, Assessment, Development, and Evaluation[19].

RESULTS

The initial search found 1,608 articles. After analysis according to the eligibility criteria, five articles comprised the final sample of the qualitative synthesis (Figure 1).

The five articles included in the research were published between 2009 and 2019[7-11]. Their sample ranged from 24 to 53 subjects, aged 21 months to 12 years, all of them with neurological disorders.

The following sialorrhea control interventions were approached: behavioral therapy[11], oral therapy motor exercises[8,10,11], chewing training[10], kinesio taping[8], botulinum toxin[7,13], and submandibular duct surgery[13]. The efficacy of these interventions was analyzed by comparing them with a placebo group or another type of intervention, assessed with the Drooling Severity and Frequency Scale[7,8,10,13], Drooling Quotient[13], Drooling Impact Scale[8], visual analog scale[13], and sialorrhea episode count[11].

The articles used different instruments and measures to assess intervention efficacy. Moreover, different interventions were used, and therefore they could not be grouped. The description of article characteristics included in the review is shown in Table 1.

None of the articles met all the methodological quality criteria. The articles that reported random sequence generation[10,11,13] used strategies such as draws and software. Only one article clearly stated the blinding of participants and personnel[7]. Three pieces of research did not present enough information

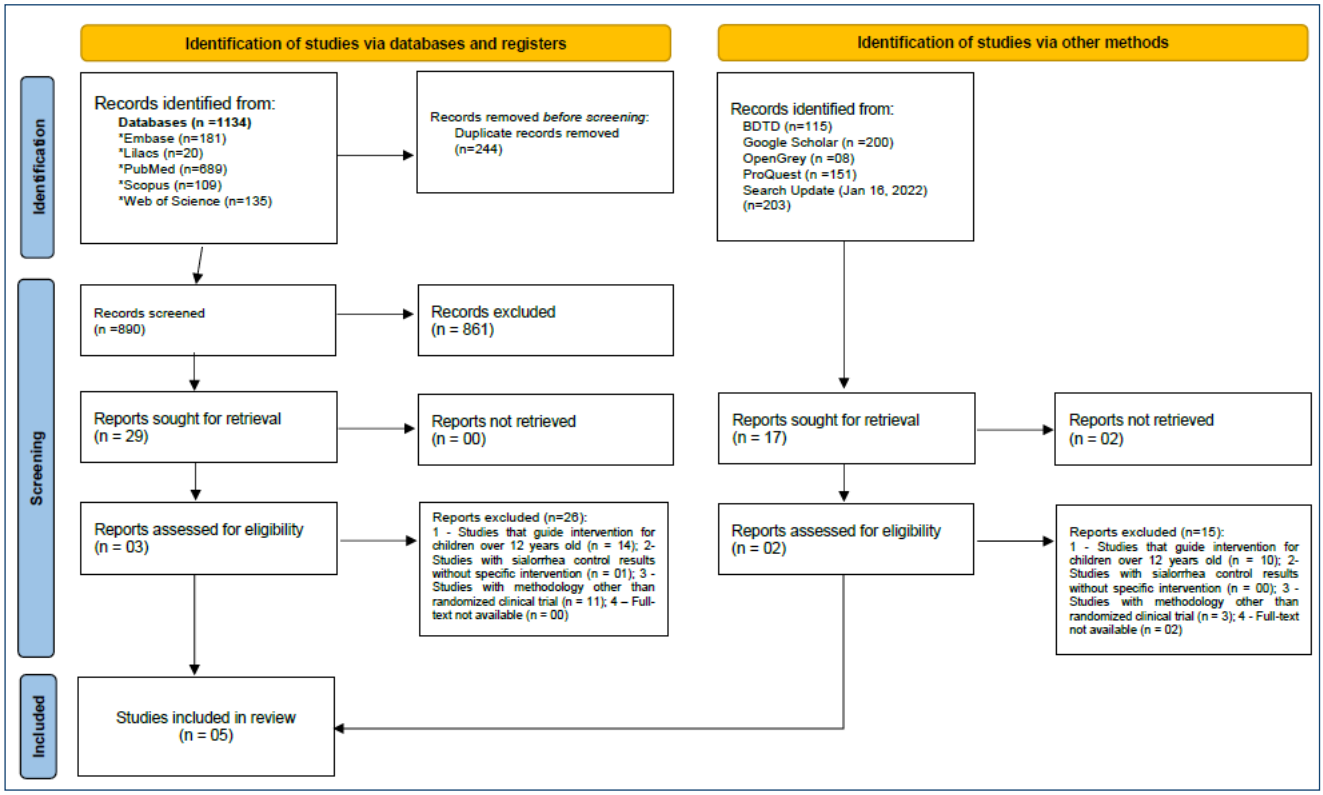

Figure 1. Flowchart of literature research and selection criteria. Source: Manuela Leitão de Vasconcelos.

Table 1. Summary of descriptive characteristics of the studies included in the review (n=5).

Authors, Years, Countries	Objectives	Samples	Interventions	Effect Measures						Conclusion
				A			B			
				I	F	Difference (p-value)	I	F	Difference (p-value)	
Alrefai et al.[7] Jordan	To investigate the efficacy and safety of injecting neurotoxin serotype A into the parotid glands to treat sialorrhea in children with cerebral palsy	Age: 21 months to 7 years S_I 24 S_F 16	A: Placebo B: Botulinum toxin	**Sialorrhea Frequency Scale (median)**						Botulinum toxin proved to be an effective and safe option in sialorrhea treatment. No intergroup comparison results were presented. The experimental group decreased 2 points in the median, while the placebo group had no variation.
				4	4	0	4	3	1 (p=0.034)	
				Sialorrhea Severity Scale (median)						
				5	5	0	5	4	1 (p=0.026)	
				Sialorrhea Total Score (median)						
				9	9	0	9	7	2 (p=0.027)	
