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DIFFICULTIES ENCOUNTERED BY NURSING STUDENTS IN CARING OF REFUGEE WOMEN: A QUALITATIVE STUDY

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ABSTRACT

Purpose: This study was done as for the purpose of manifesting the problems experienced by nursing students.

Material and Methods: This study was planned as a phenomenological type of qualitative research. Thirty-five students volunteered to participate in this research. The students were divided into five groups, seven students per group, and focus group interviews were conducted for each group.

Results: The average age of the students was 21.08 ± 0.61 years, 68.6% of the students were women, and 31.4% were men. Five themes were formed regarding the difficulties experienced by the students in the content analysis: inability to give active care because of the language barrier, displaying a sympathetic approach because of feeling mercy and thinking that she/he cannot give adequate care, fear of contagious diseases, and the inability to apply enough practice/learn in the clinical environment as a male nurse because of the perception of privacy.

Conclusion: It was determined that student nurses experienced problems during the maintenance of refugee women in particular the problem of language-related communication, women's expectations of respect for privacy, concerns about the risk of communicable disease. It may be recommended to add intercultural nursing to the academic program, increase course content on patient-nurse communication, and increase students' knowledge of asepsis/antisepsis due to infection concerns.

Keywords: Difficulty, nursing student, refugee women, qualitative study

INTRODUCTION

It is seen that many Syrians were obliged to flee from their countries and take refuge in various neighbor countries such as Lebanon, Jordan, Iraq and Turkey since the year of 2011 because of the civil war in Syria (1-5). Turkey is hosting the most crowded Syrian refugee population (nearly four and a half millions). It is stated that 75% of Syrian refugees who live in other countries as asylum seekers are women and children and most of women are at their reproductive age (1-3). Especially the women in 15-49 age group can face situations with special impacts such as disease and death during pregnancy and birth they experienced only because of their gender (even though they are physiological and social processes) in this period (3-5).

It is seen that the usage level of healthcare services of Syrian refugees who live in the camps in Turkey is high; and nearly 90% of men and 94% of women have been benefiting from these services (1,2) Studies have shown that unintended pregnancies, risky pregnancies, miscarriages, birth complications and

sexually transmitted infections, including HIV/AIDS, are among the most frequent health problems in refugees (1,2). The reported health problems encountered are primarily gynecological. Healthcare personnel should realize the requirements of migrated women, have knowledge about how to approach them, and be sensitive about cultural differences (3-6). İn this context; with increasing diversity in healthcare, nurses, as frontline care providers, are also expected to provide sensitive and culturally appropriate care to patients and families from diverse cultural and social backgrounds (7) The prejudiced and negative approach of nurses who are not well trained and equipped to provide treatment and nursing care to individuals, families or communities from other cultures can negatively affect the quality of health care (8). It is claimed that such negative attitudes in our country make compliance with treatment difficult, reduce the rate of utilization of health services, and negatively affect the physical and psychiatric health of refugees (8,9). Professional nursing aims to provide cross-cultural care that is respectful of cultural values, beliefs and lifestyles. Leininger defined transcultural nursing as "an important field of study and practice that focuses on the comparative cultural care (care) values, beliefs, and practices of individuals or groups from similar or different cultures." Providing intercultural care is the professional responsibility and moral obligation of nurses. Healthcare and nursing organizations worldwide emphasize the need to provide transcultural and sensitive care (7). Receiving culturally sensitive or intercultural care improves patients' overall well-being and makes them feel valued and respected in healthcare settings (10) It also increases patient satisfaction with healthcare and the behavior of care providers and promotes positive health outcomes (11). Therefore, it is very important for healthcare personnel to be aware of the needs of immigrant women, to have knowledge about how to approach them, and to be sensitive to cultural differences (3-6). In our country, the concept of intercultural care; In terms of nursing education, the content of theoretical and practical training curricula needs to be developed. In this context; ıt is extremely important to develop a comprehensive understanding of the challenges faced by nurses and nursing students and approaches to providing intercultural care. Because providing intercultural care is a core nursing competency. It is important to have a comprehensive understanding of the challenges

nurses and nursing students face when trying to provide cross-cultural care in clinical settings. However, there are a very limited number of studies on this subject in the literature.

Student nurses perform clinical applications for practicing and corroborating their theoretical knowledge. It is needed proper clinical environments for the effectivity of these applications. The case and practice variety that can be encountered in clinical environments creates rich learning conditions for students. In this context, it is thought that giving care to refugee patients is beneficial for the clinical learning of the student nurses. It is necessary for providing maximum benefit for students to manifest the problems they experienced while giving care to the refugee patients and to develop effective solution offers. This study was done as a phenomenological qualitative study for the purpose of manifesting the problems experienced by nursing students who will enter the profession in near future while giving care to the refugee women in clinical environments and of providing solution offers.

MATERIAL AND METHODS
Design and Sample
This study was planned as a phenomenological type of qualitative research. Phenomenology is a research type that attempts to understand or reveal the human experience (12). The subject that will be examined as a phenomenon in this study is "nursing care given to refugee women" and the experiences of nursing students about this phenomenon (12). It was aimed in this study to identify the experiences, thoughts and approaches of the students who practiced their clinical application of Gynecology-Women Health and Diseases Nursing course in the 3rd grade of the nursing department of Health College in a university in the South of Turkey in the hospital during the treatment and care practices towards refugee women.

The population of this research was composed of 102 students who study in 3rd grade of nursing undergraduate education at Health College in a university in the South of Turkey in the fall semester of 2017-2018 school year. Gynecology-Women Health and Diseases Nursing course is given in the fall semester of third school year in Health College that gives 4-year undergraduate education. The fall semester continues for 14 weeks. This lesson is studied for the entire semester through 6 hours theoretical education in school and 12 hours practical

Table 1. Interview Form

Interview Form Content
It was asked
I.   How and in what way their experiences of treatment and care towards refugee women are,
II.   What their most experienced problems/challenges or subjects in the treatment and care towards refugee women are (in terms of Gender/Religion/Language/Culture),
III.   Whether there are situations during which they behave as attentive/careful differently from Turkish patients while giving service to refugee women, and if any, what they are,
IV.   Whether there are practices or expectations they want to conduct differently from student nurses during the treatment and care towards refugee women, and if any, what they are,
V.   What kind of arrangements are needed about the services provided for refugee women, and what their recommendations about this issue.

application in the hospital each week. 35 students who accepted voluntary participation were involved to the research. It is preferential to have a homogenous group for the phenomenological research (12). It was formed groups with similar characteristics in terms of the age, gender, education semester and socio-economic conditions of the students for providing the homogeneity of the sample group. Because of that the research examines the problems experienced in care practices towards Syrian women, the students were chosen from those who made practice for 14 weeks in gynecology clinics and give care to Syrian refugee women. It was formed 7-persons homogeneously pertinent groups with the criteria determined in accordance with the purposive sample choice among the students. It was conducted focus group interviews with 5 different groups in total as of one interview for each group.

Data Collection
Focus group interviews were conducted using the semi-structured question form (Table 1). Research data was collected between January 1, 2018 and January 31, 2018 after the 14-week clinical practice ended. In the focus group interviews, one of the researchers participated as the interviewer and the other as the observer. While the observer took notes on the research, the interviewer asked the students questions about their experiences, views, and approaches about the difficulties they encountered while giving care to the Syrian refugee women. Thirty to forty-five minutes were allotted for each focus group interview. The interviews were recorded with a voice recorder with the permission of the participants, which was received with written informed consent before the interviews.

Data Collection Tools
The question form (Table 1), which included five questions prepared by academic members who were proficient and experienced in their field of Women's Health Nursing (one associate professor, one assistant professor).

Ethical Principles of the Study
Permission for this study was granted given by Faculty of Health Sciences of University and ethical approval was received from from the Hatay Mustafa Kemal University, Tayfur Ata Sokmen Medical Faculty, Clinical Research Ethics Committee for this study (Data: 02.11.2017, No: 15). Written consent was received from all students who participated in this study. In addition, it was decided that there was no ethical or scientific disadvantage in carrying out the work at the relevant center. It was said to the participants that they can stop the interviews whenever they want during the interviews and they can leave the study. It was also stated that no payment would be made to any participants and it would not be given the identities or any descriptive information that can cause the publicity of the researchers while reporting the data obtained from the study. In accordance with the data obtained from the research, the research results were shared with the Health College Directorate for developing solutions towards overcoming the problems experienced by the student nurses during treatment and care process.

Data Analysis
The records taken during the interviews were transcripted as a whole by the researchers. Content analysis technique was used in analyzing the qualitative data collected from the participants. The

Table 2. Themes Formed According to Content Analysis

Themes Formed According to Content Analysis
I.   Inadequate Treatment and Care due to Language Obstacle
II.   Responsibility to Show More Care/Show More Sympathy
III.   The Obligation to Be Careful Against Infectious Disease Risks
IV.   Cultural and Religious Barriers
V.   Arrangements
• Opening Hospitals specific to Refugees/Training towards Refugee Women (Women Health, Family Planning, Newborn Care, Personal Hygiene)
• Increasing the Number of Translators in Hospitals
• Language Courses for Health Personnel

aim in the content analysis is to gather similar data together and to interpret them by arranging them as understandable by the reader (12). While the conceptual structure of the research was specified by adopting the content analysis approach through complying with this fundamental principle, the data were supported by direct quotes from the opinions of the participants about the subject. For the purpose of increasing the validity-reliability of the research; the transcriptions of the interviews were done on the same day, themes were formed after reading the data twice on different times by two researchers in the analysis process, and it was asked to a group of participants whether the themes formed after the transcriptions and the words in the interviews were reflected true through making them read. Themes were summarized in 5 categories according to the data revealed by research questions as a result of the content analysis (Table 2). In the presentation of the findings, direct quotations were included to reflect the opinions of the participants. While presenting the quotes, the participants were defined as "Female/Male Student". Additionally, the student nurses' age was added to the end of the quotes.

RESULTS
Demographic Information
A total of 35 students were interviewed in our study. The average age of the students was 21.08 ± 0.61 years, 68.6% of the students were women, and 31.4% were men.

Data analysis in qualitative research; it includes preparing the data for analysis, coding the data, bringing the codes together and reducing them to themes, and finally presenting the data in tables or discussion. Creswell (13) coding; ıt is the process of symbolically assigning words or short sentences to research data in line with research purposes. Depending on what the researcher is looking for, the

codes may differ. Open coding was used in this research. Open coding can be defined as the process of comparing, identifying differences or similarities in the data to discover commonalities that will form categories or themes. It is generally a process performed by reading the resulting text line by line as soon as the data is collected. Sometimes coding can be done line by line or word by word, and sometimes open coding can be done in a more expanded form in the form of sentences, paragraphs or even sections. The purpose of open coding is to provide prior knowledge to build concepts and categories. Five themes were formed based on the difficulties encountered by the students from the content analysis (Table 2).

Inadequate Care Due to Language Barrier
Students stated that they could not give adequate care as they intended because they did not speak the same language with the patients. Therefore, it was determined that their experiments in this subject are not on a positive level.

"While I was giving care to refugee women, I has disagreement with them when I try to explain in Turkish because of that I do not know Arabic." (21-year-old, male student)

"We are not successful enough in this subject, namely during the treatment and care, we generally only look at one another. They do not react much. We are only giving them the treatment and leave. That is to say, we give care too, yes, but we are giving care without having any communication with the patient about these." (21-year-old, female student)

Responsibility to Show More Care/Show More Sympathy
Student nurses expressed that they paid more attention and showed more sympathy to Syrian refugee women compared to Turkish.

J Basic Clin Health Sci 2024; 8: 516-524

Erenoglu R et al. Difficulties in Caring of Refugee Women

"We do not have difficulties while giving service to Turkish patients because we have a common language. But while I was giving care to refugee patients, I try to tell a subject again and again through gestures and facial expressions; I behave more attentive and careful in this issue." (21-year-old, male student)

"I check the patient frequently, I am more attentive… Because I observed that the tendency to remove branules and to close serums by themselves of Syrian patients is much more than Turkish patients, so I always try to hinder them to do these by keeping a close watch on them. And I am a little uncomfortable about hygiene. They have grown up in a different culture." (21-year-old, female student)

"We can show more tolerance and more empathy to these patients. Because they can not understand well because of the language difference." (22 -year-old, female student)

"I Show more tolerance while I was giving service to refugee women because of that they do not understand our language. I am attentive to be more compassionate and more good humored because I think that they feel themselves embarrassed before us." (21 -year-old, female student)

"I sympathize them much more because of the trauma they experienced and I behave them in an easy fashion and good humored to provide them to feel better and to avoid a bad psychology." (21-year-old, female student)

The Obligation to Be Careful Against Infectious Disease Risks

While practicing care and treatment of refugee women, some nursing students stated that they were more attentive and careful in their physical contact with refugees compared to other patients in the same service to prevent the risk of infectious diseases.

"I am more sensitive about hygiene with them differently than Turkish patients. I wear gloves while entering the rooms of all patients, but I show a nervous approach in refugee patients. I try to be more careful because I do not know their medical story well and that I cannot ask them." (21-year-old, female student)

"I am careful about self-protection methods. When we consider that they came from a war, there is much possibility for carrying a contagious disease. For this reason, I am protecting myself differently while I am giving care and treatment." (21-year-old, female student)

Cultural and Religious Barriers

Students stated that refugee women have higher expectations regarding respect for privacy because of their religious and cultural backgrounds compared to other patients during treatment and care practices. Male students had difficulties during their gynecology internship due to this situation.

"Some refugee women say that they exactly do not want male students due to their culture and religion, I direct female friends in these situations." (22-year-old, male student)

"I observe that they generally refrain from male personnel and can refuse treatment in accordance with their customs. I also see that they have been anxious while I am giving care and applying treatment." (21-year-old, female student)

Arrangements

Students suggested that hospitals specific for refugees have to be opened. There is a need for additional training, particularly for women's health issues (women's health, family planning, newborn care, personal hygiene), for refugee women. This need has to be satisfied through different training methods, the number of translators in hospitals has to be increased for service, and healthcare personnel have to take Arabic language courses.

"I think that refugee women should receive treatment and care under another roof, namely in a different center from Turkish patients, a hospital which will give treatment in compliance with their culture, language and religion. When they are in the same place, they can understand each other better, they can feel better psychologically." (22-year-old, female student)

"It should certainly be provided a translator in each service, it should be given training to refugee women on the issues of women health, newborn care, hygiene, family planning." (21-year-old, female student)

DISCUSSION

Communication, which is a fundamental part of nursing, is an important factor with regards to patient satisfaction and qualified nursing care (14-16). In our study, the student nurses stated that they could not give adequate treatment and care as they intended, namely because they did not speak the same language as the patients. Similarly, in other studies, healthcare professionals that provided healthcare services to refugee and migrant patients stated that the most important service obstacle was "language",

J Basic Clin Health Sci 2024; 8: 516-524

Erenoglu R et al. Difficulties in Caring of Refugee Women

and therefore they had difficulties establishing communication (16-20). In a qualitative study conducted with 25 nursing students caring for refugee patients in Turkey; all of the participants mentioned the language and communication barriers in their communication with refugee patients (21). In another study conducted in our country; It has been reported that the biggest obstacle for nursing students in providing care to refugees is language and communication (22). In the research of Çamlıbel and Uslu (2023), it was found that nurses had problems especially in terms of communication during the care they gave to refugee patients (23). While our study findings correspond with the literature, our study also revealed that the student nurses could not implement the patient care they learned theoretically to practice because of the language barrier.

Professional care has five important features, compassion, competence, confidence, commitment, and conscience (24). In our study, we identified that the student nurses pay more attention and show more sympathy to Syrian refugee patients in comparison to Turkish patients for both feeling compassionate towards the patients and attempting to reduce the communication problems, which resulted from a language barrier. There are also conclusions similar with our findings reported in the literature (14). In another study that examined the experiences of the nurses caring for Syrian refugee patients, the nurses showed compassion to the patients during the care process (19). Studies have underlined that, despite nurses having negative feelings towards the refugee patients due to problems, such as language barriers and increasing work load, they still have empathetic and merciful feelings, among others, towards the patients (20,25) While empathy and compassion are proper behaviors in professional care, the mercy and expressed sympathetic approach due to this mercy can cause the nurses to go beyond their professional relationship with their patients. This situation can cause an identification with the patient, desperation, and exhaustion (26). When the positions and functions of the student nurses in the hospitals are considered, it comes to mind that they can be exploited, for example, having them make Works out of nursing by taking advantage of their compassionate and sympathetic feelings.

In Turkey, there were 330,000 respiratory tract infection cases and 50,000 diarrhea cases among 2.7 million refugees who came from Syria in 2015 (27,28). The systematic compilation study of İsenring

et al. (29) found that the most frequently encountered contagious diseases in Syrian refugees are *Leishmania tropica* and antibiotic-resistant gram-negative bacteria and colonization. In our study, some nursing students stated that they behave more attentively and carefully in their physical contact with refugee patients compared to other patients in the same service to prevent the risk of contagious diseases. While students can have concerns about contagious diseases, this concern will possibly result in the students taking different approaches towards care (less contact, wearing extra gloves/masks, etc.) and different applications to the refugee patients. This situation can result in the refugee women feeling as if they are being discriminated against. In a study examining the nursing care experiences of Syrian refugees in Turkey, nurses reported that healthcare professionals are at risk due to the infectious diseases of refugees (30). In a study conducted with nursing students in our country; Hygiene and infectious diseases are considered among the most common problems in refugee care (8). Our research findings reveal the prejudices and concerns of nursing students towards the care of refugees. At the same time, it warns us to develop solutions for the fragile points of nursing education.

Many Syrian and Arabic Muslims (both men and women) only touch people of the same gender; they feel uncomfortable exposing their bodies in clinical treatments, and therefore the healthcare professionals should ask for permission to proceed with care (31, 32). In our study, the students stated that during treatment and care practices, refugee women had higher expectations regarding respect for privacy, because of their religious and cultural characteristics, compared to other patients. Similar with our study findings, some studies reported that women demanded woman healthcare personnel during childbirth because they were Muslim, and the healthcare professionals had difficulties in the face of such situations (33, 34). In a study conducted on this subject, it was reported that Syrian refugee women were exposed to a traumatic experience due to lack of privacy during their stay in public hospitals (35). Not all nursing students are women. Thus, this situation will pose an important obstacle during the gynecology internship of male students. On the other hand, privacy is a feeling that all people experience/can experience, whether they are refugees or not. It should not be forgotten that refugee

J Basic Clin Health Sci 2024; 8: 516-524

Erenoglu R et al. Difficulties in Caring of Refugee Women

women may need more intercultural care in a country with a different culture.

İn our study, the problems experienced by the students were deeply examined, and it was demanded from the students to bring forward solutions to these problems they experienced. The students suggested that hospitals specific to refugees have to be opened; the number of translators in hospitals has to be increased; healthcare personnel have to take language education. Similar with our study findings, Gönenç et al. (19) reported that the midwife nurses and nurses suggested that separate services should be given to refugees; the personnel should be trained about this issue; service counselors should be available for solving these problems. The recommendations of the students are consonant with the literature.

Limitations of the Study

Although this study is one of the exceptional studies in which the problems experienced by nursing students who will be health personnel in the near future are evaluated in the care of refugee female patients, it was conducted with only undergraduate nursing students of a university. Consequently, the findings of the study can only be generalized fort his group. In future, similar studies can be conducted in all the schools which give nursing undergraduate education in Turkey.

CONCLUSION

It is inevitable for the students to encounter with refugee women in healthcare services given in gynecology field because of that the majority of individuals who reside in Turkey as refugees are women and children and there are necessary conditions such as pregnancy-birth in which it must be benefited from the healthcare service. For this reason, it can be said that the students who compose our study sample have been encountering with refugee women frequently and the experiences they shared created a quite rich data. In this context, knowing what these students who will start to perform the profession after one year did experience in the manner of giving trans-cultural care is important in terms of revealing the deficiencies that should be overcome in the educational system and exemplifying for other nursing schools.

The problems identified as a result of content analysis of the experiences of the students are; inability to give active care because of language obstacle, displaying

sympathetic approach because of feeling mercy and thinking that she/he can not give enough care, fear from contagious diseases, and inability to apply enough practice/learn in clinical environment as a male nurse because of the privacy perception.

It was recommended for enabling students to give enough care to refugee patients and feel themselves qualified in this issue to add trans-cultural nursing to the academic program, to perform practices in company with a translator during the clinical practice for the acute solution of the problem, and to teach different education techniques which can be used by the individuals with different languages and cultures in nursing care to the students. It was recommended to increase course contents about patient-nurse communication for enabling them to develop empathy rather than sympathy. For the purpose of prohibiting patient's feeling to be exposed to discrimination because of the healthcare personnel's concern about contagious diseases and the different approaches because of this concern, it was recommended to increase the asepsis/antisepsis knowledge of the students and to address the issues of discrimination and respect to patient dignity through practical cases as part of care ethics. Finally, for the purpose of eliminating the suffering experienced by male students as part of the privacy perceptions of refugee women, it was recommended to address the patient privacy through practical cases as part of care ethics, to incorporate the male students into practices without any privacy problem, and to compensate the incomplete practices in the clinical environment in laboratory environment.

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J Basic Clin Health Sci 2024; 8: 516-524

Erenoglu R et al. Difficulties in Caring of Refugee Women

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INVESTIGATION OF ATTITUDES OF SURGICAL NURSES TOWARDS EVIDENCE-BASED NURSING PRACTICES IN AND AFFECTING FACTORS

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ABSTRACT

Purpose: This study was conducted to investigation of attitudes of surgical nurses towards evidence-based nursing practices in and affecting factors.

Material and Methods: The sample of the descriptive and cross-sectional study consisted of 110 nurses who worked in the surgical clinics of a university hospital. Nurse Introductory Information Form and Attitude Towards Evidence-Based Nursing Questionnaire (AEBNAQ) were used to collect the data. Written permissions were obtained from the Scientific Ethics Committee, the institution where the study would be conducted, and the nurses participating in the study. SPSS (Statistical Package for Social Science) 21.0 package program was used to evaluate the data.

Results: It was determined that the average age of the nurses participating in the study was 34.03 ± 7.29 (min:22-max:58) years, 40.0% has attended a scientific meeting on evidence-based practice. The total score of the nurses was determined as 62.5 ± 8.5 (min:42- max:75) and sub-dimensions differed as in the "Evidence-Based Nursing Beliefs and Expectations" sub-dimension 29.3 ± 4.2 (min:19- max:35), in the "Evidence-Based Practicing Intention" sub-dimension 16.1 ± 2.7 (min:9- max:20), in the "Evidence-Based Nursing Emotions" sub-dimension 17.1 ± 2.5 (min:9- max:20) points. It was determined that nurses who read scientific journals, have attended scientific meetings, do graduation projects/thesis/research, need evidence in nursing practices, have information about evidence-based practices and want to participate in the training program, and the sub-dimension and total score averages of the AEBNAQ are significantly higher ($p<0.05$).

Conclusion: It was found that surgical nurses' attitudes towards evidence-based care were at a good level, with those who attended scientific meetings, followed scientific journals and were younger having higher attitudes.

Keywords: Surgery, nurses, evidence-based nursing, attitude, surgery clinic

INTRODUCTION

In health sciences, evidence is defined as the information obtained through reviews, scientific research, patient feedback, and scientific evaluation of the practice that is available to experts who are the decision makers in the health care system (1,2). There is a growing need to establish practices based on the best available evidence in all areas of

healthcare (1). Evidence-based practice is defined as the practice that combines the best available evidence with systematic research and clinical expertise wherein decisions are made by selecting, synthesizing, and concluding the results of examining a particular subject and ultimately providing the best care for patients based on experience and critical judgments (1,2,3,4,5).

Putting research results into practice is essential for evidence-based nursing practice and ensuring the maintenance of nursing care quality (5). Therefore, evidence-based nursing can be defined as the approach where nurses, as professional caregivers, use the best evidence obtained by scientific methods in making nursing care-related decisions by combining the evidence with personal experience and the preferences of the patients in healthcare environments, thereby providing the best care that patients deserve and need (1,2, 4,6).

The main aim of nursing practices is to establish an acceptable foundation for evidence-based practice and ensure that this foundation enables nursing care to be effective, rational, and dynamic and enhances clinical judgment (5, 7).

In order to develop strategies that will improve and accelerate the process of evidence-based nursing, obtaining information regarding the attitudes and views of nurses on this subject should be a priority (2,6). The importance of evidence-based practice for nurses working in surgical clinics is becoming increasingly evident with its critical role and impact in healthcare. However, comprehensive studies on evidence-based attitudes of surgical nurses are rarely found in the literature, which can be characterized as a gap (7,8,9). Evidence-based nursing enables surgical nurses to effectively use scientific evidence in patient care and to identify best practices by combining it with their clinical experience (10). This approach increases the quality of surgical care, improves patient outcomes and supports the professional development of nurses. However, more research is needed to assess surgical nurses' attitudes towards evidence-based practice and their awareness of this issue (11). These studies will be an important step to encourage surgical nurses to adopt evidence-based practice and fill the knowledge gap in this field. Therefore, the aim of this study was conducted to investigation of attitudes of surgical nurses towards evidence-based nursing practices in and affecting factors.

MATERIAL AND METHODS

The study was conducted in all surgical clinics of a university hospital. The data collection period lasted 3 months and the data were collected face-to-face. A total of 288 surgical nurses work in this hospital. The sample of the descriptive cross-sectional study consisted of nurses (n=110) working in the surgical clinics (Anesthesia and Reanimation Intensive Care Unit, Brain and Nerve Surgery Clinic, Pediatric Surgery Clinic, General Surgery Clinic, Thoracic Surgery Clinic, Eye Clinic, Gynecology and Obstetrics Clinic, Cardiovascular Surgery, Ear Nose Throat Clinic, Organ Transplant Center, Orthopedics and Traumatology Clinic, Plastic and Reconstructive Surgery Clinic, IVF Center, Urology Clinic) of a university hospital between March and September 2019.The number of samples was calculated with the data of nurses in the study, conducted by Doğan et al. (2019) (12). The impact factor was determined with the mean score of the attitude scale in this study. Using the G-Power 3.1 software, 0.36 standard deviation was determined as the smallest effect and the sample size was calculated as a total of 82 nurses with 95% power, 95% confidence interval and 0.05 margin of error. A total of 110 nurses who agreed to participate in the study were included in the sample. 38.19% of the research population was reached. Nurse Introductory Information Form and Attitude Toward Evidence-Based Nursing Questionnaire (AEBNQ) were used to collect the data.

Nurse Introductory Information Form
This form contains 14 questions regarding the sociodemographic characteristics and research activities of nurses. Sociodemographic characteristics included age; gender; marital status; educational status; years of experience in the profession, clinic, and department of work. In addition, research activities, participation in scientific meetings on evidence-based nursing, and questions about lessons are included.

Attitude Toward Evidence-Based Nursing Questionnaire (AEBNQ)
The questionnaire was first developed by Ruzafa-Martinez et al. (2011) in Spain to measure nurses' attitudes toward evidence-based nursing (13). The Turkish validity and reliability study of the questionnaire was conducted by Ayhan et al. in 2015 (14). AEBNQ contains of 15 items and three sub dimensions on a 5-point Likert scale. Eight items are

Table 1. The Score of Surgical Nurses' Attitudes Towards Evidence-Based Nursing

The minimum and maximum score that can be taken from the scale	Mean±sd	Min- Max.
AEBNQ Mean Score (15-75)	62.5 ± 8.5	42-75
Beliefs and Expectations (7-35)	29.3 ± 4.2	19-35
Practice Intention (4-20)	16.1 ± 2.7	9-20
Emotions (4-20)	17.1 ± 2.5	9-20

AEBNQ: Attitude Toward Evidence-Based Nursing Questionnaire, sd: standard deviation, Min: Minimum, Max: Maximum

scored positively (items 1, 2, 5, 7, 9, 11, 13, and 14) and seven items are scored in reverse (items 3, 4, 6, 8, 10, 12, and 15). The minimum obtainable score is 15, and the maximum obtainable score is 75. The Beliefs and Expectations for Evidence-Based Nursing Subdimension includes items 1, 2, 7, 9, 11, 13, 14 and the points that can be obtained are min:7- max:35. Evidence-Based Practice Intention Subscale includes items 3, 5, 6, 12 and the possible scores are min:4-max:20. Emotions Related to Evidence-Based Nursing Subscale; It includes items 4, 8, 10, 15 and the scores that can be obtained are min:4- max:20. Higher scores indicate more positive attitudes toward evidence-based nursing (10). In the study by Ayhan et al (2015), the Cronbach's reliability coefficient of the scale was found to be 0.86 (14), whereas in the present study it was 0.921.

All statistical analyses were conducted digitally, and SPSS (Statistical Package for Social Science) 21.0 package program was used. Descriptive statistics were presented as number and percentage and mean and standard deviation. Fisher's chi-square test was used to compare variables. The relationship between the obtained scores and other variables was evaluated using Kruskal–Wallis, Mann–Whitney U, and Spearman's correlation tests. $p < 0.05$ was accepted as statistically significant in all analyses.

Permission to use the scale was obtained from the corresponding author. Institutional permission was obtained from the chief physician of the university hospital where the research was conducted (no. 69631334-605.01). Consent was also obtained from the nurses during the research. Additionally, ethical permission was obtained from Ege University Medical Research Ethics Committee (Date: 29.05.2019, No. 19-5.2T/54).

RESULTS

The mean age of the nurses was 34.03 ± 7.29 (min:22-max:58) years, 91.8% of the were female, 87.3% nurses had a bachelor of science in nursing degree, and 40.9% were clinical nurses. When research activities of the nurses were examined, it was found that 61.8% did not read scientific journals, 78.2% attended at least one scientific meeting, 51.8% completed a graduation project/thesis/research during their undergraduate education, 91.8% needed evidence in nursing practice, 94.5% wanted to learn more about evidence-based practices, and 89.1% wanted to participate in a training program on evidence-based nursing. Mean AEBNQ score of the nurses was 62.5±8.5 (min:42-max:75). The mean score for the Beliefs and Expectations Toward Evidence-Based Nursing subdimension was 29.3±4.2 (min:19-max:35), Evidence-Based Practice Intention subdimension was 16.1±2.7 (min:9-max:20), and Emotions Related to Evidence-Based Nursing subdimension was 17.1±2.5 (min:9-max:20) (Table 1).

A significant difference was found between Evidence-Based Practice Intention and Emotions Related to Evidence-Based Nursing subdimension mean scores and total AEBNQ scores with respect to the status of participating in a scientific meeting and completing a graduation project/thesis/research. A significant difference was found in all subdimensions and total AEBNQ scores with respect to reading a scientific journal, attending a scientific meeting on evidence-based practice, needing evidence in nursing practice, wanting to learn more about evidence-based practices, and wanting to participate in a training program on evidence-based nursing ($p < 0.05$) (Table 2).

A significant relationship was found between years of experience in the profession and Beliefs and Expectations Toward Evidence-Based Nursing subdimension scores ($r = -.200$, $p = .036$) and between age, years of experience in the profession, and Evidence-Based Practice Intention subdimension scores ($r = -.227$, $p = .017$; $r = -.226$, $p = .018$, respectively) and total AEBNQ score ($r = -.201$, $p = .035$; $r = -.210$, $p = .028$, respectively). On

the other hand, a negative correlation was found between AEBNQ scores and age and years of experience in the profession. AEBNQ scores were seen to increase with decreasing age and years of experience in the profession (Table 3).

DISCUSSION

Nurses play an important role in optimally improving and developing health outcomes through evidence-based healthcare practices (6, 15). In nursing research, studies examining the knowledge, attitude, skill, and behavior of nurses toward evidence-based practice are becoming popular day by day (15).

Nursing practice in surgical clinics is constantly evolving with rapid changes in medical technology and surgical procedures. This dynamic environment requires nurses to adopt evidence-based approaches to update and effectively guide their practice. Therefore, surgical nurses can be expected to show more inclination towards evidence-based nursing

Table 2. Comparison of attitudes towards evidence-based nursing and ındependent variables

Independent Variables		n	Subdimensions of AEBNQ			AEBNQ Median(IQR)
			Beliefs and Expectations Median(IQR)	Practice Intention Median(IQR)	Emotions Median(IQR)	
Gender	Female	101	28.00(26.00-34.50)	16.00(14.50-18.00)	17.00(16.00-20.00)	62.00 (57.00-70.50)
	Male	9	28.00(27.00-32.00)	16.00(12.50-17.50)	16.00(14.50-19.50)	60.00 (56.00-68.00)
	Test Statistics		Z: -0,28 P: ,978	Z: -,659 P: ,510	Z: -,700 P: ,484	Z: -,508 P: ,611
Read scientific journals	Yes	42	31.00(28.00-35.00)	18.00(16.00-20.00)	18.50(16.00-20.00)	66.50 (60.00-73.25)
	No	68	28.00(26.00-31.00)	15.00(13.00-17.00)	16.00(15.00-19.00)	59.00 (55.00-66.75)
	Test Statistics		Z: -3,214 P: ,001	Z: -4,095 P: ,000	Z: -2,632 P: ,008	Z: -3,703 P: ,000
Attended at least one scientific meeting	Yes	86	28.50(26.00-35.00)	17.00(15.00-18.00)	18.00(16.00-20.00)	62.50 (58.00-71.00)
	No	24	28.50(26.50-31.00)	15.50(13.00-17.00)	16.00(15.00-17.75)	59.00 (53.25-63.50)
	Test Statistics		Z: -1,541 P: ,123	Z: -2,436 P: ,015	Z: -2,620 P: ,009	Z: -2,432 P: ,015
Completed a graduation project/thesis/ research	Yes	57	30.00(27.00-35.00)	17.00(15.00-19.00)	19.00(16.00-20.00)	66.00 (59.00-73.00)
	No	53	28.00(26.00-31.00)	16.00(14.00-17.50)	16.00(15.00-19.00)	60.00 (55.00-66.00)
	Test Statistics		Z: -2,160 P: 0,31	Z: -2,505 P: ,012	Z: -3,002 P: ,003	Z: -2,693 P: ,007
Attend any scientific meeting on evidence-based practice	Yes	44	32.00(28.25-35.00)	18.00(15.25-20.00)	19.00(16.00-20.00)	68.00 (62.00-74.00)
	No	66	28.00(26.00-30.25)	15.50(13.75-17.00)	16.00(15.00-19.00)	59.00 (55.75-66.00)
	Test Statistics		Z: -4,474 P: ,000	Z: -4,141 P: ,000	Z: -3,405 P: ,001	Z: -4,611 P: ,000
Took an evidence-based practice course	Yes	55	30.00(27.00-35.00)	17.00(15.00-18.00)	18.00(16.00-20.00)	65.00 (59.00-70.00)
	No	55	28.00(26.00-33.00)	16.00(14.00-18.00)	16.00(15.00-20.00)	59.00 (56.00-70.00)
	Test Statistics		Z: -1,911 P: ,056	Z: -1,886 P: ,059	Z: -,670 P: ,503	Z: -1,739 P: ,082
Needed evidence in nursing practice	Yes	101	28.00(27.00-35.00)	16.00(15.00-18.00)	17.00(16.00-20.00)	62.00 (57.00-71.00)
	No	9	25.00(22.50-29.00)	15.00(12.50-16.00)	15.00(13.50-17.00)	51.00 (49.00-62.00)
	Test Statistics		Z: -2,791 P: ,005	Z: -2,308 P: ,021	Z: -2,543 P: ,011	Z: -2,643 P: ,008
Wanted to learn more about evidence-based practices	Yes	104	28.00(27.00-34.75)	16.00(15.00-18.00)	17.00(16.00-20.00)	62.00 (57.25-70.75)
	No	6	23.00(19.75-26.50)	13.00(11.25-15.00)	15.00(13.50-16.50)	50.00 (47.25-56.75)
	Test Statistics		Z: -3,215 P: ,001	Z: -2,845 P: ,004	Z: -2,272 P: ,023	Z: -3,184 P: ,001
Wanted to participate in a training program on evidence-based nursing	Yes	98	28.50(27.00-35.00)	17.00(15.00-18.00)	17.50(16.00-20.00)	62.00 (57.00-71.00)
	No	12	26.00(22.25-27.75)	15.00(13.00-15.00)	15.50(15.00-17.75)	57.00 (49.50-59.75)
	Test Statistics		Z: -3,283 P: ,001	Z: -2,797 P: ,005	Z: -2,207 P: ,027	Z: -3,020 P: ,003

AEBNQ: Attitude Toward Evidence-Based Nursing Questionnaire, IQR: Interquartile Range, z: Mann-Whitney U

Table 3. Correlation between age, years of work in the occupation and the attitude scale towards evidence-based nursing scores

		AEBNQ	Beliefs and Expectations	Practice Intention	Emotions
Age	r_s	-,201[*]	-,186	-,227[*]	-,097
	p	**,035**	,052	**,017**	,312
Years of experience in the profession	r_s	-,210[*]	-,200[*]	-,226[*]	-,119
	p	**,028**	**,036**	**,018**	,215

AEBNQ: Attitude Toward Evidence-Based Nursing Questionnaire, r_s: Spearman's rho

principles to adapt to this variable and complex environment. This study shows that surgical nurses have positive attitudes towards evidence-based nursing. Higher AEBNQ scores observed in our study compared to other studies focusing on intensive care, internal medicine, and surgical nurses, may indicate that surgical nurses have a more positive attitude towards evidence-based nursing practices (2, 6, 7,14,16). However, this difference may be due to the fact that the study was conducted in different regions and units. This may suggest that special education and experience of surgical nurses may positively affect their attitudes towards adopting evidence-based nursing practices. In another study, it was found that Greek nurses exhibited positive attitudes and beliefs toward evidence-based practice to ensure continuity in improving the quality of care and patient outcomes (17). Similarly, another study reporting positive and strong attitudes of nurses toward evidence-based practice emphasized the importance of nurses' current knowledge regarding evidence-based practice and creating useful nursing strategies in an appropriate clinical setting in Omani (18). In this study of Al-Maskari and Patterson, although the mean AEBNQ scores were generally high, the lowest scores were obtained in the Evidence-Based Practice Intention subdimension (18). Evidence-Based Practice Intention subdimension scores are generally reported to be the lowest in most studies. In the study of Yılmaz et al. conducted with nurses working in internal and surgical clinics, the mean score for Evidence-Based Practice Intention subdimension was 7.51 ± 2.45 (2). However, it was 16.1 ± 2.7 in the present study. In Taşçı and Özer's (2023) study, it was stated that increased work intensity increased critical thinking tendency and affected the attitude towards evidence-based nursing (8). In this study, a positive relationship was found between surgical

nurses' positive attitudes towards evidence-based nursing and their desire to use it frequently. These findings suggest that the habit of frequent use of evidence-based practices of surgical nurses may positively affect their attitudes. As a result, it is seen that increasing critical thinking tendency and following information, education and scientific studies about evidence-based practices positively affect the attitude towards evidence-based nursing (8). These findings provide important guidance to support the professional development of surgical nurses and encourage the dissemination of evidence-based practices. In Himmet's (2021) study, a positive moderate relationship was found between both individual innovativeness levels and professional value levels of surgical nurses and evidence-based nursing attitudes. In other words, as both individual innovativeness levels and professional values of surgical nurses increase, their evidence-based nursing attitudes also increase (9). These results indicate that the personal characteristics, innovativeness and professional values of surgical nurses affect their tendency to adopt evidence-based practices.

In this study, the highest mean score of 29.3 ± 4.2 was obtained in the Beliefs and Expectations Toward Evidence-Based Nursing subdimension. This finding was consistent with relevant studies in the literature (17,19). Only in the study by Doğan et al. the lowest mean score was obtained in the Emotions Related to Evidence-Based Nursing subdimension (20). More than half of the surgical nurses included in the study exhibited positive attitudes and perceptions towards evidence-based practice, indicating that evidence-based practice is recognized as an important contribution to improving the quality of nursing care and patient outcomes. In another study, Durmus et al. also found that nurses had moderately positive

attitudes toward evidence-based nursing and stated that nurses' problem-solving skills affected their attitudes and perceptions regarding evidence-based nursing (20).

A significant correlation was found between years of experience in the profession and Beliefs and Expectations Toward Evidence-Based Nursing subdimension, Evidence-Based Practice Intention subdimension, and AEBNQ scores. Furthermore, there was a negative correlation between years of experience in the profession and AEBNQ scores. In other words, as years of experience in the profession increased, AEBNQ scores decreased (Table 3). This result shows that nurses with more professional experience have worse attitudes toward evidence-based practices. Similarly, Ruzafa-Martinez et al. (2011) found that nurses with more clinical experience had worse attitudes toward evidence-based practice than those with less experience (13). Unlike this study, there are also studies in which age and work experience do not affect evidence-based attitudes. These results are also supported by some studies in the literature, and it was observed that nurses' age and work experience did not affect their AEBNQ scores (6, 7, 10, 14, 22).

The findings of the study emphasize the needs of nurses working in surgical clinics for evidence-based nursing practices and their deficiencies in this field. The nurses' need for evidence in their practices shows their desire to learn more and participate in training programs. These findings reveal that surgical nurses should focus on their professional development and that they can improve the quality of patient care by adopting evidence-based practices. Therefore, it is important to provide appropriate educational and support resources to meet the needs of surgical nurses and strengthen their skills. Yılmaz and Gürler also reported that 92.5% of the nurses needed training on evidence-based practice (23). Evidence-based practice is an approach that involves aspects such as correctly questioning the current clinical problem, reviewing the relevant literature, evaluating the evidence presented in studies, and making clinical decisions based on critical thinking (22). Kilicli et al. reported that mentoring provided by nurses who are adequately equipped and experienced in evidence-based nursing is necessary to facilitate evidence-based practice (25). It is recommended that nurses be taught how to critically evaluate research evidence and use it in patient care (24).

Scientific meetings have an important place and role in increasing information sharing among nurses, enabling them to stay up-to-date and facilitating professional development (6). Another way of updating nurses with the current literature is scientific journals, which are indispensable in increasing the quality of nursing care. Studies show that nurses who read scientific journals use research results more frequently in clinical practice and exhibit good attitudes toward evidence-based nursing (6, 12). However, in the study by Dogan et al., although nearly 90% of the nurses participated in scientific meetings, no significant correlation was found between meeting participation and AEBNQ scores (12). There are studies showing that conducting research and participating in scientific meetings are factors that positively affect nurses' attitude toward evidence-based nursing practices (6, 7, 12). Consistent with the literature, rates of reading scientific journals, attending scientific meetings on evidence-based nursing, needing evidence in practice, wanting to learn more about evidence-based nursing, and willingness to participate in an education program on evidence-based nursing were very high in the present study, and a significant correlation was found between these variables and total AEBNQ and subdimension scores. In addition, although there was no correlation between completing a graduation project/thesis or scientific research during nursing education and Beliefs and Expectations Toward Evidence-Based Nursing subdimension scores, a significant correlation was found between Evidence-Based Practice Intention, Emotions Related to Evidence-Based Nursing and total AEBNQ scores. This result shows that completing a graduation project/thesis or scientific research in undergraduate education is important in improving the attitudes of nurses toward evidence-based practice.

Limitation
The results obtained in the present study are only valid for the sample where the study was conducted. AEBNQ scores were based on nurses' own statements.

CONCLUSION
Constant advances in scientific research and the findings put forward bring dynamism to and increase the quality of nursing care. In this study, as the age of the nurses and the years of experience in the profession decreased, the obtained AEBNQ scores

increased. The majority of nurses agree that nursing care is evidence-based. Furthermore, nurses believe that evidence-based practices will strengthen the duty and authority of nurses, and they want to incorporate recent research findings into their practices. Various strategies can be proposed to strengthen evidence-based approaches in nursing practice. These include revising nursing education programs to emphasize evidence-based practices, organizing continuing education and update courses, supporting resources that will provide nurses with easy access to current research findings, creating programs that encourage research, establishing platforms that encourage experience and knowledge sharing among nurses, and adopting policies that promote evidence-based practices at the institutional level. These strategies can support nurses to provide the best care to patients and improve the quality of health services by increasing their knowledge.

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DETERMINATION OF APPROPRIATE ENDOGENOUS REFERENCE GENES FOR RT-QPCR ANALYSIS IN SYRIAN (GOLDEN) HAMSTERS AND MONGOLIAN GERBILS

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ABSTRACT

Purpose: The use of hamsters and gerbils has increased significantly in a variety of fields, including biological rhythms, reproductive biology, immunology, oncology, and many others.
Material and Methods: The most stable genes in Syrian hamsters (*Mesocricetus auratus*) and Mongolian gerbils (*Meriones unguiculatus*) were assessed using 32 reference genes for normalization in RT-qPCR analysis. Adrenal, cerebral cortex, heart, hypothalamus, kidney, liver, lung and testis tissues were used to extract and purify RNAs. GeNorm was used to determine the gene expression stabilities of 14 candidate endogenous genes from each tissue that was compatible for both animals.
Results: Under our experimental conditions, we discovered that two endogenous genes are adequate for each tissue to perform RT-qPCR normalization. There were differences in the most stable genes between species and tissues.
Conclusion: We suggest that combinations of endogenous genes ought to be carefully chosen under various experimental circumstances.