Awan et al.[8] Pakistan	To determine the efficacy of KT in combination with OME to improve sialorrhea in children with cerebral palsy	Age: 4–8 years S_I 48 S_F 48	A: KT + OME B: OME	**Sialorrhea Frequency Scale (mean)**						Both groups had a significant decrease in the sialorrhea scale. In the frequency scale, group A had a final mean 0.34 lower than group B; in the severity scale, the final mean was 0.1 lower. The intergroup comparison had no statistical significance.
				3.86	2.30	1.56 (p=0.00)	3.88	2.64	1.24 (p=0.00)	
				Sialorrhea Severity Scale (mean)						
				4.00	2.47	1.53 (p=0.00)	3.25	2.48	0.77 (p=0.00)	
Bekkers et al.[13] The Netherlands	To compare the effects of submandibular duct surgery with botulinum toxin application into the submandibular glands in children with neurodevelopmental disorders	Age: 11 years S_I 57 S_F 53	A: Botulinum toxin B: Submandibular gland duct surgery	**Visual Analog Scale (mean)**						Intergroup comparison had a significant difference in VAS outcomes (p<0.001); the surgical procedure had better sialorrhea results than the botulinum toxin.
				82.1	75.0	7.1	77	45.6	31.4	
				Drooling Quotient (mean)						
				28.7	24.8	3.9	26	15	11	
Inal et al.[10] Turkey	To examine the effects of FuCT on tongue projection and sialorrhea in children with cerebral palsy	Age: 4–6 years S_I 40 S_F 32	A: FuCT B: Conventional exercises	**Sialorrhea Severity Scale (absolute number and percentage frequency)**						No significant decrease was observed regarding severity (p=0.210) and frequency (p=0.162) in the comparison of the two groups.
				Dry: 0 (0)	0 (0)	p=0.002	0 (0)	0 (0)	p=0.157	
				Mild: 0 (0)	4 (25)		2 (12.5)	3 (18.8)		
				Moderate: 6 (37.5)	8 (50)		5 (31.3)	5 (31.3)		
				Severity: 6 (37.5)	3 (18.8)		5 (31.3)	4 (25)		
				Profuse: 4 (25)	1 (6.3)		4 (25)	4 (25)		
				Sialorrhea Frequency Scale (absolute number and percentage srequency)						
				Never: 0 (0)	0 (0)	p=0.082	0 (0)	0 (0)	p=0.317	
				Occasionally: 3 (18.8)	5 (25)		1 (6.3)	1 (18.8)		
				Frequently: 7 (43.8)	8 (50)		9 (56.3)	10 (31.3)		
				Constantly: 6 (37.5)	3 (18.8)		6 (37.5)	5 (37.5)		
Sethy and Mokashi[11] India	To investigate the efficacy of reward behavioral therapy in combination with conventional therapy to control sialorrhea in children with cerebral palsy associated with mild intellectual deficit	Age: 5–12 years S_I 25 S_F 25	A: Behavioral therapy in combination with conventional therapy B: Conventional therapy	**Sialorrhea Frequency**						Behavioral therapy in combination with conventional therapy had better sialorrhea frequency results, with a final mean 15.71 points lower than the conventional therapy group. However, no intergroup comparison result was presented.
				22.17	5.67	16.5 (p=0.001)	21.85	21.38	0.47 (p=0.070)	