Keywords: Hamsters, gerbils, reference genes, endogenous genes, qPCR

INTRODUCTION

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) has been used for measuring nucleic acids (1). A powerful method for quantifying nucleotide-based molecular studies is RT-qPCR (2). A common technique for data normalization in gene expression studies is the use of expressed reference genes as endogenous controls (3). The guidelines of "Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)" indicate quantitative real-time PCR optimization methodologies (4).
RT-qPCR analyzes the comparison between reference genes and target genes to determine the level of relative gene expression (5). Gapdh, Actb, and 18S are frequently used universal targets for RT-qPCR, but their expression levels vary depending on the tissue. Additionally, a reference gene that is stable in one organism may be unstable in another, which may change the target gene's expression level (6). It is argued that the transcription level of universal reference genes may differ in organisms, tissues, or cell types (7,8). Algorithms such as GeNorm and NormFinder are commonly used for evaluating the stability of reference genes (9,10).
Syrian hamsters (*Mesocricetus auratus*) have advantageous features and are extensively used as animal models for studies in photoperiodism (11-14),

J Basic Clin Health Sci 2024; 8: 533-542

Gunduz B et al. References Genes in Hamsters and Gerbils
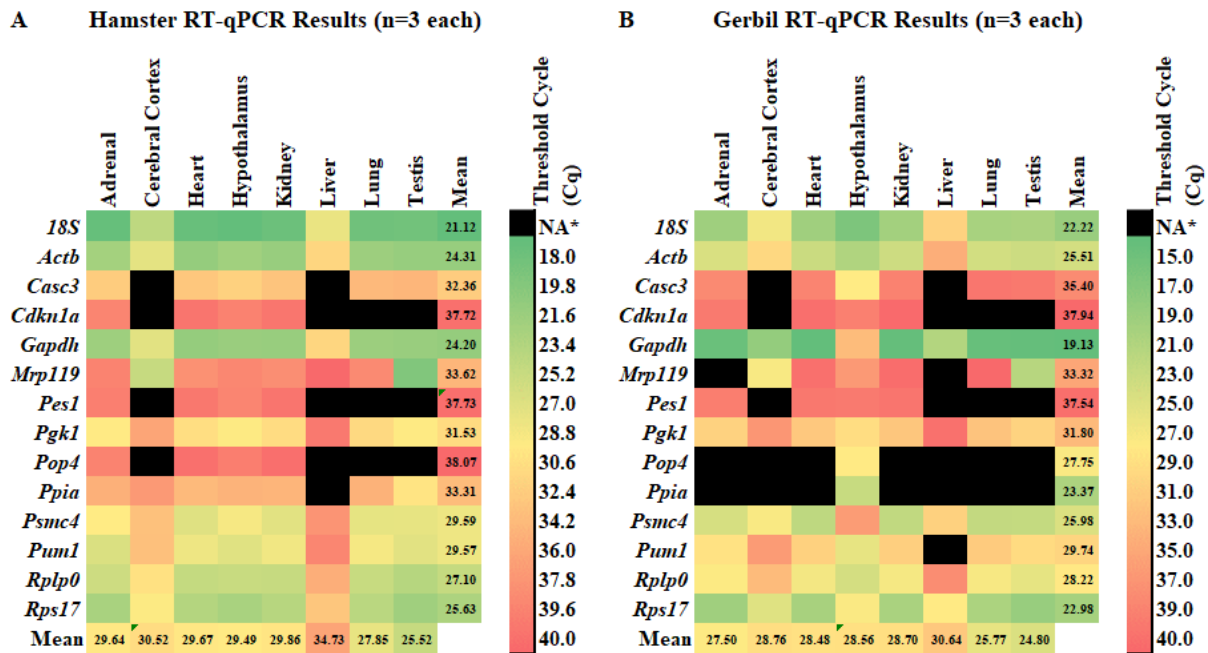
Figure 1. Heat map displays Cq values for hamster and gerbil tissues

reproduction (15,16), offspring development (17-20], infectious disease (21,22) and cancer (23). Another species used as an animal model for studies on reproduction (24,25), behavior (26-28), auditory (29,30), and inflammation (31,32) is the Mongolian gerbil (*Meriones unguiculatus*).

The limited availability of reagents for analyzing gene expression in hamsters and gerbils precludes the evaluation of transcriptional changes in physiological interactions in experimental studies of these species. In the present study, optimization of the TaqMan probe for 32 reference genes in hamster and gerbil hypothalamus was performed with RT-qPCR application. The stability rank of 14 reference genes in the adrenal, cerebral cortex, heart, hypothalamus, kidney, liver, lung, and testis was determined after analyzing RT-qPCR results with GeNorm. To properly use reference genes in future research, it was intended to identify an effective endogenous gene required for normalization in Syrian hamsters and Mongolian gerbils using RT-qPCR.

MATERIAL AND METHODS
Animals
The experiments were conducted following the ARRIVE guidelines (https://arriveguidelines.org) and guidelines of Canakkale Onsekiz Mart University and approved by the Ethical Council of Animal Research

(Date: 13.04.2011, No: 2011/04-01). The study was conducted on two species (Syrian hamster and Mongolian gerbil). The 5 male adult Syrian hamsters used in the study weighed around 100 g and were 3-4 months old. Similarly, the 5 male adult Mongolian gerbils used in the study weighed around 90 g and were 3-4 months old. There were no problems with the animals' health during the 4-week study period, so no animals were removed or added to the study. Animals were reared from birth on long photoperiod 14 L (14 h light, 10 h dark; lights off at 20:00 h) in plastic cages (16 × 31 × 42 cm) with pine shaving bedding. The lighting system was provided by cool-white, fluorescent tubes (200 lux) controlled by automated and programmable timers. Temperature of 22 ± 2°C and relative humidity of 50 ± 5% in air-ventilated rooms were provided to animals. Animals had unlimited access to water and, unless stated otherwise in the experiments, were fed (Purina Rat Chow, catalog no. 5008: fat, 17 kcal%; carbohydrate, 56 kcal%; protein, 27 kcal%) ad libitum on a 14:10 light-dark cycle. Photoperiod was provided to hamsters and gerbils both before and during the 4-week experiment. Animals were decapitated at the end of the experiment, and tissue samples were collected. As an outcome measure stability of expression in reference genes were assessed.
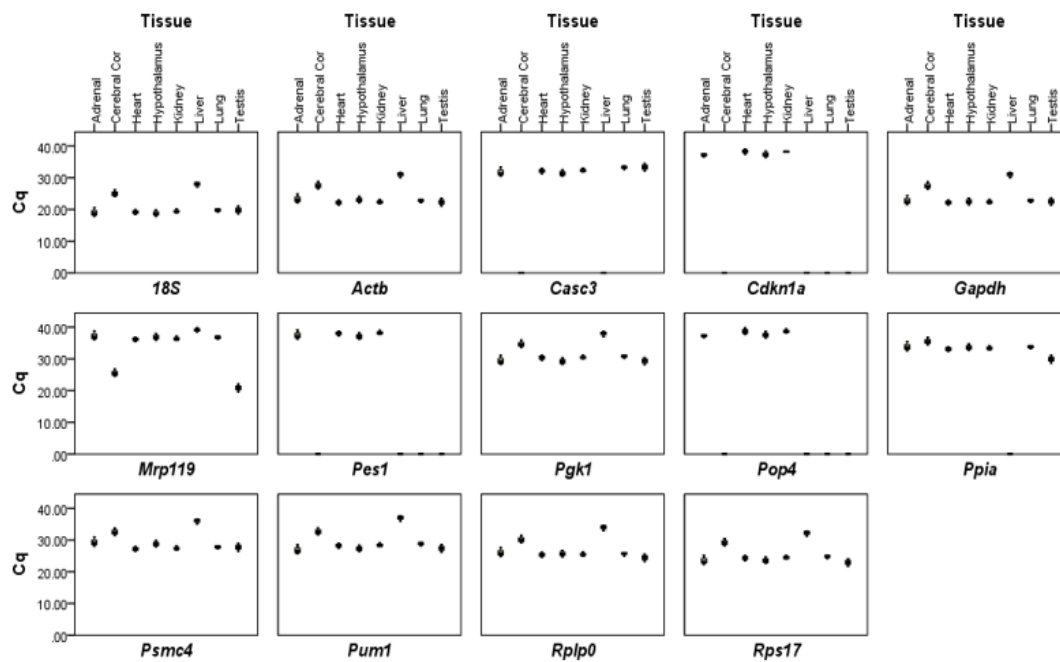
Figure 2. A boxplot chart for Cq values in hamster tissues

Tissue Collection

All animals were sacrificed by decapitation at noon (12:00 h). Immediately upon decapitation, the tissues of the adrenal cortex, cerebral cortex, heart, hypothalamus, kidney, liver, lung and testis were carefully removed without any deterioration. Tissues were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Isolation of RNA and Reverse Transcription

At the end of four weeks of ad libitum feeding, all RNAs from the adrenal cortex, cerebral cortex, heart, hypothalamus, kidney, liver, lung, and testis tissues were extracted and purified using RNA Mini Kit (PureLink) by adding DNase I (Invitrogen) in accordance with the instructions of the relevant kit. Pooling was used for each tissue and 3 randomly selected samples were studied. The samples were stored at -80ºC for a maximum of four weeks immediately after the measurement of their concentration, quality, and purity in the NanoDrop spectrophotometer (Thermo Fisher Scientific, MA, USA). In the analysis, samples with good concentration were used. Reverse transcription was performed according to the recommended procedure using the High-Capacity RNA-to-cDNA™ Kit (Applied Biosystems™). In the first step, approximately 2 μg of total RNA was added to the 20 μl reaction mixture. cDNA synthesis was carried out as in the respective

steps: 37°C for 60 min, 95°C for 5 min, and 4°C for 25 min.

Quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

RT-qPCR was performed on the StepOne instrument to measure quantitative mRNA (Applied Biosystems). Real Time PCR was performed in triplicate. In this study, there were two different approaches used. The first approach aims to understand 32 different gene probes as endogenous controls in the hypothalamus of hamsters and gerbils using a TaqMan Array Rat Endogenous Control Plate (Applied Biosystems). The second approach aims at understanding the potential of gene probes as endogenous controls in various tissues. For the second approach, gene probes are chosen based on how well they can hybridize with both animals.

To better understand their potential as endogenous genes in hamsters and gerbils, TaqMan probes for sixteen commonly used rat reference genes and sixteen human gene orthologs in rats were displayed. For each reaction, a 1:100 dilution of cDNA was applied. The reaction tube contained 5 μl of TaqMan probe master mix, 3.5 μl of RNase-free water, 1 μl of cDNA, and 0.5 μl of TaqMan probe. The reaction was carried out on a StepOne instrument (Applied Biosystems) in the following steps: 2 min. at 50°C, 10
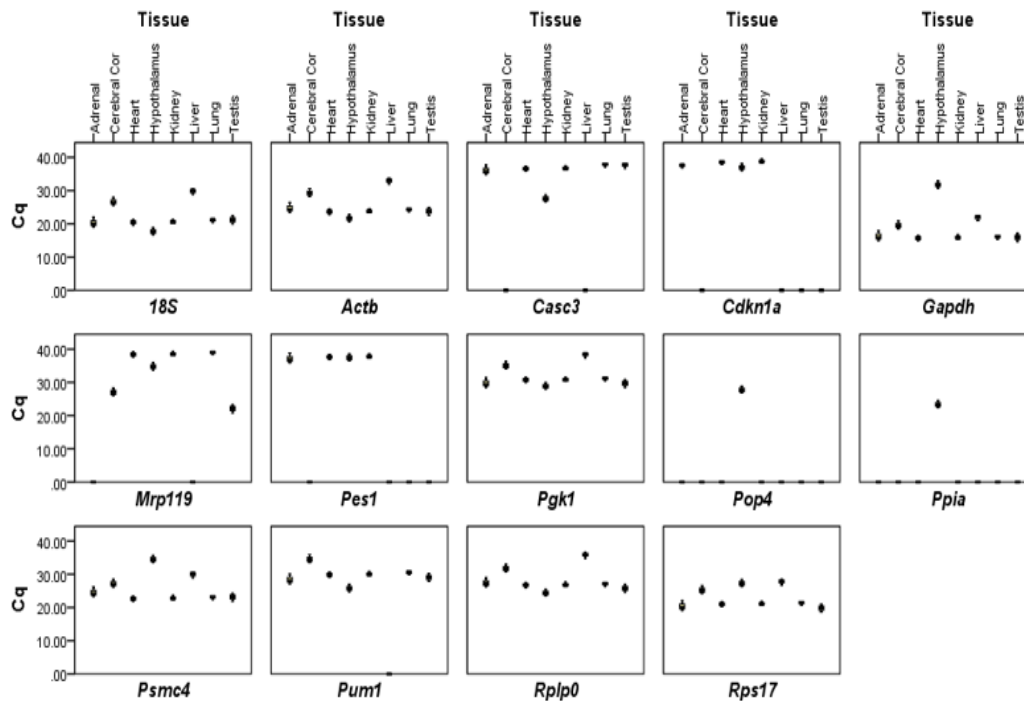
Figure 3. A boxplot chart for Cq values in gerbil tissues

min. at 95°C; 40 cycles of 15 sec. at 95°C, and 1 min. at 60°C.

Data Analysis

GeNorm (qbase+, Biogazelle) is used to determine the stability of gene expression in 14 of 32 reference genes. The mean RT-qPCR Cq value was used to determine the stability of mRNA gene expression in reference genes. GeNorm qbase+ determined the median reference stability measurement (M). The average GeNorm M value should be less than 1.5. V = 0.15 is the cutoff value for binary variation (10).

RESULTS

32 endogenous gene probes are listed in Supplementary Table 1. All gene probes were tested for their ability to be used as an endogenous control in hamsters and gerbils. While 14 of the gene probes produced a PCR product in both cases (*18S, Actb, Casc3, Cdkn1a, Gapdh, Mrpl19, Pes1, Pgk1, Pop4, Ppia, Psmc4, Pum1, Rplp0, Rps17*) 5 produced a product only in hamsters (*Gusb, Hmbs, Ppib, Rplp2, Tbp*) 5 produced a product only in gerbils (*Cdkn1b, Gadd45a, Hprt1, Rpl30, Tfrc*) and 8 produced no product at all (*Abl1, B2m, Eif2b1, Elf1, Mtatp6, Rpl37a, Ubc, Ywhaz*). Probes that produced PCR products in both species were used for further analyses in different tissues.

The heat map and boxplot charts were created for Cq values following the testing of 14 endogenous gene probes for male hamsters and gerbils on 8 different tissues (adrenal cortex, cerebral cortex, heart, hypothalamus, kidney, liver, lung and testis) using RT-qPCR (triplicate) (Figure 1, 2 and 3). All RT-qPCR reactions gave a single peak following melting curve analysis.

On male hamsters (Table 1) and male gerbils (Table 2), the average expression stability values (GeNorm M) of 14 endogenous genes are ranked from most stable (lowest GeNorm M) to least stable. To determine the optimal number of reference genes for normalization, pairwise variation (Vn/Vn+1) was used. As demonstrated, two control genes were sufficient to normalize eight tissues (Figure 4).

DISCUSSION

In this study, we evaluated the stability of gene expression in Syrian hamsters and Mongolian gerbils for 32 candidate reference genes. We demonstrated the optimal endogenous gene combination for use in 8 various tissues across two species. Additionally, the Mongolian gerbil was used as the first test subject to assess endogenous reference genes for normalization in RT-qPCR.

We evaluated mean Cq values in the hypothalamus of hamsters and gerbils. The most prevalent

Table 1. GeNorm was used to rank reference genes on hamster tissues from most stable to least stable

Ranking[a]	Adrenal	Cerebral Cortex	Heart	Hypothalamus	Kidney	Liver	Lung	Testis
1	*Psmc4*	*Rplp0*	*Pgk1*	*Pgk1*	*Casc3*	*Actb*	*Rplp0*	*Rplp0*
2	*Pgk1*	*Rps17*	*Psmc4*	*Psmc4*	*Pgk1*	*Rplp0*	*Pgk1*	*Rps17*
3	*Casc3*	*Actb*	*Pum1*	*Casc3*	*Ppia*	*Gapdh*	*Psmc4*	*Actb*
4	*Pum1*	*Gapdh*	*Rplp0*	*Pum1*	*Psmc4*	*Psmc4*	*Pum1*	*Gapdh*
5	*Rplp0*	*Psmc4*	*Casc3*	*Rplp0*	*Pum1*	*Pum1*	*Rps17*	*Psmc4*
6	*Ppia*	*Pum1*	*Ppia*	*Ppia*	*Rplp0*	*Rps17*	*Actb*	*Pum1*
7	*Mrp119*	*Pgk1*	*Pop4*	*Mrp119*	*Mrp119*	*Pgk1*	*Casc3*	*Pgk1*
8	*Pes1*	*18S*	*Rps17*	*Cdkn1a*	*Rps17*	*18S*	*Ppia*	*Ppia*
9	*Rps17*	*Mrp119*	*Mrp119*	*Pes1*	*Pes1*	*Mrp119*	*Gapdh*	*Mrp119*
10	*Actb*	*Ppia*	*Actb*	*Pop4*	*Pop4*	N[b]	*Mrp119*	*Casc3*
11	Gapdh	N[b]	*Cdkn1a*	*Rps17*	*Actb*	N[b]	*18S*	*18S*
12	*18S*	N[b]	*Gapdh*	*Actb*	*Gapdh*	N[b]	N[b]	N[b]
13	*Cdkn1a*	N[b]	*Pes1*	*Gapdh*	*18S*	N[b]	N[b]	N[b]
14	*Pop4*	N[b]	*18S*	*18S*	*Cdkn1a*	N[b]	N[b]	N[b]

[a]Depending on the results of pairwise variation, the top-ranked genes are displayed in bold [b]No available Cq data for analysis

Table 2. Ranking from most stable to least stable reference genes on gerbil tissues

Ranking[a]	Adrenal	Cerebral Cortex	Heart	Hypothalamus	Kidney	Liver	Lung	Testis
1	*Psmc4*	*Rplp0*	*Pgk1*	*Pgk1*	*Casc3*	*Actb*	*Rplp0*	*Rplp0*
2	*Pgk1*	*Rps17*	*Psmc4*	*Psmc4*	*Pgk1*	*Rplp0*	*Pgk1*	*Rps17*
3	*Casc3*	*Actb*	*Pum1*	*Casc3*	*Ppia*	*Gapdh*	*Psmc4*	*Actb*
4	*Pum1*	*Gapdh*	*Rplp0*	*Pum1*	*Psmc4*	*Psmc4*	*Pum1*	*Gapdh*
5	*Rplp0*	*Psmc4*	*Casc3*	*Rplp0*	*Pum1*	*Pum1*	*Rps17*	*Psmc4*
6	*Ppia*	*Pum1*	*Ppia*	*Ppia*	*Rplp0*	*Rps17*	*Actb*	*Pum1*
7	*Mrp119*	*Pgk1*	*Pop4*	*Mrp119*	*Mrp119*	*Pgk1*	*Casc3*	*Pgk1*
8	*Pes1*	*18S*	*Rps17*	*Cdkn1a*	*Rps17*	*18S*	*Ppia*	*Ppia*
9	*Rps17*	*Mrp119*	*Mrp119*	*Pes1*	*Pes1*	*Mrp119*	*Gapdh*	*Mrp119*
10	*Actb*	*Ppia*	*Actb*	*Pop4*	*Pop4*	N[b]	*Mrp119*	*Casc3*
11	Gapdh	N[b]	*Cdkn1a*	*Rps17*	*Actb*	N[b]	*18S*	*18S*
12	*18S*	N[b]	*Gapdh*	*Actb*	*Gapdh*	N[b]	N[b]	N[b]
13	*Cdkn1a*	N[b]	*Pes1*	*Gapdh*	*18S*	N[b]	N[b]	N[b]
14	*Pop4*	N[b]	*18S*	*18S*	*Cdkn1a*	N[b]	N[b]	N[b]

[a]Depending on the results of pairwise variation, the top-ranked genes are displayed in bold [b]No available Cq data for analysis

endogenous genes used for RT-qPCR normalization are 18S, Gadph, and Actb, which have higher expression levels than other endogenous genes in the hypothalamus of hamsters. However, only the expression of the 18S and Actb genes was noticeably higher in the hypothalamus of gerbils.

We obtained mean Cq values from the hypothalamus in the first phase of our research. The hypothalamus is thought to be the most important nervous system organizing region. All physiological systems in photoperiodic species, like hamsters and gerbils, are controlled by a circadian rhythm. The suprachiasmatic nucleus (SCN), also known as the biological clock and found in the hypothalamus, is the most significant neuron group in this rhythmic arrangement. Although rhythmic regulation in photoperiodic animals can be explained more clearly through the reproductive system, many physiological mechanisms of the organism (eating-drinking rhythm, hormonal rhythmic regulation, immunological responses, stress differences during the day, and so on) are rhythmically controlled. In photoperiodic animals, the critical photoperiod is crucial. For the Syrian hamster, this critical photoperiod is 12.5 hours, while for the gerbil, it is 10 hours (33-35). The hours above this critical photoperiod are important for normal reproduction and the proper operation of the animal's physiological systems. Nearly all of an
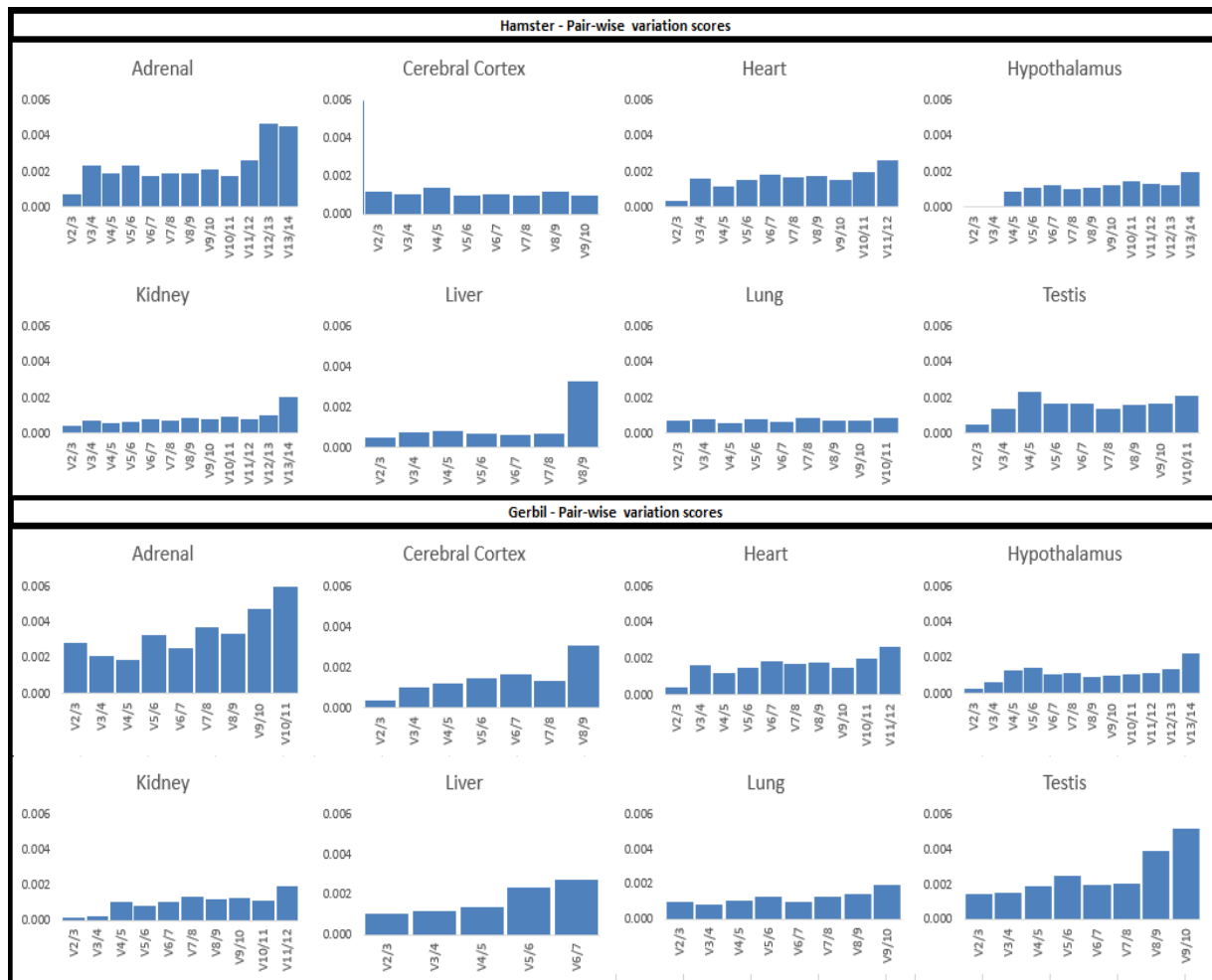
Figure 4. GeNorm V pair-wise variation scores for 8 different tissues were calculated to determine the ideal number of reference genes needed for normalization. (A) Vn/n+1 results for the hamster tissues. (B) Vn/n+1 results for the gerbil tissues. For stability, a GeNorm V threshold of less than 0.15 was chosen. The optimal number of control genes required for RT-qPCR normalization was determined to be two for all tissues in both species.

animal's physiological systems change (for example, adapting to winter conditions) and reproductive abilities decline during the hours below this critical photoperiod (36-43). The 14L photoperiod was chosen for our study because it would be the most suitable for both species. 32 endogenous genes were examined in hamster and gerbil species with distinct photoperiodic characteristics, focusing mainly on the hypothalamus.

We determined that *Psmc4/Pgk1, Rplp0/Rps17, Pgk1/Psmc4, Pgk1/Psmc4, Casc3/Pgk1, Actb/Rplp0, Rplp0/Pgk1 and Rplp0/Rps17* pairs in hamsters and *Rplp0/Pum1, 18S/Actb, Rplp0/Pum1, Pop4/Pgk1, Rplp0/Actb, Psmc4/18S, Actb/Rplp0,* and *Actb/Psmc4* pairs in gerbils, respectively, are the most stable reference genes for normalization in the following tissues: adrenal, cerebral cortex, heart, hypothalamus, kidney, liver, lung, and testis. Actb is the most commonly used reference gene for RT-qPCR normalization, but in our study, Actb was only the most stable gene in the livers of hamsters and the lungs and testis of gerbils. Furthermore, Actb was found to be the least stable in rats' hypothalamus and intestine (44). As a result, using Actb solely as a reference gene may lead to a misunderstanding of the expression levels of target genes in specific tissues. In the same tissues, stable reference genes may vary depending on the type of experiment (44). In addition, a recent study found that the gene expression of Actb varied among Syrian hamster tissues (21). In another study, different reference genes for aortic tissue in Syrian Hamsters were proposed (45). In a microarray analysis of Chinese Hamster ovary tissue, it was proposed species-specific reference genes (46). These findings

538

J Basic Clin Health Sci 2024; 8: 533-542

Gunduz B et al. References Genes in Hamsters and Gerbils

highlight the significance of selecting appropriate reference genes in future studies.

It was suggested that reference genes be used for normalization in RT-qPCR studies (10) but it is not feasible to analyze many endogenous reference genes in a study. According to our research, reference gene expression levels vary between species and tissues. According to our study's GeNorm analysis, pairwise variation results showed that two reference genes are adequate for RT-qPCR normalization in our experimental settings. It was strongly advised to choose appropriate reference genes in every experimental setting to accurately assess the target gene expression level in RT-qPCR analysis. For example, hamsters and gerbils are photoperiodic animals that are used in a variety of research studies. The limitation of the study was that only long photoperiod/normal conditions for these species were considered in the design of the study. In subsequent research, it would be appropriate to test the study under different conditions (food restriction, different ambient temperature, and different day cycles).Understanding the physiological mechanisms underlying the numerous species-specific diseases may be improved by identifying subtle variations in gene expression. Therefore, it is important to carefully choose the right reference genes for each unique experimental setting.

CONCLUSION

We compared two species for the first time in this study in terms of reference gene normalization for RT-qPCR analysis. Research revealed that the expression of reference genes may vary depending on the species and the tissue. Therefore, to identify differences that might arise because of sex differences, candidate reference genes in female Syrian hamsters and Mongolian gerbils should also be clarified. The findings pave the way for further research into the expression levels of various reference genes in various experimental settings (i.e. feeding types, and different photoperiodical conditions).

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Supplementary File

Determination of Appropriate Endogenous Reference Genes for RT-qPCR Analysis in Syrian (Golden) Hamsters and Mongolian Gerbils

Supplementary Table 1. List of 32 TaqMan endogenous control gene probes

Gene Symbol	Gene Name	Assay ID
18S	Eukaryotic 18S rRNA	Hs99999901_s1
Abl1	ABL proto-oncogene 1, non-receptor tyrosine kinase	Rn01436238_g1
Actb	Actin, beta	Rn00667869_m1
B2m	Beta-2-microglobulin	Rn00560865_m1
Casc3	Cancer susceptibility candidate 3	Rn00595941_m1
Cdkn1a	Cyclin-dependent kinase Inhibitor 1A	Rn00589996_m1
Cdkn1b	Cyclin-dependent kinase Inhibitor 1B	Rn00582195_m1
Eif2b1	Eukaryotic translation eukaryotic translation initiation factor 2B, subunit 1 alpha	Rn00596951_m1
Elf1	E74-like factor 1	Rn00585356_m1
Gadd45a	Growth arrest and DNA-damage-include, alpha	Rn00577049_m1
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	Rn99999916_s1
Gusb	Glucuronidase, beta	Rn00566655_m1
Hmbs	Hydroxymethylbilane synthase	Rn00565886_m1
Hprt1	Hypoxanthine phosphoribosyltransferase 1	Rn01527840_m1
Mrpl19	Mitochondrial ribosomal protein L19	Rn01425270_m1
Mtatp6	Mitochondrially encoded ATP synthase 6	Rn03296710_s1
Pes1	Pescadillo homolog 1	Rn01443731_g1
Pgk1	Phosphoglycerate kinase	Rn00821429_g1
Pop4	Processing of precursor 4, ribonuclease P/MRP subunit (S. cerevisiae)	Rn02347225_m1
Ppia	Peptidylprolyl isomerase A (cyclophilin A)	Rn00690933_m1
Ppib	Peptidylpropyl isomerase B	Rn00574762_m1
Psmc4	Proteasome (prosome, Macropain) 26S subunit, ATPase 4	Rn00821605_g1
Pum1	Pumilio RNA binding family member 1	Rn00982780_m1
Rpl30	Ribosomal protein L30-like	Rn01504620_g1
Rpl37a	Ribosomal protein L37a	Rn02114291_s1
Rplp0	Ribosomal protein, large, P0	Rn00821065_g1
Rplp2	Ribosomal protein, large P2	Rn01479927_g1
Rps17	Ribosomal protein S17	Rn00820807_g1
Tbp	TATA box binding protein	Rn01455648_m1
Tfrc	Transferrin receptor	Rn01474695_m1
Ubc	Ubiquitin C	Rn01789812_g1
Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	Rn00755072_m1

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PREGNANT WOMEN'S PERCEPTIONS OF PRENATAL ATTACHMENT AND BREASTFEEDING SELF-EFFICIENCY: A CROSS-SECTIONAL STUDY

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ABSTRACT

Purpose: The aim of this study is to assess pregnant women's perceptions of prenatal attachment and breastfeeding self-efficacy.
Material and Methods: The aim of this study is to assess pregnant women's perceptions of prenatal attachment and breastfeeding self-efficacy.
Results: It was found that the Prenatal Attachment Inventory (PAI) total mean score of the pregnant women was 54.44±14.67 and the prenatal breastfeeding self-efficacy scale (PBSES) mean score was 68.32±13.65. There was a positive correlation between PAI and PBSES scores of the pregnant women (p<0.0001, r=0.817).
Conclusion: Some sociodemographic and obstetric characteristics of the pregnant women were determined to affect both prenatal attachment and prenatal breastfeeding self-efficacy levels.

Keywords: Breastfeeding self-efficacy, pregnant, prenatal attachment, prenatal

INTRODUCTION

Pregnancy is a one-of-a-kind moment in a woman's life, and it is a physiological and healthy life event that she goes through during her reproductive years. The woman's acceptance of pregnancy, fetus, and parenthood continues throughout the pregnancy, and she begins to consider what she should do to give birth to a healthy baby in her second trimester and experiences a sense of creative energy in this process (1). The first significant maternal sentiments for the child during pregnancy are referred to as attachment, and they provide the groundwork for attachment theory. Attachment is a powerful bond between two individuals, and attachment that begins during the prenatal period enables the baby to continue his life with the mother reliably after delivery (2). Attachment, founded on a sense of trust between the baby and the mother, is a significant notion that develops long before birth and influences postnatal attachment (2, 3). Prenatal attachment is the cognitive, emotional, and behavioral attachment between a pregnant woman and the fetus. Infants of pregnant women with a high level of attachment during this period show healthier behaviors in a positive way (3).Feeling the baby's movements, recognizing him as an individual, and ultrasound follow-ups are all factors that increase the mother's attachment to her child throughout the prenatal period (4). Prenatal attachment is critical for the growth and health of the infant (5).

Table 1. Comparison of Prenatal Attachment Mean Scores and Socio-Demographic-Obstetric Characteristics of the Pregnant Women

Socio-Demographic Characteristics (n =320)	n(%)	X±SD	Test and p value
Education level			
Primary school	127(39.7)	51.76±16.51	
Secondary school	75(23.4)	52.41±14.04	12.170*
High school	69(21.6)	53.79±12.13	0.001‡
University	49(15.3)	65.38±7.41	
Employment status			
Employed	32(10)	63.50±10.37	3.755†
Unemployed	288(90)	53.43±14.75	0.001‡
Perceptions of Income status			
Income less than expenses	65(20.3)	50.30±15.99	19.257*
Income equal to expenses	199(62.2)	52.90±14.17	0.001‡
Income more than expenses	56(17.5)	64.67±9.65	
Gravidity			
1	124(38.8)	68.06±12.39	
2	67(20.9)	71.34±13.62	2.752*
3	56(17.5)	69.62±13.04	0.05‡
4 and more	73(22.8)	65.01±15.55	
Status of planning pregnancy			
Planned	207(64.7)	71.51±12.76	5.953†
Unplanned	113(35.3)	62.48±13.34	0.05‡
Gestational Week			
20-27 weeks	46(14.4)	75.21± 9.89	3.775†
28-40 weeks	274(85.6)	67.17±13.86	0.05‡
Gender of infants			
Girl	124(38.8)	68.95±13.62	5.853*
Boy	176(55.0)	66.84±13.66	0.05‡
Unknown	20(6.2)	77.50± 9.79	

Bold values indicate a statistically significant difference. X: arithmetic mean; SD: standard deviation. *: One-way anova test †: t Test ‡: Significance (p value)

The future of society continues with the existence of healthy individuals and the healthy birth of children. Breastfeeding is one of the most important factors in building a healthy individual and society for children. Breastfeeding, according to UNICEF, not only gives all children a better start in life but also leads to fewer healthcare expenses, healthier families, and a more competent workforce (6).To promote breastfeeding, WHO underlines that, although breast milk is the baby's first vaccination after birth, babies should only be breastfed for the first six months and up to two years (7). Breastfeeding self-efficacy shapes the mother's expectations, competence, behaviors, tasks to be undertaken, and confidence in breastfeeding. Four significant sources affect mothers' expectations of breastfeeding self-efficacy: Performance accomplishments (past breastfeeding experiences), vicarious experiences, verbal persuasion (others' encouragement), and psychological responses

(anxiety, fatigue) (8). Breastfeeding self-efficacy pertains to a mother's perceived ability to breastfeed her baby and the mother may be encouraged to breastfeed using interventions to promote breastfeeding throughout the prenatal period. The length of breastfeeding can be predicted. In their study, Şenol and Pekyiğit (9) determined that the training given during the prenatal period increased the pregnant women's perception of prenatal breastfeeding self-efficacy. In this context, assessing breastfeeding self-efficacy and providing training in the prenatal period may be beneficial in improving maternal self-efficacy in the transition to parenthood to evaluate interventions and assure the mother's orientation to breastfeeding (10).

In this case, starting midwife and nurse-led training and practices during the prenatal period contributes to the healthy progression of mother-newborn attachment, promotes breastfeeding and neonatal

Table 2. Comparison of Socio-Demographic-Obstetric Characteristics and Prenatal Breastfeeding Self-Efficacy Mean Scores of The Pregnant Women

Socio-Demographic Characteristics (n =320)	n(%)	X±SD	Test and p value
Education level			
Primary school	127(39.7)	66.14±14.89	
Secondary school	75(23.4)	65.45±12.52	12.920*
High school	69(21.6)	68.11±11.22	0.001‡
University	49(15.3)	78.69±10.05	
Employment status			
Employed	32(10)	76.09±13.01	3.450†
Unemployed	288(90)	67.46±13.46	0.05‡
Perceptions of Income status			
Income less than expenses	65(20.3)	65.01±14.07	20.342*
Income equal to expenses	199(62.2)	66.62±12.98	0.001‡
Income more than expenses	56(17.5)	78.21±10.95	
Gravidity			
1	124(38.8)	68.06±12.39	2.752*
2	67(20.9)	71.34±13.62	0.05‡
3	56(17.5)	69.62±13.04	
4 and more	73(22.8)	65.01±15.55	
Status of planning pregnancy			
Planned	207(64.7)	71.51±12.76	5.953†
Unplanned	113(35.3)	62.48±13.34	0.05‡
Gestational Week			
20-27 weeks	46(14.4)	75.21± 9.89	3.775†
28-40 weeks	274(85.6)	67.17±13.86	0.05‡
Gender of infants			
Girl	124(38.8)	68.95±13.62	5.853*
Boy	176(55.0)	66.84±13.66	0.05‡
Unknown	20(6.2)	77.50± 9.79	

Bold values indicate a statistically significant difference. X: arithmetic mean; SD: standard deviation. *: One-way anova test †: t Test ‡: Significance (p value)

care, and contributes to the development of healthy persons in all aspects in the future (11). Furthermore, Demir and Taspınar (12) found that prenatal breastfeeding education significantly affects breastfeeding success and duration, highlighting the importance of early intervention. Similarly, Selvi et al., (13) demonstrated that targeted educational interventions during pregnancy enhance breastfeeding self-efficacy in expectant mothers. These studies support the notion that prenatal education can positively influence breastfeeding outcomes, emphasizing the originality of our research in exploring specific training methodologies and their impact on maternal self-efficacy. This study assessed pregnant women's perceptions of prenatal attachment and breastfeeding self-efficacy.

MATERIAL AND METHODS
Study Design
The study was conducted as descriptive cross-sectional type. The research was approved by the Faculty of Health Sciences Ethics Committee of Atatürk University (Date: 30.12.2016, No: 2016/12/05) and institutional permission from Erzurum Provincial Health Directorate were obtained before the study. All participants provided informed consent in accordance with the Declaration of Helsinki. They were guaranteed the right to withdraw from the research at any time and the confdentiality of their personal information.

Population and Sample of the Study
The population consisted of pregnant women (being

Table 3. The Distribution of The Possible Lowest and Highest Scores, Obtained Lowest and Highest Scores and Mean Scores of The Pregnant Women for Pai and Pbses/ Correlation between scales mean scores

Scale	Possible Lowest and Highest Scores of the scales	Lowest and Highest Scores they obtained from the scales	Mean scores of the scales, X±SD
PAI	21-84	25-84	54.44±14.67
PBSES	20-100	36-98	68.32±13.65
Scales	**PBSES**		
PAI	**0.817ⵂ**		
	0.000‡		

X: arithmetic mean; SD: standard deviation. *$p<0.0001$ ‡: Significance (p value) ⵂ: Spearman's correlation analysis.

over the 20th gestational week, having a single and healthy fetus, being open to communication, having no mental disorder, and having spontaneous conception) who came to the outpatient clinic for prenatal control examinations at a Maternity Hospital in Turkey between 01 February 2017 and 29 December 2017. In the calculation in the G.Power program, the number of samples for the One-Way Anova test was calculated as 280, taking into account the effect size (d = 0.25), 5% margin of error (α = 0.05), and 95% power (1-β = 0.95). Considering that the article might cause data loss, it started with a sample of 308 pregnant women with a 10% increase and ended with a sample of 320 pregnant women. The data were obtained by conducting face-to-face interviews. It takes 15–20 min to complete each questionnaire.

Data Collection Tools
A Personal Information Form, Prenatal Attachment Inventory (PAI), and Prenatal Breastfeeding-Self-Efficacy Scale (PBSES) were employed to collect data. The study data were collected with a 13-question information form containing demographic and obstetric data.The PAI is a 4-point Likert-type scale with 21 items. Mary Muller developed the PAI in 1993 (14). The Cronbach's Alpha reliability coefficient of the scale was reported by Dereli Yılmaz and Kızılkaya Beji (15) to be 0.84. The Cronbach's alpha reliability coefficient of the scale was determined to be 0.95 in this study. The PBSES is a 5-point Likert-type scale with 20 items. Wells et al. (16) developed the scale in 2006 to determine the breastfeeding self-efficacy perceptions of pregnant women in the prenatal period. Aydın (17) conducted the scale's Turkish validity and reliability study in 2016, and

Cronbach's alpha coefficient was determined to be 0.85. The Cronbach's alpha reliability coefficient of the scale was determined to be 0.95 in this study.

Data Analysis
The SPSS 24.0 program was used to analyze the data. Percentage distributions, mean, t-test, One-Way ANOVA, and Pearson Correlation Analysis were used to assess the data. The Bonferroni test was applied as a further analysis in the One-Way ANOVA analysis.

RESULTS
Socio-demographic data of pregnant women are given in Table 1. Table 1 compares the socio-demographic and obstetric characteristics of the pregnant women with their prenatal attachment mean scores. It was found that the PAI mean scores of pregnant women with different levels of education, income, and employment status were significant (p<0.001). The PAI mean scores of the pregnant women increased as did their education levels and income levels increase. Furthermore, a significant difference was identified between the gravidity, previous pregnancy status, pregnancy planning status, gestational week, and gender of the infant and the PAI mean score (p<0.05; Table 1).
Table 2 compares the socio-demographic and obstetric characteristics of pregnant women with the PBSES mean scores. The PBSES mean scores differed significantly by employment (p<0.05), education and income status (p<0.001). Also, there was a significant difference between the planning status of the pregnancy, the gravidity, gender of the infant, and the mean score of the gestational week

and the PBSES mean score (p<0.05) were significantly different (Table 2).

The pregnant women included in the study had minimum and maximum PAI scores of 25 and 84, respectively, and their mean score was 54.44±14.67. The minimum and maximum PBSES scores were 36 and 98, respectively, and their mean score was 68.32±13.65 (Table 3).

The correlation between the scores of the pregnant women on PAI (*Mean*=54.44, SD=14.67) and PBSES (*Mean*=68.32, SD=13.65) was measured by Pearson correlation. A high, positive, and significant correlation was found between these variables (r(n)= .817, p=0.000; Table 3).

DISCUSSION

The PAI mean score of the pregnant women in this study was 54.44, which was moderate. The study's findings were similar to those of some previous studies (5, 15, 18). Contrary to the study, some studies report low PAI mean scores (4, 11).

The PBSES mean scores of pregnant women were 68.32. The present study is compatible with previous studies, reporting mean scores of 73.5, 68.08, and 73.4, respectively (19, 20). When the studies were examined, it was observed that pregnant women had high perceptions of breastfeeding self-efficacy.

The PAI mean score of the pregnant women increased as their education level and income status increased. Numerous studies have shown that attachment grows as pregnant women's education and income increase (21, 22). Based on these findings, it can be thought that pregnant women may become more conscious of and get attached to their babies as their education level rises. It can also be considered that the rise in the income level may have directed the attention of pregnant women to their babies without deviating to the socio-economic level. The present study indicated that the attachment levels of employed pregnant women were higher than those who were unemployed. In their research, Kartal and Karaman (18) found that employed pregnant women had a high level of prenatal attachment. Contrary to the study findings, some studies indicated that the prenatal attachment mean score of unemployed pregnant women was lower (4, 11). At the same time, the literature reported high levels of attachment in employed pregnant women (14, 21). The present study was found to be compatible with the literature. This may be attributed to disparities in

the social lives and economic freedom of employed pregnant women.

The study found that those who had a planned pregnancy had higher levels of attachment than those who did not. A few studies have also identified a significant correlation between planned pregnancy progression and attachment (3, 15, 23). The results of the present study are similar to the literature, suggesting that there is a positive correlation between the psychological preparedness for pregnancy, the healthy pregnancy process, and attachment levels of the pregnant woman.

As the education level of the pregnant women increased, so did their levels of PBSES. Further analysis revealed that the university graduate group caused the difference. In the study conducted by Aydın (17), it was stated that there was a significant correlation between the educational level and prenatal breastfeeding self-efficacy mean scores. When examining other studies, it was determined that the perceptions of PBSES were high in women with a high level of education, who were employed, and who had planned pregnancies (9, 20). Although the present study is compatible with the literature, it was observed that as mothers' education levels increased, they behaved more consciously, and their tendency to breastfeed increased.

Employed pregnant women had a higher mean score of PBSES than unemployed pregnant women in the study. Numerous studies have reported that as the increase in the socio-economic level of the mothers reduces anxiety, it also increases the perception of PBSES (9, 20).

A statistically significant difference was found between the pregnant women's income level and prenatal PBSES (p<0.001). Further analysis indicated that the group with more income than their expenses produced the difference. The study by Aydın (17) reported that pregnant women with a high income level had a higher perception of PBSES than pregnant women with a low income level. It is believed that providing pregnant women with a high or good income with easy access to health services and educational materials may be effective in breastfeeding self-efficacy.

It was observed that the gestational week and the gender of the fetus affected the perception of PBSES. Contrary to the study, they observed that some gestational weeks and gender affect the mean PBSES score (17, 20). Also, Baysal et al. (24)

reported in their study that the infant's gender did not affect breastfeeding. The difference between the results is considered to be associated with the characteristics of the pregnant women in the sample group. Further analysis of the infant's gender determined that the group who did not know the gender caused the difference. The high prenatal breastfeeding self-efficacy mean score of pregnant women who did not know the gender of the infant was thought to be associated with the fact that the infant's health condition was prioritized rather than gender.

When the correlation between the mean scores of the scales used in the study was assessed, it was found that there was a positive correlation between prenatal attachment and PBSE. As the attachment levels of the pregnant women increased, so did their PBSE.

CONCLUSION

It was determined that the prenatal attachment of the pregnant women was moderate, and their breastfeeding self-efficacy perceptions were at a reasonable level. Given the results of the study, it is recommended to provide counseling to pregnant women in the prenatal period. By identifying risky groups in the prenatal period of midwives and nurses providing health services to pregnant women and providing breastfeeding education by midwives and nurses in line with the needs of pregnant women, midwives, and nurses will reduce the anxiety of pregnant women in this period and increase their self-efficacy perceptions. The increased prenatal attachment is essential for the mother and infant to have a healthy postpartum period.

Practical Recommendations for Researchers and Practitioners

- Counselling and Support: Midwives and nurses should offer regular counselling sessions for pregnant women to discuss their concerns and provide emotional support.
- Tailored Education Programs: Develop and implement breastfeeding education programs that address the specific needs of pregnant women, particularly those identified as high-risk.
- Early Identification of Risk Groups: Utilize screening tools to identify pregnant women who may need additional support and education regarding breastfeeding.
- Ongoing Training for Healthcare Providers: Ensure that midwives and nurses receive continuous training on the latest breastfeeding

techniques and counselling methods to better support pregnant women.
- Enhancing Prenatal Attachment: Encourage activities that promote prenatal attachment, such as prenatal bonding exercises and parental education sessions.

By incorporating these practices, healthcare providers can enhance the prenatal attachment and breastfeeding self-efficacy of pregnant women, leading to improved outcomes for both mothers and infants.

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ADOLESCENT FOOD PARENTING QUESTIONNAIRE: EVALUATION OF TURKISH PSYCHOMETRIC PROPERTIES OF PARENT AND ADOLESCENT VERSIONS

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ABSTRACT

Purpose: This study aimed to adapt the "Adolescent Food Parenting Questionnaire: Parent and Adolescent Version" into Turkish and conduct validity and reliability studies.
Material and Methods: The research was carried out in methodological type and was carried out with 337 adolescents aged 12-16 years and their parents between June to September 2022. Data were collected with the Child and Parent Information Form and Adolescent Food Parent Questionnaire. Content validity, construct validity, internal consistency reliability, and item analysis were used to determine the questionnaire's psychometric properties.
Results: Both parent and adolescent versions of the Adolescent Food Parenting Questionnaire contain 16 items. The results of the exploratory factor analysis determined that the five-factor structure explained 50.45% of the total Variance for the parent version and 63.31% of the total Variance for the adolescent version. In addition, item factor loads for the parent version ranged from 0.32 to 0.71, while item factor loads for the adolescent version ranged from 0.37 to 0.75. As a result of confirmatory factor analysis, the goodness of fit values of the parent version were χ2/sd=2.030, RMSEA=0.055, GFI=0.94, CFI=0.95, IFI=0.95, RFI=0.87, NFI=0.90, TLI =0.93, and the adolescent version were χ2/sd=1.672, RMSEA = 0.045, GFI = 0.95, CFI = 0.96, IFI = 0.96, RFI = 0.88, NFI = 0.91, and TLI = 0.95. The Cronbach alpha value for the entire parent version was 0.86, and the Cronbach alpha values for all sub-dimensions were determined to vary between 0.60 and 0.84. The adolescent version's overall cronbach alpha was determined to be 0.88, and the adolescent version's sub-dimension Cronbach alpha values were discovered to vary between 0.63 and 0.841.
Conclusion: As a result of the research, it was determined that the "Adolescent Food Parenting Questionnaire: Parent and Adolescent Version" is a valid and reliable measurement tool to be used specifically for Turkish society.

Keywords: Adolescent, food parenting practices, nutrition, validity, reliability

INTRODUCTION

Depending on the developmental stages children go through from birth to adolescence, their nutritional requirements, eating behaviors, and lifestyles may vary (1). For various reasons, unmet nutritional needs have a lifelong impact. In addition to individual

Table 1. Participants' descriptive characteristics

Descriptive characteristics	M±SD	Min-Max
Adolescent's age	13.22+3.82	12-16
Mother's age	41.48+7.37	25-55
Father's age	45.99+7.22	28-59
	n	**%**
Number of children in the family		
1	74	22.0
2	111	32.9
3	79	23.4
4	73	21.7
Gender		
Female	198	58.8
Male	139	41.2
Mother's educational status		
Illiterate	35	10.4
Primary school	58	17.2
Middle school	67	19.9
High school	110	32.6
University	60	17.8
Master's/PhD	7	2.1
Father's educational status		
Illiterate	8	2.4
Primary school	50	14.9
Middle school	38	11.3
High school	105	31.2
University	122	36.2
Master's/PhD	7	2.1
Income		
Income=expenses	172	51.0
Income>expenses	44	13.1
Income<expenses	121	35.9
Mother's employment status		
Working	65	19.3
Housewife	272	80.7
Father's job status		
Working	276	81.9
Not working	45	13.4
Retired	16	4.7
Participants' self-evaluation of weight		
Underweight	74	22.0
Normal	199	59.1
Overweight	64	19.0
Participants' self-evaluation of height		
Short	96	28.5
Normal	197	58.5
Tall	44	13.1
Participants' self-evaluation of having an adequate diet		
No	155	46.0
No idea	182	54.0

M= Mean, SD: Standart Deviation

factors, environmental and parental factors are also mentioned in the malnutrition of children and adolescents (2-6). Dietary and physical activity patterns that develop during childhood and adolescence become behavioral patterns that can affect life as a whole, but they can also affect life following adolescence (3-5). Therefore, children and adolescents must develop healthy eating behaviors (1). Adolescence is the most intricate and vibrant transitional period in life or at least one of them. During this period, some changes, namely, developmental, social, and physical may impact the adolescent's eating behaviors, causing them to be underweight or overweight (1,7,8).

Although adolescent food consumption and lifestyle choices have become more independent, most adolescents still rely on their parents for food (4, 9, 10). But even so adolescents are likely to be

susceptible to parental food choices regarding dietary behavior, food availability, setting limits, and modeling parental behavior (2, 4). However, parents have a crucial part to play in adolescent eating behaviors (9, 10) and weight status (11-13). Parents' perspectives on food and nutrition directly or indirectly affect their children's food preferences (9, 10). There may be a direct effect on the adolescent's food preference and eating behaviors, with the food not preferred by the adolescent being offered to him/her less frequently. An adverse reaction of the parents against a food may cause the adolescent to repeat that behavior by taking the food as a model (11-13). Therefore, adolescents' eating and nutritional habits cannot be considered apart from the influence of parents, who play a significant role in their development as they are adolescents' immediate primary environment (2). Food parenting practices affect healthy behavior in children and adolescents (6).

Parents' behaviors or actions specific to child feeding and affecting child eating practices are referred to as food parenting practices (5). In other words, they are context-specific parenting actions of food and eating designed for socializing children against particular behaviors. The behaviors of eating practices between parents and children reveal the general characteristics of these interactions (3, 5).

Typical food parenting actions are limiting some food types, shaping eating behaviors as a role model, making children eat, recompensing them for affirmative eating behaviors, and identifying readily available foods at home. Recent research on the examination of the nutritional environment at home suggests that parents can positively influence their children's body weights and eating habits by presenting and modeling healthy foods (6, 14). Food-related parenting practices, such as directing children to healthy foods and restricting their intake of foods with high energy content, have been identified as important determinants of children's eating behavior and body weight (12-15).

In addition, there may be a difference between adolescents' and parents' perceptions of food parenting. For example, a parent may make a great effort to encourage the child to eat vegetables, but the child may take the example of the parent eating snacks in front of the TV (12, 16). Parents and children frequently own distinct perspectives about eating-associated behavior and weight. Therefore, it is essential to understand their views of food parenting (12).

When studies on the evaluation of parents' and adolescents' perspectives on food parenting practices were examined, it was seen that there were very few standard scales with international validity and reliability (17). Moreover, it was found that there were no measurement tools to measure adolescent food parenting practices in terms of adolescents and parents. Making international comparisons requires scales with reliability and validity. Therefore, this study was carried out to do the Turkish adaptation of the Adolescent Food Parenting Questionnaire: Parent and Adolescent Version, whose original form was in the English language, and its reliability and validity studies.

MATERIAL AND METHODS
Research Design
A methodological design was employed.

Research Population and Sample
Adolescents aged 12 to 16 from Turkey's western, central, and eastern regions and their parents participated in the study between June to September 2022. When calculating the sample size in measurement tool adaptation studies, the size is recommended to be five to ten times the total count of items on the tool (18,19). The tool to be adapted in the present study had 16 items, and thus the sample was planned to include 160 participants, which is ten times the number of items.But, the study included adolescents and their parents who could be reached from the population using convenience sampling and who volunteered to participate. The study included 337 adolescents and their parents.

Data Collection Tools
A Child and Parent Data Form, which was designed by the researchers following a review of the literature, and the Adolescent Food Parenting Questionnaire: Parent and Adolescent Version were employed.
Child and Parent Data Form: This form was designed to be filled out by parents and adolescents to collect socio-demographic data. It has two parts: family and child. The family part has items about the mother's and father's age, education level, job, economic status, and number of children. The child part has

Table 2. Participants' descriptive characteristics

Item No.	Sub-Dimensional Factor Loads				
	Autonomy Support	Coercive Control	Aperative Structure	Healthy Structure	Modeling
PI1. I educate my child about nutrition for example talking about healthy and unhealthy food.	0.71				
AI1. My parents educate me about nutrition, for example talking about healthy and unhealthy food.	0.69				
PI2. I explain why I have certain rules about eating to my child.	0.69				
AI2. My parents explain why they have certain rules about eating to me.	0.75				
PI3. There are always fruit and vegetables at home for my children to eat.				0.59	
AI3. There are always fruit and vegetables at home for me to eat.				0.55	
PI4. I sometimes give my child something to eat as a distraction.		0.32			
AI4. My parents sometimes give me something to eat as a distraction.		0.37			
PI5. I give my child feedback related to their eating habits, for example if my child eats too quickly or doesn't eat enough vegetables.	0.64				
AI5. My parents give me feedback related to my eating habits, for example if I eat too quickly or don't eat enough vegetables.	0.69				
PI6. At home my child can easily eat vegetables as they are part of our daily meals.				0.63	
AI6. At home I can easily eat vegetables as they are part of our daily meals.				0.63	
PI7. I sometimes give my child something to eat as a reward.		0.39			
AI7. My parents sometimes give me something to eat as a reward.		0.45			
PI8. I let my child snack if he/she wants to.			0.55		
AI8. My parents let me snack if I want to.			0.42		
PI9. I discuss why it is important to eat fruit and vegetables with my child.	0.56				
AI9. My parents discuss why it is important to eat fruit and vegetables with me.	0.60				
PI10. I sometimes give my child something to eat when he/she does something right, for example when doing homework.		0.42			
AI10. My parents sometimes give me something to eat when I do something right. for example when doing my homework.		0.42			
PI11. I consciously eat vegetables or fruit when my child is around.					0.52
AI11. My parents consciously eat vegetables or fruit when I am around.					0.61
PI12. I have clear rules about what my children can snack on for example 1 biscuit after school.			0.51		
AI12. My parents have clear rules about what I can snack on for example 1 biscuit after school.			0.45		
PI13. I make sure my child does not snack just before meals.			0.55		
AI13. My parents make sure I do not snack just before meals.			0.41		
PI14. I sometimes give my child a small snack as comfort.		0.39			
AI14. My parents sometimes give me a small snack as comfort.		0.46			
PI15. I try to consciously set a good example when it comes to eating fruit and vegetables.					0.55
AI15. My parents try to consciously set a good example when it comes to eating fruit and vegetables.					0.64
PI16. I have rules about when my child is allowed to eat snacks and how much.			0.61		
AI16. My parents have rules about when I am allowed to eat snacks and how much.			0.49		
P-Explained Variance (%)	30.21	9.98	5.38	2.57	2.32
A-Explained Variance (%)	34.34	9.91	8.41	5.48	5.18
P-Total Explained Variance (%)	50.45				
A-Total Explained Variance (%)	63.31				

I: Item P: Parent A: Adolescent

Table 3. Model fit indices of the parent and adolescent versions of the adolescent food parenting questionnaire (n=337)

Scales	RMSEA	GFI	CFI	IFI	RFI	NFI	TLI	χ^2	DF	χ^2/DF
Parent Version	0.055	0.94	0.95	0.95	0.87	0.90	0.93	190.832	94	2.030
Adolescent Version	0.045	0.95	0.96	0.96	0.88	0.91	0.95	157.211	94	1.672

RMSEA: Root Mean Square Error of Approximation; GFI: Goodness of Fit Index; CFI: Comparative Fit Index; IFI: Incremental Fit Index; RFI: Relative Fit Index; NFI: Normed Fit Index; TLI: Trucker-lewis Index; χ2: Chi-Square; DF: Degree of Free (References: 20, 22).

items on the child's age, gender, class, financial position, assessment of their height and weight, and having a balanced and adequate diet. Parents filled out the family data form, and adolescents filled out the child data form.

Adolescent Food Parenting Questionnaire: Parent and Adolescent Version: Koning et al. (2021) created the Adolescent Food Parenting Questionnaire: Parent and Adolescent Version to assess the food parenting practices of adolescents and their parents. There are 16 items on the questionnaire, all of which are in a five-point Likert-type evaluation structure (1 = Disagree, 2 = Disagree somewhat, 3 = Undecided, 4 = Agree somewhat, and 5 = Agree). The eighth item on the scale is reverse-scored.

Descriptive and confirmatory factor analysis was employed to analyse the construct validity of the tool. According to the explanatory factor analysis, the total explained variance was 61.6% for the parent sample (*AFPQ-p*) and 61.4% for the adolescent sample (*AFPQ-a*). The questionnaire has five sub-dimensions: autonomy support, coercive control, snack structure, healthy structure, and modeling. Items 1, 2, 5, and 9 make up the first factor, "Autonomy Support." Items 4, 7, 10, and 14 make up the second factor, "Compulsive Control." Items 8, 12, 13, and 16 make up the third factor, "Snack Structure." The third and sixth items make up the fourth factor, "Healthy Structure." The 11th and 15th items are included in the fifth factor, "Modeling." All fit indices, such as CFI, BIC, and RMSEA, were more significant than 0.80 for both the Parent and Adolescent Versions according to the confirmatory factor analysis. The questionnaire was confirmed to have validity and reliability and could be employed to assess the food parenting practices of adolescents and their parents (17). Cronbach's alpha coefficient was utilized to assess the reliability of the questionnaire. The alpha values of the sub-dimensions for parent and adolescent versions were found as follows: 0.79 - 0.82, autonomy support (AFPQ-p; AFPQ-a); 0.85-0.83, compulsive control (AFPQ-p; AFPQ-a), 0.79-0.75, snack structure (AFPQ-p; AFPQ-a); 0.78 and 0.88, healthy structure (AFPQ-p; AFPQ-a); 0.69-0.74, modeling (AFPQ-p; AFPQ-a). Both parents and adolescents responded to the survey. Results indicated that the questionnaire developed was good and dependable and that it could be employed to assess the food parenting practices of adolescents and their parents (17).

Research Steps

To achieve the linguistic equivalence of the Adolescent Food Parent Questionnaire, the researchers and three Turkish-native English linguists, fluent in both languages, cultures, and terminology, translated the scale items into Turkish. The researchers created the Turkish form of the scale by selecting the most appropriate expressions from the translations of the Adolescent Food Parenting Questionnaire. Regarding language and content validity, the English and Turkish versions were presented to 11 experts (Child Health and Disease Nursing, Psychiatric Nursing, Nutrition, and Dietetics specialists who speak English). The experts were asked to assess the consistency between the original form and the Turkish translation of the scale and do the content validity evaluation of the items. The Davis Technique was employed to assess content validity. The necessary corrections were made based on the raters' suggestions. Two linguists translated the approved scale items back into English. They had not seen the English copy of the questionnaire. So as to determine the clarity and comprehensibility of scale items, a pilot study was conducted on 20 parents and 20 adolescents who were not involved in the sample group. Adolescents and parents stated after the pilot application that the Adolescent Food Parenting Questionnaire statements were clear and understandable. Accordingly, the scale items were finalized. A questionnaire involving the data collection forms was created on Google Forms, and data were collected online. The adolescents and their parents first submitted consent about participation in the study on the first page of the online questionnaire and then begun to respond to the items. No personal information or emails were collected from the participants.

Ethical Considerations

For the adaptation of the Adolescent Food Parenting Questionnaire to Turkish, the permit of the scale owner who improved the scale was gotten via email. Ethical approval was obtained from Hakkari University Scientific Research and Publication Ethics Committee (Date: 02/06/2022, Decision No:2022/58-1) before starting the research practice. The written consent of the participants was taken after they were apprised about the goal of the research.
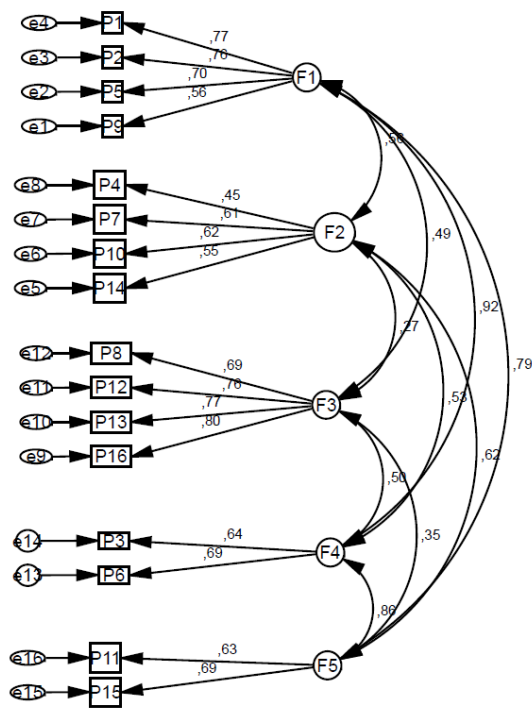
Figure 1. Confirmatory factor analysis of the parent version of the adolescent food parenting questionnaire.
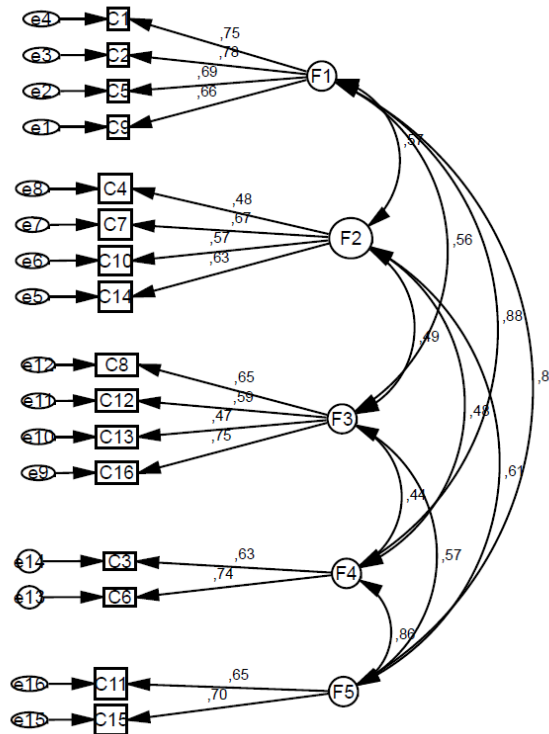
Figure 2. Confirmatory factor analysis of the adolescent version of the adolescent food parenting questionnaire.

Statistical Analysis

Data were analysed on the SPSS 24.0 and AMOS 20.0 software packages. Mean, standard deviation, percentage statistics, and frequency values were employed to present descriptive data. The following methods were employed for the Turkish adaptation of the Adolescent Food Parenting Questionnaire: exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) for construct validity; item-total correlation tests for the language content validity index; Davis technique; Cronbach's alpha value for internal consistency. The predictive level of the independent variables on the parent and adolescent version scores of the scale for determining food parenting practices between adolescents and their parents was evaluated by linear regression analysis. A confidence interval of 95% and a $p<0.05$ significance level were taken as criteria in the analyses.

RESULTS

Descriptive Characteristics of Children and Parents

The mean age was found as 41.48±7.37 for the mothers, 45.99±7.22 for the fathers, and 13.22±3.82

for the adolescents. Of the parents, 32.9% (n=111) had two children. Of the adolescents in the study, 58.8% (n=198) were female, 41.2% (n=139) were male, 59.1% (n=199) evaluated their height and 58.5% (n=197) weight as normal, and 54.0% (n=182) did not know enough about adequate and balanced nutrition. Also, 32.6% (n=110) of the mothers were high school graduates, 36.2% of the fathers (n=122) were university graduates, 80.7% (n=272) were housewives, and 81.9% (n=276) of fathers had a job. Regarding financial status, 51.0% (n=172) of the parents stated that their income was equal to their expenses (Table 1).

Validity Analysis Results of Adolescent Food Parenting Questionnaire

Eleven experts in the field were consulted for the form generated in the study, and a validity analysis was done to evaluate the scores provided by them. The item-level content validity index (I-CVI) varied between 0.89 and 1.00, and the scale-level content validity index (S-CVI) was 0.98 for the parent form. The I-CVI value varied from 0.99 to 1.00 and the S-CVI was 0.99 for the adolescent form.

The construct validity of the Adolescent Food Parenting Questionnaire was assessed with EFA and CFA.

The adequacy of the study sample for factor analysis was examined using Bartlett's Test of Sphericity (BTS) and Kaiser-Meyer-Olkin (KMO) analyses. The test results of the parent version were 0.871 (KMO) and 1848.530 (BTS), which were statistically significant (p<0.05). The EFA results expressed that the parent and adolescent versions of the Adolescent Food Parenting Questionnaire contained five factors (autonomy support, compulsive control, snack structure, healthy snacking), and the total explained variance ratio for the five-factor version in the parent version was 50.45%. The study indicated that the KMO test score of the adolescent version was 0.894, and the BTS test score was 1686.278, which were statistically significant (p<0.05). The explained variance ratio for the total adolescent version of the five-factor structure was 63.31 percent. The variance rates and factor load values explained for the sub-dimensions of the scale in the parent and adolescent version are given in Table 2.

CFA was utilized to interpret the construct validity of the Turkish-adapted version of the Adolescent Food Parenting Questionnaire. The fit indices obtained from the analysis were RMSEA=0.055, GFI=0.94, CFI=0.95, IFI=0.95, RFI=0.87, NFI=0.90, TLI=0.93, $\chi2$=190.832, DF=94, $\chi2$/DF=2.030 for the parent version and RMSEA=0.045, GFI=0.95, CFI=0.96, IFI=0.96, RFI=0.88, NFI=0.91, TLI=0.95, $\chi2$=157.211, DF=94, and $\chi2$/DF=1.672 for the adolescent version (Table 3).

According to the CFA results, the factor loading values of the parent version of the scale ranged between 0.56-0.77 for autonomy support, 0.45-0.62 for coercive control, 0.69-0.80 for snack structure, 0.64-0.69 for healthy structure, and 0.63-0.69 for

modeling (Figure 1). The factor loading values of the adolescent version of the scale varied were 0.66-0.78 for autonomy support, 0.48-0.67 for coercive control, 0.47-0.75 for snack structure, 0.63-0.74 for healthy structure, and 0.65-0.70 for modeling (Figure 2).

Reliability Analysis Results of the Adolescent Food Parenting Questionnaire

Cronbach's alpha for the total parent version was 0.86. The alpha coefficients for the sub-dimensions were 0.79 for autonomy support, 0.64 for coercive control, 0.84 for snack structure, 0.62 for healthy structure, and 0.60 for modeling. Cronbach's alpha values for the first and second halves were 0.78 and 0.72, respectively. The Spearman-Brown coefficient was 0.88, the Guttman split-half coefficient was 0.87, and the split-half analysis indicated that the correlation coefficient between the two halves was 0.78 (Table 4). Hotelling's T^2 was identified to be 114.190, F=7.295, and p=0.000.

Cronbach's alpha value of the total adolescent version was 0.88. The alpha coefficients of the sub-dimensions were 0.81 for autonomy support, 0.67 for coercive control, 0.70 for snack structure, 0.64 for healthy structure, and 0.63 for modeling. Cronbach's alpha values of the first and second haves were 0.80 and 0.74, respectively. The Spearman-Brown coefficient was 0.86, the Guttman split-half coefficient was 0.86, and the split-half analysis showed that the correlation coefficient between the two halves was 0.75 (Table 5). Hotelling's T^2 was 133.983, F=8.560, and p=0.000.

The item-total scale score correlations of the parent version varied between 0.31 and 0.65, and the correlations were between 0.34 and 0.70 for the item-sub-dimension score (p<0.001). The item-total scale score correlations of the adolescent version ranged from 0.36-0.67, and the correlations ranged between

Table 4. Reliability analysis results of the parent version and sub-dimensions of the adolescent food parenting questionnaire (n=337)

	Total Scale	Autonomy Support Sub-Dimension	Coercive Control Sub-Dimension	Snack Structure Sub-Dimension	Healthy Structure Sub-Dimension	Modeling Sub-Dimension
Cronbach α	0.86	0.79	0.64	0.84	0.62	0.60
First Half Cronbach α	0.78					
Second Half Cronbach α	0.72					
Spearman-Brown	0.88					
Guttman Split-Half	0.87					
Correlation Between Two Halves	0.78					

0.37 and 0.67 for the item-sub-dimension score ($p<0.001$) (Table 6).

A significant relationship was found between the adolescent food parenting questionnaire ($p<0.01$) between the adolescent version and the parent version ($r=.715$).

When examining which independent variables predicted adolescent and parent food practices scale scores, it was determined that adolescents' age, gender, class, economic status, adolescent's body weight, adolescent's height, mother and father's age, and mother and father's education level significantly predicted the scale score ($p<0.05$). It was determined that these independent variables explained 33.6% of the adolescent scale score ($R^2=0.336$, $p<0.05$) and 27.3% of the parent scale score ($R^2=0.273$, $p<0.05$) (Table 7). When the independent variables were examined one by one, it was determined that the only variables that significantly predicted the parent scale score were grade, adolescent's age and mother's age, respectively ($p<0.05$). In the adolescent form, only the age of the adolescent was found to be a significant predictor ($p<0.05$).

DISCUSSION
Validity Analysis of the Adolescent Food Parenting Questionnaire

The translation, expert evaluation, back translation, and pilot implementation procedures were followed to adapt the scale to Turkish. The first step in adapting a scale to another society is language validity. After the translation, expert evaluation, back translation, and pilot phases were completed, the final Turkish version was created (18-21). The content validity index was computed using the opinions of eleven experts so that content validity could be assessed. The consequences of the original study by Koning et al. (2021) could not be compared to our results because content validity was not provided in that study (17).

EFA is performed to establish the construct validity of a scale (18, 20, 22, 23). It was determined that content validity was achieved in this study ($p<0.05$).The consequences of the original study by Koning et al. (2021) could not be compared to our consequences because content validity was not performed in that study. Since information on sample size was not given, the consequences of the original study by Koning et al. (2021) could not be compared with our consequences (17). In this study, it was determined that the total variance explained for the parent and adolescent version was over 40% (19, 22, 24). Koning et al. (2021) also found that the total variance value explained for the parent and adolescent version was over 40% (17). These results are similar to our study. In this study, factor loadings for the parent and adolescent versions were determined to be above 0.30 (20, 21). In our study, when all factor loadings in both parent and adolescent versions were examined, it was determined that only three items were below 0.40, and these items are thought to have lower factor loadings because food parenting practices by parents and adolescents are not common behaviors. Factor loadings were found to be 0.30 or higher for both the parent and adolescent versions in the original study by Koning et al. in 2021 (17). Our results and these results are similar. As a result of EFA, it was determined that the sub-dimensions of the scale can adequately measure food parenting practices and adequately measure the conceptual structure in Turkish culture.

In this study, it was determined that the goodness of fit index values for the parent and adolescent versions showed an acceptable level of agreement in CFA (20,22). When the goodness of fit indices for both the parental version and the adolescent version were

Table 5. Reliability analysis results of adolescent food parenting questionnaire adolescent version and sub-dimensions (n=337)

	Total Scale	Autonomy Support Sub-Dimension	Coercive Control Sub-Dimension	Snack Structure Sub-Dimension	Healthy Structure Sub-Dimension	Modeling Sub-Dimension
Cronbach α	0.88	0.81	0.67	0.70	0.64	0.63
First Half Cronbach α	0.80					
Second Half Cronbach α	0.74					
Spearman-Brown	0.86					
Guttman Split-Half	0.86					
Correlation Between Two Halves	0.75					

examined in the original study by Koning et al. (2021). The CFA outcomes indicated that the data were consistent with the model, the structure determined by EFA was confirmed, the sub-dimensions were compatible with the scale, and that the items were adequately related to their sub-dimensions.

Reliability Analysis of the Adolescent Food Parenting Questionnaire

In Likert-type measurement tools, the reliability criterion known as Cronbach's alpha coefficient is employed to assess the internal consistency of a scale (19, 22, 24). In this study, the Cronbach's alpha coefficient of all sub-dimensions except three sub-dimensions of the scale and the Cronbach's alpha coefficient as a result of split-half analysis were found to be above 0.70 and the scale was found to be highly reliable (20,21,23). Cronbach's alpha coefficients of the three sub-dimensions are also above 0.60. When we look at the literature, in many sources, the Cronbach alpha reliability coefficient of the sub-dimensions being over 0.60 indicates that it is an acceptable reliability coefficient (19, 20, 22, 24). It is thought that the Cronbach's alpha coefficients in the sub-dimensions are within acceptable limits due to the fact that food parenting practices of a few items in this sub-dimension are uncommon behaviors. The

original study conducted by Koning et al. (2021) indicated that Cronbach's alpha reliability coefficients for both the parent and adolescent versions and sub-dimensions were higher than 0.60 (17). These results are similar to our study. Since information about the split-half analysis was not included in the original study by Koning et al. (2021), no comparison could be made with our results (17).

Item-total score analysis indicates whether the items on a scale measure the concept to be measured (19, 22). It is recommended that the item-total score correlation be at least 0.30 (21, 23). In this study, the correlations between the items in the parent version and the adolescent version with both the total scale score and the total sub-dimension score were found to be greater than 0.30. The results of this study revealed that the items were pertain to both the scale and the sub-dimensions, the scale adequately measured the subject, and that the reliableness of the items on the scale was high. The item-total score analysis of the scale and its sub-dimensions was not presented in the original study developed by Koning et al. (2021) (17), so no comparison could be made with our study results. As a result of the reliability analysis, it was determined that it was able to adequately show the food parenting practices

Table 6. Item scale total score and sub-dimension total score correlations of the adolescent food parenting questionnaire parent and adolescent versions (n=337)

Subscales	Items	Item-Total Score Correlation (r)* Parent-Adolescent	Item-Subscale Total Score Correlation (r)* Parent-Adolescent
Autonomy Support	I1	0.65-0.62	0.65-0.67
	I2	0.63-0.67	0.64-0.64
	I5	0.59-0.61	0.62-0.61
	I9	0.49-0.53	0.49-0.59
Coercive Control	I4	0.31-0.36	0.34-0.37
	I7	0.38-0.44	0.45-0.52
	I10	0.38-0.41	0.47-0.45
	I14	0.37-0.44	0.41 -0.48
Snack Structure	I8	0.48-0.42	0.63-0.51
	I12	0.44-0.44	0.68 -0.47
	I13	0.48-0.39	0.69-0.38
	I16	0.52-0.48	0.70-0.58
Healthy Structure	I3	0.48-0.50	0.45 -0.47
	I6	0.57-0.56	0.45 -0.47
Modelling	I11	0.47-0.56	0.43-0.46
	I15	0.51-0.58	0.43-0.46

* p<.001

Table 7. Independent Variables that predict the scores of the adolescent food parenting questionnaire parent and adolescent versions

Variables-P	Beta	Standard Error	β*	t**	p	%95 CI Lower	%95 CI Upper
Constant	49.286	10.694		4.609	0.000	27.958	70.614
Adolescent age	1.111	0.318	0.395	3.488	0.000	0.476	1.746
Adolescent's class	0.736	0.208	0.423	3.530	0.000	0.320	1.151
Economical situation	2.849	1.717	0.185	1.659	0.102	-0.576	6.273
Adolescent's body weight	-0.011	0.070	-0.017	-0.150	0.881	-0.151	0.130
Mother's age	-0.572	0.275	-0.333	-2.080	0.041	-1.120	-0.024
Father's age	-0.133	0.285	-0.075	-0.469	0.641	-0.701	0.434
Mother's education level	0.775	1.548	0.062	0.501	0.618	-2.313	3.863
Father's education level	1.063	1.665	0.080	0.639	0.525	-2.257	4.384

P: R*** 0.579, R2**** 0.336, F***** 4.422, p=0.000, DW****** 2.158

Variables-A	Beta	Standard Error	β*	t**	p	%95 CI Lower	%95 CI Upper
Constant	20.729	9.545		2.172	0.032	1.804	39.653
Adolescent age	0.494	0.218	0.249	2.270	0.025	0.062	0.926
Adolescent's gender	3.497	2.007	0.150	1.742	0.084	-0.483	7.477
Economical situation	0.716	1.424	0.044	0.503	0.616	-2.107	3.539
Adolescent's body weight	0.008	0.072	0.011	0.105	0.917	-0.135	0.150
Adolescent's height	0.020	0.017	0.111	1.150	0.253	-0.014	0.054
Mother's age	-0.085	0.252	-0.069	-0.339	0.736	-0.585	0.415
Father's age	0.409	0.235	0.340	1.740	0.085	-0.057	0.875
Mother's education level	2.656	1.364	0.193	1.947	0.054	-0.048	5.360
Father's education level	-1.172	1.552	-0.085	-0.755	0.452	-4.249	1.905

A: R*** 0.522, R2**** 0.273, F***** 4.423, p=0.000, DW****** 1.611

Abbreviations: *β, Standartized Beta; ** t, t-test value; ***R, correlation co-efficient; **** R², R Square; *****F, Anova Value, ******DW, Durbin-Watson, P: Parent, A: Adolescent

between adolescents and their parents and that the items were related to each other.

In this study, it was observed that the adolescent's age, grade, and mother's age were significant predictors for both adolescent and parent scale scores. It was observed that as the age and grade of the adolescents increased, the scale scores also increased. Additionally, it was determined that there was a decrease in the scale score as the age of the parent increased. In the literature, it is seen that the factors affecting food parenting practices between adolescents and their parents are the gender of the adolescent, the education level of the parent, parenting styles, the family's illness experience and parental motivation (25-27). In this study, it is thought that the increase in positive nutrition scores, especially with increasing age and grade, may be due

to the adolescent's increased awareness of nutrition, the diversification of nutritional preferences offered by the family, the fact that they have nutritional preferences in different environments, and the increase in communication with their parents about nutrition. It is thought that the decrease in the scale score as the mother's age increases may be due to the fact that she has more up-to-date information about nutrition, is able to empathize with her child because she is closer in age, and can offer more appropriate nutritional choices to the adolescent (26-29).

Limitations
The limitation of this study is that the convenience sampling method was used, which means that only those who agreed to fill in the questionnaire were

included in the study. The inability to compare and interpret the results in the intercultural dimension is another limitation. There are no studies on the adaptation of the original scale to different cultures.

CONCLUSION

According to the analyses and evaluations conducted in this study, the Adolescent Food Parenting Questionnaire: Parent and Adolescent Version is a reliable and valid measure for the Turkish sample. Researchers can identify adolescents' and parents' food parenting practices using this scale, reduce negative behaviors such as unhealthy food consumption and improper eating habits, and develop programs to address these issues. Additionally, they might detect the rising prevalence of overweight and obesity in adolescents before it happens. They can also conduct cross-cultural comparative studies by using this scale.

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EXPECTATIONS OF MOTHERS WITH INFANTS IN THE NEONATAL INTENSIVE CARE UNIT FOR HEALING CARE PROCESSES: A QUALITATIVE STUDY

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ABSTRACT
Purpose: The study aimed to describe the expectations of mothers whose infants were in the neonatal intensive care unit regarding the healing care processes.
Material and Methods: This study is descriptive qualitative research conducted with mothers whose infants were in a private hospital's neonatal intensive care unit. Data were collected between October and November 2022 by using purposive sampling method. Semi-structured interviews were conducted with 11 mothers via an audio recorder. The inductive thematic analysis method was used.
Results: The analysis identified three main categories and seven sub-themes: "humanistic healing care behaviors, healing physical environment and supports." In the theme of humanistic healing care behaviors, mothers stated that open and honest information sharing was necessary; in the healing care environment, the organization of the neonatal intensive care unit and noise control were necessary. The support theme mentioned the effect of family and home health personnel support on healing processes. The themes were created based on Watson's human caring theory.
Conclusion: The analysis identified three main categories and seven sub-themes: "humanistic healing care behaviors, healing physical environment and supports." In the theme of humanistic healing care behaviors, mothers stated that open and honest information sharing was necessary; in the healing care environment, the organization of the neonatal intensive care unit and noise control were necessary. The support theme mentioned the effect of family and home health personnel support on healing processes. The themes were created based on Watson's human caring theory.
Keywords: infant, mothers, nursing, Watson's human caring theory.

INTRODUCTION

Some infants are treated in the Neonatal Intensive Care Unit (NICU) for various reasons in the postpartum period. Spending the first days of her/his life in intensive care may be challenging for the infant and her/his family. Incubators and devices for diagnosis and treatment in NICUs can present a frightening sight for parents (1). This can be even more challenging, especially for mothers who bond with their neonate during the prenatal period. Uncertainties about what awaits them in the future, as well as the treatments received by the infant during the intensive care process, are a major source of stress for mothers. The stress of the parents may increase continuously due to the uncertainty of the infant's prognosis (2). It is known that high-stress

level hinders the ability of parents to make healthy decisions and their participation in the care of the infant (3).

Mothers with infants in NICU have many needs in the postpartum period, such as their health issues, newborn care, and home care after intensive care. Studies in the literature report that families need information about the treatment process of the infant and whether he/she receives good care (4, 5). A study conducted in Jordan reported that parents need information about their children and need holistic care in case of a loss. It has also been reported that the expectations of the families and the care given by the nurses do not match and that this area needs to be developed (3). For this reason, it is essential to determine the expectations of mothers about the process and their needs to alleviate the stress they experience and improve their coping skills. At this point, nurses have important responsibilities.

Neonatal nurses are essential in creating a family-centered healing care environment in NICUs. The mothers' seeing the NICU for the first time after birth, the excessive amount of devices in the unit, the treatments received by the infant and the mothers' inability to hold their infants affect the healing care processes (6). At this point, it is vital to support the needs of parents who have an infant in the NICU to express their feelings and experiences. Nurses need to evaluate newborns holistically and determine their mothers' feelings, their views on infant care, and their expectations to provide effective care in NICUs.

Watson's Theory of Human Caring provides a guiding framework for conceptualizing healing care processes. Healing processes are defined as creative problem-solving processes. Watson emphasized the importance of regulating the care environment in healing processes (7).

The nurse has an important role in the healing process. According to Watson, the nurse is at the center of the individual-nurse relationship that produces therapeutic results in the interpersonal care process (8). Caring behaviors recommended to be used to create a healing process in nursing practices within the framework of Watson's human caring theory are touching, artistic expressions, play, eye contact, smiling, and active listening (7). Healing consists of 10 processes, each interacting with the others. In the NICU, healing processes are essential in guiding care and directing holistic, developmental, and family-centered care. This way, the life chances increase, neurodevelopmental support is provided,

and the family participates in the care (6). Studies assess different stages of healing processes in different populations (9, 10). There are studies about the expectations of mothers who have infants in the NICU (3, 4). It is seen that there are qualitative studies, especially about the supportive care practices that mothers expect from nurses (11), expectations in terms of needs and communication (12), and their actual experiences. There are also qualitative studies on some healing processes in studies on family-centered care in the neonatal intensive care unit (13, 14).

However, this study is thought to contribute to the literature as it investigates mothers' expectations and experiences regarding healing care processes based on Watson's human caring theory. The experiences and expectations of the mothers with their infants in the NICU are essential in providing evidence for safe and effective clinical practices.

MATERIAL AND METHODS
Objective
This study aims to determine the expectations of mothers whose infants are hospitalized in the NICU from the healing environment based on Watson's human caring theory. The main research question of this study is: What are the expectations of the mothers whose infants are hospitalized in the NICU? The detailed interview questions of the study are provided in Table 1.

Design and Participants
The study was conducted in a qualitative research design with mothers whose infants were hospitalized in the NICU of a private hospital between October 19 and November 19, 2022. Healing care, by its very nature, requires in-depth and comprehensive examination. For this reason, a qualitative research design was preferred to reveal the expectations of mothers whose infants were hospitalized in the NICU. This study was conducted and written considering the Consolidated Criteria for Reporting Qualitative Research (COREQ). The components of qualitative rigor (Credibility, Transferability, Dependability, and Confirmability) were considered to make sure confidence in the methods.

The study was carried out in the NICU of a private hospital in Ankara, the capital city of Turkey. The NICU is a unit with 13 incubators. Families were able to visit their infants two days a week (Tuesday and

Table 1. Interview Questions of The Study

IInterview questions
1. What are your expectations from nurses responsible for your infant's care?
a) What are your expectations from nurses regarding the infant's care and treatment process?
b) What are your expectations from nurses in terms of communication?
2. What are your expectations and wishes regarding the physical environment of the hospital/NICU?
3. What are your expectations and requests from your family during the intensive care process?
4. What social supports (peer, friend, community, spirituality) do you expect during the intensive care process?

Thursday) and received information by phone on other days.

The purposive sampling method was used in this study. In qualitative research, there is no definite rule regarding the number of people to be included in the research. It was aimed to reach data saturation in the in-depth interviews. The saturation point is reached when there is no more new information in the interviews (15). Interviews were continued until the data became repetitive and were concluded upon reaching data saturation after interviewing 11 mothers. The interviews were listened and codes were determided by the researchers. Since no new codes were created and data saturation was achieved, and interviews of 11 mothers were analyzed in the research.

The acceptance criteria of mothers in the study were determined as follows:

1) Those who agree to participate in the study will be selected voluntarily,
2) Those who have a premature infant or an infant who has been in intensive care for at least a week due to a health problem,
3) Those who are over the age of 18,
4) Those who do not have problems in terms of communication and do not have any physical or mental health problems.

Ethical Considerations

Ethical approval was obtained from the Lokman Hekim University, Non-Invasive Clinical Research Ethics Committee (Date: 18.12.2022, Decision No: 2022/169) before starting the study. Consent regarding participation in the study was obtained from each participant The study was conducted by the principles of the Declaration of Helsinki.

Data Collection

Qualitative data were collected during semi-structured interviews conducted by the researchers. Before the interview, a descriptive characteristics form was used, which asked about the demographic characteristics of the mother and the infant (a total of 3 questions, including the mother's age, the infant's gestational age, and the length of stay in NICU). Mothers were asked to fill out the form. Additionally, information about the infants was checked from medical records.

The interviews were held in the mother's waiting room when the mothers came to see their infants. The mother's waiting room is for one person as it is a place where mothers who want to express their milk can express their milk and wait. For this reason, the interviews were held in a quiet environment where the conversation would not be interrupted. Mothers who wanted to participate in the study were asked when they would be available for an interview, and interviews were held in the mother's waiting room at the date and time given by the mothers. Mothers especially see their infants between 13 and 14 in the afternoon. That is why the interviews were held at these hours. Only the first researcher who will conduct the interview participated in the interview. Four semi-structured questions were asked one by one to each mother.

Interview questions were sent to three experts in pediatric nursing and qualitative research methods for peer review. No changes were made to the questions due to peer review. In addition, a sample interview was conducted with two mothers to test the semistructured interview questions. After sample interviews, it was understood that the first question was general. As a result, two sub-questions were created: mothers' expectations from nurses in infant care and their expectations from nurses regarding communication. The study did not include the two mothers with whom we conducted a sample interview.

Each interview lasted an average of 45-60 minutes. Researchers reviewed their notes with each mother before the end of the interview. The interviews were recorded with a voice recorder. The mothers were

Table 2. Sociodemographic Characteristics of Mother and Infants

Sample	Age	Infant's Gestational Age (week)	Length of Stay in Neonata Intensive Care Unit (days)
Participants 1	33	33 weeks, three days	20
Participants 2	27	38 weeks	8
Participants 3	23	38 weeks, two days	21
Participants 4	25	36 weeks, four days	19
Participants 5	25	36 weeks, three days	18
Participants 6	28	35 weeks, two days	21
Participants 7	26	38 weeks	14
Participants 8	25	36 weeks	17
Participants 9	27	35 weeks, four days	19
Participants 10	30	37 weeks, one day	21
Participants 11	26	38 weeks, two days	23

informed before the recording started, and the research purpose and consent form were given in the envelope in advance. Those who accepted to participate in the study were included in the study. The questionnaire prepared by the researchers to determine the descriptive characteristics of mothers and infants was filled in by the researchers before the interview.

Data Analysis
The data obtained from the interview form were analyzed using inductive thematic analysis. While thematic analysis offers a systematic and flexible approach, it guides the objective analysis of data (16). First, the interviews in the audio recordings were listened to and transcribed. The researchers transcribed the interview records themselves to prevent data loss. The written interviews were coded sentence by sentence to make their meanings more understandable. A "code list" was created using the data included in the coding. Later, themes that can explain these codes under certain categories were created. Six stages of the thematic analysis were followed: familiarization with the data, assigning preliminary codes, searching for themes, reviewing themes, defining and naming themes, and producing the report (16). All categories and themes were discussed with all researchers to complete the analysis. Thematic analyses were carried out independently by two researchers to ensure the consistency of the data. The themes and expressions coded for the validity of the data were presented to two other experts in the pediatric nursing field. Researchers and consulted experts were required to be experienced in qualitative work and to have at least one SCI article prepared and published using the individual/focus interview technique.

Research Team and Reflexivity
The first author is a female pediatric nursing lecturer (assistant professor) who completed her doctorate in neonatal nursing. The second author is a female lecturer in the field of pediatric nursing. At the same time, the second author previously worked as an intensive care nurse in an accredited hospital for one year. The authors have received courses and education in qualitative research. As a result, there are studies on qualitative research methodology in international indexed journals. The first author conducted interviews and had a PhD at the time of the interviews. There was no relationship between the interviewer and any of the participants. Reflexivity was used in all phases of data collection and analysis. The primary author was transparent with the participants about her career as a mother and an academic working in neonatal nursing. Since the second researcher had one year of experience working in the pediatric intensive care unit, the first author carried out the data collection phase for reflexivity. The emphasis was to be aware of one own perspective and, with different perspectives, read the data and actively enhance reflexivity. The perspectives of the two authors were discussed in the analysis and reporting.

Rigor
In this study, the components of qualitative rigor as Credibility, Transferability, Dependability and Confirmability were used to provide confidence about methodology of the study (17).

Table 3. Themes and Sub-themes of The Study

Themes	Sub-themes	Codes	f
1. Humanistic Healing Care Bahviours	Clear and honest information sharing	Factual knowledge sharing	11
		Responding sensitively to questions	10
		Communication that will make me feel safe	8
		Eye contact	8
	Instilling faith and hope	Sharing experiences	6
		Establishing eye contact	8
		Psychological support	11
2. Healing physical environment	Design/organization of neonatal intensive care unit	Private areas or single rooms reserved for mother and infant	9
	Noise control	Noise control of medical devices	11
3. Supports	Family support	Spousal support	11
	Support of healthcare professionals	Nurse and doctor support	10
	Spiritual support	Praying	6
		Questioning the meaning of life	5

Credibility: Transcripts of all interviews were analyzed separately by two researchers and similarities and differences were evaluated. After a few examinations, the statements of the participants were quoted in the results section.

Transferability: Demographic characteristics of mothers and infants were tabulated in the study so that readers could evaluate whether the study findings applied to their populations.

Dependability: Researchers decided on each stage of the research process together. Peer review on interview questions and content was sought. A pre-application was made with a mother to ensure reliability in advance.