A: group A; B: group B; I: initial measure; F: final measure; S_I: initial sample; S_F: final sample; KT: kinesio taping; OME: oral motor exercises; FuCT: functional chewing training; DQ: Drooling Quotient; VAS: visual analog scale. Source: Manuela Leitão de Vasconcelos.

on the blinding of outcome assessment[7,8,11], while two were classified as low risk[10,13]. Regarding incomplete outcome data, two studies were classified as high risk[7,13] because of frequent losses, which were unbalanced between the groups; two studies did not present enough information[8,11]; and one was classified as low risk[10]. Concerning selective reporting, one article did not make clear which outcomes would be assessed, characterizing high risk[11], one study did not present enough information to assess[8], and three were classified as low risk[7,10,13] (Figure 2).

Given the few articles included in the analysis, publication bias could not be assessed with a funnel plot. However, the inclusion of LILACS with languages other than English, the broad search strategy, and the search in the gray literature diminish the likelihood of such bias occurring.

The certainty of the evidence of the following outcomes was assessed: total score of sialorrhea frequency and severity[7]; sialorrhea frequency[7,8,10]; sialorrhea severity[7,8,10]; sialorrhea episode count[11]; Drooling Quotient[13]; and Drooling Impact Scale[13]. They were classified as very low (the frequency and the severity of sialorrhea), low (sialorrhea episode count), and moderate (the total score of the sialorrhea frequency and severity scale, Drooling Quotient, and the impact of sialorrhea).

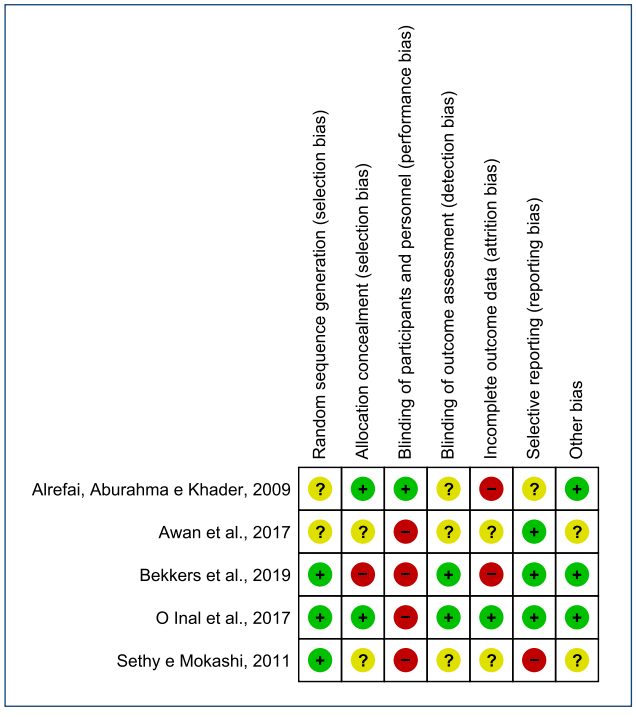

Figure 2. Assessment of the risk of bias in the studies included in the synthesis, assessed with ROB1. *Studies were assessed with ROB1. Green indicates a low risk of bias, yellow indicates an unclear risk, and red indicates a high risk of bias. Source: Manuela Leitão de Vasconcelos.