Confirmability: Spare questions were asked for each interview question to clarify that question considering the impact of the results due to the perceptions of the researchers. The final form was summarized, and the participant was expected to approve of the situation. The opinions of two faculty members who are experts in their fields were taken for the themes and sub-themes created by two researchers. Different children made citations to increase conformability.

RESULTS

While the age range of mothers varies between 23-33, fathers' age varies between 24-33 years. Most mothers are high school graduates, and most fathers are university graduates. The gestational age at which mothers give birth is between 33.3 and 38.2 weeks, and the majority were primiparous. Detailed sociodemographic characteristics of the participants are shown in Table 2.

Themes

After the content analysis, the expectations of the mothers regarding the healing care processes were determined as three themes: humanistic healing care behaviors, healing physical environment and supports, and seven sub-themes explaining these. Themes, sub-themes and codes explaining the themes are given in Table 3.

Main Theme 1: Humanistic Healing Care Behaviours

The theme of humanistic healing care behaviors consists of two sub-themes: open and honest information sharing and instilling faith and hope. Mothers emphasized that important components of interpersonal communication such as eye contact, being respected as an individual, feeling safe, nurse sensitivity, and feeling understood are very important in the healing processes.

Sub-theme-1: Being clear and honest in information sharing

All of the participants stated that they expected clear and real information from the nurses about the health status of their infants. Mothers emphasized that nurses' being sensitive to their questions, making them feel understood, and establishing eye contact with them had a healing effect.

"…My biggest expectation is that when I ask questions about my infant's health, the nurses give clear information in a way that I can understand. Since I don't see my infant all the time, the clear and honest information provided by the nurses makes me very comfortable at home…" (P1,33 years, 33 gestational weeks)

"…Especially as the time to see my infant is coming to an end, more questions come to my mind. Nurses may not allow questions or continue breastfeeding because time is running out. Nurses need to be a little more flexible, reassuring, and supportive when answering questions…" (P3,23 years, 38 gestational weeks)

Sub-theme 2: Instilling faith and hope

The mothers stated that the nurses' tone of voice, eye contact, and sincere behavior instilled hope in them while communicating with them, and this increased their belief that their infants were well taken care of and would be well.

"It was very bad to see my infant in the NICU among many devices. A nurse talked about the healing and discharge of families who had a similar situation… Especially the nurse's suggestions about believing and being strong changed a lot in our lives.….." (P8, 25 years, 36 gestational weeks)

"…In this process, one needs a small ray of hope... Sometimes, this can be the reassuring voice of the nurse, and sometimes, it can be a look…" (P5, 25 years, 36 gestational weeks)

Main Theme 2: Healing Physical Environment

Most of the mothers stated that they wanted to spend time with their infants in a more comfortable room away from people and noise. They stated that especially the sounds of medical devices coming from a large number of incubators affect the heart and respiratory rates of their infants. The physical environment is an important factor in establishing a healing care environment.

Sub-theme 3: Design/organization of neonatal intensive care unit

Most mothers want a single room or a suitable environment where they can spend time with their

"My infant is in a large room with many incubators. My infant's weights two kilos, but the infant in the incubator next to him is four kilos and looks more active. This made me very sad, and my milk was running low. I wish there were partitions between incubators or single rooms, especially during the visiting hours of mothers." (P4, 25 years, 36 gestational weeks)

"As two mothers, we went to see our infants at the same time. We both tried to breastfeed our infants. The other mother had a hard time breastfeeding and when she saw my infant suckling she cried and fainted. Therefore, it would be better for infants to be in single rooms so that mothers do not compare and influence each other's infants.…" (P7, 26 years, 38 gestational weeks)

Sub-theme 4: Noise control

All mothers stated that noise control is important in providing a healing environment for maternal and infant health.

"My infant is being treated in an incubator in a large room. When there is too much noise from other infant's medical devices during visiting hours, my infant's heart rate and breathing are negatively affected. I think noise control is essential for my infant's health." (P1, 33 years, 33 gestational weeks)

Main Theme 3: Supports

In the third main theme, all of the mothers stated that they received support and/or the support sources they expected were important in the healing processes. In this theme, the sources of support that mothers expect are family, spirituality, and healthcare professionals.

Sub-theme 5: Family support

All of the mothers stated that they especially expected support from their spouses and that this was very important for them on the way to recovery. They also emphasized that within the scope of family support other than their spouses, it is important for their families to ask few questions and to be empathetic rather than accusing.

"The biggest support came from my husband during this period. With my partner's support, I feel better and I think it reflects on our infant as well." (P6, 28 years,35 gestational weeks)

"My wife was with me at every stage of the process. While waiting to see our baby, every moment …" (P4, 25 years, 36 gestational weeks)

Sub-theme 6: Support of healthcare professionals

All of the mothers reported that their biggest source of support was healthcare professionals. They reported that the healthcare professionals who care and treat their infants provide social support in terms of information support and emotional support.

"Knowing how they take care of my infant in intensive care is the most important thing for me. It's so nice to see they take better care of our infants than mothers. This support of the healthcare professionals, who I can get information about during visiting hours and on the phone, who understand me and take care of my infant, is very valuable." (P2, 27 years, 38 gestational weeks)

"The support of doctors and nurses is so essential… Their support affects me both psychologically and affects my breastfeeding and my whole family …" (P11, 26 years,38 gestational weeks)

Sub-theme 7: Spiritual support

Mothers stated that one of their greatest supports during the stressful intensive care process is to cling to prayer and faith. They also reported that this period caused them to question the meaning of life and increased the importance of spiritual support.

"It is very sad to see my infant in intensive care while dreaming of holding my infant. I began to question the meaning of life. Clinging to my faith and praying was my biggest support during this period." (P10, 30 years, 37 gestational weeks)

"I questioned the meaning of life a lot. I could not hold my baby to my heart's content. I always asked why. However, now I realize my life's meaning is my baby, and I will stand up straighter for him…" (P9,27 years, 35 gestational weeks).

DISCUSSION

This study, based on Watson's human caring theory, examined the expectations regarding the recovery care processes of mothers whose infants are in the NICU in Turkish society. Revealing the expectations of mothers regarding healing care behaviors and examining the effects of healing environments and supports are the strengths and new aspects of our study.

We concluded that mothers had expectations from nurses such as facilitating access to real information, providing clear and understandable information, and instilling faith and hope. These expectations of mothers are based on basic human values such as respect, appreciation, compassion, empathy, and eye contact. Therefore, mothers whose infants are hospitalized in the NICU expect humanistic care

behaviors from nurses. This finding supports previous studies emphasizing that humanistic care (respect, sustainability of communication, eye contact, valuing, etc.) is essential in pediatric healthcare (9, 18). This is related to the healing processes of humanity and devotion that correspond to Watson's humanistic care. According to Watson, humanistic healing behaviors are an indicator of higher-quality care (7). The findings of our study emphasize behaviors that prioritize basic human values in care, and in this sense, it is compatible with the international basic care framework. Considering that maternal and infant health will affect each other, it is thought that nurses' healing care behaviors will contribute to both maternal and infant health.

The mothers in our study want to have a single-family room where they can spend more time with their infants and if possible, away from incubators. Mothers stated that the sounds of many incubators and medical devices cause stress in mothers and infants and negatively affect the communication between mother and infant. Mothers expect an environment during their visit that is as quiet as possible, where no one will disturb them, and where they can have close contact with their infants. It has been reported that in an environment where many incubators are together, sharing mothers with other mothers and comparing infants' development negatively affect the health of mothers and infants. Although the design of NICUs is related to hospital policy and capacity, nurses should lead the creation of a quiet, well-adjusted environment where mother and infant will spend time together and will not be interrupted by others. Nightingale's Environmental Theory and Watson's healing environment support the findings of our study within the scope of creating a remedial care environment. In many countries, NICUs are large spaces where more than one incubator can fit, but one or more single family rooms can also be provided. Others may only have units with more than one single family room (19, 20). In a meta-analysis study evaluating the effectiveness of the design of NICUs, newborns staying in a single-family room had a lower incidence of infant sepsis and higher breastfeeding rates at discharge compared to newborns staying in a standard neonatal unit. It has been determined that family-centered care and skin-to-skin contact are more common in the NICU, which has a family room design where infant can stay with the mother (21). Studies have reported that mothers with infants in an open NICU have higher levels of

stress than mothers staying with their infants in a single-family room (19, 22). Our study is similar to the existing literature although the number of studies evaluating the design of NICUs is limited in the literature. Creating the healing care environment to meet the expectations in terms of maternal and infant health is compatible with pediatric nursing practices and philosophy in terms of the implementation of family-centered care.

Family, healthcare professionals and spiritual support were determined as the sources of support that mothers experienced and expected. A meta-synthesis study examining the experiences of mothers with newborn in the NICU emphasized the importance of family support and the support of health professionals for mothers (23). Our study, on the other hand, concluded that mothers' hopes and beliefs are one of the biggest supports in the intensive care period. Some studies in the literature report that mothers gain a serious power when they think about divine power, are hopeful and feel comfortable, and this reflects positively on the relationship between mother and infant (24, 25). Only a limited number of studies have highlighted the spiritual support needs of mothers with infants in NICUs (24, 25). One of the most important sub-themes of healing supports is spiritual support in this study. One of the most important sub-themes of healing supports is spiritual support in this study. Healing care processes are one of the important health areas that mothers should be supported.

The mothers stated that they were worried about the development of their infants, breastfeeding and communication between them during their visits and that they needed the support of healthcare professionals. They think that the support of nurses is particularly important for their concerns. Heo & Oh (2019) stated in their study that parents of preterm infants need effective communication with the team (26). One of the most important roles of the nurse in this process is to guide the parents in the care of the infant and to support their parenting roles. Studies have emphasized the change in the parenting roles of mothers and have determined that they need accurate and understandable information the most (6, 27). This situation can be associated with mothers feeling inadequate due to not being able to fulfill their parenting roles and having to leave their infants alone among many technological tools whose function they do not know. We can say that family, health professionals, and spiritual support provide healing supports for mothers and their infants. With these supports, it is possible to improve and support the health of mothers and therefore infants.

Limitations

One of the limitations of this study is that most of the participants are primiparous mothers, and the experiences and expectations of multiparous mothers may be different. Therefore, there is a need for future studies that examine the expectations of primiparous and multiparous mothers in depth. Moreover, the fact that all of the children in the study had respiratory problems is thought to be reflected in the expectations of the mothers.

CONCLUSION

This study examined the expectations of mothers whose infants were hospitalized in the NICU within the framework of Watson's Theory of Human Caring. Mothers expect humanistic healing care behaviors from nurses. Moreover, they expect a healing environment where mothers will participate in the care of their infants, where different mothers will not be adversely affected when they see each other, where noise control is provided, and where there are more aesthetic single rooms. The biggest sources of support for mothers during this period are their spouses, families, healthcare professionals, and spirituality. In line with these results, reviewing the organization of NICUs, increasing awareness of health personnel about healing behaviors, and mobilizing mothers' support resources will be effective in the implementation of healing care processes.

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THE EFFECT OF SOFT TISSUE STRUCTURE ON THE PSYCHOSOCIAL IMPACT OF DENTAL AESTHETICS IN WOMEN AND MEN

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ABSTRACT

Purpose: This study aimed to determine the impact of soft tissue structure on the psychosocial effects of malocclusions in women and men.
Material and Methods: The severity of malocclusion was determined in 84 individuals with a Class I skeletal pattern. The Psychosocial Impact of Dental Aesthetics Questionnaire was administered to determine the malocclusion perceptions of the patients. Arnett's soft tissue analysis was performed to determine the soft tissue structure. The difference between the sociodemographic factors and severity of malocclusion averages of men and women was statistically examined. Subsequently, the impact of Arnett's soft tissue analysis parameters on the Psychosocial Impact of Dental Aesthetics Questionnaire scores of each gender was evaluated.
Results: No statistically significant association was observed between women and men related to the sociodemographic factors and the severity of malocclusions. The number of Arnett's soft tissue analysis parameters affecting the Psychosocial Impact of Dental Aesthetics Questionnaire scores was higher in men than women. Women were more psychologically affected by malocclusions, while men were affected by soft tissue structure changes, and women were affected by dental factors.
Conclusion: Although the soft tissue structure did not affect the perception of malocclusions in women, women experienced a more severe psychological effect of malocclusion.

Keywords: esthetics, malocclusion, psychosocial, soft tissue, questionnaires.

INTRODUCTION

The harmony of hard and soft tissues in the orofacial system determines face aesthetics. Hard tissue movement due to orthodontic treatments or orthognathic surgery may affect the soft tissue structure, and the structure of the soft tissue may also affect treatment planning. Thus, soft tissue cephalometric analyses are as essential as hard tissue cephalometric analyses for orthodontic management, especially in complex cases. Arnett's soft tissue cephalometric analysis (STCA) is one of the most recent methods of measuring soft tissue structure, which evaluates the physical relationship between soft and hard skeletal tissues independent of the skull base (1).

In psychology and cognitive sciences, perception is defined as the process of receiving, interpreting, selecting, and organizing sensory information (2). According to Gestalt psychology, organisms tend to perceive elements in space by grouping objects according to their proximity (3). Thus, patients' perceptions of malocclusion may be affected by the

alignment of the teeth and anatomical variations in the surrounding soft tissue. According to our clinical observations, patients with thicker soft tissues seemed to have fewer aesthetic complaints related to malocclusions.

The practical implications of understanding the impact of STCA parameters on the psychosocial impact of malocclusions could significantly improve treatment outcomes and patient satisfaction in orthodontic practice. Therefore, it is essential to evaluate the patient's perception of malocclusion and the severity of malocclusion objectively. The Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ) is a specific psychometric oral health-related quality of life scale that can determine the psychosocial impact of malocclusion on young adults with high reliability (4). To objectively assess the severity of malocclusions, the dental component of the Index of Orthodontic Treatment Need (IOTN) is frequently used (5). To the best of our knowledge, no study has assessed the effect of the STCA parameters on the psychosocial impact of malocclusions on women and men seeking orthodontic treatment. Therefore, this study aimed to determine the impact of the STCA parameters on the total and subscale PIDAQ scores of women and men with skeletal Class I malocclusion who accepted undergoing orthodontic treatment.

MATERIAL AND METHODS
Study Population
This prospective cohort study included 84 individuals (52 women and 32 men) between the ages of 16 and 30 who accepted undergoing orthodontic treatment at the Orthodontic Clinic of the Faculty of Dentistry of Uşak University.

Inclusion Criteria
The sample was selected according to the following inclusion criteria: aged 16 to 30 years, ANB value ranging between 0 and 4, skeletal Class I malocclusion without any craniofacial anomaly, no history of undergoing orthodontic treatment, and any systemic or mental illnesses.

Exclusion Criteria
The exclusion criteria were previous history of orthodontic or cosmetic procedures, systemic diseases, syndromes, cleft lip/palate, poor-quality cephalograms, history of trauma to the jaw and face,

patients with skeletal malocclusions, patients younger than 16 years old and older than 30 years of age.

Sample Size
Since no previous study has examined the effect of STCA parameters on PIDAQ scores, Cohen's (1988) correlation test was performed with a moderate effect size of 0.5 (6). The sample size was calculated at a 95% confidence level using G. Power v. 3.1.9.2, and a minimum sample size of 84 was necessary for a theoretical power of 80%.

Determining the Severity of Malocclusions
Clinical examinations were performed by two trained investigators (D.A.U. and S.T.Y.) using the dental health component of the IOTN-DHC to determine the nature and severity of the malocclusion (5). The inter- and intra-rater reliability values were 0.86 (weighted kappa) and 0.92, respectively.

Determination of Perception of Orthodontic Malocclusions
The Turkish version of PIDAQ was administered to all patients (4). The Turkish version of PIDAQ comprises four subscales, divided according to one positive and three negative domains: aesthetic attitude (AA), which evaluates the aesthetic concerns of the patient (three items); psychological impact (PI), which evaluates the negative feelings regarding one's dental appearance (six items); social impact (SI), which identifies potential problems that may arise in social situations (eight items); and dental self-confidence (DSC), which assesses the impact of dental aesthetics on self-image (six items). A five-point Likert scale was used to record the responses. The responses ranged from 0 (not at all) to 4 (very strongly), with one positive and three negative domains. The items in the DSC were scored in reverse mode to ensure that the same direction of scoring was used for all questionnaire items, and a consistent measure of impact was obtained. Cronbach's alpha reliability analysis was applied to the total/subscale PIDAQ scores to evaluate the consistency and reliability of the questions in the questionnaire. Cronbach's alpha reliability analysis determined that the total/subscale PIDAQ scores were sufficiently reliable, with Cronbach's alpha reliability coefficients ranging between 0.756 and 0.942.

Soft Tissue Lateral Cephalometric Analysis
Cephalometric radiographs were acquired with the lips and head in a natural position (7). The first researcher (D.A.U.) performed STCA (Table 1) (1) at one-month intervals using AudaxCeph Advantage software (Audax d.o.o., Ljubljana, Slovenia). The variability between the first and second measurements was evaluated using the Pearson moment product correlation test. A high intraclass correlation coefficient (0.95) was obtained for the measurements.

Study Design
Firstly, the IOTN-DHC grades and sociodemographic data of women and men, including education level, residence, parental education level, average family income, and age, were compared in this study. Then, the impact of the STCA parameters on the total/subscale PIDAQ scores was determined for each gender.

Ethical Considerations
This prospective cohort study was approved by Uşak University Faculty of Medicine, Clinical Research Ethics Committee (Date: 06.01.2021, Approval Number: 05-05-10) and the study was conducted according to the principles of the Declaration of Helsinki. Every single individual who agreed to participate was informed of the research procedures, and written consent was obtained from all subjects. In the case of minors, their parents provided written consent.

Statistical Analysis
The normality of the data distribution was assessed using the Shapiro–Wilk test. The Mann–Whitney U test was used to compare the means of two independent groups with normally distributed data. Spearman's correlation was used to evaluate the relationship between continuous variables that were non-normally distributed. For categorical data analysis, Pearson's chi-square test was used when the sample size was sufficient, and Fisher's exact test was used when the sample size was not sufficient. All statistical analyses were performed using IBM SPSS 25 software. The significance level was set at $p < 0.05$.

Table 1. Definition of STCA parameters used in this study

Dental factors	
Overbite	The extent of vertical overlap of Mx1 over Md1
Overjet	The extent of horizontal overlap of Mx1 over Md1
Soft Tissue Structures	
ULT	Upper vermilion thickness
LLT	Lower vermilion thickness
Pog-Pog'	Soft tissue thickness at the pogonion
Me-Me'	Soft tissue thickness at the menton
NLA	Angle formed by the nasal base and the upper lip
ULA	The angle formed by the line passing through the Sn' and upper lip anterior to the TVL
Facial Lengths	
N'-Me'	Vertical distance from N' to Me'
Sn'-Me'	The distance between the Sn' and menton
Sn'-ULI	Vertical distance from the Sn' to the inferior border of the upper lip
LLS-Me'	Vertical distance from the superior border of the lower lip to Me'
ULI-Mx1	Distance from inferior border of the upper lip to Mx1
Sn'-Mx1	Distance from Sn' to Mx1
Md1-Me'	Distance from the Md1 to Me'
TVL Projections	
A'-TVL	Horizontal distance from A' to TVL.
ULA-TVL	Horizontal distance from ULA to TVL
Mx1-TVL	Distance from Mx1 to TVL.
Md1-TVL	Distance from Md1 to TVL
LLA-TVL	Horizontal distance from LLA to TVL
B'-TVL	Horizontal distance from B' and TVL
Pog'-TVL	Distance from Pog' to TVL.

Abbreviations: ULT, upper lip thickness; LLT, lower lip thickness; Pog-Pog', soft tissue pogonion thickness; Me- Me', soft tissue menton thickness; NLA, nasolabial angle; ULA, upper lip angle; N'-Me', total face height; Sn'-Me', lower face height; Sn'-ULI, upper lip length; LLS-Me', lower lip length; ULI-MxI, Mx1 exposure; Sn'-MxI, maxillary height; Md1-Me', mandibular height; A'-TVL, soft tissue point A; ULA-TVL, upper lip anterior; Mx1-TVL, maxillary incisor 1; Md1-TVL, mandibular incisor 1; LLA-TVL, lower lip anterior; B'-TVL, soft tissue point B; Pog'-TVL, soft tissue pogonion.

RESULTS
Due to the lack of a sufficient sample size, IOTN grade 1 was combined with IOTN grade 2 and renamed "1 and 2." No statistically significant relationship was observed between men's and women's sociodemographic factors and IOTN grades (Table 2). No statistically significant difference was observed between the total/subscale PIDAQ scores of men and women (p>0.05), except for their PI scores (p>0.05); the PI of the existing malocclusion was more severe in women than that in men (p<0.05, Table 3).

Correlations Related to Women
A weak negative correlation was observed between the subscale AA scores and overbite, and a moderately negative correlation was observed between the subscale AA scores and maxillary exposure (Table 4). A moderately positive correlation was observed between the subscale SI scores and Mx1-TVL, while a weak positive correlation was observed between the subscale SI scores and Md1-TVL (Table 4).

Table 3. Comparison of the total and subscale PIDAQ scores of women and men

	Gender	Mean	SD	p
PIDAQ	Women	53.7885	18.45114	0.075
	Men	46.4063	17.84134	
DSC	Women	15.2885	5.33702	0.337
	Men	13.5938	5.95065	
SI	Women	10.5000	5.55719	0.107
	Men	8.5000	5.29150	
PI	Women	12.4423	2.43676	0.001*
	Men	9.5938	4.03900	
AA	Women	15.5577	7.36024	0.596
	Men	14.7188	6.41186	

*p<0.05. Mann Whitney U test. Abbreviations: PIDAQ, Psychosocial Impact of Dental Aesthetics questionnaire; AA, aesthetic attitude; PI, psychological impact; SI, social impact; DSC, dental self-consciousness.

Correlations Related to Men
A moderately negative correlation was observed between ULT and the subscale SI and AA scores, as well as PIDAQ scores. A strong negative correlation was observed between ULA and the subscale DSC and PIDAQ scores, and a moderately negative correlation was observed between ULA-TVL and the subscale DSC and PIDAQ scores. A moderately negative correlation was observed between the subscale SI scores and ULA.

Table 2. Comparison of the sociodemographic characteristics and IOTN-DHC grades of women and men

			Women	Men	Test Statistics	p
Age	≤ 19	%	67.9	32.1	2.207[1]	0.137
	≥ 20	%	51.6	48.4		
Education Level	High school	%	57.7	42.3	.283[1]	0.595
	University	%	63.8	36.2		
Mother's Educational Status	Primary education	%	60.6	39.4	1.119[2]	0.972
	Secondary education	%	61.5	38.5		
	High school	%	56.3	43.8		
	Bachelor	%	66.7	33.3		
	Master's degree	%	100.0	0.0		
Father's Educational Status	Primary education	%	68.4	31.6	1.310[1]	0.860
	Secondary education	%	65.0	35.0		
	High school	%	53.6	46.4		
	Bachelor	%	64.3	35.7		
	Master's degree	%	66.7	33.3		
Residence	Rural	%	70.0	30.0	1.171[1]	0.557
	Suburban	%	66.7	33.3		
	Urban	%	57.1	42.9		
Income status	1	%	63.2	36.8	.947[1]	0.890
	2	%	60.0	40.0		
	3	%	50.0	50.0		
	4	%	75.0	25.0		
IOTN-DHC Grades	1 and 2	%	9.6	12.5	2.352[2]	0.515
	3	%	25.0	12.5		
	4	%	57.7	62.5		
	5	%	7.7	12.5		

*p<0.05. [1]Pearson Ki Kare. [2]Fisher's Exact test. Abbreviations: IOTN; Index of Orthodontic Treatment Need; DHC, Dental Health Component.

Table 4. Relationship between the STCA parameters and the total and subscale PIDAQ scores of women

		PIDAQ	Rho	DSC	Rho	SI	Rho	PI	Rho	AA	Rho
Overjet	p	0.723	0.050	0.842	0.028	0.468	0.103	0.697	0.055	0.955	0.008
Overbite	p	0.154	-0.201	0.324	-0.140	0.311	-0.143	0.417	-0.115	**0.033***	**-0.297**
ULT	p	0.252	-0.162	0.271	-0.156	0.056	-0.267	0.915	-0.015	0.508	-0.094
LLT	p	0.201	-0.180	0.287	-0.150	0.054	-0.269	0.726	-0.050	0.252	-0.162
Pog-Pog'	p	0.577	0.079	0.355	0.131	0.947	-0.009	0.329	0.138	0.703	0.054
Me-Me'	p	0.547	-0.085	0.693	-0.056	0.243	-0.165	0.387	0.122	0.418	-0.115
NLA	p	0.473	0.102	0.425	0.113	0.538	-0.087	0.062	0.261	0.361	0.129
ULA	p	0.474	0.101	0.408	0.117	0.210	0.177	0.096	-0.233	0.400	0.119
N'-Me'	p	0.584	-0.078	0.822	-0.032	0.290	-0.150	0.467	-0.103	0.956	0.008
Sn'-Me'	p	0.668	0.061	0.864	0.024	0.877	0.022	0.344	0.134	0.529	0.089
Sn'-ULI	p	0.783	-0.039	0.824	0.032	0.393	-0.121	0.712	-0.053	0.908	0.016
LLS-Me'	p	0.698	-0.055	0.832	-0.030	0.384	-0.123	0.953	-0.008	0.931	0.012
ULI-MxI	p	0.057	-0.265	0.208	-0.177	0.225	-0.171	0.087	-0.240	**0.019***	**-0.324**
Sn'-MxI	p	0.443	-0.109	0.540	-0.087	0.515	-0.092	0.842	-0.028	0.477	-0.101
Md1-Me'	p	0.690	-0.057	0.932	0.012	0.247	-0.164	0.514	-0.093	0.973	0.005
A'-TVL	p	0.806	0.035	0.933	-0.012	0.209	0.177	0.660	-0.063	0.966	0.006
ULA-TVL	p	0.635	0.067	0.962	0.007	0.151	0.202	0.451	-0.107	0.684	0.058
Mx1-TVL	p	0.064	0.258	0.242	0.165	**0.002***	**0.419**	0.753	0.045	0.152	0.201
Md1-TVL	p	0.302	0.146	0.505	0.095	**0.031***	**0.299**	0.987	-0.002	0.561	0.082
LLA-TVL	p	0.473	0.102	0.568	0.081	0.173	0.192	0.786	-0.039	0.584	0.078
B'-TVL	p	0.680	0.059	0.402	0.119	0.497	0.096	0.792	-0.037	0.857	0.026
Pog'-TVL	p	0.673	0.060	0.345	0.134	0.739	0.047	0.981	0.003	0.763	0.043

*p <0.05. Bold data are statistically significant. Spearman correlation. Abbreviations: ULT, upper lip thickness; LLT, lower lip thickness; Pog-Pog', soft tissue pogonion thickness; Me-Me', soft tissue menton thickness; NLA, nasolabial angle; ULA, upper lip angle; N'-Me', total face height; Sn'-Me', lower face height; Sn'-ULI, upper lip length; LLS-Me', lower lip length; ULI-MxI, Mx1 exposure; Sn'-MxI, maxillary height; Md1-Me', mandibular height; A'-TVL, soft tissue point A; ULA-TVL, upper lip anterior; Mx1-TVL, maxillary incisor 1; Md1-TVL, mandibular incisor 1; LLA-TVL, lower lip anterior; B'-TVL, soft tissue point B; Pog'-TVL, soft tissue pogonion; PIDAQ, Psychosocial Impact of Dental Aesthetics Questionnaire; AA, aesthetic attitude; PI, psychological impact; SI, social impact; DSC, dental self-consciousness; STCA, soft tissue cephalometric analysis; TVL, true vertical line.

Table 5. Relationship between the STCA parameters and the total and subscale PIDAQ scores of men

		PIDAQ	Rho	DSC	Rho	SI	Rho	PI	Rho	AA	Rho
Overjet	p	0.853	0.034	0.161	0.254	0.399	-0.154	0.831	0.039	0.816	-0.043
Overbite	p	0.634	0.087	0.320	0.181	0.634	-0.088	0.528	0.116	0.968	0.007
ULT	p	**0.024***	**-0.399**	0.051	-0.348	**0.026***	**-0.392**	0.537	-0.113	**0.026***	**-0.393**
LLT	p	0.316	-0.183	0.384	-0.159	0.267	-0.202	0.851	-0.035	0.286	-0.195
Pog-Pog'	p	**0.047***	**-0.354**	**0.020***	**-0.408**	0.434	-0.143	0.647	-0.084	**0.030***	**-0.384**
Me-Me'	p	0.689	-0.074	0.846	-0.036	0.735	-0.062	0.328	0.179	0.122	-0.279
NLA	p	0.920	-0.018	0.585	0.100	0.619	-0.091	**0.035***	**-0.373***	0.601	-0.096
ULA	p	**0.001***	**-0.542**	**0.001***	**-0.540**	**0.017***	**-0.419**	0.432	-0.144	0.090	-0.305
N'-Me'	p	0.950	0.012	0.544	0.111	0.918	-0.019	0.825	-0.041	0.718	-0.067
Sn'-Me'	p	0.320	-0.182	0.828	0.040	0.528	-0.116	0.135	-0.270	0.248	-0.210
Sn'-ULI	p	0.192	-0.237	0.263	-0.204	0.765	-0.055	0.218	-0.224	0.205	-0.230
LLS-Me'	p	0.199	-0.233	0.488	-0.127	0.679	-0.076	0.167	-0.250	0.172	-0.247
ULI-MxI	p	0.151	0.260	0.246	0.211	0.180	0.243	0.475	0.131	0.205	0.230
Sn-MxI	p	0.888	0.026	0.356	0.169	0.848	0.035	0.522	-0.117	0.859	-0.033
Md1-Me'	p	**0.043***	**-0.360**	0.253	-0.208	0.304	-0.188	0.290	-0.193	**0.019***	**-0.412**
A'-TVL	p	0.754	0.058	0.855	-0.034	0.550	0.110	0.134	0.271	0.531	0.115
ULA-TVL	p	**0.004***	**-0.490**	**0.004***	**-0.491**	0.069	-0.326	0.236	-0.216	0.122	-0.279
Mx1-TVL	p	0.872	0.030	0.919	-0.019	0.448	0.139	0.855	-0.034	0.370	0.164
Md1-TVL	p	0.705	0.070	0.710	-0.068	0.226	0.220	0.921	-0.018	0.193	0.236
LLA-TVL	p	0.363	-0.166	0.057	-0.340	0.717	0.067	0.638	-0.087	0.873	-0.030
B'-TVL	p	0.510	-0.121	0.099	-0.297	0.499	0.124	0.252	-0.209	0.886	0.026
Pog'-TVL	p	0.061	-.399	**0.012***	**-0.440**	0.558	-.107	0.132	-0.272	0.223	-.222

*p < 0.05. Bold data are statistically significant. Spearman correlation. Abbreviations: ULT, upper lip thickness; LLT, lower lip thickness; Pog-Pog', soft tissue pogonion thickness; Me-Me', soft tissue menton thickness; NLA, nasolabial angle; ULA, upper lip angle; N'-Me', total face height; Sn'-Me', lower face height; Sn'-ULI, upper lip length; LLS-Me', lower lip length; ULI-MxI, Mx1 exposure; Sn'-MxI, maxillary height; Md1-Me', mandibular height; A'-TVL, soft tissue point A; ULA-TVL, upper lip anterior; Mx1-TVL, maxillary incisor 1; Md1-TVL, mandibular incisor 1; LLA-TVL, lower lip anterior; B'-TVL, soft tissue point B; Pog'-TVL, soft tissue pogonion; PIDAQ, Psychosocial Impact of Dental Aesthetics Questionnaire; AA, aesthetic attitude; PI, psychological impact; SI, social impact; DSC, dental self-consciousness; STCA, soft tissue cephalometric analysis; TVL, true vertical line.

between pog-pog' and the subscale AA and DSC scores, as well as PIDAQ scores. A moderately negative correlation was observed between Md1-Me' and the subscale AA and PIDAQ scores. A moderately negative correlation was observed between the subscale DSC scores and Pog'-TVL. A moderately negative correlation was observed between the subscale PI and NLA. The relevant data are presented in Table 5.

DISCUSSION

Sociodemographic factors, such as age, socioeconomic status, and the severity of malocclusion, can affect the psychosocial impact of malocclusions (8-12). In the present study, the IOTN-DHC grades and sociodemographic data of both men and women were similar, which facilitated the evaluation of the effect of gender on the relationship between STCA parameters and malocclusion perception. The financial aspect of orthodontic treatment may lead to an error in understanding the perception of malocclusion. At our clinic, the financial burden of treatment was a primary reason for rejecting orthodontic treatment; therefore, patients who rejected orthodontic treatment were not included as a control group. We aimed to enroll a similar number of men and women at the time of sample size calculation; however, the number of male and female participants could not be equalized. Since the two groups were homogeneous regarding the PIDAQ scores, IOTN grades, and sociodemographic data, it was determined that the differences between the sample sizes of the two groups would not affect the results.

IOTN has two separate components: IOTN-DHC, which assesses the objective need for treatment, and IOTN-AC, which assesses the aesthetic component of the perceived need for treatment (5). IOTN-AC is used to determine the need for orthodontic treatment when the IOTN-DHC grade of the patient is 3. The IOTN-AC score is evaluated by dental professionals in the original IOTN. However, previous studies have indicated that the scoring of IOTN-AC by orthodontists differs from that of laypeople (13-15). Moreover, some studies have shown that the IOTN-AC component may reduce the need for orthodontic treatment (15,16). Therefore, the IOTN-AC component was not used in this study.

According to the data obtained from this study, the anatomical features of the face that can be considered attractive (according to the literature) are less affected by the psychosocial impact of malocclusion. NLA plays an important role in the perception of facial profile attractiveness (17). Sinno et al. determined that a steeper NLA is ideal in North American men, whereas a wider NLA is suitable in Asian and Caucasian men (18). Consistent with these findings, in our study, the malocclusions' psychological effects were weaker in Caucasian men with a wider NLA. Many studies have stated that a profile with a protruding nose and chin and less protruding lips is considered suitable in men (19-24). However, some studies have reported that similar to the profile of women, fuller lips and a convex profile are considered more attractive in adolescents and young men due to the influence of fashion magazines (25,26). Consistent with these studies, DSC increased in adolescents and young men when the pogonion was positioned backward relative to TVL. Similarly, the negative effects of the existing malocclusions decreased in adolescents and young men when the values of ULT, ULA-TVL, and ULA increased. An increased displayed length of maxillary teeth at rest is characteristic of younger individuals (27). In line with this, the present study showed that increasing the value of Mx1 exposure reduced the aesthetic concern associated with malocclusion in women.

Although the severity of the malocclusion and the sociodemographic data of women and men were similar in the present study, the psychological effect of malocclusion was more severe in women. The fact that women are psychologically more affected by malocclusions may be due to the societal pressure applied on women for centuries to appear attractive (28); in contrast, men are not judged based on such aesthetic standards (29). Apart from societal pressure, the inherent differences between men and women may also cause the psychological effect of malocclusion to be more severe in women. Directing the perception formed by the sense of sight to a specific stimulus is known as selective attention, whereas directing it to more than one stimulus is known as split attention (30). Since women tend to place greater emphasis on facial appearance than men (31), combined with the lower number of stimuli affecting the face of women (only STCA parameters related to the positions of the teeth), they may experience increased selective attention, thereby amplifying the PI of malocclusion.

Contrary to our expectations, soft structures such as upper lip and pogonion thickness and projections of

the upper lip and pogonion only affected the PIDAQ scores of men. Indeed, men and women have a developmental discrepancy regarding their cognitive processing of faces. Men tend to gain spatial relations abilities by scanning a wider area (32-34) and integrating the internal and external facial features as a whole rather than as individual parts (35). Men's tendency to perceive the whole from a broader perspective may cause them to be affected by soft tissue structures when evaluating malocclusion. On the other hand, since women scan a narrower area, they are more likely to detect changes in the position of the teeth. Similarly, according to the data obtained in this study, changes in dental positions affected the SI of malocclusions and aesthetic anxiety in women only.

Limitations

This study has some limitations. Adolescent and young adult patients were included in the study; however, their characteristics did not reflect those of younger or older populations. Moreover, only the STCA parameters related to the lower third of the face were evaluated in the present study. Midface anomalies were not evaluated as they are seen more frequently in patients with skeletal problems. Therefore, the impact of the soft tissue structure of the midface on the psychosocial effect of malocclusion should be examined in patients with skeletal malocclusions. Lastly, soft tissue compensation might be different in different skeletal malocclusions. Thus, more extensive studies, including larger patient populations of different ages and with skeletal malocclusions, should be performed in the future.

CONCLUSION

In conclusion, the findings of this study suggest that the number of STCA parameters affecting the total and subscale PIDAQ scores was higher in men than in women. Soft structures, such as upper lip and pogonion thickness and projections of the upper lip and pogonion to the TVL, were found to affect men's subscale and total PIDAQ scores. In contrast, STCA parameters related to the positions of the teeth were found to affect the subscale PIDAQ score of women. Nevertheless, the psychological effect of malocclusion was found to be more severe in women.

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EFFECT OF ASSISTIVE ROBOTIC TECHNOLOGIES ON QUALITY OF LIFE AND FUNCTIONAL INDEPENDENCE IN INDIVIDUALS WITH SPINAL CORD INJURY

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ABSTRACT

Purpose: To investigate the effect of assistive robotic technologies on quality of life, functional independence, and perceived fatigue level in individuals with spinal cord injury (SCI).
Material and Methods: This research involved a cohort of 25 patients who had been diagnosed with SCI. To assess their progress, clinical assessments were administered both at the commencement and completion of a six-week robotic rehabilitation treatment regimen. The evaluations encompassed the use of the Spinal Cord Independence Measure (SCIM III) to measure their performance in daily living activities and mobility. Additionally, the quality of life was assessed using the World Health Organization Quality of Life Scale – Short Form (WHOQOL-BREF) scale, while the levels of fatigue experienced during rehabilitation were gauged using the Modified Borg Scale (RPE).
Results: The participants' average age and BMI were 40.72±1.28 kg/m2 and 23.43±0.57 year. Statistically significant differences were found in self-care (p=0.006) and mobility (p=0.004) values of SCIM III scale compared to pretreatment values. WHOQOL-BREF General health status, Physical health, Psychological, Social relations and Environment sub-parameters all showed statistically significant differences compared to pre-treatment values (p<0.001). There was a significant decrease in the RPE value to determine the level of fatigue during exertion in robotic walking training (p<0.001).
Conclusion: Assisted robotic rehabilitation approaches increased individual independence, quality of life and reduced fatigue during exertion in Individuals with SCI. We think that assisted robotic approaches applied in addition to traditional rehabilitation provide additional benefits in increasing the level of independence and quality of life of individuals with SCI in daily life and reducing fatigue during exertion.

Keywords: daily living activities, robotic rehabilitation, spinal cord injury, quality of life.

INTRODUCTION

Spinal cord injury (SCI) often experiences a significant decrease in their quality of life (QoL) due to lack of voluntary muscle control, which severely affects their independence. After sustaining a SCI, individuals typically face an irreversible motor and sensory impairment, resulting in symptoms like spasticity, muscle paralysis, atrophy, pain, and gait

disorders (1). SCI has profound consequences for the entire body, affecting systems such as the musculoskeletal, respiratory, cardiovascular, gastrointestinal, genitourinary, metabolic, skin, and neurologic systems (2).The SCI often prevents individuals from fulfilling their daily activities and may have a detrimental effect on their overall QoL (3). The QoL is closely linked to the fulfilment of personal needs, having control over one's neighborhood and having the freedom to choose. Research findings show while compared to the general population, individuals having SCI experience a significant decrease in their QoL (4). However, it is important to note that adjusting to living with severe impairment is a complex journey and life fulfilment trajectories may differ between various subgroups of individuals with SCI (5). To advance the field of rehabilitation, assistive technologies should focus on harnessing individuals' potential and promoting their social engagement and successful reintegration into society, ultimately enhancing their QoL.

Some of the innovations examined in these studies include neuro-prostheses, hybrid systems, orthotic devices, robotic aids, limb supports, virtual reality, virtual reality, reinforced exoskeletons, brain-computer interfaces, and portable devices for electronic assistive (6-11). This innovation hold promises for applications in aiding individuals in tasks, facilitating rehabilitation, mobility and enhancing brain connectivity. These studies have shed light on the effectiveness, adoption and perceived equity of using assistive technologies for behavioural management, diagnostic interventions and collaborative self-management among people with SCI (8, 9). Studies underline how the continued development and expanding use of these technologies offers an excellent opportunity to improve people with disabilities' QoL (6, 11). Furthermore, augmented exoskeletons and brain-computer interfaces demonstrated their utility and value as assistive technologies and educational interventions during the rehabilitation phase for individuals with disabilities resulting from quadriplegia. Nonetheless, particular challenges and areas requiring further work also come to the fore. There are limitations in the application of brain-computer interfaces, and a substantial knowledge gap exists concerning brain connectivity following SCI, which could significantly influence the selection of appropriate assistive technologies.

Robotic-assisted walking training was introduced at the end of the 1990s and provides a number of benefits (12). It allows you to increase the frequency and overall duration of training sessions while retaining the natural gait pattern. The practice of task-specific gaits enhances the sensory response related to typical movement and has the potential to induce changes in the relevant motor centres (13). The speed of movement, level of assistance and amount of weight support can be regulated to create a challenging environment for individuals to practice stepping. Robotic rehabilitation is recognized as potentially enabling SCI individuals to lead a healthier and more active gait and increase their physical activity levels, and is a promising approach for restoring functional gait and improving locomotor skills (14). After evaluating the literature in our country and other countries, we aimed to contribute to the rare studies conducted in Türkiye on robotic rehabilitation and traditional rehabilitation combined treatment. In addition, in our study that we conducted only with patients at certain levels of neurological injury, we aimed to minimize the effect of neurological differences between the levels of the patients on the treatment process. Similar studies conducted on specific levels in the future will allow us to gain more information about the symptoms and consequences of SCI depending on the levels. In our study, we planned to investigate the effect of the individual fatigue level that occurs during rehabilitation on robotic rehabilitation training. It appears that the number of studies conducted in this context is very limited. In line with the goals we set in our study, we aimed to analyze the effect of assistive robotic technologies on the health QoL, functional independence and perceived fatigue level in individuals with SCI.

MATERIAL AND METHODS

In this study, 25 individuals diagnosed with SCI participated. Our study is a single group and robotic rehabilitation, and traditional neurological rehabilitation were applied in the same process. When classifying spinal cord injuries, it is determined whether the injury is complete or incomplete and the American Spinal Cord Association Impairment Scale (AIS), which indicates the degree of impairment, is determined. This scale is a standardized neurological examination used by the rehabilitation team to assess the sensory and motor levels which were affected by

Table 1. Demographic information of the participants

Variables	Value Mean±SD or n (%)	
Age, Year	40.72±1.28	
Gender Male/Female	18 (%72)	7 (%28)
BMI (Kg/m^2)	23.43±0.57	
Duration of injury (Years)	2.60±1.66	
Neurological Level	T2	3 (12)
	T3	1 (4)
	T6	1 (4)
	T8	7 (28)
	T9	1(4)
	T10	1 (4)
	T12	11 (44)

BMI (Kg/m2): Body Mass Index, n: number of people, , SD: standard deviation, T: thoracic spine level

the spinal cord injury. Inclusion criteria: those diagnosed with spinal cord injury, thoracic (T1-T12) and lumbar (L1-L5) level injuries according to AIS were included in the study. Those with conditions that may prevent their participation in robotic rehabilitation (infection, incontinence, open wound in the lower extremity, etc.), additional orthopedic diseases and cognitive problems were excluded from the study.

Rehabilitation Program
A traditional rehabilitation programme aimed at increasing the functionality and general health levels of the patients during their inpatient treatment was applied; exercises were performed to increase general endurance and aerobic capacity, improve range of motion in the body and limbs, increase muscle strengthen and improve mobility. The standard exercise program performed here consists of stretching, strengthening, functionality, balance and coordination exercises performed on the meth. The aim was to improve functionality and mobility by selecting frequently used activities in daily life activities to solve the problems in the areas where the patients had problems. All this traditional rehabilitation programme was applied for 6 weeks, 5 days a week and 45 minutes a day.
The Lokomat® (Volketswil,Switzerland) is a robotic gait orthosis designed for use in neurorehabilitation, with the aim of automating locomotor functions. Consisting of a system that supported the user's human body weight and combined with a treadmill. This setup mimics the biomechanics of lower limb movement during above-ground walking and can be complemented by an interactive reality system. Lokomat® could be categorised into an exoskeleton

robot. In such robots, linear electric motors drive knee and hip movements, guiding an orthosis attached to the user's body. In addition, during the swing phase, a foot lift mechanism produces passive dorsiflexion of the ankle (15). Supports a bilaterally symmetrical walking pattern by encouraging each individual to actively propel each limb when stepping on the treadmill. Lokomat® uses a previously programmed walking pattern that mimics normal walking kinematics. These include synchronisation of the walking cycle, cooperation between extremities and joints, and proper distribution of the load on the limbs to facilitate effective rehabilitation (16). The participants in our study participated in training on different games, from simple to difficult levels, for 6 weeks, 3 days a week, 30 minutes a day, using the Lokomat® lower extremity robotic device. The aim of the games was to improve mobility, increase lower extremity functionality, and improve QoL and improve mobility and daily activities.
During the initial session, clinical assessments were conducted, which included gathering personal information such as gender, age, height, weight, educational background, and the level of injury through direct questioning. Various assessment tools were then used at both the beginning and end of the six-week robotic rehabilitation and traditional neurological rehabilitation treatment program. The Scale of Spinal Cord Independence (SCIM III) was used to assess the participants' performance in activities of daily living and mobility, World Health Organization Quality of Life Scale (WHOQOL-BREF) was used to measure the participants' quality of life, and the Modified Borg Scale (RPE) was used to
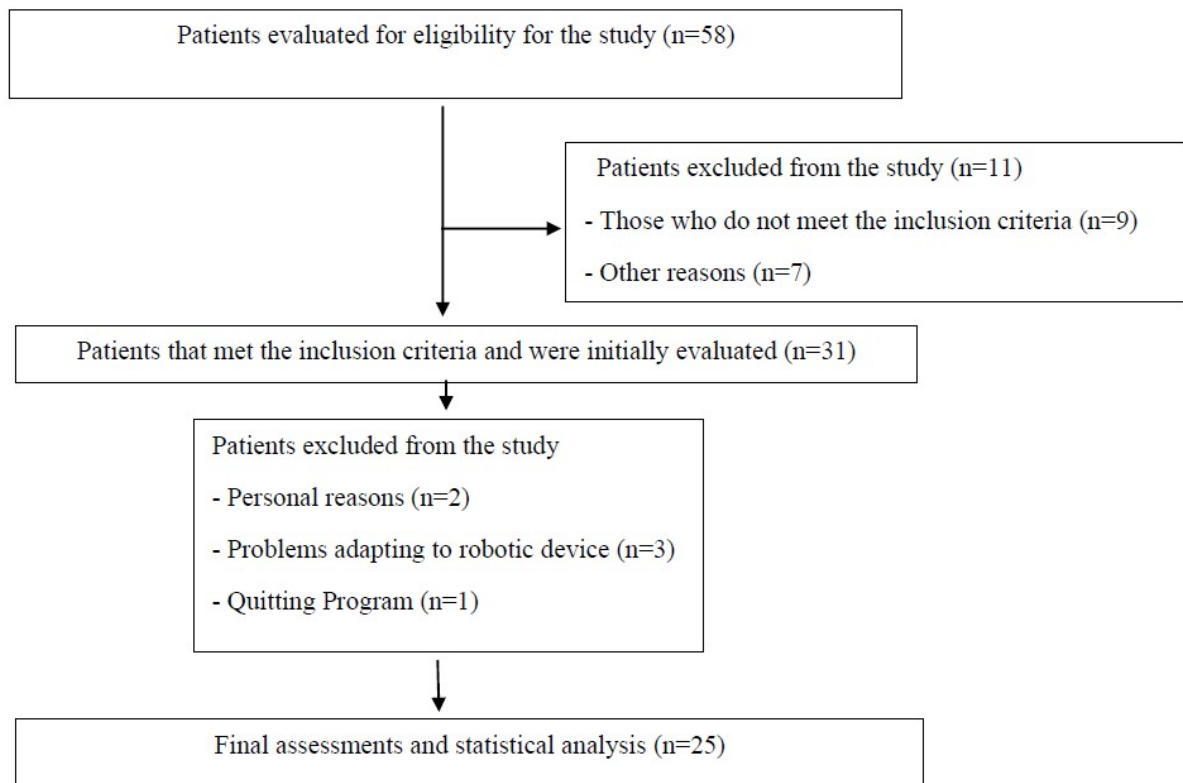
Figure 1. CONSORT diagram

measure the level of fatigue experienced during the rehabilitation process.

Spinal Cord Independence Measure (SCIM III)
The SCIM III was developed to address three specific areas of functioning in individuals with SCI. Evaluates the person's ability in self-care tasks like feeding, self-grooming, bathing and dressing, as well as control over breathing and sphincter management. Additionally, it evaluates a patient's mobility skills, both in terms of bed and transfers and indoor/outdoor movement. Furthermore, the SCIM III serves as a diagnostic tool for clinicians to identify aims and objectives of care for individuals with SCI. It's notable for its user-friendly nature, as the scoring system is self-explanatory and doesn't require a manual to guide clinicians through the scoring process. The SCIM III scores are quantified on a scale that spans from 0 to 100. While a score of zero means complete dependence, a score of one hundred means complete freedom. Each subscale score within the SCIM III evaluation corresponds to this 100-point scale. In particular, the sub-scale for self-care ranges from 0 to 20, the subscale for respiratory and sphincter administration ranges from 0 to 40, and the

subscale for mobility ranges from 0 to 40. This allows for a detailed assessment of an individual's functional independence in various aspects of daily living and mobility (17). The Turkish validity and reliability of the scale was determined by Kesiktas et al (18)

World Health Organization Quality of Life Scale (WHOQOL-BREF)
WHOQOL-BREF is a condensed version of WHOQOL-100 and both were published in 1995, developed by WHO. This comprehensive questionnaire was created through a collaborative effort spanning several years and involving 15 centers worldwide. The questions in the WHOQOL-BREF reflect the perspectives of individuals with and without health problems and the views of health professionals, utilizing a variety of statements related quality of life, health and well-being. The WHOQOL-BREF is a self-administered survey that comprises 26 questions. Investigates individuals' perceptions of themselves regarding their health and well-being during the previous fortnight. The answers to these questions are evaluated according to a 1-5 Likert scale, with a score of 1 meaning "disagree" or "strongly disagree" and a score of 5 meaning

J Basic Clin Health Sci 2024; 8: 580-589

Yildiz A et al. Assistive Robotic Technologies and Spinal Cord Injury

Table 2. Comparison of baseline and post-treatment parameters

	Baseline	Post-treatment	P	Cohens-d
	Mean±SD	Mean±SD		
SCIM III self-care (0-25)	10.16±3.13	12.32±2.06	0.006*	0.815
SCIM III breathing and sphincter control (0-35)	24.04±6.51	24.36±3.87	0.406	0.060
SCIM III mobility (0-40)	9.76±4.72	8.32±3.88	0.004*	0.333
SCIM III total (0-100)	43.52±12.97	45.00±8.27	0.076	0.136
WHOQOL-BREF- General health condition (%)	48.31±11.72	57.31±7.83	<0.001*	0.903
WHOQOL-BREF- Physical health (%)	41.88±10.17	49.67±6.79	<0.001*	0.901
WHOQOL-BREF-Psychological (%)	38.57±9.55	45.85±6.26	<0.001*	0.902
WHOQOL-BREF- Social relations (%)	57.96±14.07	68.77±9.39	<0.001*	0.904
WHOQOL-BREF- Environment (%)	64.20±15.72	75.96±9.03	<0.001*	0.917
RPE (6-20)	15.68±2.98	8.92±2.77	<0.001*	2.350

SCIM: Spinal Cord Independence Measure, RPE: The Modified Borg Scale, WHOQOL-BREF: The World Health Organization Quality of Life Scale – Short Form, SD: Standard deviation, d: Cohen effect size *Shapiro-Wilk test; significance was accepted as p<0.05.

"strongly agree" or "strongly agree". WHOQOL-BREF assesses four different domains, each of which has specific aspects: Psychological well-being, Physical health, social relationships and Environment. In addition, there are two separate questions that directly question the person's general health perception and general QoL (19). The Turkish validity and reliability of the scale was determined by Eser et al (20).

The Modified Borg Scale (RPE).
The Modified Borg Scale, also known as the Rate of Perceived Exertion (RPE), is a method used to assess the intensity of physical activity based on personal sensations like increased heart rate, breathing rate, sweating, and muscle fatigue. It ranges from 6 to 20, with 6 indicating no exertion and 20 indicating maximal effort. Typically, an RPE of 12 to 14 is considered moderate intensity. Monitoring RPE helps adjust exercise intensity in real time to match fitness goals and comfort levels, making it a valuable tool for gauging and managing workout intensity (21).

Statistical Analysis
The sample size was calculated using the G-power program. The SCIM III score in the reference article was calculated with a 1% margin of error and a confidence interval of 0.99, and it was deemed sufficient to include 23 people in the study (22). Analyzing the data obtained in the study "SPSS

(Statistical Package for Social Sciences) (SPSS 22.0, SPSS, Chicago, IL)" statistical programme was used, p<0.05 (two directional) values are statistically considered significant. Study data whether it shows normal distribution using the Shapiro-Wilk test evaluated. The study data were analyzed as normal distribution was found to be appropriate. The study in the statistical analysis, the evaluation variables taken are minimum, maximum, average (Mean), standard deviation (SD) and percentage (%) were defined with values. To compare the values of the patients before and after treatment, Paired Sample t-test was used. According to Cohen's d value, if the d value was less than 0.2, the effect size was considered weak, if the d value was 0.5, the effect size was considered moderate and if the d value was greater than 0.8, the effect size was considered strong (23).

Ethical Considerations
The study received ethical approval from the Bakirkoy Dr. Sadi Guest Training And Research Hospital Clinical Research Ethics Committee (Date: 19.06.2017, Decision No: 2017-06-24), and conducted in accordance with the principles set out in the Declaration of Helsinki.

RESULTS
The research involved a cohort of 25 individuals with SCI for various reasons. The majority of these people consist of male participants (%72). Detailed demographic information and the neurological levels

of the participants can be found in Table 1. Among the SCI individuals included in the study, 12 were AIS A (48%), 6 were AIS B (24%), 4 were AIS C (16%) and 3 were AIS D (12%). Furthermore, the mean duration of injury of the participants was 2.60±1.66 years.

Statistical analysis revealed notable findings in this study. In particular, an improvement of 2.16 points in the self-care subscore (p= 0.006) of the SCIM III scale and a 1.44-point improvement in the mobility sub-score (p= 0.004) were achieved. It was observed that there was a significant difference compared to the pre-treatment values of these two sub parameters. However, significant difference was not observed in respiratory and sphincter control sub-parameters (p=0.406). Additionally, when the WHOQOL-BREF scale was examined, an improvement of 7.79 points in the physical health sub-score (p<0.001), 7.28 points in the psychological sub-score (p<0.001), 11.76 points in the environmental sub-score (p<0.001), 10.81 points in the social relations sub-score (p<0.001) and 9.00 points in the general health sub-score (p<0.001) was achieved. All sub-parameters showed statistical differences compared to pre-treatment values. This suggests changes in the participants' perceived QoL across these domains. Additionally, a significant decrease with a 6.76 point improvement in the RPE value, which is used to evaluate the level of fatigue during effort in robotic walking training, is a remarkable result (p<0.001). These findings are synthesized in Table 2, which provides a comparative analysis of pre- and post-treatment parameters. In our study, the SCIM III mobility parameter had a low effect size (<0.5) in the Cohen-d effect size values of the parameters that were significant when comparing the pre- and post-treatment values; Our other parameters were found to have a high effect size (>0.8) (see table 2).

DISCUSSION

This study includes an attempt to assess independence, quality of life, activity level and fatigue during activity in individuals with SCI using robotic-assisted physiotherapy in combination with conventional physiotherapy. In our study, specialised assessment tools were used to assess independence in daily living and disease-specific clinical status, which are specific to individuals with SCI. The potential of developments in the area of assistive technologies is both extensive and varied, as demonstrated by the many studies that have

examining their effectiveness and clinical applications. We found a remarkable improvement in SCIM III self-care and mobility levels, similar to the results of robotic-assisted rehabilitation in the literature. While a positive increase was observed in other parameters (breathing and sphincter control and total score), this increase was not statistically significant. We think that robotic walking aids used in SCI rehabilitation increase the independence of the individual. These advancements hold great promise for various applications, including assisting individuals in tasks, aiding in rehabilitation, enhancing mobility, and improving brain connectivity.

Research in this area has illuminated the efficacy, uptake and value of using assistive technologies as therapeutic interventions and means of self-management among persons with SCI (10). Considering the literature, it is seen that assistive robotic devices are used in many areas and in many subjects. When we look at similar studies on this subject; the pilot study revealed that robotic-assisted ambulation positively affected individuals with SCI. They noted that participants showed improvements in walking, mobility, overall quality of life, increased self-confidence and decreased dependence on caregivers, and that exoskeleton technology has the potential to improve both the physical and psychological health of people with SCI (24). In the clinical trial, it was observed that robotic-assisted gait training can lead to significant improvements in motor function, particularly for individuals with incomplete SCI. It was stated that the participants in the group receiving robotic-assisted gait training showed significant improvements in motor recovery significantly improved gait in comparison to the control group (25). In another study found that robotic gait training had a positive impact on cardiovascular condition in individuals with SCI. Participants experienced improved cardiovascular fitness and circulation as a result of the training. The results indicated that this form of rehabilitation not only enhances mobility but also contributes to overall health. However, the study recognized the need for tailored approaches for varying degrees of SCI and emphasized the importance of long-term studies to fully understand the cardiovascular benefits (26). Conducted in another study explored the psychological impact of assistive technologies on the QoL for individuals with SCI. The results indicated that these technologies can have a positive effect on mental well-being by increasing independence and

self-esteem. The study emphasized that assistive technologies could mitigate psychological challenges associated with SCI, same as depression and feelings of helplessness (27). Robotic-assisted gait training, specifically using the Lokomat system, has shown positive effects on motor impairments including walking speed, walking distance, effort, range of motion and locomotion in persons with SCI (28). It has been stated that improved functional independence, measured by SCIM III, is associated with sitting balance and wheelchair skills in full-time wheelchair users with SCI (29). Individuals with SCI, especially those with paraplegia, show good functional autonomy in self-care, respiration and incontinence management, but may experience limitations in mobility, particularly in tasks such as climbing stairs and transferring from the ground to a wheelchair (30). In two different robotic walking rehabilitation studies that evaluated using SCIM, both studies reported positive improvements in ADL independence, but no significant results were found (31, 32). As a result of the evaluation we made using SCIM in our study; While there was an improvement in breathing and sphincter control and total score at the end of the treatment, no significant results were observed. A statistically significant difference was found in the self-care and mobility sub-scores. Moreover, low effect size for SCIM III mobility and high effect size for SCIM III self-care were observed after the treatment. If we examine it from this dimension, we see that we obtain more positive and meaningful results as a result of not working. It can be thought that robotic walking training applied in addition to the traditional neurological rehabilitation program may have a positive and significant contribution to ADL activities, depending on physical characteristics.

The integration of robotic-assisted gait training alongside conventional rehabilitation has demonstrated favorable outcomes for individuals with subacute complete SCI (33). They stated that as a result of four studies examined on QoL in the review on robotic walking training, both standing upright and the use of robotic walking devices consistently improved health-related QoL measurements (34). In another more comprehensive review, 12 articles on QoL were examined and as a result, it was stated that robotic walking training had positive effects on QoL (2). A recent study similarly stated that lower extremity robotic walking training may have potential benefits in terms of QoL and daily living activities in SCI patients (35). This combined approach has been shown in the literature to have beneficial effects on the functional independence, mobility, and overall QoL of these individuals. Similarly, in our study, statistically significant differences were observed in all sub-parameters of the WHOQOL-BREF scale. Considering these significant differences, it can be concluded that assistive robotic devices have a positive impact on the QoL of individuals with SCI. Especially when we evaluated the size of the effect size after the comparison of the results, it was seen that we obtained a high effect size in all parameters. We think that this shows that the effectiveness of the treatment is high.

The perceived level of fatigue was another parameter evaluated in our study. In the preliminary study conducted by Sale et al. on Wearable Robot Technologies on individuals with SCI, they found the Borg scale values as 3 ± 3.464 and T1 1.667 ± 1.155. They stated that they recorded a 36% improvement in RPE values (36). Corbianco et al., in their study comparing lokomat and exoskeleton robotic rehabilitation applications, stated that they found the RPE value to be 4-5/10 and that there was no difference between the two different robotic rehabilitation applications (37). McIntosh et al.'s studies reported that perceived exertion was on average "moderate" (mean 3.1 and 3.0) at both the midpoint and end points of a robotic rehabilitation session (38). Escalona et al., in their study using robotic exoskeleton, found the median RPE value to be 3.2, similar to the literature (39). In our study, we recorded an average improvement of 6.76 points after the intervention compared to the pre-treatment RPE values. When we look at the effect size, we see that we obtained a high effect size result. With the training, we achieved a similar improvement in perceived exertion levels as in the literature. In the literature, it is seen that the number of studies evaluating RPE after robotic rehabilitation is limited and the sample size of these studies is low. One of the advantageous aspects of our study is that we have a larger sample size compared to the literature. It is predicted that fatigue levels in individuals with SCI will decrease as the level of exertion increases. Therefore, it can be concluded that the use of assistive robotic devices may contribute to the reduction of fatigue in this population.

Limitations

Studies in this area often face challenges in recruiting a sufficiently large and diverse sample of individuals with SCI. Restricted sample size may make generalising findings to a wider population difficult, while lack of variation may limit the applicability of findings to a diverse subsample of individuals with SCI. SCI can differ greatly in terms of severity and extent of injury and specific impairments. That heterogeneity may make it difficult to reach broad conclusions about the effects of assistive robotic technologies, because what works for one individual may not work for another.

To establish the true impact of assistive robotic technologies, it is crucial to conduct well-designed comparative studies with appropriate control groups. However, finding suitable control groups can be challenging.

The successful integration of assistive robotic technologies often depends on proper training and adaptation. Studies should consider the learning curve and adaptation period required for users to achieve optimal benefits.

CONCLUSION

Assisted robotic rehabilitation approaches increased individual independence, QoL and reduced fatigue during exertion in Individuals with SCI. We think that assisted robotic approaches applied in addition to traditional rehabilitation provide additional benefits in increasing the level of independence and QoL of individuals with SCI in daily life and reducing fatigue during exertion.

Main points: The use of assistive robotic technologies can help in regaining some level of independence and performing daily activities, which can positively affect psychological and emotional well-being.

Assistive robotic technologies such as exoskeletons, wheelchair-mounted robotic arms, or adaptive computer interfaces can assist with mobility, self-care, and communication with spinal cord injuries.

The use of assistive robotic technologies can help in regaining some level of independence and performing daily activities, which can positively affect psychological and emotional well-being.

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Yildiz A et al. Assistive Robotic Technologies and Spinal Cord Injury

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INTERACTION BETWEEN PHYSICAL PERFORMANCE AND HEALTH METRICS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME

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ABSTRACT

Purpose: The study aimed to investigate the potential correlation between physical performance and health metrics in patients with obstructive sleep apnea syndrome (OSA), while also comparing variations in health metrics (such as obesity, hypoxia, quality of life, etc.) and physical performance across different severities of OSA.
Material and Methods: Eighty-four participants undergoing polysomnography with the suspected presence of OSAS were included. Participants completed the Epworth Sleepiness Scale (ESS), 36-Item Short Form (SF-36), Hospital Anxiety-Depression Scale (HADS) questionnaires. Physical performance was assessed using the 30-Second Sit-to-Stand Test (30CST). Participants were categorized into two groups to compare health metrics based on the severity of OSA.
Results: Body mass index, neck circumference, ESS scores, hypoxia levels, emotional well-being, and energy levels of SF-36, differed statistically according to the severity of OSA. While there was no correlation between 30CST scores and social functioning (r=.125, p=.290), all other SF-36 subheadings and 30CST were significantly correlated (p<.05). 30CST variance is predicted significantly by SF-36 (p<.001), HADS (p<.001), ESS (p<.001), obesity (p=.001), and hypoxia (p=.011).
Conclusion: These results indicate that the physical performance of patients with OSA is correlated with health metrics, including quality of life, daytime sleepiness, anxiety-depression, hypoxia, and obesity, irrespective of the severity of apnea-hypopnea.

Keywords: apnea; quality of life; depression; obesity; physical performance.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a common sleep disorder characterised by repeated episodes of collapse of the upper airway during sleep (1, 2). The incidence in the general population ranges from 9% to 38%, it is seen more in men than in women, and it increases with age (3). The main complaints of patients are snoring, fatigue, decreased

cognitive activity and excessive daytime sleepiness (3, 4). Snoring, fatigue, decreased cognitive activity and excessive daytime sleepiness are the main complaints of patients. In addition to these disease effects, nocturnal low oxygen saturation and reduced attention span may affect physical performance in the OSAS population (5-7).

A limited number of studies have shown that physical performance is affected (8-11) or not (12) in patients with OSAS. Physical performance assessment is related to obstructive sleep apnea syndrome (OSAS) because OSAS can impair cardiovascular and muscular function (13), leading to reduced physical performance (14). Assessing physical performance is necessary to understand the extent of these impairments, monitor the effectiveness of treatments, and tailor interventions to improve overall health and quality of life in individuals with OSAS. However, no study on functional performance was encountered. Methods used to evaluate functional performance include the six-minute walk test (15), timed up and go test (16), and sit-to-stand performance test (17, 18). Sit-to-stand performance is achieved by the continuous and effective integration of the central nervous system with vestibular, visual, musculoskeletal, and proprioceptive information (17, 19). According to the data of Byun et al., patients with OSAS had a higher incidence of peripheral vestibular disorders than subjects without OSAS (20). Furthermore, it was shown that a higher incidence of vestibular dysfunction has been observed in patients with moderate to severe OSAS compared with patients with mild OSAS (12, 21). Considering physical performance and vestibular connectivity, in patients with OSAS, it is essential to take into account the contributing factors to impairment. First, excessive daytime sleepiness (EDS), which is the main factor in patients with OSAS, increases the risk of accidents and injuries by causing loss of attention (22, 23), and fatigue (24). Indeed, another factor is nocturnal hypoxia experienced by OSAS patients may cause neurophysiological changes by causing damage to the brain stem centers (25). Additionally, it's crucial to focus on chronic nasal congestion and inadequate oxygen intake, which contribute to hypoxia in OSAS patients (26). Understanding these interconnected factors can provide insights into the mechanisms underlying impairment in physical performance and vestibular function in OSAS, thereby guiding more effective assessment and management strategies.The chronic hypoxia

experienced by OSAS patients can damage the cerebellum and vestibulocochlear nuclei, which are the signal centers of physical performance (27). Also, the current review results reported that OSAS may cause falls by damaging the vestibulo-ocular reflex, visual acuity, cognitive functions (27), and muscle function, which are essential physiological functions (28, 29). Chronic hypoxia in sleep disorders causes neurological damage, impairing cognition, while also increasing the risk of cardiovascular diseases through vascular remodeling and metabolic disturbances, heightening susceptibility to obesity and diabetes (30). Despite all these risk factors, no definite consensus on physical performance loss has been reached yet in OSAS patients, and a standard clinical assessment profile has not yet been established for physical performance assessment in the OSAS population. Explaining the relationship between the loss of physical performance and OSAS is necessary because it helps clinicians and researchers understand the potential consequences of OSAS beyond just sleep disturbances. This understanding underscores the importance of comprehensive assessments and interventions to address not only sleep-related issues but also the broader impact of OSAS on physical health and functioning, such as increased risk of falls (7) due to impairments in various physiological functions such as vestibulo-ocular reflex, visual acuity, cognitive functions, and muscle function (25-30). It also highlights the need to establish standardized protocols for the assessment of physical performance in people with OSAS in order to guide clinical management effectively.

The link between obesity and OSAS is significant, as obesity contributes to the development and exacerbation of OSAS. Excess weight, especially around the neck and throat area, can lead to airway obstruction during sleep, resulting in episodes of apnea or hypopnea (31). Considering the fact that obesity is an important cause of OSAS (32), the risk of falling (7), and vestibular issues (20, 21, 33) of the patients, examining the functional physical performance test (16) which includes an activity that is frequently performed in daily routine such as sitting and standing, maybe a holistic assessment approach for physical performance risk factors in OSAS. The physical performance has been reported to be influenced by multiple physiological and psychological processes (18), representing a specific transfer skill rather than a surrogate measure of lower
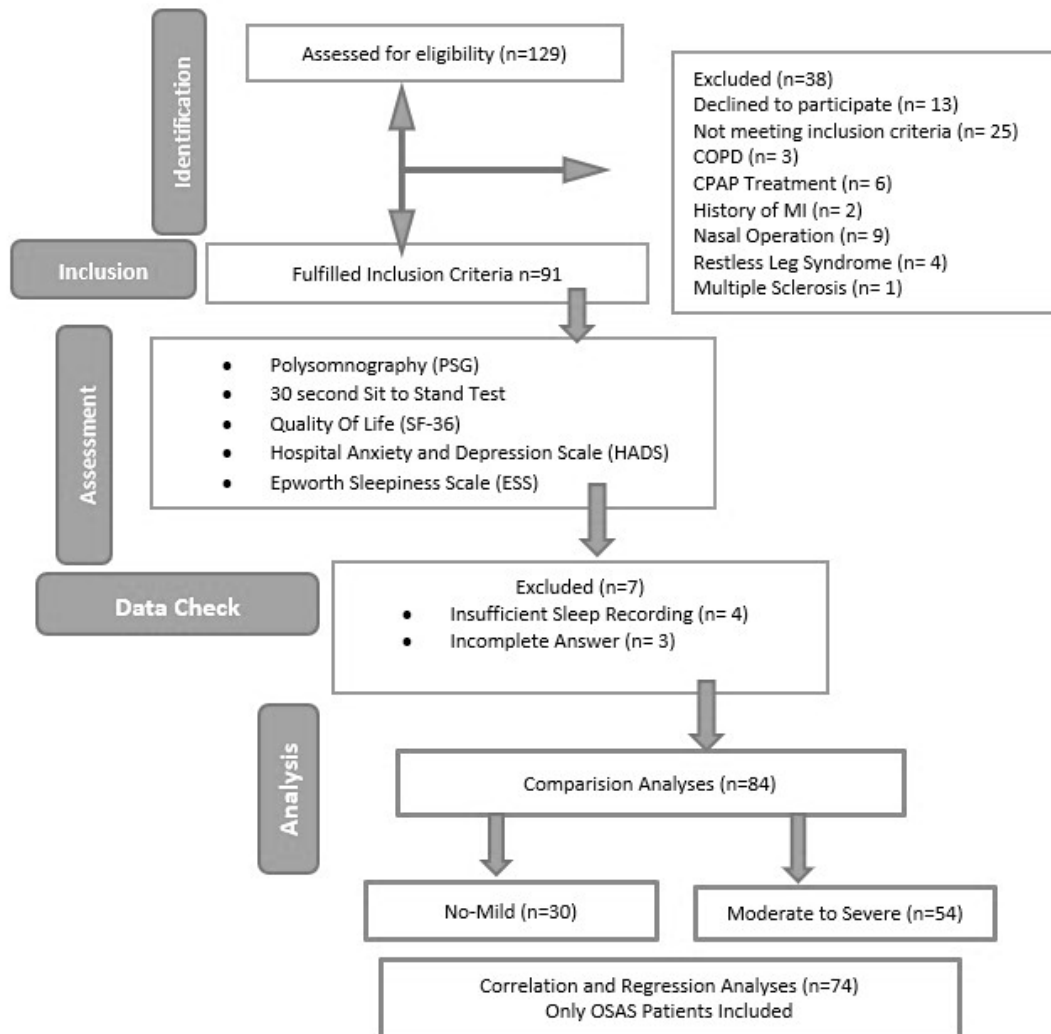
Figure 1. Study flow

extremity strength (17). Also, when the close association of obesity alone (21) or sarcopenic obesity (12) with OSAS and the effect of obesity on postural balance (22) are combined, it is concluded that physical performance examinations may be important in patients with OSAS.

Previous studies have reported that disease severity is not associated with the level of patients' anxiety-depressive symptoms (ADS) (34), EDS correlates weakly with depression and anxiety, and EDS complaints are higher in severe OSAS patients (35, 36). Although studies are showing that the prevalence of depression and anxiety is high in OSAS patients (37), conflicting results have been reported in the literature (38, 39). Decreased deep sleep duration, increased arousal index, and a high ratio of sleep duration with oxygen saturation below 90% to overall

sleep duration in OSAS patients increase EDS and depressive symptoms, thus impairing health-related quality of life (QoL) (40). Untreated OSAS patients have a worse QoL than the general population (5, 41-43). However, there was no correlation between apnea severity usually measured by apnea–hypopnea index (AHI), and QoL of OSAS patients (6, 42, 44). Depression (6), age (45), obesity (35, 36, 46), the severity of hypoxia (42) and EDS (47) have been reported to be the main factors affecting patients' health-related QoL. Unlike the above studies, no study was found that investigated the physical performance and related health metrics in OSAS patients.

The primary objective of this study is to compare the physical performance, QoL, ADS, EDS, and obesity in terms of the severity of OSAS. Subsequently, the

study aims to investigate potential relationships between physical performance and QoL, ADS, EDS, obesity, and hypoxia in patients with OSAS. It was hypothesized that greater severity of OSAS will be associated with poorer physical performance, lower QoL, increased EDS and ADS, and higher obesity rates. Additionally, we hypothesized that there will be significant correlations between physical performance and each of the aforementioned factors, among patients with OSAS.

MATERIAL AND METHODS
Study Design
The study included adults who had undergone a complete sleep assessment in the sleep centre. Informed consent was obtained from all participants. They were all assessed while awake, one hour after their follow-up in the laboratory of the sleep centre. The neck circumference was measured at the level of the cricoid cartilage, and the waist circumference was measured at the level of the navel with a measuring tape. Body height (cm) and weight (kg) were recorded, and BMI (kg/m2) was calculated. Epworth Sleepiness Scale (ESS), Hospital Anxiety Depression Scale (HADS), and 36-Item Short Form (SF-36) questionnaires were completed after polysomnography follow-up. Finally, the physical performance assessment of the participants was performed using the 30-Second Sit to Stand Test (30CST).

Participants
Individuals were included in the study if they were 18 years of age or older, had a body mass index (BMI) of <40 kg/m2, and were willing to participate. Exclusion criteria included (i) any tonsillar or pharyngeal disease (including neuromuscular diseases), nasal polyps, or conchal obstruction due to other causes, (ii) a history of systemic disease causing general health impairment, (iii) other defined sleep disorders, (iv) pathology on PA chest X-ray (asthma, COPD, bronchiectasis, etc.), (v) use of sleeping pills or alcohol. The number of participants was determined using a power analysis conducted in the G*Power software package (version 3.1.9.2). Specifically, the linear multiple regression (fixed model, R^2 deviation from zero) model setting was utilized with the following parameters: effect size (f^2) of 0.30, significance level (α) of 0.05, number of predictors set to 8, and a total desired sample size of

84. This analysis confirmed that the chosen sample size was adequate, with a statistical power $(1 - \beta)$ greater than 0.95. Maintaining a large sample size was an intentional effort to enhance the study's statistical power and influence, ensuring robustness and reliability of the findings.

Of the 129 participants invited to the study, 13 refused to participate and 25 patients did not meet the inclusion criteria, leaving 91 participants who were assessed.

Polysomnography Recording and Protocol
The study included individuals assessed by video recording and overnight polysomnography (Neuron-Spectrum-5, Neurosoft Sleep Systems, Ivanovo, Russia). Standard leads were used, including 6-channel electroencephalography, electrocardiography, bilateral electrooculography, 1-channel chin electromyography, thermistor and nasal pressure transducer for airflow, piezo bands for chest and abdominal wall motion, body position sensor, a pulse oximeter and microphone recording of snoring. Sleep stages were scored in 30-second epochs following the criteria outlined by the American Academy of Sleep Medicine (AASM). All data from the sleep study were recorded. According to the AASM scoring guidelines, OSAS was defined as normal if the AHI was < 5 events/hour, mild if the AHI was 5-15 events/hour, moderate if the AHI was ≥ 15-30 events/hour, and severe if the AHI was ≥ 30 events/hour (48). After all data were collected, participants were examined in two groups according to their AHI scores, as no-mild (n= 30) and moderate or higher (n= 54).

Epworth Sleepiness Scale
The Turkish version of the Epworth Sleepiness Scale (ESS) was used to assess sleepiness level of participants (49). The ESS is an 8-item self-report questionnaire assessing sleepiness during 8 different activities Each question is scored on a scale of 0 to 3 points. Excessive daytime sleepiness (EDS) is indicated by a high ESS score. The cut-off for subjective daytime sleepiness is a scale score >10 (50).

Sit-to-Stand Performance
The 30-Second Sit-To-Stand Test (30CST) was used to assess the functional physical performance of the

Table 1. Demographic, clinical features, and polysomnographic findings of participants

Characteristics	No-Mild OSAS Mean (SD)	Moderate to Severe OSAS Mean (SD)	MD	t/Z	95% CI Lower	Upper
Age (year)	46.00 (9.38)	43.78 (11.35)	2.22	0.91	-2.62	7.07
BMI (kg/m2)	28.87 (2.94)	32.00 (5.09)	-3.12	-3.09	-5.14	-1.11
Waist Circum. (cm)	105.13 (10.55)	109.50 (12.58)	-4.37	-1.61	-9.76	1.03
Neck Circum. (cm)	39.00 (3.54)	41.65 (3.65)	-2.65	-3.22	-4.29	-1.01
ESS Score	3.80 (3.76)	8.78 (6.68)	-2.98	-2.25	-5.61	-0.35
Sleep Study						
AHI (event/hour)	5.89 (3.21)	40.62 (19.66)	-34.74	-9.58	-41.95	-27.52
TB (min)	445.10 (31.29)	443.64 (35.28)	1.46	0.19	-13.91	16.82
TSPT (min)*	394.71 (49.80)	393.02 (70.95)	1.69	-0.76	-27.42	30.80
TST (min)	346.33 (69.06)	336.39 (53.07)	9.94	0.74	-16.88	36.77
SOL (min)*	24.11 (15.59)	35.69 (61.18)	-11.57	-0.02	-34.25	11.10
PSL (min)	27.28 (15.26)	27.56 (20.32)	-0.28	-0.07	-8.74	8.19
SEI (TST/TB) %	77.85 (14.44)	75.46 (10.92)	2.38	0.85	-3.18	7.95
SEI (TSP/TB) %	88.54 (7.77)	91.15 (6.57)	-2.61	-1.63	-5.79	0.57
SQI	104.34 (35.32)	117.05 (29.27)	-12.71	-1.77	-27.00	1.58
RSQI*	20.02 (11.66)	21.99 (7.90)	-1.97	-1.85	-6.23	2.29
REM SL (min)	172.72 (82.76)	162.60 (90.87)	10.12	0.51	-29.78	50.03
N1%*	3.93 (3.67)	4.05 (3.31)	-0.13	-1.05	-1.69	1.44
N2%	74.01 (8.80)	71.54 (9.13)	2.47	1.21	-1.61	6.56
N3%	7.65 (6.05)	9.20 (6.59)	-1.56	-1.07	-4.46	1.34
REM%	14.42 (6.61)	15.20 (7.77)	-0.78	-0.46	-4.12	2.56
Baseline SaO$_2$ %	95.20 (2.04)	92.33 (2.76)	2.87	4.98	1.72	4.01
Mean SaO$_2$% in Sleep	93.33 (1.95)	87.74 (6.42)	5.59	4.64	3.20	7.99
Minimal SaO$_2$ % in Sleep	84.53 (4.50)	66.04 (19.52)	18.50	5.10	11.29	25.71
Average HR*	80.33 (20.34)	84.22 (29.02)	-3.89	-0.93	-15.79	8.02
Minimum HR*	40.13 (11.91)	42.41 (28.35)	-2.27	-0.37	-13.09	8.54
Maximum HR	167.67 (15.88)	161.81 (22.70)	5.85	1.25	-3.46	15.16
Sit-to-Stand Performance						
30CST	12.93 (3.97)	13.11 (3.87)	-0.178	-0.2	-1.947	1.592
Quality of Life (%)						
Physical functioning	63.7 (29.8)	54.3 (25.0)	9.37	1.46	-3.41	22.15
RL due to physical health	38.9 (43.6)	45.0 (39.6)	-6.11	-0.64	-25.25	13.03
RL due to emotional problems	45.7 (40.1)	37.8 (44.4)	7.91	0.83	-10.97	26.78
Energy	52.6 (22.2)	38.0 (14.5)	14.59	3.23	5.61	23.57
Emotional well-being	61.8 (19.9)	48.0 (23.6)	13.78	2.84	4.14	23.42
Social functioning	74.5 (20.5)	65.8 (31.3)	8.70	1.54	-2.55	19.96
Pain	60.7 (29.4)	51.5 (33.9)	9.24	1.31	-4.85	23.33
General health	50.2 (23.3)	45.0 (21.8)	5.19	1.00	-5.15	15.52
Depression and Anxiety						
Depression Score	7.4 (3.8)	8.5 (4.8)	-1.06	-1.11	-2.95	0.83
Anxiety Score	8.2 (4.8)	10.0 (5.6)	-1.85	-1.60	-4.15	0.45

Data are shown as means (SD). MD: Mean difference. No OSAS: Apnea-Hypopnea Index < 5. Mild OSAS: Apnea-Hypopnea Index: 5-15. Moderate OSAS: Apnea-Hypopnea Index: 16-30. Severe OSAS Apnea-Hypopnea Index > 30. BMI: Body Mass Index. Circum: Circumference. ESS: Epworth Sleepiness Scale. AHI: Apnea-Hypopnea Index. TB: Time in Bed. TSPT: Total Sleep Period Time. TST: Total Sleep Time. SOL: Sleep Onset Latency. PSL: Persistent Sleep Latency SEI: Sleep Efficiency Index. SQI: Sleep Quality Index. RSQI: Relative Sleep Quality Index. SL: Sleep Latency. N1: Light Sleep. N2: The Second Stage of Sleep. N3: Deep Sleep. REM: Rapid Eye Movement. HR: Heart Rate. SaO2: Oxygen Saturation. 30CST: 30 Second Sit To Stand Test. RL: Role limitations.
*Mann-Whitney U Test.

assessing lower limb strength, balance, and mobility. Specific physical attributes measured in this test often include:

•   Lower Limb Strength: The ability to stand up from a seated position requires sufficient strength in the muscles of the legs, particularly the quadriceps, hamstrings, and glutes.

•   Balance: Maintaining stability while transitioning from sitting to standing and vice versa involves balance control, which relies on coordination between muscles and sensory feedback.

•   Mobility: The ease and fluidity with which an individual performs the sitting to standing movement can indicate their overall lower limb mobility, including factors such as flexibility and joint range of motion (51). An armless chair with a seat height of 43 cm was used for the test. Participants were seated with their backs to their sides, their feet about shoulder distance apart, and their arms crossed Participants were then instructed to perform as many full squats as possible within 30 seconds. Performing less than 10 sit-ups in 30 seconds is an indication of poor performance (52, 53).

Quality of Life

The Turkish version of 36-Item Short Form was used to assess quality of life (54). It is a reliable, valid and widely used self-report measure of health status. There are 36 questions covering eight areas. These are physical function (10 items), social function (2 items), role limitation due to physical problems (4 items) and emotional problems (3 items), mental health (5 items), vitality (4 items), pain (2 items) and general health (5 items). The scores of the items for each dimension were coded and converted to a scale ranging from 0 (worst health) to 100 (best health) by proportional calculation. The survey was completed by the patients and scores were calculated and recorded for eight subdomains.

Anxiety and Depression

The Hospital Anxiety and Depression Scale (HADS) in the native language of the participants was used (55). It is a self-report scale for the assessment of individuals at risk of anxiety and depression. The HADS consists of two subscales, one measuring anxiety with seven items and another measuring depression with seven items. These are scored separately. High-risk individuals are defined as those who score 10 or more on the anxiety subscale and 7 or more on the depression subscale. Participants

were asked to read the questionnaire carefully and answer the questions according to the answer that was most appropriate for them, and then the scores were recorded.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 21.0 software (SPSS Inc, Chicago, IL, USA). The normality was checked with the Shapiro-Wilk test. The Chi-Square test was used for the categorical analysis of nominal data. Descriptive data for variables were presented in the form of mean and standard deviation, percentiles and frequencies. The Independent Samples T-Test was used to assess variability differences among groups based on the presence and severity of OSAS, taking into account the normal distribution of data (SF-36, HADS, 30CST, ESS, and BMI). The Mann-Whitney U Test was applied for analysing data that did not show a normal distribution (total sleep period time, sleep onset latency, relative sleep quality ındex light sleep period, and heart rate). The correction for multiple comparisons was performed for p-values that reached a statistically significant difference. Pearson correlation analysis was used to analyse the relationship between physical performance, AHI, hypoxia, and the independent variables of QoL, ADS, EDS, and obesity. Correlation coefficient (r) values of 0.00-0.24, 0.25-0.49, 0.50-0.74, and 0.75-1.00 were accepted to present weak, moderate, strong, and very strong correlations, respectively. Using multivariate linear regression analysis, the predictive level of QoL and ADS for physical performance was investigated. The predictive ratio of ESS, hypoxia, and BMI to physical performance was analysed using simple linear regression analysis. Control for VIF (<3), linear relationship, extreme values (Cook's Distance, Mahal Distance), normal distribution of residuals, and homoscedasticity of changes were checked. The significance has been evaluated at a level of $p<.05$.

Ethical Considerations

The study was approved by the Istanbul Medipol University, Non-Interventional Research Ethics Committee (Date: 16.04.2020, Decision No: 314), and followed the standards of the Declaration of Helsinki. Participants were recruited from the respiratory department and sleep centre of Bitlis State Hospital.

Table 2. Interaction Between Physical Performance and Health Metrics in Patients with Obstructive Sleep Apnea

Sit-To-Stand Performance						
	Unstandardized		Standardized		Adjusted R^2	$F(8, 65)$
Quality of life	B	SE_B	β	t	0.396	6.98
Constant	8.90	1.34		6.62		
Physical functioning	0.04	0.02	0.31	2.09		
Role limitations due to physical health	-0.01	0.01	-0.10	-0.68		
Role limitations due to emotional problems	0.01	0.02	0.07	0.45		
Energy	0.07	0.03	0.36	2.26		
Emotional well-being	0.05	0.03	0.29	2.06		
Social functioning	-0.01	0.02	-0.04	-0.30		
Pain	0.04	0.02	0.29	1.89		
General health	0.00	0.02	0.00	0.02		
Depression and Anxiety	B	SE_B	β	t	Adjusted R^2	$F(2, 73)$
Constant	17.15	0.86		19.89	0.266	14.196
Depression Score	-0.11	0.13	-0.122	-0.88		
Anxiety Score	-0.34	0.11	-0.442	-3.17		

	Unstandardized						
Excessive Day Time Sleepiness	B	SE_B	t	p	R	R^2	$F(1, 72)$
Constant	15.65	0.51	30.69	<.001	0.612	0.375	43.213
ESS Score	-0.39	0.06	-6.57	<.001			
Obesity						R^2	$F(1, 72)$
Constant	22.81	2.89	7.89	<.001	0.364	0.133	11.005
BMI (kg/m^2)	-0.30	0.09	-3.32	.001			
Hypoxia						R^2	$F(1, 72)$
Constant	8.84	1.77	4.99	<.001	0.294	0.087	*6.825*
Min. SaO$_2$ (%)	-0.06	0.02	-2.61	.011			

ESS: Epworth Sleepiness Scale. BMI: Body Mass Index. Kg: Kilogram. m2: Square Meter. Min SaO2: Minimal Arterial Oxygen Saturation. **p<.05.

RESULTS

The data of three people who had missing answers in the survey results and four people who had problems with their PSG records were not included in the analysis.

Comparative analyses were performed using data from a total of 84 participants with a mean age of 44.6±10.8 years, and 54 men (64%). Regression analyses were performed with the data of 74 OSAS patients with AHI> 5 events/hour. The clinical characteristics, demographic features, and polygraphic sleep study results of the participants are summarized in Table 1. Of those involved in the study; 11.9% were simple snorers (AHI < 5); 23.8% had mild OSAS (AHI=5-15); 26.2% had moderate OSAS (AHI=16-30); 38.1% had severe OSAS (AHI > 30), and 66.7% of the study participants never smoked (p<.05). Overall participants were categorized as no-mild (n=30), and moderate to severe (n=54) OSAS groups, according to AHI results.

In comparison to the no-mild OSAS group, the moderate to severe OSAS group exhibited statistically significant lower mean differences in BMI (-3.12 kg/m2), neck circumference (-2.65 cm), ESS score (-2.98 points), and AHI (-34.74 events/hour) (p<.05). The baseline (2.87%), mean (5.59%), and minimal (18.50%) SaO2 scores were higher in the no-

mild group than in the moderate to severe OSAS group (p<.001). Energy (14.59%), and emotional well-being (13.78%) QoL parameters were higher in the no-mild group than in the moderate to severe OSAS group (p<.05). All other QoL subheadings of participants did not differ according to the presence or severity of apnea (p>.05). There were no significant differences in other demographic characteristics and polysomnographic findings between the two groups (p>.05).

As a result of the multivariate linear regression analysis (Table 2), a significant regression model, $F_{(8, 65)}=6.98$, p<.001, was found, at the same time it was determined that 40% of the physical performance variance (R2adjusted= .396) was explained by the quality of life sub-variables. Accordingly, physical functioning (β =.31, t (65)=2.09, p=.041, pr2=.06), energy (β=.36, t (65)=2.26, p=.027, pr2=.07), and emotional well-being (β=.29, t (65)=2.06, p=.043, pr2=.06) variables of QoL predict physical performance variable positively and significantly. No significant regression pattern was observed between other QoL variables (p>.05).

A multivariate linear regression analysis (Table 2), was performed to predict physical performance with ADS. As a result of the analysis, a significant regression model, $F_{(2, 71)}= 14.196$, p<.001, was found. At the same time, it was determined that 27% of the physical performance variance (R^2 adjusted=.266) was explained by ADS. Accordingly, anxiety predicts physical performance negatively and significantly (β= -.44, t (71)=-3.17, p=0.02, pr2=.12). Depression, on the other hand, does not significantly predict physical performance (β= -0.12, t(71)= -0.87, p=.383, pr2= .01).

Using simple linear regression analysis, a significant regression model ($F_{(1, 72)}= 43.213$, p<.001) was found in the analysis results of EDS predicting physical performance, and it was found that 38% of physical performance variance (R^2= .375) was explained by EDS. Accordingly, EDS predicts physical performance negatively and significantly (β = -.39, t (72)= -6.57, p<.001).

A significant regression model ($F_{(1, 72)}=11.0$, p=.001) was found in the analysis results of BMI predicting physical performance, and 13% of physical performance variance (R2=.133) was explained by BMI. Accordingly, BMI predicts physical performance negatively and significantly (β = -.30, t (72)=-3.32, p=.001).

A significant regression model ($F_{(1, 72)}= 6.83$, p=.011) was found in the analysis results of desaturation predicting physical performance, and it was found that 9% of physical performance variance (R^2= .087) was explained by desaturation. Accordingly, desaturation predicts physical performance negatively and significantly (β= -.06, t(72)= -2.61, p= .011).

A significant regression model was not formed in the analysis results of AHI's predictive physical performance (p= .211).

DISCUSSION

The objective of the research was to investigate the potential relationship between physical performance and health metrics among individuals diagnosed with obstructive sleep apnea (OSA). Additionally, the study aimed to analyse the differences in health metrics such as obesity, hypoxia, quality of life, etc., and physical performance across varying severities of OSA. Based on the study results, the physical performance is associated with hypoxia, anxiety-depression, daytime sleepiness, obesity, and especially with the pain and physical dimensions of QoL of patients with OSAS. However, the physical performance levels of the participants were within normal limits and did not differ according to the presence and severity of the disease.