DISCUSSION

This review investigated the efficacy of different intervention methods to manage sialorrhea in children. Treatment efficacy was assessed by comparing before and after intervention with different assessment instruments. Although all of them were compared before and after the interventions, only three articles presented comparisons between groups[8,10,13], which reflected a risk of bias and the quality of evidence.

Sialorrhea assessment instruments are useful to diagnose it, define therapy procedures, and monitor interventions. The literature describes various assessment instruments for the general population[3]. However, the assessment of children, especially those with neurological disorders, must consider their skills before choosing which instrument will be used, since for some methods, it is necessary for the child to spend a period without swallowing as well as knowing how to spit.

The Drooling Severity and Frequency Scale was the most often used instrument to assess sialorrhea[20], using which the examiner and/or caregiver directly observe and classify the saliva according to its frequency and severity. This instrument is important because it considers the circadian variation and the interference of factors such as hunger, thirst, fatigue, anxiety, and oral infections[3].

Sialorrhea management interventions included in this review sample range from behavior-based strategies to surgical interventions[11,13]. The literature indicates that interventions must begin with less invasive methods and progress toward more invasive ones if children do not respond to the treatment[8,10]. The least invasive therapeutic strategies include speech and language therapy and behavioral therapy[8,10,11].

This sample used the following sialorrhea management strategies: speech-language-hearing therapy, behavioral therapy, botulinum toxin injection, and surgical intervention.

The least invasive therapeutic interventions in our sample were speech and language therapy and behavioral therapy. Considering that sialorrhea results from poor oral control and inefficient swallowing[10], improving this function is supposed to positively impact sialorrhea management. In this way, speech and language therapy is one of the first intervention options to manage sialorrhea.

In speech and language therapy, stimuli are used to adjust orofacial muscle tone and improve intraoral sensitivity, as well as oral motor exercises and chewing and swallowing training[8,10,11].

Inal et al.[10] showed a significant decrease in the severity scale for the group treated with functional chewing training, improving tongue movement and consequently swallowing; however, the comparison between the groups did not show a significant difference.

Some resources are generally used as support in speech-language-hearing therapy. A study[8] investigated the efficacy of kinesio taping to help manage sialorrhea. It was proved to be effective, especially when used in combination with oral motor exercises.

Sethy and Mokashi[11] investigated the effectiveness of conventional speech and language therapy and behavioral therapy. The results showed that behavioral therapy is effective when combined with conventional therapy, as children must have motor skills in order to swallow. Moreover, children must have preserved cognition to understand the rules, follow commands, and thus benefit from this strategy[11].

Botulinum toxin injection into salivary glands was another therapeutic strategy contemplated in our sample. It is used as a strategy when conservative therapies do not control sialorrhea[13]. Considering that parotid and submandibular glands are responsible for producing the greatest volume of saliva, they are targeted in botulinum toxin intervention[7]. Studies indicate that this procedure is safe and effective to control sialorrhea[7,21]. However, they also highlight some side effects such as thickened saliva, xerostomia, and worsened swallowing function[7].

Finally, surgical intervention is the last resource because it is the most invasive strategy. The literature describes various surgical techniques such as salivary gland resection and submandibular duct relocation. Research in the sample compared the effect of this surgery with botulinum toxin application. Results indicate a greater efficacy of the surgical procedure in question, but they call attention to the risks involved in surgery, even if they are minimal[13].

Considering all sialorrhea management strategies, the individuality of each condition stands out. The strategy to be used must be decided by a multiprofessional team based on careful assessment and analysis of a variety of information, such as comorbidities, responses to other treatments, and the risk and benefit of each intervention. Moreover, combining therapies may be feasible and help avoid more invasive procedures[8,10,11].

The evidence of outcomes ranged from moderate to very low, as there were limitations, inconsistencies, and imprecisions, e.g., not describing how randomization, allocation, and blinding were made. Some of them justified non-feasible blinding due to the different procedures being compared; also, most articles had significant drop-outs.

Interventions generally indicate decreased sialorrhea in the outcomes. However, in intragroup comparison, these variations were significant only regarding botulinum toxin[7], oral motor exercises and kinesio taping combined with oral motor exercises[8], and behavioral therapy[11] in combination with conventional therapy. These results suggest the possibility of positive effects of such interventions; however, in comparison between groups, only the research comparing botulinum toxin with surgery[13] presented significant differences between the groups, as surgery controlled sialorrhea more effectively.