Some of the previous studies reported that while physical performance is not affected by disease severity in mild and moderate-severe OSAS patients, only severe OSAS causes abnormal vestibular responses (12), and functional capacity decreases as the severity of apnea increases (9, 10, 12). Unlike these studies, Demir et al. stated that OSAS patients had more balance impairments compared to their healthy peers, especially in cases where head rotation was added or the floor was narrowed when the eyes were closed (8). Also decreased pulmonary function was associated with lower physical performance in OSAS patients (15). Moreover, physical performance is affected by disease severity in OSAS patients, and the factors of this are decreased attention due to impaired sleep quality, increased daytime sleepiness, and low oxygen saturation (8). Results of this study showed that OSAS patients received different responses on physical performance and postural balance according to the type of assessment method. Some of the responses worsened or unchanged according to the

severity of the disease. The observed associations of health metrics with physical performance suggest that functional physical performance evaluations should be included in the assessments of this patient population.

A 1% decrease in oxygen desaturation could cause a 0.59-point decrease in the physical function score of QoL and a 0.13-point decrease in EDS scores according to Huang et al(42). Asghari et al. also showed that physical component scores in the QoL were correlated at a low level with mean oxygen saturation (44). The level of hypoxia was better in individuals with simple snoring and mild OSA than in moderate to severe OSAS patients in the current study. As the severity of the disease increased, hypoxia (9%) slightly worsened. The QoL was more effective (40%) than increased daytime sleepiness (38%) in predicting physical performance regardless of the severity of apnea in patients with OSAS. Similarly, in previous studies, researchers did not observe a difference in QoL according to AHI (6, 44), and only the physical component scores, one of the sub-headings of QoL, were lower in OSAS patients than in their healthy peers (44). Lee et al. reported that hypoxia was associated with obesity, QoL, and the amount of daytime sleepiness was similar to the presented study physical performance results. But unlike the findings of this study, they reported that hypoxia only interacts with the physical components of QoL (6). In light of these, we conclude that by examining the level of physical performance, or hypoxia detailed information can be taken about vestibular function or other sub-headings of QoL, especially the physical components.

In OSAS patients, the prevalence of depression comorbidity is between 7-63%, and for anxiety disorder, it is between 5% and 11-70% (39). Lee et al. showed that both anxiety and depression were independently associated with a lower QoL in patients with OSAS (56). In contrast, the findings of the current study indicate that the severity of OSAS was not related to the level of depression-anxiety experienced by the OSAS patients. Similarly, Asghari et al. reported that the severity of OSAS was not associated with the severity of depression-anxiety (35). Moreover, according to Pamidi et al., higher depression scores are more effective than OSAS severity in predicting QoL and daytime sleepiness in patients with OSAS (32). According to these results, we could say that the relationship between OSAS severity and anxiety along with depression is not yet

clear. To our knowledge, the relationship between physical performance and mental health of OSAS patients is investigated for the first time with our study. The correlation of depression-anxiety level for physical performance was found 27% and it shows that instead of associating the severity of OSAS with the depression and anxiety states of the patients (57), the relationship between physical performance and mental state can be examined. The lower level of depression-anxiety, the better the physical performance of the OSAS patients can be observed. In this population, considering that physical performance slightly affects depression and anxiety symptoms, improvements in mental health can be observed with attempts to improve physical performance. Moreover, Yosunkaya et al. stated that depressed patients with OSAS had worse QoL and increased daytime sleepiness as their depressive state worsened (40). The prediction of daytime sleepiness in physical performance was at the level of 38% in the current study. The effects of increased daytime insomnia comorbidity on physical performance in OSA have demonstrated once again with these results. To minimize the risk of trauma and accident, combined interventions to reduce daytime sleepiness and QoL improvement can be prioritized in the primary preventive treatment phase.

Obesity is the main factor affecting OSAS patients' health-related QoL (35, 46). It is also an important factor in not being able to take better results in physical performance tests (58). Supporting this, Pływaczewski et al. previously reported that obesity is associated with lower physical performance in patients with OSAS (15). In this study, the result of higher BMI in the moderate to severe OSAS group than in the no-mild group was expected. However, while it was thought that the effect of obesity on the physical performance of OSAS patients would be much higher, it did not play an active role as much as other health metrics (quality of life, daytime sleepiness, anxiety, depression, etc.). Unlike the previous literature, the predictive effect of obesity on the physical performance of patients with OSAS was investigated in this study and concluded that it was only 13%. The reason for these results may be not including a specific BMI classification in the study. Another reason may be that the functional physical performance test chosen for the study was not sufficient to reflect the effect of obesity in this patient population. However, with our study results showing that obesity has a small effect on physical

performance in patients with OSAS, we could say that approaches to obesity management should be included in the routine clinical follow-ups of patients.

Limitations

The main limitations are the inability to make a physical performance assessment which suggests that more objective outcome measures should be included in further studies to examine the effect of disease severity on physical performance. Further studies should be conducted in a larger sample by including different functional physical performance tests in the assessment. Also, further research can be done with functional tests that require more effort to examine the effects of hypoxia and obesity on physical performance.

CONCLUSION

To conclude, the results of this study reveal that OSAS patients experienced more physical and mental health limitations, the amount of daytime sleepiness they experienced increased, and their energy worsened or vice versa. In these patients, if functional physical performance can be improved with therapeutic options, the patients experienced role limitation, worsened emotional well-being, and energy levels, the level of depressive and anxiety symptoms, and the amount of daytime sleepiness can be reduced.

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THE ROLE OF PERFUSION MAGNETIC RESONANCE IMAGING IN THE DISCRIMINATION OF BENIGN VS MALIGN CHARACTER OF MUSCULOSKELETAL TUMORS

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ABSTRACT

Purpose: There may be difficulties in diagnosing musculoskeletal system tumors with Magnetic Resonance Imaging (MRI). Advanced MRI imaging techniques may contribute to noninvasive diagnosis. The aim of our study was to evaluate the performance of perfusion magnetic resonance imaging quantitative data in the differentiation of benign and malignant musculoskeletal tumors.

Material and Methods: Thirty-six histologically proven patients were included in the study retrospectively. All patients underwent 1.5-T perfusion MRI (magnetic resonance imaging) with T1 mapping and diffusion MRI. Four quantitative and one semiquantitative parameter were obtained for each tumor using the Toft pharmacokinetic model. ADCmean and ADCmin values were calculated from apparent diffusion coefficient (ADC) maps.

Results: Eighteen of 36 patients (50%) had malignant tumors, and 18 had benign tumors. There were 26 soft tissue tumors and 10 bone tissue tumors. Among malignant and benign tumors, the best performance was found in the Ktrans, Kep, Ve values. (p=0.011, p=0.013, p=0,035).

Conclusion: Ktrans and Kep values, which are indicators of increased permeability in the tumor, are noninvasive parameters for determining the malignant character of the tumor. The 'Ve' value is an indicator of the extracellular volume of the tumor. Ktrans, Kep and Ve maps can also guide the biopsy.

Keywords: ADC, MRI, Kep, Ktrans, ROI, Ve.

INTRODUCTION

Noninvasive characterization of musculoskeletal tumors can be difficult with conventional imaging methods because of their overlapping radiological appearances and their rarity (1). Multiparametric DC-MRI (dynamic contrast-magnetic resonance imaging) and DWI (diffusion weighted imaging) provide information about tissue perfusion, vascularization, vascular permeability, interstitial space value, and cellularity of the lesion (2). DC-MRI; gives a graph of

Table 1. Soft tissue tumor histological subtypes

Soft tissue			
Benign		**Malign**	
Schwannoma	4	Sarcoma	1
Vascular tumors	4	Synovial sarcoma	2
Pseudotumor*	1	Mesenchymal tumor	4
Desmoid tumor	1	Round cell tumor	1
Others**	6	Squamous cell tumor	1
Tenosynovial giant cell tumor	1		
Total: 26			

*Pseudotumor: Gout. **Solid fibrous tumor, neurofibroma, lipomatous tumor

Table 2. Bone tumor histological subtypes

Bone Tumors			
Benign		**Malign**	
Fibrous histiocytoma	1	Osteosarcoma	1
Brown tumor	1	Round cell tumor	1
		Multiple Myeloma	1
		Chordoma	1
		Chondrosarcoma	2
		Metastasis***	2
Total: 10			

***bladder transitional cell cancer, lung adenocarcinoma

the signal intensity over time. It allows the measurement of values such as capillary permeability of tissue, blood flow, blood volume, and extracellular-extravascular volume fraction with quantitative measurements (3).

Permeability MRI quantitative values provide important clues in determining the noninvasive characterization of the tumor. Since the Ktrans value reflects the capillary permeability and the aggressiveness of the tumor, it is the most emphasized parameter in the literature in determining the malignant vs. benign character (4).

Clinical application is not simple because the obtained quantitative parameters are very sensitive depending on the technique used and require

additional time to daily MRI practice and long post-processing procedures (5). There have been studies in the literature that it can be used as a biomarker in many tumor groups. Although DC-MRI parameters are thought to be useful in the differentiation of benign and malignant musculoskeletal tumors, there are not enough studies on this subject in the literature (6). The diffusion coefficient "ADC" determined in DWI allows us to predict the cellularity and malignant potential of the tumor (7).

The aim of this study was to evaluate the correlation of histopathological diagnosis with parameters ADC, Ktrans, Kep, Ve, Vp, and IAUC (incremental area under the curve) obtained from multiparametric MRI in the differentiation of benign and malignant soft tissue and bone tumors.

MATERIAL AND METHODS
Ethical Considerations
This study was approved by Dokuz Eylul University, Non-Interventional Research Ethics Committee (Date: 14.09.2020, Decision No: 2020/21-19).

Population
In this single-center study, between July 2018 and December 2020, patients diagnosed with bone and soft tissue tumors as a result of biopsy or excision and imaging with DC-MRI were retrospectively scanned, and 36 patients were identified. This study was approved by Dokuz Eylul University, Non-Interventional Research Ethics Committee (Date: 14.09.2020, Decision No: 2020/21-19). All patients were over the age of 18. Informed consent was waived due to the retrospective nature of the study. Patients whose MRI images are not suitable for evaluation due to metallic artifacts (prosthesis, etc.) and patients who received treatment before MRI for the tumor for their current diagnosis were excluded. Thus, the study population consisted of 10 bone and 26 soft tissue tumor patients (Tables 1, 2).

MRI Protocol
All patients were captured on a 1.5 A Tesla MRI device (Philips Healthcare/Philips Medical Systems B. V, The Netherlands), DWI and DC-MRI sequences were taken in addition to routine sequences.

In dynamic imaging, the shooting was taken with TR: 4.01 msec, TE: 1.94 msec, matrix: 192x192, GE (T1 Single Shot Turbo Field Echo) sequence, and the shooting time was approximately 6 minutes. The contrast agent gadoterat meglumin (Dotarem,
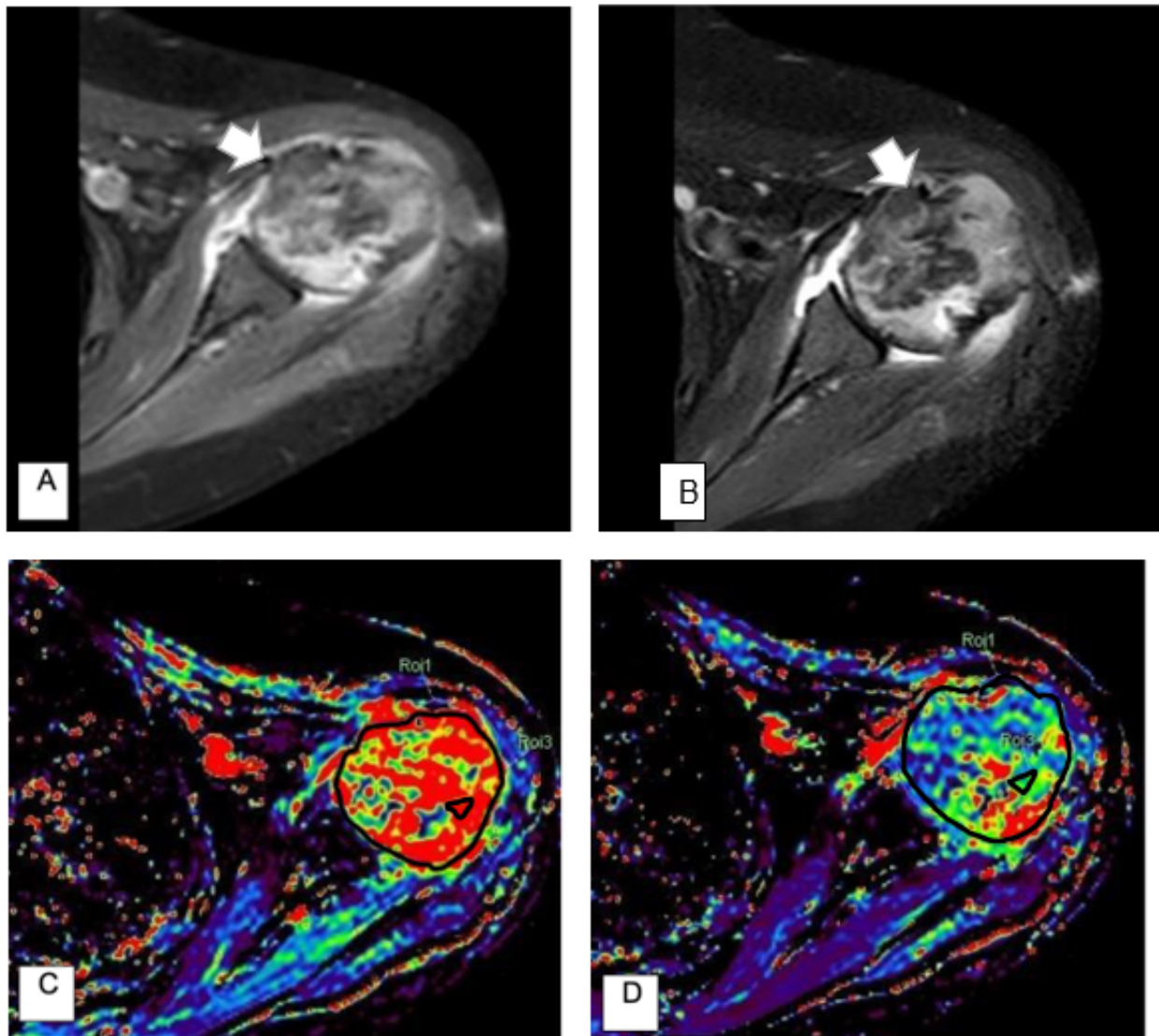
Figure 1. Measurement samples of T2W, postcontrast T1W and DC-MRI quantitative parameters of a patient with osteosarcoma. **A:** Postcontrast T1 weighted images (left arrow) **B:** On T2 weighted images, lesion located in the humeral epiphysometaphysis causing cortical destruction (right arrow) **C:**Ktrans, **D:** Kep maps, measurements were made with the ROI placed on the widest (ROI 1) section and the narrow ROI (ROI 3) (highlighted by black drawing).

Guerbet Medicine Medical Materials and Devices Industry and Trade Inc.) was administered intravenously from the forearm with an automatic injector at a dose of 0.2 mmol/kg at a rate of 2 ml/s. After the contrast agent injection, 20 ml of saline was injected.

To provide sufficient data for pharmacokinetic analysis data, the examination will typically continue for more than 5 minutes (3-4 min for Kt alone, 6 min for Ve and Vp). High temporal resolution is essential (3-6 seconds) to obtain high-quality images and to clearly show the hemodynamic process in the tissue. The temporal resolution can be enhanced using the parallel imaging method. High temporal resolution

may require sacrificing the signal-to-noise ratio (SNR). Mapping DC-MRI dynamic parameters requires an optimal balance between temporal and spatial resolution and volumetric area to be imaged and SNR. In diffusion-weighted imaging, b=500 s/mm2 and b=1000 s/mm2 values were used.

Analysis of images
Images of all patients were transferred to the workstation (IntelliSpace Portal V8.2.20820, Philips Medical Systems, Netherlands), and all measurements were made at the workstation. DC-MRI images were obtained by means of dynamic series and T1 maps. AIF (arterial input function);

Table 3. DC-MRI data of malignant and benign tumor tissue comparison

	Malign Tumor		Benign Tumor		P value
	Median	Minimum-Maximum	Median	Minimum-Maximum	
Wide ROI Ktrans (min -1)	45,3	0,009-366,06	34,42	0,001-50,4	0,018
Standart ROI Kep (min -1)	578,32	0,005-1485,74	222,96	0,001-589,92	0,013
Standart ROI Ktrans	254,86	0,011-1,850	39,86	0,001-404,74	0,011
Wide ROI Ve	308,96	0,023-452,63	20,45	0,00-109,77	0,035

modeled using an intermediate mode provided by the software. Diffusion images and ADC maps of each B value were used to evaluate the diffusion-weighted images. Necrotic and solid areas of the tumor were determined with the help of available T2W (weighted), non-contrast and contrast-enhanced T1W (weighted), images.

Two different measurements were made for the quantitative analysis of the images. First, for the evaluation of DC-MRI, with the help of T1W and T2W images, areas of necrosis were avoided ,and the widest section of the tumor was determined. Ktrans, Kep, Ve, Vp ,and iAUC measurements were made with the free ROI placed in this section. In the second method, in lesions larger than 1 cm in diameter, three standard ROIs of approximately 0.35 square centimeters, which do not intersect with each other as much as possible, were used from the most solid areas of the tumor. In lesions smaller than 1 cm (with approximately 0.35 square centimeters ROI), Ktrans, Kep, Ve, Vp, iAUC ,and ADC data from the ADC map were measured and recorded from DC-MRI sections. The highest Ktrans, Kep, Ve, Vp ,and iAUC values and the lowest ADC values were chosen to reflect the solid part of the tumor in lesions with three measurements. A single measurement was used in patients who could not have three measurements. In addition, DC-MRI data of normal tissue were obtained with ROIs placed in normal tissues.

Pharmacokinetic analysis was performed according to the Toft model (8). The contrast agent in the vein passes into the EES depending on the concentration difference and permeability. The volume transfer constant (Ktrans) reflects the rate of flow of gadolinium contrast from the blood plasma into the extracellular-extravascular space (EES). In cases where permeability is limited, Ktrans becomes an indicator of permeability (9). It has been reported that blood flow and vascular permeability may increase in cancer angiogenesis compared to normal tissue, and therefore, Ktrans may be higher in primary tumor localization (10). Kep is the flow rate constant between the plasma and the EEB. Ve is the volume of the EES cavity, and Vp is the plasma volume. Vp; indicates how much of the unit tissue volume is the plasma volume. The area under the signal intensity time curve (IAUC) does not require AIF and is an independent parameter from pharmacokinetic modeling calculations. It reflects both tumor perfusion and permeability (11).

For the evaluation of diffusion, measurements were made with the ROI placed similarly in the same section, and ADCmean, min, and max data were recorded.

Measurements were made by two radiologists at different times, one with 5 years of experience (s, s.) and the other with 3 years of experience (s, e.a.). The observer with 5 years experience was accepted as standard while analyzing benign and malignant tumor differentiation. Interobserver compliance was assessed.

Statistics

The Kolmogorov-Smirnov test was performed to determine whether the data were normally distributed. The Spearman correlation test was used

Table 4. Concordance of interobserver DC-MRI data valuation

Compared metrics	SCC	95% CI
$G1_{Ktrans}$ -$G2_{Ktrans}$	0.918	0.840 to 0.958
$G1_{Kep}$ -$G2_{Kep}$	0.955	0.913 to 0.977
$G1_{Ve}$ -$G2_{Ve}$	0.936	0.875 to 0.967
$G1_{Vp}$ -$G2_{Vp}$	0.731	0.476 to 0.862
$G1_{iAUC}$ -$G2_{iAUC}$	0.665	0.348 to 829

SCC: Standard correlation coefficient; CI: Confidence interval

to evaluate the correlation of nonnormally distributed data. The Mann-Whitney U test was used to evaluate the differences between DC-MRI and DWI data in the differentiation of benign vs. malignant tumors. In addition, the Wilcoxon signed rank test was used to determine whether there was a significant difference between DC-MRI data from tumor tissue and DC-MRI data from normal tissue. The chi-square test was used to compare qualitative data.

The intraclass correlation coefficient (ICC) was used to evaluate the correlation of DC-MRI and diffusion parameters between the two observers.

SPSS 24 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and the results with $p<0.05$ in all analyses were considered statistically significant.

RESULTS

In measurements made with standard and wide ROIs, in Ktrans and Ve values with large ROIs, a statistically significant difference was observed in malignant lesions compared to benign lesions in Ktrans and Kep values performed with standard ROIs ($p<0.05$) (Table 3). There was no significant difference between benign and malignant tumors in the diffusion MRI parameters ADCmean, ADCmin and ADCmax. The sensitivity and specificity of the cut-off values in the ROC analysis remained below 50%.

In the measurement of DC-MRI data, in the measurement of Ktrans, Kep, Ve, and Vp, the agreement between the observers was excellent-good, and the iAUC was moderate (Table 4).
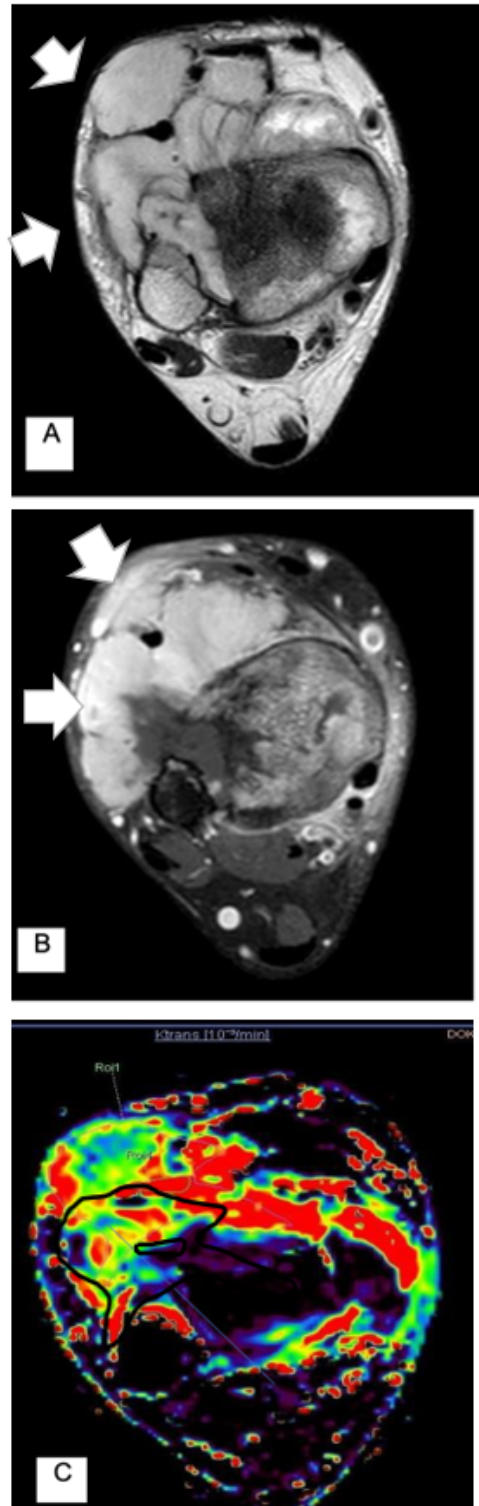
Figure 2. Measurement samples of T2W, postcontrast T1W, DC-MRI quantitative parameters of a patient with osteosarcoma. A: T2W B: Mass lesion in the ankle that destroys bone and extends into soft tissues on postcontrast T1W images (white arrows) C: Illustration of the measurement of dynamic contrast-enhanced MRI quantitative parameters with large (ROI 1) and standard ROIs (ROI 4) (highlighted by black drawing).
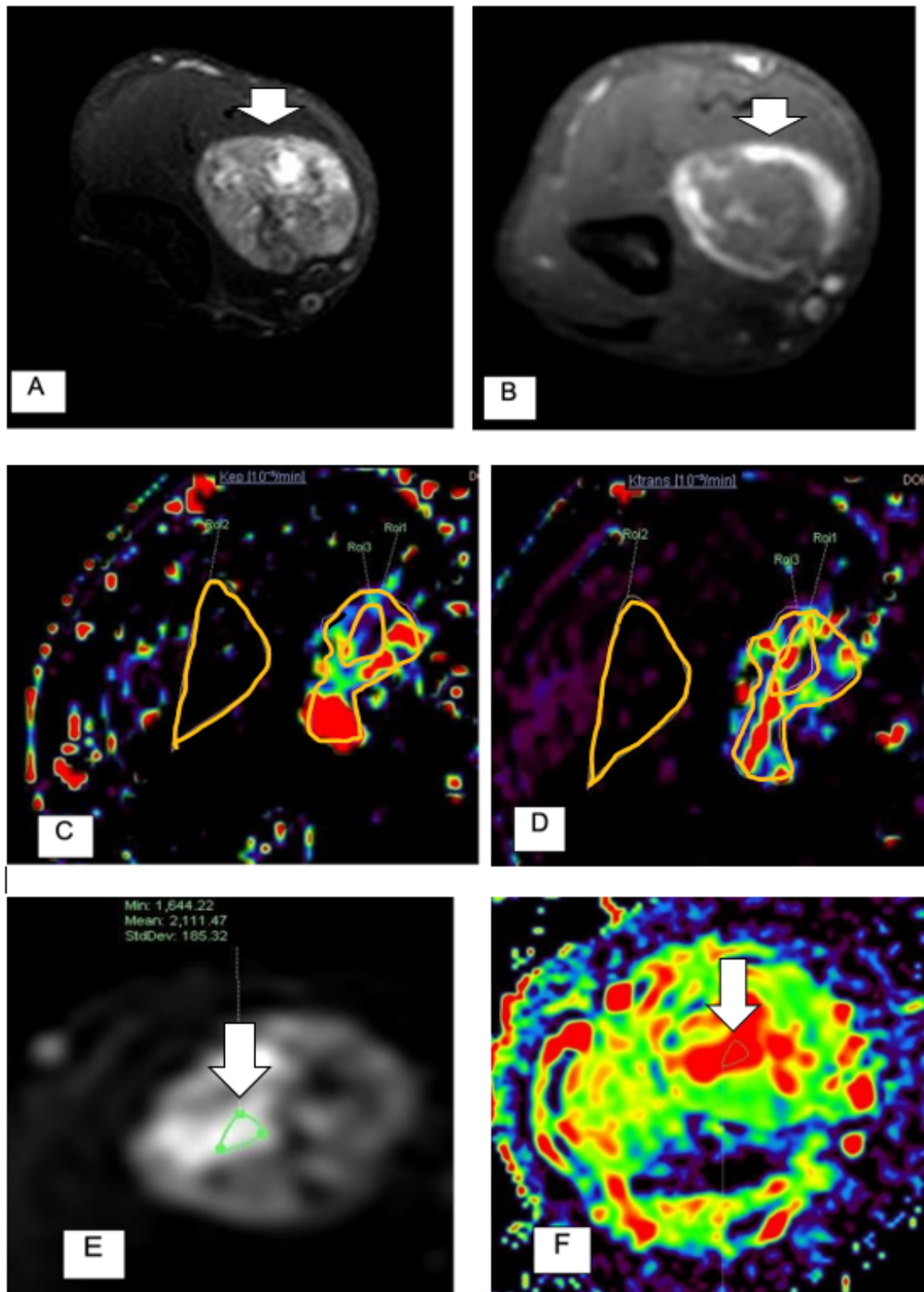
Figure 3. Measurement samples of T2W, postcontrast T1W, DC-MRI quantitative parameters of the patient with osteosarcoma and diffusion-weighted images **A:** T2W **B:** Deeply located heterogeneous mass lesion between muscle planes on postcontrast T1W images (white arrows) **C and D**: Wide and wide range of dynamic contrast-enhanced MRI quantitative parameters display of measurement with a standard ROI (ROI 3). (ROI 2: normal tissue, ROI 1: large ROI from the tumor) (highlighted by yellow drawing) **E:** Diffusion weighted image (left arrow) **F:** ADC (apparent diffusion coefficient); measurements were made from the area where diffusion restriction was observed in the lesion (right arrow)

DISCUSSION

In our study, we found Ktrans and Kep values in standard ROI in musculoskeletal tumors to be significantly higher in the malignant group and Ktrans and Ve values to be higher in our measurements with large ROI. Interobserver correlation in Ktrans, Kep, Ve and Vp values was good-excellent.

The findings show that determining quantitative permeability values, which are closely related to neovascularization of the tumor, will be beneficial in addition to conventional MRI findings in making a non-invasive diagnosis. It can also guide the biopsy by creating permeability maps such as Ktrans, Kep and Ve. Since there is no clear consensus regarding the measurement, we wanted to show two different measurement results. In the standard ROI, Ktrans and Kep values, which show the permeability of the tumor, reflect the measurement made from the most solid part of the tumor; We found the Ve value, which is a quantitative indicator of extracellular volume, to be significantly higher in the wide ROI, reflecting the largest area of the tumor.

Clinical application of perfusion MRI does not require extra doses of gadolinium and does not increase the cost of conventional MRI examination for standard characterization of musculoskeletal tumors. For this reason, it is thought that its routine use will contribute to the diagnosis.

The results of studies conducted on musculoskeletal tumors, generally with soft tissue tumors, have shown that the use of quantitative parameters to examine tumor perfusion may be useful (12).In studies conducted with the soft tissue tumor group, Ktrans and Kep values, which are associated with tumor aggressiveness and neoangiogenesis, were found to be significantly higher in the malignant tumor group (13,14,2).

Although we obtained a significant difference in permeability quantitative values in the malignant group, we obtained low sensitivity and specificity. The limitations of the study are due to the small number of patients, the fact that the patient population is a very heterogeneous group including both bone and soft tissue tumors, the lack of a standardized protocol of DC-MRI applied in the same way in every center and the chosen pharmacokinetic model. It is thought that there are factors affecting the perfusion image quality and causing interpatient variation in quantitative perfusion parameters depending on the technique. Many factors during data acquisition and analysis, such as the characteristics of the MRI device, T1

analysis method, AIF measurement method, ROI and parameter selection, and pharmacokinetic model variability, were considered. It can affect the reliability of the results of studies with MRI, which causes different results in different institutions (6). Intraobserver compliance was not measured, which may be considered as a limitation.

DWI, in addition to contributing to the diagnosis of musculoskeletal tumors, it also provides an idea about the structure and behavior of the tumor. Diffusion MRI makes great contributions to early diagnosis, staging and evaluation of response to treatment (15). In our study, no significant difference was observed in the ADCmean and ADCmin values in the group in which the majority (n=26) of 36 patients had soft tissue tumors (p>0.05). Some studies have shown a correlation between ADC values and tumor grade (16). High ADC values in tumors with dominant necrotic or cystic components may reduce the relationship between ADC and cell density. High ADC values can be detected in bone tumors with a high chondroid matrix and soft tissue tumors with a high myxoid matrix, even if they are malignant (17).

Limitations

We also showed in our study that there may be overlaps in diffusion studies in musculoskeletal tumors. More meaningful results can be obtained in a more homogeneous patient group.

The distinction between benign and malignant soft tissue and bone tumors, which have a very heterogeneous spectrum, is not always easy. In addition to conventional magnetic resonance imaging and other imaging modalities, it has become possible to obtain more data about these lesions with newly developed techniques.

CONCLUSION

Our work has shown that the use of perfusion MRI quantitative data can be useful in the differential diagnosis of tumors, similar to many studies in the literature. DC-MRI quantitative parameters have the potential to be a noninvasive biomarker in determining the malignant character of the tumor. In future studies, it is thought that the contribution of the method will be clearer with the standardization of measurement methods and techniques and a wider patient group.

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FALL RISK, POSTURAL CONTROL, MUSCLE STRENGTH AND COGNITIVE FUNCTION IN PATIENTS WITH LIVER CIRRHOSIS

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ABSTRACT

Purpose: Falls are common in liver cirrhosis. Our aim was to investigate fall risk, postural control, muscle strength and cognitive function in cirrhosis, compare the results to healthy controls and investigate the inter-relationships.
Materials and Methods: Twenty-four patients (12 males, 12 females) and 24 healthy controls (11 males, 13 females) were enrolled. Fall risk was assessed with Falls Efficacy Scale (FES) and Timed Up and Go Test (TUGT). Postural control was assessed using posturography. Quadriceps Femoris and Tibialis Anterior strength were assessed with dynamometer. Cognitive function was evaluated with Stroop test. Disease severity was assessed with Child-Pugh and Model for End-Stage Liver Disease scores.
Results: Postural sway velocity (PSV) on foam surface eyes open (FSEO), FES score and TUGT duration were higher in patients ($p<0.05$). TUGT duration was correlated with disease severity. Lower extremity muscle strength was lower in patients ($p<0.05$). Dynamic postural control was deteriorated in patients ($p<0.05$). Lower extremity muscle strength was correlated with PSV-FSEO and FES score ($p<0.05$).
Conclusion: Patients with liver cirrhosis present significant decrease in muscle strength independent from disease severity, associated with static postural control on an unstable surface and fear of falling. Dynamic postural control is significantly deteriorated in liver cirrhosis, not in relation to muscle strength.

Keywords: Fall risk, postural control, muscle strength, cognitive function, liver cirrhosis

INTRODUCTION

The World Health Organization defines a fall as "an event which results in a person coming to rest inadvertently on the ground or floor or other lower level" (1). These inadvertent events mainly occur due to impairments in postural control, which is the ability to maintain the body position in space during static and dynamic conditions dependent on the interaction between visual, vestibular and somatosensory systems (2). Falls are common in patients with liver cirrhosis, a diffuse hepatic process characterized by fibrosis and structurally abnormal nodules (3,4)

Previous studies presented postural control problems in liver cirrhosis (5-7). The majority of these studies mostly focused on minimal hepatic encephalopathy (HE), clinically manifested as cognitive impairment (5-7). HE is a brain dysfunction caused by liver insufficiency and/or portosystemic shunts. Hyperammonaemia, systemic inflammation and oxidative stress are the critical factors in the pathophysiology of HE. It has a broad spectrum of neuro-psychiatric manifestations ranging in severity from subclinical alterations to coma (8).
There are many other factors possibly affecting

postural instability in liver diseases such as muscle mass loss, hyponatremia, psychoactive drugs and sleep problems (9). However, most of the studies investigated only HE as a predictor of postural instability and falls in patients with liver cirrhosis (5-7, 10). Only in one study by Frith et al., decreased lower extremity muscle strength was associated with increased fall risk in a subgroup of patients including primary biliary cirrhosis (3).

Protein-energy malnutrition due to dietary restrictions, malabsorption, intestinal protein loss and hypermetabolism leads to loss of muscle mass in cirrhosis (13). Muscle strength also decreases as a result of skeletal muscle mass loss and mitochondrial dysfunction including reduced number of mitochondria and diminished mitochondrial oxidative capacity (14). The strength of different muscle groups decrease in patients with liver cirrhosis including respiratory, upper and lower extremity muscles (15-17).

In spite of the studies exploring fall risk, postural control problems and their relation to cognitive dysfunction, no studies have investigated the relationship between muscle strength and these parameters in patients with liver cirrhosis. Therefore, our study was designed to assess fall risk, postural control, muscle strength and cognitive function in patients with liver cirrhosis, to compare the results to healthy controls and to investigate the relationships between these parameters.

MATERIALS AND METHODS
Participants
Twenty-four outpatients were recruited from Department of Gastroenterology, Dokuz Eylul University Hospital, diagnosed as liver cirrhosis by clinical, analytical, and ultrasonographic findings or by liver biopsy (18). Exclusion criteria were: ≥ 65 years of age, severe neurologic or vestibular disease that can affect postural control, history of severe lower extremity musculoskeletal injury and/or surgery, alcoholic liver cirrhosis, diabetes mellitus, active alcohol consumption (in previous three months), severe co-morbidities (e.g., cardiac, pulmonary, renal, psychiatric). We also recruited 24 healthy controls with similar age and gender without any known chronic diseases and previous lower extremity injury and/or surgery. Subjects with active alcohol intake were also excluded from the control

The study was approved by Non-invasive Research Ethics Board of Dokuz Eylul University (Date: 01.03.2012, Decision No: 2012/08-06) and performed following the ethical guidelines of 1975 Declaration of Helsinki. All patients and healthy controls gave written consent before participation.

Data Collection
Demographic and clinical features of the patients had been collected from the patients and patient records. Age, gender, body weight and height were recorded as demographic features while etiology of liver cirrhosis, Child-Pugh score, Model for End-Stage Liver Disease (MELD) score and time since diagnosis were recorded as clinical features (19).

Assessment of fall risk
Turkish version of Falls Efficacy Scale (FES) was used to measure fear of falling and fall risk based on the patient's perceived self-efficacy at avoiding falls during activities of daily living (20). The scale consists of 10 items. Each item is scored from 1 (very confident) to 10 (not confident at all). Total score is between 10 and 100, higher scores indicating higher fall risk. Timed Up and Go Test (TUGT) was used to assess dynamic balance function and functional mobility. The patient was asked to rise from a standard arm chair, walk 3 meters away, return and sit down again. The time in seconds was recorded by a chronometer. The test was repeated for three times and mean duration of three measurements was used for the analysis. Scores more than 13.5 seconds were interpreted as high fall risk (21). Mean durations to complete the test were compared between the groups, as higher durations indicating higher fall risk. History of falls including the number of falls in the previous one year, hospital admissions due to falls and fall-related injuries were also recorded for each subject in the groups.

Postural control assessment
Postural control was objectively assessed using the Balance Master system continuously monitoring the position and movement of the center of gravity (CoG). During assessments subjects stand on the force platform measuring the vertical forces. The computer analyses the data from the force platform and prints a graphical and a numerical report (22).
"Modified Clinical Test of Sensory Interaction on Balance" (mCTSIB) was performed to assess

J Basic Clin Health Sci 2024; 8: 611-619

Yıldırım M et al. Falls in Patients with Liver Cirrhosis

Figure 1. Modified Clinical Test of Sensory Interaction on Balance

Figure 2. Limits of stability Test

postural control under different sensory conditions and "Limits of Stability" (LoS) test was performed to assess dynamic postural control. mCTSIB quantifies the position of CoG in the upright position under four different conditions including firm and foam surfaces with eyes open (EO) and eyes closed (EC). Postural control was assessed as the sway velocity of the CoG and indicated by postural sway velocity (Fig 1).

LoS, quantifies the maximum distance that a patient can displace his CoG in a given direction. It involves shifting the weight to target points. The measurements include reaction time, CoG movement velocity, directional control, end point excursion and maximum excursion (Fig 2).

Reaction time is the time between the command to move and the initial movement, expressed in seconds. Movement velocity is the average speed of CoG movement (degrees/second). End point excursion is the distance of the first movement toward the target which is the point at which the first movement stops. Maximum excursion is the maximum distance achieved during the trial. Directional control is the comparison of the amount of intended movement to the extraneous movement.

Lower extremity muscle strength
The maximal isometric muscle strength for Quadriceps Femoris and Tibialis Anterior of each leg were assessed using a hand-held dynamometer (Powertrack II, J-Tech Medical, USA). Quadriceps Femoris strength was measured in sitting position with 90 degrees knee flexion by applying the force on full knee extension. Tibialis Anterior strength was measured in supine position with ankles slightly hanging out of bed by applying the force to the end point of ankle dorsi-flexion. The testing procedures were repeated for three times for each muscle with 90 second-intervals and the best value was recorded for the analysis (23).

Cognitive function
We used a color-word version of the Stroop test to evaluate cognitive function. This test has been used previously in patients with cirrhosis in order to evaluate particularly "anterior attention system" which was hypothesized to be more sensitive to early stages of minimal HE compared to posterior attention system (24). The test is based on assessing the abilities of selective selection, response inhibition, and executive control under congruent and incongruent conditions. Congruent stimuli consisted of the words displayed in the same color indicated by the word. Incongruent stimuli consisted of the words naming colors displayed in a different color from that indicated by the word. Our test included four tasks: (1) Naming the colors printed in black, (2) Naming the colors printed in a rectangular shape (neutral condition), (3) Reading the colors printed in incongruous colors, (4) Naming the colors printed in incongruous colors (incongruent condition).

"Stroop Effect" was calculated (Forth task duration-Second task duration) for the interpretation of the test results. We also recorded the number of errors during the incongruent condition in order to use for analysis (25).

Table 1. Demographic features of the groups

	Patient Group	Control Group	p
Age *(years)*	39 (35-46)	38 (35-44.75)	0.620
Gender, *male/female (%)*	12 (50) / 12 (50)	11 (45.8) / 13 (54.2)	0.773
Height *(cm)*	167 (158-170)	168 (164.25-180)	0.098
Body Weight *(kg)*	70 (62.50-74)	73.5 (64.25-83-75)	0.297
BMI* *(kg/m^2)*	24.63 (22.25-27.45)	25.95 (23.19-27.08)	0.797

*BMI: Body Mass Index; Mann Whitney U Test, Chi-Square Test
Values expressed as median (interquartile ranges) or number of patients (%)

Table 2. Disease characteristics of liver cirrhosis

Etiology of cirrhosis	Number of patients	%
Viral Hepatitis	9	37
Cryptogenic	4	17
Autoimmune Hepatitis	3	13
Wilson Disease	3	13
Budd-Chiari Syndrome	2	8
Primary Biliary Cirrhosis	1	4
Primary Sclerosing Cholangitis	1	4
Overlap Syndrome	1	4
Clinical Scores	**Median**	**25th -75th**
Child-Pugh	7	5-9
Model for End-Stage Liver Disease	13	8.25-16
Time since diagnosis *(years)*	5	2-9.50

Values expressed as median and interquartile ranges or number of patients (%)

Table 3. Comparison of fall risk assessments between the groups

	Patient Group	Control Group	p
Falls Efficacy Scale Score *(10-100)*	13 (10-35.75)	10 (10-12.50)	**0.027**
Timed Up and Go Test Duration *(sec)*	8.5 (7-10.75)	6 (5-6)	**0.001**

Mann-Whitney U Test, Values expressed as median and interquartile ranges (25th-75th)

Table 4. Comparison of postural control measurements between the groups

	Patient Group	Control Group	p
mCTSIB *(°/sec)*			
EO firm surface	0.30 (0.26-0.30)	0.30 (0.30-0.30)	0.769
EC firm surface	0.30 (0.30-0.40)	0.35 (0.30-0.40)	0.877
EO foam surface	0.70 (0.52-0.70)	0.55 (0.50-0.60)	**0.047**
EC foam surface	1.25 (1-1.47)	1.10 (1-1.37)	0.334
LOS			
Reaction Time *(sec)*	1.03 (0.93-1.24)	0.72 (0.59-0.90)	**0.001**
Movement Velocity *(°/sec)*	2.80 (2.37-3.37)	4.15 (3.10-5.62)	**0.001**
Maximum Excursion *(%)*	76.63 (68.28-81.75)	86.62 (76.10-91.50)	**0.005**
End point Excursion *(%)*	96.37 (89.03-99.87)	101.31 (96.15-103.65)	**0.006**
Directional Control *(%)*	81.50 (77-84.75)	84 (82.25-87.00)	**0.014**

mCTSIB: Modified Clinical Test of Sensory Interaction on Balance, EO: Eyes Open, EC: Eyes Closed; LOS: Limits of Stability, Mann-Whitney U Test,
Values expressed as median and interquartile ranges (25th-75th)

Table 5. Comparisons of lower extremity muscle strength between the groups

	Patient Group	Control Group	p
Quadriceps Femoris *(R)*	18.25 (16.12-27.16)	34 (27.87-44.5)	**0.001**
Quadriceps Femoris *(L)*	18.08 (14.58-25.20)	30.66 (25.16-41)	**0.001**
Tibialis Anterior *(R)*	13.83 (10.95-15.62)	20.75 (16.20-26)	**0.001**
Tibialis Anterior *(L)*	11.83 (9.75-15.50)	19.50 (14.70-23.91)	**0.001**

R: Right, L: Left; Mann-Whitney U Test; Values expressed as median and interquartile ranges (25th-75th)

Statistical Analysis

SPSS software version 22 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Descriptive statistics and frequencies were used for demographic and clinical features. Normality of the data distribution was tested by Shapiro–Wilk test. Medians and interquartile ranges (25th–75th percentile) were used for descriptive analyses of quantitative variables. Fall risk assessments, posturographic measurements, lower extremity muscle strength and cognitive function of the groups were compared with Mann-Whitney U Test.

Spearman's rank correlation coefficient rho (r) was used to identify the relationships between fall risk (FES scores and TUGT duration), posturographic measurements (mCTSIB and LoS), lower extremity muscle strength (Quadriceps Femoris and Tibialis Anterior) and cognitive function (Stroop effect and number of errors during incongruent condition). The strength of correlations was classified as very weak (r=0–0.19), weak (r=0.2–0.39), moderate (r=0.40–0.59), strong (r=0.6–0.79), and very strong (r=0.8–1). p values <0.05 were considered as statistically significant.

RESULTS

No significant difference was found in terms of the demographic features between the groups (Table 1). Table 2 shows the clinical characteristics of liver cirrhosis including etiology of the disease, Child-Pugh score, MELD score and time since diagnosis.

FES score and TUGT duration were significantly higher in patients with liver cirrhosis compared to healthy controls (p=0.027 and p=0.001, respectively) (Table 3). There were no subjects within the two groups having TUGT score >13.5 seconds indicating high fall risk. Four subjects in patient group and one subject in control group declared one or more times of falls in the previous one year (p=0.156). One subject in patient group was admitted to hospital having a fracture due to a fall.

Postural sway velocity for EO condition on foam surface assessed by mCTSIB was significantly higher in patients with cirrhosis compared to healthy controls (p=0.047) (Table 4).

Patient group showed slower reaction times in comparison to control group during LoS test (p=0.001). Movement velocity was also significantly slower in-patient group (p=0.001) whereas the percentages of directional control, maximum excursion and end point excursion were significantly

lower than the control group (p=0.014, p=0.005 and p=0.006, respectively) (Table 4).

Quadriceps Femoris and Tibialis Anterior muscle strength were significantly lower for both left and right sides in patient group compared to the control group (p<0.01) (Table 5).

There was a significant difference in terms of Stroop effect between the groups (p=0.004). The time to complete the task was 33 seconds (27-47) in the patient group, whereas it was 26 seconds (21-31) in the control group. No significant difference was found in terms of number of errors during incongruent condition between the groups (p=0.063).

Correlations between the parameters in patients with liver cirrhosis are shown in Table 6.

There was no significant correlations between muscle strength and LoS test parameters.

Cognitive function assessed by Stroop effect and number of errors did not correlate with any of the postural control measurements, fall risk assessments and lower extremity muscle strength (p>0.05).

Clinical disease scores (MELD and Child-Pugh) and time since diagnosis were not significantly correlated with postural control and lower extremity muscle strength. TUGT duration was moderately and positively correlated with MELD score (r=0.414, p=0.044) and with Child-Pugh score (r=0.471, p=0.020).

DISCUSSION

In our study, in which we have investigated fall risk, postural control, muscle strength and cognitive function in patients with liver cirrhosis, we found impairments in postural control under the conditions in which the somatosensory system had been compromised. Dynamic control of posture was deteriorated which had been considered as an indicator for fall risk. Our patients showed increased fear of falling and a clinically assessed fall risk associated with disease severity. Lower extremity muscle strength bilaterally decreased and was correlated with both fear of falling and postural control on an unstable surface in patients with cirrhosis. Cognitive function of patients with cirrhosis was also impaired in comparison to healthy controls, but did not show any correlations with fall risk, postural control and muscle strength.

Previous studies demonstrated deterioration in postural control using posturographic measurements in patients with liver cirrhosis (5-7). Aref et al. investigated postural control using dynamic

posturography and found significantly weaker performances in cirrhotics compared to healthy controls (5). In another posturographic study, Schmid et al. found deterioration in postural control in alcohol induced and non-alcohol induced cirrhosis (6). In both of these studies, patients with cirrhosis showed impairments in sensory organization tests indicating the inability to organize sensory information appropriately to maintain balance (5,6). In a recent study, Urios et al. performed mCTSIB and LoS tests similar to our study and found lower stability on foam surface in eyes open condition (7). Patients with minimal HE in that study showed lower global scores in eyes open condition while the deterioration was higher on foam surface compared to patients without minimal HE (7). Aforementioned studies indicated an association between postural control disturbances and cognitive impairment in cirrhosis. Although we have detected a significant decrease in cognitive function evaluated with Stroop test, which had been commonly used for patients with chronic liver diseases, we did not find a relationship between cognitive function and neither postural control nor fall risk.

Postural sway velocity of our patients and the controls were similar, while all sensory systems were available for maintenance of balance. However, on foam surface with eyes open, postural control of the patients significantly deteriorated suggesting the difficulty in integrating somatosensory information in the presence of visual information. Interestingly, when the eyes were closed, the deterioration in postural control disappeared. Oppositely, maintenance of balance is expected to be hindered in the absence of visual information. Contrary to Aref et al.'s and Schmid et al.'s findings, indicating the increase in postural instability parallel to the progression of liver disease (5,6), we did not find any relationships between postural control and clinical disease scores. However, disease scores were positively correlated with TUGT duration suggesting a disease severity-dependence in clinically assessed fall risk. Although there were not any recurrent fallers in our patient group, the duration to complete TUGT was significantly higher in patients than the controls. FES score was also significantly higher in our patients than healthy controls indicating an increased fear of falling and fall risk based on the patient's perceived self-efficacy at avoiding falls during daily life.

Postural sway velocity during standing on a foam surface with eyes open was significantly correlated with all lower extremity muscle strength values except left side Tibialis Anterior in patient group. Although the relationship between postural control and lower extremity muscle strength has been shown in different populations (26, 27), no data exists in liver cirrhosis. Only in one study, Frith et al. recorded the duration of 5 times sit-to-stand test in order to

Table 6. Correlations between the parameters in patients with liver cirrhosis

	QF-L		QF-R		TA-L		FES Score		TUG		PSV-foam EO		Child-Pugh		MELD	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
QF-L			.951	<.001	.785	<.001	-.634	.001	-.307	.145	-.518	.009	.133	.535	.257	.225
QF-R	.951	<.001			.809	<.001	-.603	.002	-.403	.051	-.574	.003	.004	.987	.111	.607
TA-L	.785	<.001	.809	<.001			-.563	.004	-.081	.707	-.425	.039	.021	.923	.146	.496
FES	-.634	.001	-.603	.002	-.563	.004			.082	.703	.148	.491	-.209	.328	-.250	.239
TUG	-.307	.145	-.403	.051	-.081	.707	.082	.703			.599	.002	.471	.020	.414	.044
PSV-foam EO	-.518	.009	-.574	.003	-.425	.039	.148	.491	.599	.002			.029	.893	-.100	.642
CP	.133	.535	.004	.987	.021	.923	-.209	.328	.471	.020	.029	.893			.896	<.001
MELD	.257	.225	.111	.607	.146	.496	-.250	.239	.414	.044	-.100	.642	.896	<.001		1

QF: Quadriceps Femoris, L: Left, R: Right, TA: Tibialis Anterior, FES: Falls Efficacy Scale, TUG: Timed Up and Go, PSV: Postural Sway Velocity, EO: Eyes Open, CP: Child-Pugh, MELD: Model for End stage Liver Disease

estimate lower extremity muscle strength indirectly and concluded that lower limb strength was associated with number of falls in patients with primary biliary cirrhosis (3). However, the authors included only one etiology of cirrhosis and interpreted a functional test's results rather than measuring muscle strength using direct methods. Similar results have been shown in previous studies indicating diminished lower extremity muscle strength in patients with alcoholic liver cirrhosis, which was an exclusion criteria in our study (28,29). However, there is no study addressing the relationship between muscle strength, postural control and fall risk in this patient group. Our results showed a correlation between lower extremity muscle strength, static postural control and fear of falling.

The inability to voluntarily shift the body toward LoS may lead to instability during dynamic daily living activities such as reaching for objects and walking (30). Therefore, it is important to test this ability in order to measure dynamic postural control and determine fall risk during daily life among populations with higher fall incidence. Our patient group presented impairments in overall performance on LoS test compared to control group which was not in association with cognitive function. Similar to our findings, not only patients with minimal HE but also without minimal HE got lower global LoS scores indicating high risk for falls independent from cognitive impairment in Urios et al.'s study (7). Reaction time was significantly longer in our patient group suggesting the possible psychomotor slowing (31). End point excursion, representing the distance of the first movement toward the target, was smaller in our patient group indicating that patients with cirrhosis could not stop their initial movement as far as the healthy controls possibly suggesting the disturbances in the perception of safety limits. Movement velocity was also slower in our patient group showing the insecure in CoG displacement in order to maintain balance during dynamic activities. Not surprisingly, the maximum distance achieved during the trials defined as maximum excursion was also smaller in our patients. The percentage of directional control was also smaller in patients than the controls indicating the inability to control the movement of CoG through the targeted directions. These findings suggest that special attention is necessary to restore the dynamic balance abilities in patients with cirrhosis in order to prevent fall risk.

We have some limitations in this current study. Although, we have assessed fall history including number of falls during the last one year, hospital admissions and fall related injuries, we could not detect any important differences between the patients and the controls. Therefore, we could not divide our patients into groups such as non-fallers and recurrent fallers. Indeed, this point is much more important for elderly patients with cirrhosis. However, we believe that relatively younger patient population in our study, contrary to other studies investigating postural control in cirrhosis, is the strength of our study as postural control, muscle strength and cognitive function possibly deteriorate with older age. In spite of the exclusion of elderly patients and the absence of recurrent fallers, our findings indicated higher fall risk, impaired postural control and decreased muscle strength not dependent on the deterioration of cognitive function in patients with liver cirrhosis. Our findings should be considered in future studies

CONCLUSION
Patients with liver cirrhosis presented significant increase in fall risk represented by the deterioration in dynamic postural control independent from muscle strength loss. The decrease in lower extremity muscle strength was associated with postural control on an unstable surface and fear of falling. Contrary to the general findings, cognitive function did not affect postural control and fall risk in our patient group who presented significant decline compared to healthy controls. Therefore, we believe that individually planned exercise programs to increase lower extremity muscle strength and functional activities including standing or walking, especially on irregular surfaces or under challenging conditions, would help to restore static and dynamic postural control strategies in patients with liver cirrhosis, no matter if they clinically present higher fall incidence or not.

J Basic Clin Health Sci 2024; 8: 611-619

Yıldırım M et al. Falls in Patients with Liver Cirrhosis

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THE INTERPRETATION OF COMPUTED TOMOGRAPHY PULMONARY ANGIOGRAPHY EVALUATION OF COMPLIANCE BETWEEN EMERGENCY MEDICAL ASSISTANTS AND RADIOLOGISTS

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ABSTRACT

Purpose: Computed tomography pulmonary angiography (CTPA) is an effective method far the evaluation of patients with suspected pulmonary thromboembolism (PTE) and should be interpreted quickly and accurately. We aimed ta determine the agreement between emergency medicine assistants (EMAs) and radiology assistants (RA) and specialists (RS) in the evaluation of CTPA
Materials and Methods: 11 EMAs, one RA and one RS participated in the study. 100 CTPA images were re-reported. The participating RA evaluated 100 CTPAs and 11 EMAs evaluated 20 randomised CTPAs aut of 100. The Kappa statistic was used ta assess agreement, and the Douglas G. Altman classıfıcatıon (K<0.20 poor, K: 0.21-0.40 fair, K: 0.41-0.60 moderate, K: 0.61-0.80 good, K: 0.81-1.00 very good) was used ta grade consistency.
Results: The agreement between EMA and RS was 79.1% far the presence of PTE, 59.1% far the location, 84.5% far the parenchymal fınding, 70.0% far the name of the parenchymal fınding. Moderate agreement was found between EMA and RS in the assessment of the presence of PTE (Kappa: 0.590)
Conclusion: There is moderate agreement between EMA and RS in CTPA evaluation.

Keywords: Computed tomography pulmonary angiography, emergency medicine resident, radiologist

INTRODUCTION

PTE develops when the proximal part of a venous thrombus ruptures and causing obstruction of the pulmonary arteries or their branches. Venous thromboembolism is the 3rd most common acute cardiovascular syndrome (1) The annual incidence is between 39-115 per 100000 (2). The wast range of symptoms might delay diagnosis and treatment. Mortality is 25-30% in untreated patients and 2-8% in treated patients (3). Prevailing usage of CTPA has caused increase on diagnosis rates.

Computed tomography (CT) can be considered as one of the most significant devices (4). It is frequently used for PTE in emergency departments.

CTPA visualises thrombi in the pulmonary artery branches up to the subsegmentary level and shows the lung parenchyma, mediastinal structures, pleura and chest wall pathologies (5). The sensitivity and specificity of CTPA for PTE are 83% and 96%, respectively (6). Correct interpretation requires a high level of knowledge and familiarity with the clinical situation. Especially in developing countries, due to technical problems, difficulties in accessing radiology physicians, lack of official reporting and sloppy verbal interpretations, it is essential for emergency physicians to be able to evaluate their imaging studies. In the literature, there are studies evaluating the knowledge and skills of emergency physicians in CT evaluation, but there is no study specifically on CTPA evaluation.

We aimed to determine the agreement between EMA and RA and RS in CTPA evaluation.

MATERIALS AND METHODS

Our study was conducted using CTPA images taken in the emergency department between 1 May 2021 and 30 April 2022 after obtaining ethics committee approval.

12 EMAs that have been employed more than 1 year and finished their rotation in radiology department.11 volunteer EMAs, one RA and one RS participated. Participating EMAs and RAs were informed about the study and consent for participation was obtained.

All patients who underwent CTPA imaging between 1 May 2021 and 30 April 2022 were screened using the hospital information management system. 1826 patients underwent CTPA imaging. Of these, 74 (4.0%) were not reported and 52 (2.8%) were not evaluated due to technical issues. Of the remaining 1700 patients, 111 (6.5%) had filling defects compatible with PTE and 1589 did not. 50 images each with and without PTE findings were selected. This selection was based on the frequency of PTE in different locations, with a higher incidence of PTE in the main pulmonary arteries, which was considered a major error.

From 1 to 100 images were enumbered conformoring to the aquisation date. The normal and abnormal CTPAs were known only by the study authors. The EMAs participating in the study were sorted according to the alphabetical order of their names and coded with letters. CTPAs numbered from 1-100 were written from top to bottom on the EMAs coded with letters. Each EMA was randomised to evaluate 20 images each. All images were re-reported by the RS and the reports were accepted as gold standard. PTE was detected in 3 of the 50 CTPAs that were considered normal before re-reporting. Therefore, the participants evaluated 47 normal and 53 abnormal CTPAs, which was different than planned.

Identity and clinical information of the patients were not given to the participants in the study. All 100 images, including coronal, axial and sagittal sections, were shown to the RA and 20 images to the EMAs using Picture Archiving and Communication Systems from the monitors in the emergency department. No assistance from any other assistant or specialist was obtained during the evaluation of the images. Participants were asked to write down each pathology seen, its type and localisation on the study form.

The study data recording and evaluation form was used for the records. The pathologies written by the EMAs and RA on the evaluation form and the pathologies in the RS report were marked as "present" or "absent" on the evaluation form. The assessment of "presence of pathology" or "absence of pathology" by both parties was considered concordant; the assessment of "presence of pathology" by one party and "absence of pathology" by the other party was considered discordant.

In case of "suspected pathology" in the RS report, this was not included in the concordance assessment.

All statistical analyses of the data were performed in SPSS 25.0 for Windows with 95% confidence interval and 0.05 significance level. Nominal and ordinal data were described by frequency analysis. Age averages were described with mean and standard deviation values. Kolmogorov Smirnov test was performed for normality analysis of age distribution. Since the distribution was normal, Independent Sample t-test was used to analyse the difference between the groups with and without PTE. Fischer's Exact test was used to analyse the difference between the group with and without PTE in terms of gender. The Kappa statistic was used in order to evaluate the agreement with the aquired data. Douglas G. Altman classification (K<0.20 poor, K: 0.21-0.40 fair, K: 0.41-0.60 moderate, K: 0.61-0.80 good, K: 0.81-1.00 very good) was used for consistency grading. Spearman's rho correlation analysis was performed for the relationship between

Table 1. Age and gender distribution of the groups

	Pulmonary thromboembolism		p value
	Absent (n=47; %47)	**Present (n=53; %53)**	
Age, mean ± SD	60.02±17.02	66.57±15.39	0.046[a]
Gender, n (%)			0.110[b]
Male	19 (40.4)	29 (54.7)	
Female	28 (59.6)	24 (45.3)	

   a.   Independent sample t-test, b. Fischer's Exact Test, SD: Standard Deviation.

Table 2. Comparison of EMA assessments with RS report

	Compatibility (%)	Kappa	Douglas G. Altman Classification
Pulmonary thromboembolism	% 79,5	0,590	Moderate
Truncus	% 97,2	-0,12	Poor
Right main pulmonary	% 91,3	0,768	Good
Left main pulmonary	% 91,8	0,740	Good
Right lobar	% 79,5	0,523	Moderate
Left lobar	% 84	0,570	Moderate
Right segmentary	% 75,4	0,476	Moderate
Left segmentary	% 81,3	0,583	Moderate
Right subsegmentary	% 65,9	0,298	Fair
Left subsegmentary	% 70,9	0,353	Fair
Parenchymal finding	% 86,8	0,719	Good
Atelectasis	% 88,6	0,236	Fair
Ground-glass opacification	% 89	0,593	Moderate
Consolidation	% 90,4	0,577	Moderate
Effusion	% 89	0,634	Good

emergency medicine resident professional seniority and compliance

RESULTS

Between 01.05.2021 and 30.04.2022, PTE was absent in 47% and present in 53% of the BTPAs selected for the study from the BTPA images taken in the emergency department.The mean age of the group without PTE (60.02±17.02) was statistically significantly lower than the mean age of the group with PTE (66.57±15.39) (p<0.05). While 40.4% of the group without PTE was male and 59.6% was female, 54.7% of the group with PTE was male and

45.3% was female. Between the groups, no major difffernce with ot without PTE in terms of gender is examined. (p>0.05) (Table 1).

The ages of the non-PTE group were between 20 and 89 years, while the ages of the PTE group were between 30 and 93 years. The age range of the non-PTE group was 69 years and 63 years in the PTE group.

Among the EMAs in the study, 36.4% had 1 to 2 years, 36.4% had 2 to 3 years and 27.3% had 3 to 4 years of professional experience. PTE agreement between EMA and RS was 79.1%, location agreement was 59.1%, the agreement of the presence of parenchymal finding was 84.5% and

J Basic Clin Health Sci 2024; 8: 620-625

Perver G et al. Interpretation of Computed Tomography Pulmonary Angiography

Table 3. Comparison of RA assessments with RS report

	Compatibility (%)	Kappa	Douglas G. Altman Classification
Pulmonary thromboembolism	% 98	0,960	Very good
Truncus	% 100	1,00	Very good
Right main pulmonary	% 100	1,00	Very good
Left main pulmonary	% 100	1,00	Very good
Right lobar	% 100	1,00	Very good
Left lobar	% 100	1,00	Very good
Right segmentary	% 100	1,00	Very good
Left segmentary	% 100	1,00	Very good
Right subsegmentary	% 98	0,960	Very good
Left subsegmentary	% 99	0,980	Very good
Parenchymal finding	% 100	1,00	Very good
Atelectasis	% 100	1,00	Very good
Ground-glass opacification	% 100	1,00	Very good
Consolidation	% 100	1,00	Very good
Effusion	% 100	1,00	Very good

the agreement of the parenchymal finding was 70.0%.

Moderate agreement was found between EMA and RS in the evaluation of the presence of PTE. The highest agreement was seen in the right main pulmonary artery. Good agreement was found in the left main pulmonary artery and moderate agreement was found in the right and left lobar arteries. Agreement was moderate in bilateral segmentary arteries and poor in bilateral subsegmentary arteries. Good agreement was observed between EMAs and RS in parenchymal findings. Agreement in parenchymal findings was lowest in the atelectasis finding and highest in the effusion finding. Moderate agreement was found between RS and EMAs for ground glass and consolidation (Table 2).

There was 73.75% agreement for the presence of PTE in those with a professional seniority of 1-2 years. The degree of agreement was moderate (Kappa: 0.473, Douglas G. Altman K: 0.41-0.60 moderate). In the same group, very good agreement was found in the evaluation of parenchymal findings (Kappa:0.813, Douglas G. Altman K: 0.81-1.00 very good). There was 86.25%

and good agreement for the presence of PTE in EMAs with a professional seniority of 2-3 years (Kappa: 0.722, Douglas G. Altman K: 0.61-0.80 good). There was good agreement for parenchymal findings (Kappa: 0.691, Douglas G. Altman, K: 0.61-0.80 good). In EMAs with a professional seniority of 3-4 years, the agreement was moderate for PTE and good for parenchymal findings (Kappa: 0.564 and 0.625, Douglas G. Altman, K: 0.41-0.60 moderate, K: 0.61-0.80 good). No truncal PTE was detected in any CTPA in the groups with 2-3 years and 3-4 years of seniority, so the kappa value could not be calculated. According to Spearman's rho correlation analysis, the professional experience of the EMAs did not show a statistically significant effect on compliance assessment.

Agreement between RA and RS was 98.0% for PTE, 97.0% for location, 100.0% for the presence of a parenchymal finding and 100.0% for the name of the parenchymal finding. Excellent agreement was seen in all localisations (Table 3).

DISCUSSION

In the literature, different agreement rates have been reported between EMAs and RS in the

evaluation of radiological imaging. In a study by Aydın et al. in which 5 EMA and RA were performed, good agreement was found (Kappa: 0.773) (7). The lower agreement rate compared to this study may be because of the difference in training and experience between the evaluators and the large percentage of false negatives. In our study, false negatives were common in subsegmentary arteries. Güven et al. found that the rate of CT interpretation by emergency physicians for PTE was 89.4% and the agreement with the final results was moderate (Kappa: 0.590) (4).

Hochhegger et al. compared the agreement rates between RAs and emergency physicians for PTE detection (8). In this study, the agreement between RAs and RSs was found to be very good, and between RSs and emergency physicians was found to be moderate, similar to our study.

In a study by Cervini et al. in which 840 CTPAs were examined in two different centres, the preliminary interpretation of the on-call RA and the RS report were compared, and 90% agreement was found (P = .76, 95% confidence interval, 0.71-0.81) (9). In our study, the agreement between RA and RS was 98% for the presence of PTE. In the study by Cervini et al. the agreement between RA and RS was lower than in our study due to the multisite comparison of cTPAs and the inclusion of a large number of patients and RA.

In our study, the highest agreement was 94.8% for left subsegmentary arteries, while the lowest agreement was 65.9% for right subsegmentary arteries. One of the reasons for the low agreement in subsegmentary arteries compared to other locations may be the lack of careful evaluation due to the perception that the presence of PTE in subsegmentary arteries, which are usually caught incidental in daily practice, do not give clinical findings and have a low hospitalisation rate, is not important.

Although the agreement rate in truncal evaluation was 97.2%, the kappa value was -0.12. In our study, 4 PTEs detected in the trunk in 100 CTPAs were not detected by EMAs, while 2 false positive truncal PTEs were detected. Although the correct interpretation of CTPAs, 98.2% of which were negative in terms of truncal PTE, increased the percentage of agreement, it can be said that EMAs are insufficient to evaluate PTE in the trunk. This is one of the unexpected results of the study. In a study in which thrombus locations were evaluated

in CTPA, PTE in the trunk was found to be only 3.0% and 2.4% in Duru et al. study (10), (11). Because of its infrequent occurrence, it can be said that EMAs are not accustomed to this anatomical location and cannot make an accurate assessment. PTE in the trunk may be confused with PTE in the main pulmonary arteries. This is not because PTE was not recognised, but because the location was misjudged.

In our study, the agreement in terms of the presence of parenchymal findings was 84.5%, while the agreement in terms of the parenchymal findings was 70%. It can be said that the finding seen more prominently in some images with more than one parenchymal finding causes other findings that will not lead to a change in the treatment process to be ignored.

In the studies of Arhami et al. and Perron et al. no relationship was found between the number of years spent in the profession and radiological evaluations, similar to our study (12), (13).

Limitations

Since CTPAs were scanned retrospectively, our study is not a study reflecting the clinical environment. In practice, in cases of intermediate cases, the diagnosis and treatment process of patients progresses by consulting the specialists. This study was conducted only for the evaluation of EMAs.

CONCLUSION

In our study, the agreement between EMA and RS was 79.1% for the presence of PTE, 59.1% for the location, 84.5% for the presence of parenchymal finding and 70.0% for the name of the parenchymal finding. Moderate agreement was found between EMA and RS in the assessment of the presence of PTE. There was poor agreement in the pulmonary trunk, good agreement in bilateral main pulmonary arteries, moderate agreement in bilateral lobar and segmental arteries and poor agreement in bilateral subsegmental arteries. Good agreement was observed in the evaluation of parenchymal findings. There was poor agreement for atelectasis, moderate agreement for ground glass and consolidation, and good agreement for effusion. Agreement between RA and RS was 98.0% for PTE, 97.0% for location, 100.0% for parenchymal findings and 100.0% for the name of the parenchymal finding.

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CAROTIS INTIMA-MEDIA THICKNESS, CORONARY CALCIUM SCORE AT DIFFERENT STAGES OF CORONARY ARTERY DISEASE

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ABSTRACT

Purpose: Coronary Artery Calcium Score (CACS) and Carotid Artery Intima-Media Thickness (CIMT) are surrogate markers for atherosclerosis. CACS is a recognized indicator of coronary artery disease (CAD), but CIMT's role in CAD diagnosis is debated. This study aimed to assess how well CIMT and CACS predict CAD presence and severity as detected by coronary computed tomography angiography (CCTA).

Materials and Methods: In the study, 88 participants (57 CAD and 31 controls) underwent coronary angiography and CACS calculation using computerized tomography and CIMT measured according to the guidelines. Patients with CAD were classified by CACT results and further subdivided by CACS into three groups: Group I (<100), Group II (100-300), and Group III (≥300). The relationship between CIMT and CAD groups with zero Agatston scores, as well as the control group, was also examined.

Results: The CACS had 82% sensitivity and 100% specificity for predicting CAD, excluding CAD with 75.6% specificity. A CIMT max cut-off of ≥0.78 mm showed 76% sensitivity and 54% specificity for CAD. A CIMT max cut-off of ≥1.03 mm had 93% specificity but only 35% sensitivity, while ≤0.59 mm excluded CAD with 96% specificity but just 10% sensitivity. Patients with CIMT levels between 0.59 mm and 1.03 mm may need further testing to assess CAD risk accurately.

Conclusion: The CACS is more sensitive than CIMT in predicting CAD, and CIMT is not helpful when the CACS is zero. Determining an optimal CIMT cutoff for CAD prediction is challenging, and patients with CIMT between 0.59 mm and 1.03 mm may require additional testing.

Keywords: Carotis intima media thickness, coronary artery disease, coronary artery calcium score, agatston score

INTRODUCTION

Cardiovascular disease (CVD) represents the most prevalent cause of mortality and morbidity, with low- and middle-income countries bearing the majority of the associated burden. Amongst the various forms of CVD, ischaemic heart disease represents the primary cause of mortality, accounting for 38% of CVD-related deaths in women and 44% in men (1).

It is estimated that approximately half of all deaths related to CVD occur in individuals without a prior history of heart disease. This highlights the necessity of accurately assessing an individual's risk in the absence of symptoms to prevent these fatalities (2). The combined thickness of the intimal and medial layers of the carotid artery is determined through a test known as carotid intima-media thickness (CIMT), which serves to indicate the presence of widespread atherosclerosis (3). CIMT is typically quantified by measuring the distance between the echogenic media-adventitia layer and the echogenic lumen-intima layer using B-mode ultrasound images (4). It is regarded as a marker for forecasting the initial stages of atherosclerosis prior to a history of CVD. CIMT is linked to cardiovascular events and outcomes (5). CIMT serves as an indirect marker of coronary atherosclerotic burden. A number of clinical studies have demonstrated the relationship between CIMT and the occurrence, degree and extent of coronary artery disease (CAD) (6, 7).

Coronary computed tomography angiography (CCTA) is a non-invasive imaging technique that has been demonstrated to have high sensitivity for the identification of coronary artery disease (CAD). The indications for the use of CCTA as a Class I upgrade (8) in the diagnostic work-up of patients with stable symptoms and a low to intermediate risk for obstructive CAD have recently seen a significant increase in utilisation. The primary methodology employed in numerous investigations examining the correlation between carotid CIMT and CAD is invasive coronary angiography (ICA). These studies typically utilise lumenographic criteria and involve individuals with high-risk CAD. However, recent research suggests that, with regard to measuring atherosclerotic burden, CCTA is more accurate than ICA (9, 10).

In most of the studies investigating the relationship between CIMT and CAD, the extent of CAD was determined by ICA. In these studies, the CAD group typically consisted of individuals diagnosed with more than 50% coronary artery stenosis, whereas the control group consisted of individuals with less than 50% stenosis. However, it is important to note that patients with less than 50% stenosis and no luminal narrowing due to eccentric plaques with atherosclerosis may not fully represent a normal group without coronary atherosclerosis, especially in studies using ICA lumenographic criteria (6, 11,

12). The inclusion of patients with atherosclerosis in the control groups of these previous studies makes the interpretation of CAD prediction data unreliable. Furthermore, the determination of CAD severity by ICA is a methodology that needs to be questioned, as it does not take into account the presence of eccentric plaques that do not result in significant intraluminal plaque burden (9, 10, 13, 14).

In the present study, we used CCTA to investigate the association between CIMT, CACS, and CAD. This method provided more accurate data than ICA, allowing us to differentiate between patients with CAD and those with normal coronary arteries. The aim of the study was to assess the relationship between CIMT, CACS, and atherosclerosis in two groups: individuals with normal coronary arteries, as confirmed by CCTA, and patients with CAD identified by CCTA, who presented with soft, calcific, or mixed plaques at various stages of atherosclerosis, with differing Agatston scores and severity.

MATERIALS AND METHODS

A cohort of 128 consecutive patients with symptomatic chest pain was prospectively evaluated using carotid ultrasonography to assess CIMT and CCTA between September 2023 and January 2024. From this cohort, we excluded individuals with a history of known CAD (n=18), established non-ischemic cardiac conditions (n=16), contraindications to contrast media (n=1), impaired renal function defined as a creatinine clearance rate < 60 ml/min (n=2), and cardiac arrhythmias (n=3).

All participants underwent carotid ultrasonography and CCTA on the same day. The study excluded pregnant women, individuals with atrial fibrillation or age below 35, those using statins, individuals with renal dysfunction, and those with known coronary or peripheral artery disease

Of the 128 participants, 88 were included in this study. Among them, 31 participants with normal coronary arteries and an Agatston score of "0" constituted the control group, while 57 participants with coronary atherosclerosis of varying severity and calcification levels constituted the CAD group.

Demographic data such as sex, age, height, weight, and blood pressure were collected for all patients. In addition, clinical and laboratory data were also collected with patient consent, which includes information on diabetes mellitus (DM) medication,

J Basic Clin Health Sci 2024; 8: 626-633

Okan T. et al. Intima Media Thickness and Coronary Artery Disease

smoking history, hyperlipidemia, family history of CAD, and levels of glycated hemoglobin (HbA1c), lipid profile, and creatinine. The study protocol was approved by the Ethical Committee of Bakırcay University, written informed consent was obtained from all patients.

CIMT Measurements

CIMT images were obtained by an experienced radiologist using high-resolution carotid ultrasound with a linear transducer (>7.5 MHz) at the same radiology centre where CCTA was performed. Following standard protocols, longitudinal B-mode images of the left and right carotid arteries were acquired to assess each common carotid artery (CCA). The mean and maximum CCA intima-media thickness (IMT) was measured within a 10 mm segment proximal to the carotid bulb. These measurements were performed using automated IMT measurement software on a Hitachi Arietta 850 ultrasound machine, and the assessments were independently verified by both an experienced radiologist and a cardiologist.

Computed Coronary Angiography

A 128-slice single-source computed tomography (CT) scanner (Somatom Go Top; Siemens Healthcare, Forchheim, Germany) was used to perform CCTA in all patients. The Agatston score was used to measure the CACS on the same CT scanner. CCTA was independently interpreted by an experienced radiologist and cardiologist. The patients were then divided into three groups based on their Agatston scores. Group I patients had a slightly increased risk, group II patients had a moderately increased risk, and group III patients had a moderate-to-severe increased risk. The Agatston scores of the patients fell between 0 and 99, between 100 and 299, and above 299, respectively. This classification system has been previously reported in the literature (2,15).

Statistical Analysis

A power analysis was conducted using G*Power 3.0.10 for Windows, with an α value of 0.05 and a study power of 0.90.

The statistical analysis was conducted using IBM SPSS Statistics, version 29 (IBM Corp., NY, USA). A Student's t-test was employed to ascertain whether there were any significant differences in the means of continuous numeric variables between the

groups in question. A chi-square test was employed for the comparison of categorical variables. The Mann-Whitney U test was employed to assess the existence of statistically significant differences in CACS and CIMT levels between the two groups. In instances where there were more than two groups, the Kruskal-Wallis test was employed, followed by a post-hoc analysis with Bonferroni adjustment for multiple comparisons. A receiver operating characteristic (ROC) analysis was employed to calculate the area under the curve (AUC) and 95% confidence intervals for the purpose of evaluating the predictive value of CIMT for the presence and severity of CAD. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

There were no discernible variations in age, sex, body mass index (BMI), or smoking status between the CAD and control groups. Lipid profiles, blood pressure during both systolic and diastolic phases, and blood glucose and creatinine levels were also comparable (Table I). The study demonstrated a statistically significant relationship between the CACS of the patient group and the mean and maximum CIMT values of the right and left CCA. In particular, compared with low- and intermediate-risk patients (Group I and Group II), patients classified as high-risk with a CACS (Agatston score) higher than 300 (Group III) showed significantly higher CIMT values. Table II presents an overview of this relationship. According to the CACS, this study points to a possible link between elevated CIMT levels and an increased risk of coronary artery disease. In patients with a substantial CACS, high CIMT readings may be indicative of a higher cardiovascular risk.

Notably, Table III shows that there was no statistically significant difference in the CIMT values of patients with CAD who did not have CAC (Agatston=0) compared to the control group who had normal coronary arteries. Of the left and right CCA IMT readings.

In the study, CACS had 82% sensitivity and 100% specificity in predicting CAD, while also excluding CAD with 75.6% specificity.

The highest value (CIMT max) of the CIMT measurements showed the strongest statistically significant correlation for predicting the presence of CAD. Regarding CIMT Max Max value, a cut-off of 0.78 mm was identified as the most suitable for CAD

prediction, offering 76% sensitivity and 54% specificity. Alternatively, accepting a CIMT Max Max cut-off of ≥ 1.03 mm yielded 93% specificity but reduced the sensitivity to 35%. Conversely, a CIMT Max Max ≤ 0.59 cut-off enabled CAD exclusion with 96% specificity but decreased sensitivity to 10%. The study suggests that patients with CIMT Max Max levels between 0.59 mm and 1.03 mm may require additional diagnostic testing to accurately assess CAD risk. ROC curve analysis was used to determine the cutoff value of CIMT for CAD prediction (Figure 1).

DISCUSSION
In this study, we examined how well carotid CIMT and CACS predict the existence and severity of CAD detected by CCTA. Our research verified a correlation between CIMT, CACS, and the degree of CAD, as determined by CCTA. Similar to our findings, Pathokata et al. discovered a high association between CACS and CIMT and the severity of CAD, as determined by Gensini and SYNTAX Scores (16).

The CACS, which measures calcium deposits on arterial walls, is a crucial imaging biomarker for

Table 1. Baseline characteristics of study groups

	Coronary Artery Disease (n: 57)	Control (n: 31)	p
Female %	%31.5 (n: 18)	%35.4 (n: 11)	0.330
Age (years)	57.01±7.49	58.03±6.92	0.535
BMI (kg/m²)	27.43±3.23	27.07±3.88	0.640
Glucose (mg/dL)	110.75±38.62	99.76±37.80	0.209
Cholesterol (mg/dL)	224.21±49.69	235.48±41.77	0.286
LDL (mg/dL)	140.64±44.84	151.38±40.09	0.269
HDL (mg/dL)	53.36±10.55	54.45±17.18	0.891
Triglyserides (mg/dL)	196.33±118.53	149.09±86.36	0.054
Creatinin (mg/dL)	0.86±0.19	0.78±0.16	0.051
Smoking %	35.08%	29.03%	0.354
Diabetes Mellitus %	26.32%	22.58%	0.469
Systolic BP (mmHg)	134.50±17.81	131.32±12.80	0.514
Diastolic BP (mmHg)	78.96±11.67	77.38±8.96	0.514

Table 2. Statistical relationship between CAD groups based on CACS (Agatston score) and CIMT

	Group I Agatston<100 (n: 29)	Group II 100<Agatston<300 (n:12)	Group III Agatston≥300 (n: 16)	p#	p##	p###	p
CIMT (Mean Right) mm	0.65±0.23	0.64±0.15	0.84±0.30	0.986	0.001	0.001	0.005
CIMT (Max Right) mm	0.80±0.27	0.84±0.19	1.08±0.37	0.829	0.001	0.032	0.007
CIMT (Mean Left) mm	0.65±0.14	0.68±0.14	0.84±0.29	0.843	0.001	0.072	0.043
CIMT (Max Left) mm	0.85±0.18	0.87±0.18	1.13±0.39	0.780	0.001	0.018	0.026
CIMT (Max Mean) mm	0.70±0.21	0.72±0.11	0.94±0.36	0.811	0.001	0.048	0.020
CIMT (Max Max) mm	0.89±0.18	0.95±0.17	1.24±0.43	0.837	0.001	0.029	0.012

CIMT Max Max: The highest CIMT value of both carotid arteries, CIMT Max Mean: The highest mean CIMT values of both carotid arteries, p#: p value for Group I and Group II, p##: p value for Group I and Group III, p###: p value for Group II and Group III, p: comparison p value for three groups

Table 3: Comparison of CIMT between CAD group with zero Agatston value and normal coronary artery group

	Coronary Artery Disease with Agatston: 0 (n: 10)	Control group (n: 31)	p
IMT (Mean Right) mm	0.64 ± 0.25	0.60±0.10	0.747
IMT (Max Right) mm	0.78±0.26	0.73±0.13	0.925
IMT (Mean Left) mm	0.67±0.19	0.62±0.10	0.787
IMT (Max Left) mm	0.84±0.19	0.79±0.13	0.735
IMT Max Mean mm	0.70±0.22	0.64±0.11	0.791
IMT Max Max mm	0.90±0.20	0.80±0.15	0.276

J Basic Clin Health Sci 2024; 8: 626-633

Okan T. et al. Intima Media Thickness and Coronary Artery Disease
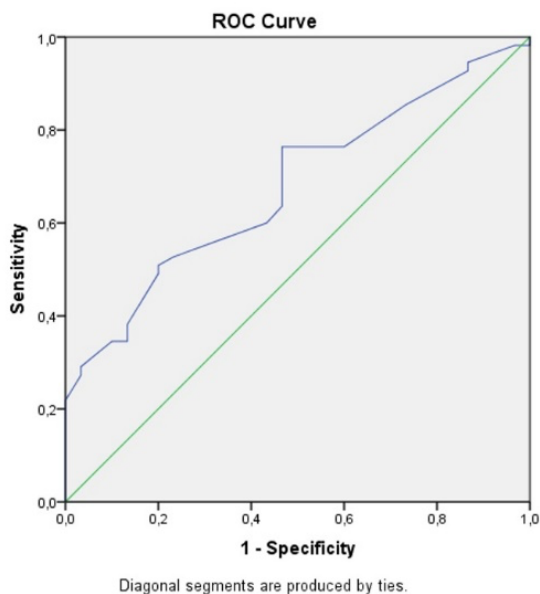

Figure 1. The cutoff value of CIMT for CAD prediction

heart health. Low-dose radiation (<1 mSv) scans without contrast enhancement are frequently used for evaluate it (9). Microcalcifications, a characteristic of susceptible (high-risk) plaques and indicator of active inflammation, are the form in which CACS is found in the early stages of atherosclerosis (17). On the other hand, the Agatston score, also known as the CACS, quantifies the extent of coronary calcium deposition typically observed in the later stages of atherosclerosis and associated with a stable plaque phenotype. Although CACS is an effective marker of total atherosclerotic plaque burden, it has a weak correlation with the degree of luminal stenosis. While there has been debate as to whether coronary artery calcium reflects plaque stability, the current consensus is that a high CACS identifies a vulnerable patient rather than indicating unstable plaques or vessels. An elevated calcium score is associated with an increased risk of major adverse cardiac events (MACE). One study demonstrated that a two-fold increase in the calcium score was associated with a 15 to 35% increased risk of major coronary events and a 18 to 39% increased risk of any coronary event (5,18,19). CACS is currently recommended for low- and intermediate-risk asymptomatic patients as IIb in the ESC guidelines and IIa in the ACC/AHA guidelines. (9).

In line with earlier research, we did not find a significant correlation between the CACS and the degree of coronary artery stenosis in our investigation. This phenomenon may be attributed to the existence of calcified deposits exhibiting minimal calcification but significant luminal narrowing, or alternatively, to the presence of dense calcifications with eccentric localization that do not result in notable luminal narrowing (2,18,19). A false-negative rate of 18% for CAD was observed in 10 patients with non-calcific soft plaques not detected by CACS among the 57 CAD patients in our study. The presence of calcium deposition in the coronary artery wall signifies permanent structural alterations that culminate in plaque stability of plaque (18,19). In contrast, theoretically soft, non-calcified plaques are more likely to rupture (vulnerable plaques). It is unfortunate that CACS is unable to identify non-calcified plaques and cannot always reliably rule out the possibility of a coronary event (20,22).

As CACS is unable to predict the disease in the CAD group with a CACS of zero, CIMT determination may prove to be a valuable diagnostic method for predicting the development of CAD. However, when our study analysed patients with a CACS value of zero, no statistically significant difference was found between the mean and maximum CIMT values of both carotid arteries in the CAD group and the non-CAD control groups.

In their study, Lester and colleagues examined 89 patients, aged between 36 and 59 years, with a CACS of zero. In their study, 47% of patients exhibited a calcium score of zero, indicative of coronary atherosclerosis based on CIMT. The authors proposed that while CIMT and CACS are both markers of subclinical atherosclerosis, CAC typically manifests later in the course of CAD. Consequently, the reliability of this method may be questionable, particularly in younger individuals with a CACS of zero. In this demographic, CIMT may offer a more valuable insight into the presence of CAD (23). It is possible that other factors may contribute to the inconsistencies observed in the predictive efficacy of CIMT in patients with non-calcified soft plaques. One potential explanation for this discrepancy is that the CAD group with a CACS of zero in our patient cohort was older than the patient group in the Lester et al. trial. Additionally, it is conceivable that the sample size was inadequate to yield definitive conclusions. Our findings suggest that CIMT measurement is an ineffective diagnostic technique, similar to CACS, in the early stages of

J Basic Clin Health Sci 2024; 8: 626-633

Okan T. et al. Intima Media Thickness and Coronary Artery Disease

atherosclerosis. However, some studies have reported that it is an effective method for predicting CAD with soft, non-calcific plaques, the initial phase of coronary atherosclerosis, and that it allows the prediction of early CAD characterised by the presence of soft, non-calcific vulnerable plaques, especially in young patients (23–25). Similarly, Dokumaci et al. reached the same conclusion (26). The mean and maximum values of CIMT for both carotid arteries were significantly higher in the group with an Agatston score of 300 and above, as well as in the group exhibiting the most severe and extensive atherosclerosis, compared to patients with atherosclerosis scoring below 300, according to our study, which showed that CAD patients were divided into three groups based on the Agatston score. This outcome is in line with the higher CIMT associated with CACS on CCTA (12, 27,28).

In our study, the inability of CIMT to predict patients with zero CAD or a lower CACS level in the early atherosclerosis stage may be compensated for by its capacity to predict patients with an Agatston score above 300 who are at high risk of MACE. This may render CIMT a valuable tool in the identification of high-risk CAD patients for risk stratification, although not as a diagnostic method (19,29,30).

The diagnostic utility of CIMT for CAD has been a topic of considerable debate. The increased CIMT observed in the carotid artery has been linked to coronary artery disease (CAD) in several studies conducted over the past few decades. However, this relationship has not been confirmed by other investigations (31-34). In their evaluation of the correlation between CIMT and the severity of CAD, Lisowska et al. reported a diagnostic sensitivity of 91% and a specificity of 65% (36). In accordance with the findings of another study, CIMT is an effective method for excluding severe CAD in patients undergoing cardiac valve surgery. A study found that CIMT had 100% sensitivity and 50% specificity for diagnosing CAD (37). As demonstrated by Kanadasi et al., the specificity and diagnostic sensitivity were 86.4% and 14.3%, respectively (33). The CIMT cutoff value was 1 mm, with an associated sensitivity and specificity of 31.91% and 90.52%, respectively, according to the findings of Zhang et al. (38). The ESC guidelines do not recommend carotid ultrasound IMT for cardiovascular risk assessment (Class III). The results of our study support this recommendation. Specifically, the inability to determine an effective

and reliable cut-off value for CIMT in CAD prediction, and the observation that CIMT values with high specificity for CAD exhibit very low sensitivity for the disease, corroborate the guidelines (8).

Limitations

The present study is limited by the relatively small size of the cohort and the fact that the findings represent a single-centre experience. Consequently, external validation is required. However, this pilot study has the potential to initiate a new line of research that could lead to more comprehensive studies in the future.

CONCLUSION

The degree and severity of CAD on CCTA can be predicted using both the CACS and CIMT measures; however, the CACS has a higher sensitivity. In patients with a calcium score of zero, CIMT is not as useful in predicting CAD as CACS. Predicting CAD is difficult when CIMT measurements need to be set at the ideal cut-off value. More diagnostic testing is required to accurately predict CAD in patients with CIMT levels between 0.59 mm and 1.03 mm, more diagnostic testing is required.

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THE ROLES OF IMMUNE MOLECULES IN THE ACUTE POST-TRANSPLANT PERIOD

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ABSTRACT

Purpose: Renal transplantation is a therapeutic choice that enhances the quality of life for patients suffering from end-stage renal failure. The objective of this study was to ascertain the alterations in the levels of immune molecules following transplantation and to examine the correlation between these changes and the medical records of the patients.

Materials and Methods: The gene expression of an immune molecule panel (FOXP3, TNF-α, IFN-γ, IL-18, IL-6, IL-17a, IL-12a, IL-2, IL-10, and TGF-β) in peripheral blood specimens of 30 kidney transplant patients was determined by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) method with SYBR Green Dye. The serum proteins were quantified using Enzyme-Linked ImmunoSorbent Assay (ELISA).

Results: TGF-β exhibited the most significant alteration in gene expression levels compared to the levels before transplanting ($p < 0.05$). A strong association was seen between the change in IFN-γ levels and the estimated glomerular filtration rate (eGFR) values of the patients ($p < 0.05$).

Conclusion: The cytokine expression alterations may provide information on patients' clinical condition. Individualized immune scanning after transplantations may contribute to personalized treatment of each patient. The communication between the laboratory and the clinics is important for the accurate consultation of the patients.

Keywords: kidney transplantation, real-time pcr, cytokine, gene expression

INTRODUCTION

Organ transplantation is a vital treatment that saves the lives of people who are suffering from organ failure at an advanced stage, yet its success is often jeopardized by the host immune response, which can lead to graft rejection. The balance between pro-inflammatory and regulatory pathways is pivotal in determining whether the transplanted organ is accepted or rejected. Understanding these immunological changes is critical for improving post-transplant outcomes (1,2).

Cytokines are key mediators in the immune response, and their levels post-transplantation can significantly influence graft survival. Tregs (regulatory T cells) require the transcription factor Forkhead box P3 (FOXP3) for both their growth and operation, which help maintain immune tolerance to the graft. Elevated levels of FOXP3-expressing Tregs are often associated with reduced incidence of graft rejection and improved transplant outcomes (3).

In contrast, Tumor necrosis factor-alpha (TNF-α) and Interpheron-gamma (IFN-γ) are pro-inflammatory cytokines that play a role in the development and propagation of immune responses that might result in graft rejection. TNF-α is known for its role in promoting inflammation and apoptosis, while IFN-γ enhances the immune response by activating macrophages and increasing antigen presentation, thereby exacerbating graft rejection (4,5). Recent studies have highlighted the complex role of Interleukin 17a (IL-17a) in transplant immunology, where it can contribute to both graft rejection and tolerance depending on the context (6).

Additional cytokines like IL-6, IL-18, and IL-12a play a role in the pro-inflammatory response, contributing to the activation and differentiation of T helper cells, which are critical in orchestrating the immune response (7,8). On the other hand, IL-10 and Transforming growth factor-beta (TGF-β) are central to immune regulation, with IL-10 acting as an anti-inflammatory cytokine and TGF-β playing a dual role in promoting both immune tolerance and, paradoxically, fibrosis in the graft (9,10).