Some methodological limitations must be considered. First, different sialorrhea assessment methods were used, and even though the Drooling Severity and Frequency Scale was used in four out of the five articles, they presented the results differently. Moreover, confounding factors, such as the severity of neurological disorders, may have influenced estimates, as few studies were included, while most of them were removed because of the study design or participants' ages. Also, given the few articles in the sample and their heterogeneous methodology, it was not possible to conduct a meta-analysis.

CONCLUSION

The studies that comprised the sample reported different interventions and outcome assessments. Considering the heterogeneous designs and the methodological limitations that impact the quality of evidence, the efficacy of the interventions could not be verified. However, most of them reported positive effects.

AUTHORS' CONTRIBUTIONS

MLV: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft. **DGPC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **GÂSA:** Conceptualization, Formal Analysis, Investigation, Supervision, Validation, Visualization, Writing – review & editing. **TCCAS:** Formal Analysis, Writing – review & editing. **LMALF:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **KVMT:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **CMA:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **LP:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

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REVIEW ARTICLE

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Biological action of melatonin on target receptors in breast cancer

Paulo Celso Pardi[1], José Antonio Orellana Turri[1*], Luiza Helena Costa Moreira Bayer[1], Gabriela Bezerra Nóbrega[2], José Roberto Filassi[2], Ricardo dos Santos Simões[1], Bruna Salani Mota[2], Isabel Cristina Espósito Sorpreso[1], Edmund Chada Baracat[1], José Maria Soares Júnior[1]

INTRODUCTION

Melatonin is a hormone involved in the body's circadian rhythms, acting through receptors and distinct second messenger pathways to regulate the cell cycle, proliferation, survival, apoptosis, DNA repair, and tumor suppression[1].

In vitro and in vivo studies showed that melatonin may prevent DNA damage and tumor growth and be related to the modulation and gene expression of estrogen, leading to a protective effect against breast cancer due to its antioxidant, immunomodulatory, and anticarcinogenic properties[1-6].

High artificial exposure to light at night is related to an increased risk of breast cancer [relative risk (RR)=1.17, 95% confidence interval (CI): 1.11–1.23], and the risk of breast cancer was reduced by 14% after melatonin treatment (RR=0.86, 95%CI 0.78–0.95), with a linear dose–response trend (p=0.003)[7,8].

Some studies suggest that melatonin's antioncogenic properties are due to its angiogenesis and apoptosis properties, which prevent tumor growth in breast cancer cells[2]. Another mechanism is the inhibition of human breast cancer growth by inhibiting tumor metabolism through phospho-activation of the receptor kinases Ak strain transforming (AKT), extracellular signal-regulated kinase (ERK1/2), and transcription factors[4,9].

This review seeks to synthesize the available studies and evidence related to the influence of melatonin on breast cancer to better understand this hormone's role in the prevention, treatment, or control of this disease[9,10].

METHODS

It was a narrative review of melatonin receptors in breast cancer. The search was performed in the PubMed database between 2018 and 2023. The descriptors used were melatonin; N-acetyl-5-methoxytryptamine; breast neoplasms; breast cancer; mammary cancer receptors; estrogen; estrogen receptor (ER); ER-alpha; ER-beta; progesterone; progesterone receptor (PR); human epidermal growth factor receptor 2 (HER2); apoptosis; programmed cell death; antineoplastic agents; anticarcinogenic agents; and antioxidants.

LITERATURE REVIEW

Melatonin is a molecule composed of three essential components: an aromatic indole ring, an acetamide side chain, and a methylene group[11]. Melatonin is crucial in regulating circadian rhythms and sleep-wake cycles. In the context of breast cancer, melatonin receptors have gained significant attention due to their potential role in modulating tumor development and progression[11-14].

Melatonin affects target cells by binding to specific receptors, such as melatonergic receptors (MT1 and MT2 receptors), which are transmembrane G-protein-coupled proteins[15,16]. Activation of these receptors can lead to an intracellular signaling cascade, resulting in several biological effects[17-19]. Melatonin also acts in cell cycle regulation by regulating the progression of the cell through the phases of cell division, inhibiting cell cycle progression in cancer cells, and preventing uncontrolled cell growth[16,20]. Melatonin may affect the expression of hormone receptors, such as ER and PRs, essential in regulating cell growth and proliferation in breast cancer[21]. Melatonin increases antineoplastic immunity by reducing telomerase activity and inhibiting the fatty acid uptake and metabolic pathways of fat and the angiogenesis through vascular endothelial growth

[1]Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Departamento de Obstetrícia e Ginecologia, Laboratório de Investigação Médica em Ginecologia Estrutural e Molecular (LIM-58), Disciplina de Ginecologia – São Paulo (SP), Brazil.
[2]Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Departamento de Obstetrícia e Ginecologia, Setor de Mastologia, Disciplina de Ginecologia – São Paulo (SP), Brazil.
*Corresponding author: antonioturri@usp.br
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factor (VEGF) messenger ribonucleic acid (mRNA) in MCF-7 cells, and inhibits proliferation, invasion, and migration[22,23].

Epidermal growth factor and insulin-like growth factor 1 receptors

Melatonin can induce programmed cell death, or apoptosis, in cancer cells, reducing tumor size and proliferation by inhibiting the production of growth factors related to cancer development such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1)[24,25].

Estrogen and progesterone receptors

Approximately 60–75% of breast cancers express estrogen receptor alpha (ERα). Melatonin affects the expression and activity of the estrogen receptors, which regulate breast cell growth and division. Melatonin has been shown to decrease the expression of ER-alpha and ER-beta and reduce estrogen binding to these receptors in vitro and in animal studies[26].

Melatonin modulates estrogen signaling in estrogen synthesis by reducing the gonadotropin action, disrupting the activation of estradiol receptors on breast tumors, regulating the enzymes involved in the biosynthesis of estrogens in other tissues, resulting in reduced estrogen-dependent tumor growth, and potentially reducing the risk of hormone receptor-positive breast cancer[27].

Progesterone is another crucial hormone in breast cell development and proliferation. Melatonin may interfere with the action of progesterone receptors (PR), reducing their expression and activity in breast cancer cells[26,28].

HER 2 receptors

Approximately 20–30% of breast tumors have HER2-positive receptors, a growth factor promoting cell division. Preclinical studies indicate that melatonin may affect HER2 expression by decreasing its activity and inhibiting tumor growth[29,30].

MT1 and MT2 melatonin receptors

MT1 receptors are widely expressed in various tissues, including the breast, while MT2 receptors are mainly found in the brain and retina. Both receptors have distinct but overlapping roles in regulating cellular processes and influencing breast cancer biology[31].

MT1 is associated with the G-receptor protease family, and MT2 is related to the hydrolysis of phosphoinositide and calcium. Activation of MT1 receptors in breast cancer cells inhibits cell proliferation, induces cell cycle arrest, and promotes apoptosis. These actions are mediated by inhibiting specific signaling pathways involved in cell growth and survival, such as the PI3K/AKT and ERK/MAPK pathways. MT1 activation also helps suppress the formation of new blood vessels (angiogenesis) within tumors, thereby limiting their supply of nutrients[32].

In estrogen receptor alpha (ERα)-positive human breast cancer, melatonin, via the MT1 receptor, suppresses ERα mRNA expression and ERα transcriptional activity. Some studies suggest that MT2 activation may also increase the invasiveness and metastasis of breast cancer cells. This conflicting role of MT2 receptors in breast cancer requires further investigation to understand their underlying mechanisms[32-34].

Synergic effect of melatonin with anticancer chemotherapy and radiotherapy

The role of statins in combination with melatonin has been extensively investigated regarding its risk reduction in specific cancer types. Pravastatin, a statin medication, is a widespread chemotherapy used to treat high blood cholesterol levels and prevent heart attacks and strokes. The chemopreventive effects of pravastatin in combination with melatonin in a breast cancer experimental model were evaluated. Pravastatin alone suppressed tumor frequency by 20.5% and average tumor volume by 15% compared with controls. The combined administration of the drugs decreased tumor frequency by 69%[35].

Doxorubicin is one of the most common chemotherapy drugs used to control breast cancer. Chemotherapeutic resistance, particularly to doxorubicin, represents a significant impediment to successfully treating breast cancer and is linked to elevated tumor metabolism, tumor overexpression, and/or activation of various families of receptor- and non-receptor-associated tyrosine kinases. Doxorubicin and other chemotherapy drugs are frequently employed as the initial treatment for individuals dealing with metastatic breast cancer or endocrine resistance. Similar to many other chemotherapy agents, doxorubicin often prompts resistance among patients. The capacity of melatonin to hinder the activation and expression of these kinases adds substantial backing to the emerging concept of melatonin functioning as a circadian-regulated kinase inhibitor[36].

The synergistic use of doxorubicin and melatonin holds promise as a prospective approach to treating breast cancer, demonstrating combined antitumor and anti-apoptotic influences while regulating calcium influx and its associated ion channel receptors. The results suggest that melatonin not only enhances the actions of doxorubicin through the activation of calcium ion channel receptors and the promotion of apoptosis but also triggers the demise of breast cancer cells[36].

Likewise, the emergence of resistance to tamoxifen and alternative endocrine treatments has emerged as a significant obstacle within endocrine therapy. This issue is pronounced,

with an estimated 30–50% of patients harboring estrogen receptor-positive breast tumors exhibiting inherent resistance. Furthermore, while most patients initially respond, the eventual development of acquired resistance to tamoxifen is nearly universal[6].

Augmenting cancer cells' susceptibility to radiation is a paramount objective within clinical radiobiology. The oncostatic effects of melatonin hold particular significance for estrogen-dependent mammary tumors. The impact of co-administering ionizing radiation and melatonin on proteins engaged in estrogen biosynthesis within breast cancer cells has undergone thorough scrutiny. Preliminary treatment with melatonin prior to radiation significantly diminishes the presence of active estrogens at the cancer cell level, reducing the activity and expression of proteins integral to estrogen synthesis by 50%. Furthermore, melatonin elicits a twofold alteration in p53 expression in comparison to radiation treatment alone[22].

DISCUSSION

It is crucial to recognize that the impact of melatonin on individual receptor types can exhibit variations contingent on factors such as the cancer stage, concurrent genetic modifications, and patient-specific elements. There is a substantial amount yet to be unveiled regarding the interplay between melatonin and receptors in breast cancer. Consequently, further research is imperative to unravel these mechanisms and their complexities[27,32,34].

Given that night-time melatonin significantly suppresses tumor kinase signaling, one could consider melatonin a broadly based "circadian-regulated broad kinase inhibitor" that exhibits potent antimetabolic, antiproliferative, and progressive/metastatic activity in breast cancer[12,37].

Breast cancer patients could potentially encounter varying levels of nocturnal light exposure due to factors such as stress, sleep deprivation, or extended night shift work, resulting in disruptions to their circadian rhythms and a decrease in melatonin production[9]. This factor could contribute to inherent and potentially acquired resistance to multiple chemotherapy agents. Consequently, there arises the potential for a novel approach involving the administration of chemotherapy in a manner optimized for circadian rhythms, in conjunction with supplemental melatonin therapy, for breast cancer patients. It is a possibility that needs further studies to prove it.

CONCLUSION

Melatonin action may act on cancer development and may reduce the risk of breast cancer. However, further studies are necessary.

AUTHORS' CONTRIBUTIONS

PCP: Conceptualization, Writing – original draft, Writing – review & editing. **JAOT:** Conceptualization, Writing – original draft, Writing – review & editing. **LHCMB:** Writing – original draft, Writing – review & editing. **GBN:** Writing – original draft, Writing – review & editing. **RSS:** Writing – original draft, Writing – review & editing. **ICES:** Writing – original draft, Writing – review & editing. **ECB:** Conceptualization, Writing – original draft, Writing – review & editing. **JMSJ:** Conceptualization, Writing – original draft, Writing – review & editing. **BSM:** Conceptualization, Writing – original draft, Writing – review & editing. **JRF:** Writing – review & editing.

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Where it reads:
The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine on May 31, 2023 (OMU KAEK 2023/178).

It should read:
The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine on November 14, 2019 (OMU KAEK 2019/744).

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