Despite advances in immunosuppressive therapies, the challenge of graft rejection persists, highlighting the need for a deeper understanding of the immune dynamics post-transplantation. This study aims to investigate the alterations in the levels of FOXP3, IFN-γ, TNF-α, IL-6, IL-18, IL-17a, IL-12a, IL-2, IL-10, and TGF-β in patients following transplantation. Additionally, it seeks to correlate these changes with the clinical outcomes documented in patients' medical records. By integrating cytokine profiles with clinical data, this research aims to identify potential biomarkers that could predict transplant outcomes and guide personalized therapeutic strategies.

MATERIALS AND METHODS
Sampling

Blood samples of 30 patients were collected from three different hospitals at certain intervals (before transplantation, 1 month and 3 months after transplantation). The ready platform was used to calculate the patients' estimated glomerular filtration rate (eGFR) values at the time of blood sampling using the brief Modification of Diet in Renal Disease (MDRD) formula (1).

Signed informed consents were obtained from all patients. In compliance with the Declaration of Helsinki, our Institutional Non-Interventional Clinical Research Ethics Committee approved the study (Date: 22.02.2019, Decision No: 02).

RNA Isolation and cDNA Synthesis

RNAs were isolated by GeneJet Whole Blood RNA Purification Mini Kit (Thermo Scientific, Vilnius, Lithuania). Prior to isolation, the pellets were produced using 1X concentrated ACK (ammonium-chloride-potassium) buffer for the red blood cell lysis. The purification method was performed according to the manufacturer's instructions. The quantity and purity of the RNA samples were measured on a Nanodrop (Thermo Scientific, USA) instrument. The absorbance ratios (A260/A280) between 1.9 and 2.1 were accepted as pure and the samples with 5 ng/µl and above concentration were included in the study. Complementary DNA (cDNA) was synthesized from RNA samples by RevertAid First Strand Synthesis Kit (Thermo Scientific, Lithuania). The procedure was performed according to the manufacturer's instructions. The cDNA samples were stored at -20 °C until their usage.

Quantitative Real-time PCR (qRT-PCR)

IDT Integrated DNA Systems (https://www.idtdna.com/pages) and NCBI primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) online platforms were used to design the primers for the target gene amplification.

Table 1. The primer sequences used for gene expression analysis

Name	Sequence	Length (bp)
β-Aktin [2]	F: 5'-CTTCCTGGGCATGGAGTCCTG-3'	21
	R: 5'-GGAGCAATGATCTTGATCTTC-3	21
IL-2	F: 5'-CTCACCAGGATGCTCACATTTA-3'	22
	R: 5'-CCTCCAGAGGTTTGAGTTCTTC-3'	22
IL-6	F: 5'-GGAGACTTGCCTGGTGAAA-3'	19
	R: 5'-CTGGCTTGTTCCTCACTACTC-	21
IL-10	F: 5'-CCTTGCTGGAGGACTTTAAG-3'	20
	R: 5'-TCTTGGTTCTCAGCTTGGGG-3'	20
IL-12A	F: 5'-GATGTACCAGGTGGAGTTCAAG-3'	22
	R: 5'-GCCTGCATCAGCTCATCAATA-3'	21
IL-17A	F: 5'-CTCATTGGTGTCACTGCTACT-3'	21
	R: 5'-GGGAAGTTCTTGTCCTCAGAAT-3'	22
IL-18	F: 5'-GAAGAGGAAAGGAACCTCAGAC-3'	22
	R: 5'-GGTTCAGCAGCCATCTTTATTC-3'	22
IFN-γ	F: 5'-CTGCCAGGACCCATATGTAAA-3'	21
	R: 5'-GTCACTCTCCTCTTTCCAATTCT-3'	23
TNF-α	F: 5'-CCAGGGACCTCTCTCTAATCA-3'	21
	R: 5'-TCAGCTTGAGGGTTTGCTAC-3'	20
TGF-β	F: 5'-TTGATGTCACCGGAGTTGTG-3'	20
	R: 5'-TCCACTTGCAGTGTGTTATCC-3'	21
FOXP3	F: 5'-CAAGTTCCACAACATGCGAC-3'	20
	R: 5'-ATTGAGTGTCCGCTGCTTCT-3'	20

F: Forward, R: Reverse, IL: interleukin, IFN-γ: Interferon-gamma, TNF-α: tumor necrosis factor-alpha, TGF-β: Tumor growth factor-beta, FOXP3: Forkhead box P3, bp: base pair

Table 2. The demographic features of the patients

Patients		n (%)
Patient average age		46.1
Donor average age		49
Patients Gender	Female	15 (%53.6)
	Male	13 (%46.4)
Donor Gender	Female	11 (%39.3)
	Male	17 (%60.7)
Patient Blood group	A Rh+	15 (%53.6)
	A Rh-	1 (%3.5)
	B Rh+	4 (%14.3)
	0 Rh+	4 (%14.3)
	0 Rh-	4 (%14.3)
Donor Blood group	A Rh+	15 (%53.6)
	A Rh-	1 (%3.5)
	B Rh+	4 (%14.3)
	0 Rh+	4 (%14.3)
	0 Rh-	4 (%14.3)

The primers for the reference β-actin gene were obtained from a study (2). The sequences of the primers were given in Table 1. The qRT-PCR analysis was conducted with the SYBR Green dye (Ampliqon, Odense, Denmark) on the Thermo Scientific PikoReal Real-Time PCR System (Thermo Scientific, Vantaa, Finland). The qRT-PCR protocol was followed according to the manufacturer's instructions. The results were evaluated according to ΔΔCt method.

Enzyme Linked Immunosorbent Assay (ELISA)
The samples with most significant changes in gene expression of IFN-γ, IL-17A, IL-12A, IL18, IL-10, and TGF-β were analyzed for their protein levels in the patients' sera samples. The BT-LAB ELISA kits (Zhejiang, China) were used to perform ELISA tests. The procedure was conducted according to the manufacturer's instructions. The optical density of each well was determined at 450 nm by Thermo Scientific Multiskan go (Thermo Fisher Scientific, Vantaa, Finland).

J Basic Clin Health Sci 2024; 8: 634-641

Kocyigit AÖ et al. Immune Roles After Kidney Transplantation

Table 3. The immunological complications of the patients at the post-operative first and third months

Patient No	Complication	
	1st Month	3rd Month
P3	Urinary infection, DSA+	NC
P4	Urinary infection	NC
P5	TIN	TIN
P6	NC	Incisional hernia
P9	NC	Urinary infection
P12	Urinary infection	NC
P15	NC	Fever, leukopenia
P25	NC	Urinary infection
P28	Acute TIN	TIN

DSA: Donor Specific Antibody, NC: No Complication, ACR: Acute Cellular Rejection, TIN: Tubulointerstitial Nephritis

Statistical Analyses

Demographic and clinical average values were calculated by Microsoft Office Excel 2016 program. The statistical evaluations of the gene expression results were conducted with IBM SPSS Statistics 25 Software Program using paired t test. p values <0.05 were accepted as significant. HeatMap and scatter plot and bar graphs were created by GraphPad Prism 9.3.1 Software Program.

RESULTS

Demographic and clinical features of the patients

In total, 28 patients were evaluated because a patient had a primary non-functional kidney after transplantation and the other had biopsy-proven cellular rejection during the first month. The allografts were removed by nephrectomy in these patients. Because P28 rejected the allograft 15 months after transplantation, the patient was evaluated as without rejection on the post-transplant first and third months. The allograft was removed from P10 by nephrectomy, who lost renal function and returned to hemodialysis due to transplant renal arteria stenosis (TRAS) three months after transplantation. These patients were transplanted from a deceased donor (Figure 1). The demographic information of the patients was given in Table 2. Post-transplant maintenance treatment included Tacrolimus or Cyclosporine, Mycophenolate Mofetil or Azathioprine, and steroids.

Two patients (6.6%) had no alloimmunization (pregnancy, blood transfusion, and previous transplantation status). Prior to transplantation, all patients tested negative for crossmatch and PRA.

Some of the patients had complications in the post-operative 1st and 3rd months (Table 3).

The relationship between gene expression profiles and clinical outcomes in patients

The ΔΔCt method was used to calculate the results. Fold change of <1 and >1 was interpreted as a decrease and an increase, respectively.

The post-transplant first and third month mean ΔCt values did not differ significantly from the pre-transplant values (p>0.05) (Figure 2a). We discovered a significant difference between pre- and post-transplant expression levels of patients individually. The greatest number of significant differences were observed in P9 when compared to pre-transplantation (in 11 immune molecules, 8.4%). While seven (5.3%) of these results were obtained in the first month, four (3%) were obtained in the third month. In the 15th and 18th patients, there was no statistically significant change in gene expression of the immune molecules (p>0.05).
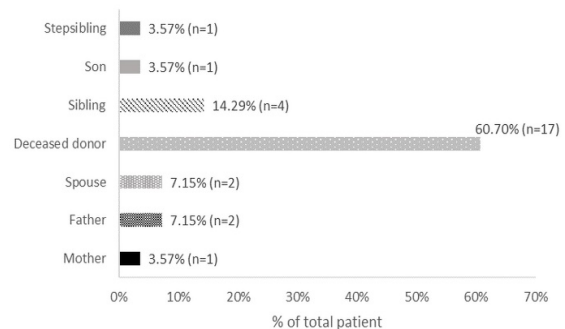

Figure 1. The relationship between the recipients and donors.

J Basic Clin Health Sci 2024; 8: 634-641

Kocyigit AÖ et al. Immune Roles After Kidney Transplantation

TGF-β gene expression levels had the highest number of statistical significance (p<0.05) including 65.5% (n=19) of the patients. Subsequently, the gene expression levels of IFN-γ (n=15, 51.7%) and IL-10 (n=13, 44.8%) had the greatest number of significant differences (p<0.05). IL-2 (n=3, 10.3%) was found to have the least number of statistically significant gene expressions (Figure 2b). The percentage of statistically significant (p<0.05) results was higher in the first month (n=75, 25.9%) than in the third month (n=56, 19.3%).

The relationship between cytokine serum levels, gene expression levels, and the clinical picture of the patients

The serum levels of IL-10, IL12A, IL-17A, IL-18, TGF-β, and IFN-γ were determined by ELISA method. The correlation between serum levels and gene expression levels was positive but not statistically significant (p<0.05). There was a significant difference in IL-10 serum levels between the pre-transplant and first post-transplant month (p=0.025, t=3.148). They were also strongly and positively correlated (r=0.839, p=0.005). When the patients were evaluated individually, however, there were significant differences (Figure 2c).

DISCUSSION

IL-2 is a well-known pro-inflammatory cytokine released from T cells. Kutukculer et al. found a significant increase in post-transplant IL-2 levels compared to pre-transplant by ELISA method in their sera samples (3). However, there was no significant difference in IL-2 gene expression levels like the other investigations (4,5). IL-6 is a pro-inflammatory cytokine involved in the activation of cellular and humoral immune responses. It prevents the development of regulatory T (Treg) cells, whereas it induces acute phase reactions, B-cell maturation and differentiation, and the differentiation of cytotoxic T cell. Omrani et al. compared the serum IL-6 levels of the kidney-transplanted patients with a control group, and found association with allograft rejection (7). In contrast, Waiser et al. reported that IL-6 levels were not an indicator of the rejection (8). Nevertheless, treatment approaches are being considered to prevent the interaction of the cytokine IL-6 and its receptor, IL-6R, to prevent kidney damage following kidney transplantation (6). We determined
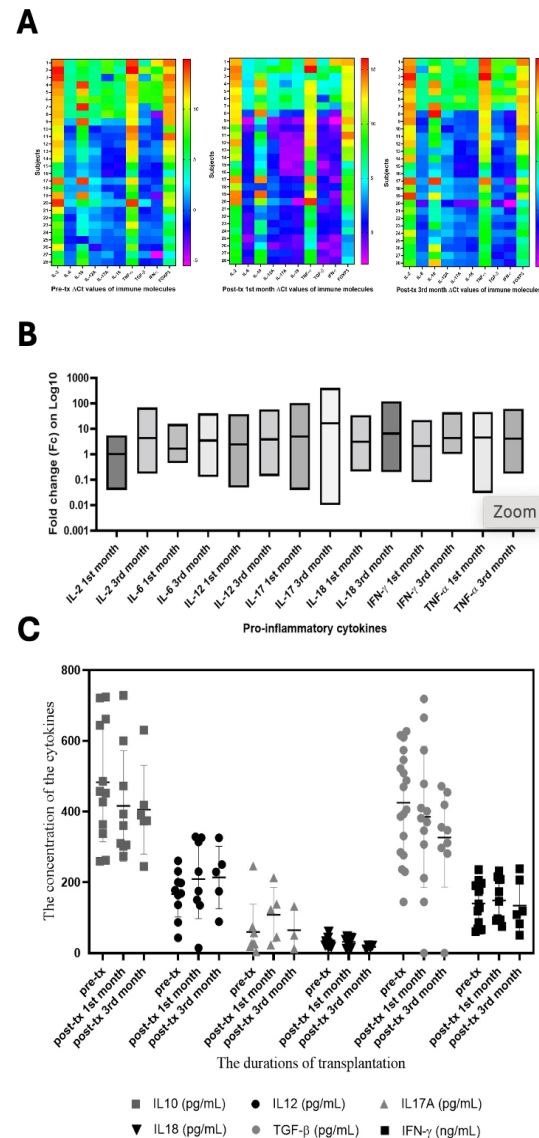
Figure 2. The alterations in gene and protein expression levels A) The alterations in ΔCt values at first and third months after transplantation compared to pre-transplantation were analyzed with HeatMap analysis. The values were normalized to β-actin. The fold changes (Fc) were calculated according to ΔΔCt method using these ΔCt values. Colors turning to red and purple at first and third months indicate the increase and decrease in expression, respectively. B) The fold changes of the immune molecules on the 1st and 3rd month compared to their pre-transplantation expression. Each line in the boxes indicates the mean values. The value above 10 indicates an increase and the value below 10 indicates a decrease. C) The pre-transplant, post-transplant 1st month and 3rd month serum levels of the patients are shown on dot plot graph. Each symbol indicates an individual. IL-10, IL-18, and TGF-β decreased, whereas IL-12 increased compared to pre-transplant values. IL-17A serum level increased in the first month but decreased in the third month after transplantation. The serum levels of IFN-γ were similar to pre-transplant values.

J Basic Clin Health Sci 2024; 8: 634-641

Kocyigit AÖ et al. Immune Roles After Kidney Transplantation

significant changes in gene expression and serum levels compared to pre-transplantation levels. IL-12 is another pro-inflammatory cytokine that activates NK and cytotoxic T cells. Benett et al. reported that IL-12 serum levels of pre- and post-transplant samples were similar in kidney transplanted patients (9). We observed significant alterations in gene and serum levels but did not determine a correlation with the clinics. The most common member of the IL-17 cytokine family is IL-17A. Th17 cells produce this cytokine, which has a pleiotropic effect. In our study, we found significant differences between pre- and post-transplantation gene expression levels. However, we could not find a correlation with the recipients' immune responses. Haouami et al., on the other hand, proposed that elevated IL-17A mRNA and protein levels could predict early acute organ rejection (10). As another pro-inflammatory cytokine IL-18 plays a role in both innate and adaptive immune responses (11). In their animal models, Wyburn et al. discovered that IL-18 expression levels increased significantly during acute organ rejection (12). There were significant differences in gene expression and serum levels of our patients after transplantation. IFN-γ is also a pro-inflammatory cytokine, which plays crucial roles in organ transplantations, viral infections, autoimmune responses, and adaptive immune responses (13). There were significant changes in gene expression and serum levels after transplantation in our study cohort. According to Nazari et al., patients with acute organ rejection episodes had higher levels of IFN-γ gene expression than patients who did not have this episode.14 Spivey et al. reported that patients with antibody-mediated organ rejection produced more IFN-γ than the control group (15). TNF-α is another pro-inflammatory cytokine that contributes to organ rejection following kidney transplants (16). In our study cohort, there were significant decreases and increases in gene expression of patients after transplantation.

IL-10 is an anti-inflammatory cytokine that regulates B and T cell functions and decides the initiation or prevention of the immune response (17). Gao et al. investigated if IL-10 was associated with cardiovascular and/or all-cause mortality after kidney transplantation (18). They found that IL-10 serum levels were independently associated with cardiovascular and all-cause mortality after the transplantations. In our study cohort, there were

statistically significant results after transplantation. TGF-β plays important roles in cell proliferation, differentiation, and apoptosis. It is the primary cause of fibrosis in all chronic kidney diseases (19). Hribova et al. observed that TGF-β gene expression was elevated in acute organ rejection patients after examining 174 biopsy samples (20). In our study, we observed significant changes in its expression levels; however, there was not a full rejection condition in our study group. Another study found that the expression levels of TGF-β may be affected using tacrolimus and cyclosporine A as immunosuppressive drugs (21). In our study, there was no significant correlation between the drug usage and the levels of TGF-β.

FOXP3 is an important regulatory transcription factor for the regulatory T cell function. Treg cells play crucial roles in the control of immune response to the allograft and development of transplant tolerance. In a study, FOXP3 gene expression levels were high in stable patients, but low in patients with chronic organ rejection (22).22 In contrast, Bunnag et al. investigated the FOXP3 gene expression levels in 83 kidney biopsy materials and concluded that the levels of FOXP3 were not associated with the kidneys' post-transplant condition. They did believe, however, that the FOXP3+ cells would infiltrate the allograft over time and stabilize the inflammation areas (23). Muthukumar et al. compared FOXP3 gene expression levels in 36 acute rejection patients, 18 chronic kidney nephropathy patients, and 29 stable kidney transplanted patients. They reported that patients with acute organ rejection had lower levels of FOXP3 (24). Hayato et al. found that FOXP3 mRNA levels decreased soon after kidney transplantation and then increased in peripheral blood samples from 272 kidney transplanted patients (25). We determined significant differences between pre- and post-transplant expression levels. The limitations of this study were: 1) There was a small scale of study cohort, 2) We assessed the patients individually, 3) There was no active acute-rejection episode sample.

CONCLUSION
In conclusion, the pro-inflammatory, anti-inflammatory, and regulatory effects of immune molecules in kidney transplantation are significant. We observed significant changes in the expression of all the cytokines compared to pre-transplantation

levels of the patients, although there was no significant association with their clinical symptoms. However, the number of individuals was insufficient for the statistical assessment. It would be beneficial to follow up the patients for their immune responses after transplantation. Hereby, the communication between the laboratories and the clinics becomes even more important to provide the recipients' samples accurately. The expression values or features of the study group may be assessed due to their different immune responses in different people. Accordingly, individualized immunotherapies may be considered as a protective, preventive, and/or therapeutic way for the recipients to improve the transplantation outcomes.

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EXAMINATION OF COACHES' DAYTIME SLEEPINESS LEVELS IN TERMS OF GENDER, PHYSICAL ACTIVITY LEVEL AND WAIST CIRCUMFERENCE

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ABSTRACT

Purpose: This study aims to examine coaches' daytime sleepiness levels in relation to gender, physical activity level, and certain anthropometric variables.
Materials and Methods: The study involved 330 coaches (173 women) from various sports in Istanbul. Daytime sleepiness was measured using the Epworth Sleepiness Scale, and physical activity levels were assessed with the International Physical Activity Questionnaire (IPAQ) short form. Anthropometric data (body weight, height, waist circumference) were self-reported.
Results: Female coaches had higher Epworth Sleepiness Scale scores than male coaches ($p < 0.05$). Male coaches had higher IPAQ scores, waist circumferences, and BMIs compared to female coaches ($p = 0.00$). There was no correlation between daytime sleepiness and BMI ($r = .066$) or waist circumference ($r = -.050$) ($p > 0.05$). A significant negative correlation was found between physical activity level and daytime sleepiness ($r = -113, p < 0.05$).
Conclusion: The study found a significant negative correlation between physical activity and daytime sleepiness, highlighting the importance of physical activity for sleep health. The young age of participants may limit the generalizability of these results, suggesting a need for further research with older adults.

Keywords: Physical fitness, Epworth Sleepiness Scale, trainer, gender

INTRODUCTION

Sleep is very important for the health of all living things. As well as improving immune functions and regulating appetite, sleep is also effective in other physiological processes that affect health. Moreover, sleep significantly affects health behaviours that require motivation and self-control, such as physical activity and healthy eating habits (1). In a study by Bediz and Günay (2), poor sleep quality during the COVID-19 pandemic resulted in a 58% increase and a 15% decrease in food consumption, respectively, among male para-athletes from swimming, soccer, and track and field.The common types of sleep problems frequently encountered in society are disorders such as insomnia, narcolepsy, restless legs syndrome and sleep apnea (3). Insomnia is characterised by an inability to fall asleep and stay asleep. Insomnia can also occur as a condition in which a person wakes up several hours early and is unable to continue sleeping. Insomnia may also manifest itself in a person as excessive sleepiness during the day, which can lead to functional

impairment in the person's daily activities (3). Narcolepsy is a condition in which excessive daytime sleepiness occurs, as well as sudden muscle weakness. Restless legs syndrome is defined as a condition in which involuntary and unpleasant sensations occur in a person's legs (3). Sleep apnea is a sleep disorder characterised by repeated pauses in breathing during sleep. These pauses inhibit the person's normal breathing during sleep and cause short-term insomnia (3).

Daytime sleepiness is a feeling of excessive sleepiness during the day and outside normal sleeping hours. This condition can make it difficult for a person to continue their normal daily activities and can negatively affect their performance. Daytime sleepiness can be caused by a variety of reasons, which may include inadequate sleep, sleep disorders, stress, depression, anxiety, the side effects of medications, or other health problems. Long-term daytime sleepiness may reduce a person's quality of life, and a person may require treatment (4). Sleep deprivation can have negative effects on functionality at work, at school, when driving, and in social life, and can also lead to various diseases. Some disorders resulting from sleep deprivation include heart disease, kidney disease, high blood pressure, diabetes, stroke, obesity and depression (5). Furthermore, sleep deprivation poses a major safety risk, especially in some professions. For example, sleep deprivation may increase the risk of accidents in individuals such as pilots, truck drivers, shift workers and medical assistants (6). Lack of sleep in students may lead to decreased academic performance, mood imbalances, and general health problems (7), while sleep deprivation in teachers may have a negative impact on their teaching performance (8). Sleep deprivation in military personnel can cause problems such as attention and focus problems, decreased decision-making skills, slower reaction times, and physical and mental fatigue (9). Sleep deprivation in physicians not only carries a risk for the patients they care for, but also has a negative impact on their own health (10). As well as in physicians, inadequate sleep in nurses can negatively affect both their own health and the health of the patients they care for (11).

The risk of insomnia increases in individuals who do physical activity but have high obesity levels. Therefore, it can be argued that improving body composition will increase sleep quality (12). However, some studies also emphasise that physical activity is not necessarily associated with sleep quality in all populations, and state that the relationship between physical activity and subjective sleep duration depends on age and gender (13). It is striking that apart from a few studies examining the sleep health and daytime sleepiness levels of coaches as a professional group, there are not enough studies on this subject (14-18). Although the Centers for Disease Control and Prevention (CDC) recommend at least 7-9 hours of sleep per night for adults (19), some studies report that coaches do not get enough sleep and suffer from sleep disorders (15,18,20).

Therefore, due to the fact that there are not enough studies on the sleep health of coaches in our country, this study aimed to examine the daytime sleepiness levels of coaches in terms of gender, physical activity level and some anthropometric variables. The hypotheses of the study are: 1) as waist circumference increases, daytime sleepiness level also increases, 2) as physical activity level increases, daytime sleepiness level decreases, and 3) daytime sleepiness levels of female coaches are higher than those of male coaches.

MATERIALS AND METHODS
Population and Sample
The sample size of the study was determined by the proportional sample size formula (18). Accordingly, it was determined that 186 participants out of a total of 350 coaches working in the Bağcılar district would be sufficient to conduct the study, with a 95% confidence interval and 5% margin of error. The sample of the study consists of 330 coaches (173 women and 157 men) working in various sports clubs in Bağcılar, Istanbul.

The criteria for inclusion in the study were determined as being at least 18 years of age, having a coaching certificate, having at least 2 years of coaching experience, and being an active coach. Those under the age of 18, those who did not have a coaching certificate and those who were not actively coaching were excluded from the study. The study was approved by Trakya University, Faculty of Medicine Non-Interventional Clinical Research Ethics Committee with protocol number TÜTF-GOBAEK 2023/186 (Date: 24.04.2023, Decision No: 07/26). Throughout the study, all practices were carried out in accordance with the Declaration of Helsinki.

Data Collection Tools
The study data were collected using Google Forms.

J Basic Clin Health Sci 2024; 8: 642-650

Kezer D. et al. Daytime Sleepiness Levels of Coaches

Personal Information Form

Personal information of the participants was collected with the form created by the researcher. This form includes questions about participants' gender, age, height, weight, sports branch, previous experience in sports, and number of years spent in coaching.

International Physical Activity Questionnaire (IPAQ)

The participants' physical activity levels were determined with the short form of the International Physical Activity Questionnaire (IPAQ), whose Turkish validity and reliability study was conducted by Öztürk (21). The questionnaire was developed to report the physical activity levels of people in daily life based on self-reports. The questionnaire basically includes questions about physical activity lasting at least 10 minutes during the day in the last week. In the questionnaire, participants are asked how many days and for how long during the last week they have participated in vigorous, moderate and walking activities, each lasting at least 10 minutes (21, 22).

Application Protocol: Physical activity level is determined by the metabolic equivalent of task (MET) method. The reference MET value is set at 3.5 ml/kg/min. According to this formula, the amount of oxygen consumed by the individual at rest is 3.5 ml per kg per minute. Three different references are used to determine the MET level in the scale. Accordingly, vigorous physical activity is defined as 8 METs, moderate physical activity is defined as 4 METs, and walking activity is defined as 3 METs. It is determined how many days and how many minutes a week the coaches exercise in these three groups, and the total MET value is calculated by summing the MET values for the three different physical activity levels (22,23).

Epworth Sleepiness Scale (ESS)

This is a questionnaire whose Turkish validity and reliability study was conducted by İzci et al. (24), and which consists of 8 questions to assess a person's daytime sleepiness. The scale is based on self-report, and includes questions about the individual's general daytime sleepiness. It assesses sleep status and tendency to doze off during certain activities of daily life (sitting and reading, watching television, sitting inactive in a public place, as a passenger in a car, lying down to rest in the afternoon, sitting and talking to someone, sitting quietly after lunch without alcohol, and in a car while stopped for a few minutes in traffic)

(24). The evaluation range of the questions in the scale is between 0-3 points, and the maximum score that can be obtained is 24 points. During the evaluation phase, the participants' responses are collected and if the result is between 2-10 points, the rate of sleepiness is normal, whereas if the result is higher than 10 points, this indicates a high rate of sleepiness (24,25).

Measurement of Body Weight, Height, and Waist Circumference

The participants' body weight and height were determined based on their own statements (self-reports). Prior to the study, the participating coaches were explained how to measure their waist circumference in a video prepared by the researcher. In this video, the points to consider when measuring waist circumference were explained. For waist circumference measurement, the use of an inelastic tape measure was recommended, and the measurement was requested to be taken in the standing position at the narrowest level between the lower rib border and the uppermost border of the iliac bone, at the end of a normal exhalation (26). According to the World Health Organization (27), waist circumference should be less than 80 cm for women and 94 cm for men. A waist circumference over 88 cm in women and over 102 cm in men is considered to be an increased cardiometabolic risk factor (26).

Statistical Analysis

Data analysis of the study was carried out using the SPSS 22.0 program. The coaches' demographic characteristics were analysed with descriptive statistics. The obtained results are presented as arithmetic mean ± standard deviation ($\bar{x}$ ± SD). The Kolmogorov-Smirnov test was applied to determine whether the data showed a normal distribution, and it was determined that the data did not show a normal distribution. Therefore, non-parametric analyses were performed in the study. The Mann-Whitney U test, one of the non-parametric tests, was carried out for pairwise group comparisons, while Spearman's rho correlation analysis was performed to determine the correlation between parameters. The significance level for this study was set at $p < 0.05$.

RESULTS

The coaches' demographic and anthropometric characteristics, the comparison of their waist

J Basic Clin Health Sci 2024; 8: 642-650

Kezer D. et al. Daytime Sleepiness Levels of Coaches

Table 1. Comparison of demographic and anthropometric characteristics according to gender

	Gender	X ± SD	Mean Rank	Sum of Ranks	U	Z	p
Age (years)	Female	34.37 ± 4.91	155.37	26878.50	11827.500	-2.032	0.042*
	Male	35.81 ± 6.11	176.67	27736.50			
Height (m)	Female	1.74 ± 0.46	128.25	22188.00	7137.000	-7.457	0.000***
	Male	1.82 ± 0.47	206.54	32427.00			
Weight (kg)	Female	64.37 ± 11.86	123.42	21351.00	6300.000	-8.359	0.000***
	Male	76.96 ± 12.88	211.12	32934.00			
Waist Circumference (cm)	Female	73.26 ± 11.66	132.20	22870.00	7819.000	-6.671	0.000***
	Male	81.58 ± 12.69	202.20	31745.00			
BMI (kg/m^2)	Female	21.79 ± 4.38	139.68	24165.50	9114.500	-5.160	0.000***
	Male	24.11 ± 4.25	193.95	30449.50			
Experience in Sports (years)	Female	7.53 ± 3.21	173.14	29953.50	12258.500	-1.535	0.125
	Male	7.01 ± 3.42	157.08	24661.50			
Coaching Experience (years)	Female	3.88 ± 2.86	161.62	27959.50	12908,500	-0.785	0.433
	Male	4.66 ± 4.19	169.78	26655.50			

BMI: Body Mass Index. *** p<0,01

Table 2. Comparison of waist circumference and body mass index levels according to gender

	Gender	X ± SD	Mean Rank	Sum of Ranks	U	Z	p
BMI (kg/m^2)	Female	21.79 ± 4.38	139.68	24165.50	9114.500	-5.160	0.000***
	Male	24.11 ± 4.25	193.95	30449.50			
Waist Circumference (cm)	Female	73.26 ± 11.66	132.20	22870.00	7819.000	-6.671	0.000***
	Male	81.58 ± 12.69	202.20	31745.00			

BMI: Body Mass Index

Table 3. Comparison of sleepiness levels according to gender

	Gender	X ± SD	Mean Rank	Sum of Ranks	U	Z	p
Sleepiness Level	Female	8.01 ± 5.48	176.26	30493.00	11719.000	-2.156	0.03*
	Male	6.83 ± 5.40	153.64	24122.00			

circumference and body mass index levels, and the comparison of their sleepiness levels according to gender are given in Tables 1-3, respectively. The mean physical activity levels according to gender, the correlation of waist circumference and body mass index with sleepiness levels, and the correlation between physical activity levels and sleepiness levels are explained in Tables 4-6, respectively.

The age, height, weight, waist circumference, BMI, length of experience in sports and coaching experience of the coaches participating in the study were analysed according to gender using the Mann-Whitney U test. According to the results, a significant difference was found between genders in terms of age, height, weight, BMI and waist circumference measurements ($p < 0.05$). When the source of the significant difference was examined, it was determined that the difference originated from male coaches, except for experience in sports.

The BMI and waist circumference measurements of the coaches participating in the study were analysed according to gender using the Mann-Whitney U test. According to the results, a significant difference was found between genders in terms of BMI and waist circumference measurements ($p < 0.05$). When the source of the significant difference was examined, it

J Basic Clin Health Sci 2024; 8: 642-650

Kezer D. et al. Daytime Sleepiness Levels of Coaches

Table 4. Mean physical activity levels according to gender

Gender	X ± SD MET-min / week	Mean Rank	Sum of Ranks	U	Z	p
Female	4614.96 ± 3666.414	151.28	26172.00	11121.000	-2.842	0.00***

Table 5. Correlation of sleepiness levels with waist circumference and body mass index

		Sleepiness Level
BMI (kg/m^2)	r	0.066
	p	0.230
Waist Circumference (cm)	r	-0.050
	p	0.363

BMI: Body Mass Index

Table 6. Correlation between physical activity levels and sleepiness levels.

		Sleepiness Level
Total Physical Activity (MET-min / week)	r	-.113*
	p	.039*

* $p < 0.05$

was determined that this originated from male coaches in both measurements.

The sleepiness levels of the coaches participating in the study were analysed according to gender using the Mann-Whitney U test. According to the results, a significant difference in sleepiness level was found between genders (p < 0.05). When the source of the significant difference was examined, it was determined that this originated from female coaches. The physical activity levels of the coaches participating in the study were analysed according to gender using the Mann-Whitney U test. According to the results, a significant difference was found between genders in total physical activity MET levels (p < 0.05). When the source of the significant difference was examined, it was determined that that this originated from male coaches.

No significant relationship was found between the coaches' sleepiness levels and their BMI (r = .066) or waist circumference (r = -.050) (p > 0.05).

A significant negative relationship was found between the coaches' physical activity levels and their daytime sleepiness levels (r = -.113) (p < 0.05).

DISCUSSION

This study aimed to examine coaches' daytime sleepiness levels in terms of gender, physical activity level and some anthropometric variables. The main findings of the study are: a) there is a significant

difference between male coaches and female coaches in favour of male coaches in terms of physical activity level (p < 0.05); b) though not at pathological levels, daytime sleepiness levels of female coaches are higher than those of male coaches (p < 0.05); c) there is a significant negative relationship (p < 0.05) between the coaches' physical activity levels and their daytime sleepiness levels (r = -.113); d) no significant relationship was found between the coaches' sleepiness levels and their BMI (r = .066) and waist circumference (r = -.050) (p > 0.05). Based on the main findings of the study, it can be understood that study hypotheses 2 [as physical activity level increases, daytime sleepiness level decreases (Table 6)] and 3 [daytime sleepiness levels of female coaches are higher than those of male coaches (Table 3)] can be confirmed, while study hypothesis 1 [as waist circumference increases, daytime sleepiness levels also increase (Table 5)] cannot be confirmed.

It is reported that regular physical activity improves sleep quality, reduces sleep onset latency, increases total sleep quality, and is effective in controlling sleep disorders such as insomnia (29).

The American College of Sports Medicine (ACSM) recommends moderate-intensity activity for at least 30 minutes five days a week (150 minutes a week) or high-intensity activity for at least 20 minutes three

J Basic Clin Health Sci 2024; 8: 642-650

Kezer D. et al. Daytime Sleepiness Levels of Coaches

days a week (60 minutes a week) for every adult individual (29).

Cuppett and Latin (30) evaluated the physical activity levels of 1200 certified athletic trainers using the Baecke Questionnaire of Habitual Physical Activity and found that 16% of the athletic trainers did not meet the physical activity level recommended by the ACSM. Additionally, in the study by Cuppett and Latin (30), it was determined that female trainers had higher physical activity scores than male trainers. In the same study, the mean ages of female trainers and male trainers were found to be 31.1 ± 6.8 years and 33.9 ± 8.6 years, respectively. Another result obtained in their study was that trainers aged 36 and over were less active than younger trainers (230). Although the male and female coaches in our study were 34-35 years old on average, age-related physical activity was not assessed.

In another study, in which 255 certified athletic trainers were evaluated in terms of general health and fitness habits, it was determined that 7% of the trainers were sedentary, while 42% met the physical activity level recommended by ACSM (31).

In our study, the physical activity levels of the coaches were evaluated with the International Physical Activity Questionnaire Short Form (IPAQ-SF) and both male and female coaches were assessed as physically active (≥3.000 MET-min/week) according to the IPAQ-SF assessment guideline (32). In our study, unlike the study by Cuppett and Latin (30), it was determined that the physical activity levels of male coaches were higher than those of female coaches.

It is known that increases in body fat mass lead to overweight or obesity and negatively affect sleep quality and duration (33). In a Wisconsin Sleep Cohort Study conducted with seven hundred participants, it was determined that a 10% increase in body weight increased the apnea-hypopnea index (AHI) score by 32%, whereas a 10% loss in body weight decreased the AHI score by 26% (34).

Although the body mass index (BMI) is not a perfect method for determining overweight or obesity, it is widely preferred by healthcare professionals because it is a practical method (35). However, in recent years, some researchers have stated that waist circumference (WC) is more effective than BMI in determining central obesity and health risks (26, 36-38). Davidson and Patel (39) stated that WC is more effective than neck circumference or BMI in detecting sleep disordered breathing (SDB). In our study,

although both the BMI values and waist circumferences of male coaches were higher than those of female coaches, both groups had normal BMI and waist circumference values (Table 1) (27).

The inability to detect a significant relationship between sleepiness levels and waist circumference or body mass index in our study may be due to the young age of the coaches or the fact that their BMI and waist circumference values were within normal limits. In the study by Groth et al. (31), the mean BMI values of male and female trainers were determined as 25.78 (kg/m2) and 27.97 (kg/m2), respectively (30). Groth et al. (31) found that 53% (n = 72) of female trainers had a BMI in the healthy range, 25% (n = 34) were overweight, and 22% (n = 31) were obese. Of the male trainers, 26% (n = 31) had a healthy BMI, 50% (n = 60) were overweight, and 24% (n = 29) were obese.

In their study, Torres-McGehee et al. (15) examined the energy availability, mental health and sleep patterns of 47 certified athletic trainers from the southeastern US region (males = 23, age = 29.8 ± 8.5 years; females = 24, age = 28.9 ± 7.9 years). It was reported that 39 trainers participated in physical activity while 8 did not participate, and that 42 out of 47 coaches complained of sleep disorders. In their study, while the trainers' sleep quality was assessed with the Pittsburgh Sleep Quality Index, the trainers were only asked whether they participated in physical activity and it was not evaluated whether the level of physical activity was sufficient (15).

In a study conducted with the participation of 2.020 athletic trainers, Winkelmann et al. (18) stated that in the last week, 228 (18.6%) of the trainers had not done any physical activity, 455 (37%) participants had exercised less than 3 times, and 546 (44.4%) had exercised 3 or more times.

The American Academy of Sleep Medicine (AASM) and Sleep Research Society (SRS) state that adults should sleep at least 7 hours regularly every night (40). Although Winkelmann et al. (18) stated in their study that the trainers slept 5-8 hours every night, the fact that the data were collected based on a web-based survey rather than a standard data collection tool makes it difficult to interpret the results. In the same study, it was stated that 29 trainers slept 4 hours or less per night (2.4%), 1,117 coaches slept 5-8 hours (90.9%), and 83 coaches (6.8%) slept more than 8 hours.

In a study conducted with the participation of three basketball coaches and 12 female basketball players,

J Basic Clin Health Sci 2024; 8: 642-650

Kezer D. et al. Daytime Sleepiness Levels of Coaches

both the Epworth Sleepiness Scale (ESS) scores and the Insomnia Severity Index of the coaches were found to be 4±2. These scores show that coaches aged 42 ± 15 years and with a mean BMI of 29 ± 7 (kg/m2) did not have a risk of sleep apnea or daytime sleepiness (16).

Another finding of our study is that, although not at pathological levels (Epworth Score > 10), daytime sleepiness levels of female coaches were higher than those of male coaches (8.01 ± 5.48 vs. 6.83 ± 5.40). Since no study could be found that evaluated coaches' daytime sleepiness levels in terms of gender, this finding of our study was compared with results obtained from the normal population. In this context, Sanford et al. (41), in a study conducted with the participation of 703 people between the ages of 20 and 98 from the American community, stated that the Epworth Sleepiness Scale (ESS) score did not differ in terms of gender or age. On the other hand, Miura and Honma (42) stated that premenstrual female Japanese students had higher ESS scores than their male peers (12.1 ± 4.8 vs. 9.4 ± 4.5). Similar to the results of the study by Miura and Honma (42), Putilov et al. (43) stated that excessive daytime sleepiness levels of female Russian university students were higher than those of their male peers.

According to another finding of our study (Table 6), as the coaches' physical activity levels increased (IPAQ Score increased), their daytime sleepiness levels decreased (ESS decreased). McClain et al. (13) stated that physical activity and daytime sleepiness levels depended on gender (men had lower daytime sleepiness levels than women) and age, and that as the level of physical activity increased in young (20-39) and elderly (≥60) individuals, the level of daytime sleepiness decreased, but this was not the case for middle-aged (40-59) individuals.

In a study conducted with the participation of 876 Brazilian female adolescents (16.4 ± 1.2 years), it was stated that the level of physical activity was inversely related to the Pediatric Daytime Sleepiness Scale (PDSS) score (44). Mahfouz et al. (45) also reported that mean Pittsburgh Sleep Quality Index (PSQI) scores were higher for physically inactive students than for moderately or highly active students.

In practice, the daytime sleepiness level, physical fitness level, and body composition of trainers should be monitored by health authorities or the relevant government department to prevent chronic diseases and increase job productivity during their service.

To our knowledge, this is the first study that attempts to explain the relationship between coaches' daytime sleepiness levels and their physical activity levels, body mass indices and waist circumferences. Since no other study could be found in the literature that attempted to explain the association between coaches' daytime sleepiness levels and their physical activity levels, body mass indices, and waist circumferences, we aimed to compare the results obtained from our study with those from the literature as much as possible

Limitations

The data obtained in this study explain the relationship between daytime sleepiness levels and gender, physical activity levels and waist circumference of coaches working in only one district of Istanbul. In order to generalize the results, coaches from different districts of Istanbul or different provinces of Turkey should be invited to participate in the study, and the study should be repeated with the participation of a larger number of coaches. One of the most important limitations of the study is that the coaches' height, weight and waist circumference were determined based on the coaches' own self-reports, without being measured by the researchers. This also applies to the scales used to determine physical activity level and daytime sleepiness. Completing the scales in question face-to-face instead of online (Google Forms) may produce different results.

CONCLUSION

The aim of this study was to examine coaches' daytime sleepiness levels in terms of gender, physical activity level and some anthropometric variables. İn line with the aim of the study, the coaches' daytime sleepiness levels were compared in terms of physical activity level, gender and waist circumference. Though not at a pathological level, female coaches had higher levels of daytime sleepiness. Male coaches' physical activity levels were higher than those of female coaches. There was no significant relationship of daytime sleepiness level with BMI or waist circumference. However, there was a negative significant relationship between physical activity level and daytime sleepiness level. In order to better understand the relationship between daytime sleepiness level and physical characteristics, gender and physical activity level, the study should be

conducted with a larger sample and with the participation of coaches from different age groups.

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IMPACT OF YOGA INTERVENTION ON PHYSICAL FITNESS PARAMETERS IN PREADOLESCENT CHILDREN: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Purpose: This study aims to assess the impact of yoga on physical fitness among healthy primary school children using a randomized control design.
Material and Methods: A total of 52 children, aged 8-13, were randomly assigned to either the Yoga Group (n = 27, mean age = 10.48 ± 1.25 years) or the Control Group (n = 25, mean age = 11.20 ± 1.50 years). The participants underwent evaluation using the EUROFIT Test Battery as the primary outcome measure The Yoga Group engaged in 45-minute yoga sessions twice a week for a duration of 10 weeks, while the Control Group received no intervention. Outcome assessments were repeated at the conclusion of the 10-week intervention period.
Results: Significantly positive enhancements were observed in the Yoga Group across various components of the EUROFIT Test Battery, including balance, speed, flexibility, muscle endurance, and cardiovascular endurance.
Conclusion: These findings demonstrate that yoga interventions contribute to improvements in physical fitness among preadolescent children. Consequently, incorporating yoga exercises into the school curriculum is recommended, given their efficacy, minimal resource requirements, and positive impact on sports readiness.

Keywords: School aged children, muscle stretching exercises, physical endurance

INTRODUCTION

The escalating prevalence of sedentary lifestyles during childhood is acknowledged to be associated with a myriad of health issues. Recent reports from prominent organizations, such as the World Health Organization, underscore a rapid surge in complications linked to physical inactivity, notably childhood overweight and obesity (1). This burgeoning concern possesses substantial potential to detrimentally impact overall health, cognitive proficiency, psychological well-being, and social behavior in children (2). Establishing an active behavioral lifestyle and cultivating exercise engagement during childhood emerges as pivotal in averting the aforementioned problems.

Childhood represents a pivotal phase for the acquisition of motor skills. Following the development of gross motor skills, an active lifestyle progressively

J Basic Clin Health Sci 2024; 8: 651-659

Bagkur M et al. The Effect of Yoga on the Fitness of Children

Figure 1. Study Design

evolves and is enhanced through the implementation of sports-specific fundamental motor skill patterns and systematic repetitions. Especially during childhood, a critical developmental period for neuromotor mechanisms, exercises incorporating coordination, strength, balance, agility, and speed transform this phase into a window of opportunity alongside physiological development (3,4). The rate of learning and development during this period diminishes with age, underscoring its significance as the golden age (5). The pre-adolescent phase is recognized as crucial for joint range of motion and flexibility development, ensuring movement patterns are executed with precision at wide angles and mitigating future injury risks (6).

Yoga is conceived as a holistic practice encompassing physical postures ('asanas'), breathing exercises ('pranayama'), and relaxation techniques. These components, constituting physical activity, breathwork, and mindfulness meditation, contribute to the comprehensive nature of yoga (7). Asanas involve a diverse array of body poses, where individuals maintain a position for a designated duration or transition smoothly between postures, now recognized as a crucial intervention for enhancing physical health. Consistent yoga practice has demonstrated effectiveness in augmenting muscle strength, endurance, and cardiopulmonary fitness in children, while also mitigating stress, anxiety, and enhancing overall well-being (8,9).

Research on children underscores the mental and emotional health benefits of yoga, attributable to its meditative aspects and breathing exercises. A review by Khunti et al. emphasizes the positive impact of school-based yoga interventions on children's mental health (10). While numerous articles explore therapeutic applications of yoga, studies on its effects on the sports readiness and physical fitness of healthy primary school children remain limited.

Consequently, our study aims to investigate the influence of yoga practice on the physical fitness of primary school children.

MATERIAL AND METHODS
Study Design
This investigation is a randomized controlled trial designed to assess the physical fitness levels among primary school-age children subjected to diverse yoga interventions, comparing them with the outcomes observed in a control group devoid of such interventions. The study encompasses a 10-week intervention phase, during which participants undergo evaluation at two specific time points: Initial assessment (M1), conducted one week preceding the commencement of the exercise program, and subsequent evaluation (M2), executed one week post the conclusion of the program. Notably, assessments for both the control and yoga groups are conducted during the same week and at identical time slots to mitigate potential performance variances (Figure 1). Subjects were instructed to exhibit their utmost performance during the performance assessments. The research protocol received approval from Scientific Research Ethics Committee of Near East University (Date: 21.11.2019, Decision No: 2019/74-938) and participants, as well as their familial representatives, provided informed consent.

Participants
The investigation was conducted between March and June 2020 within the "Wellbeing Center," where an
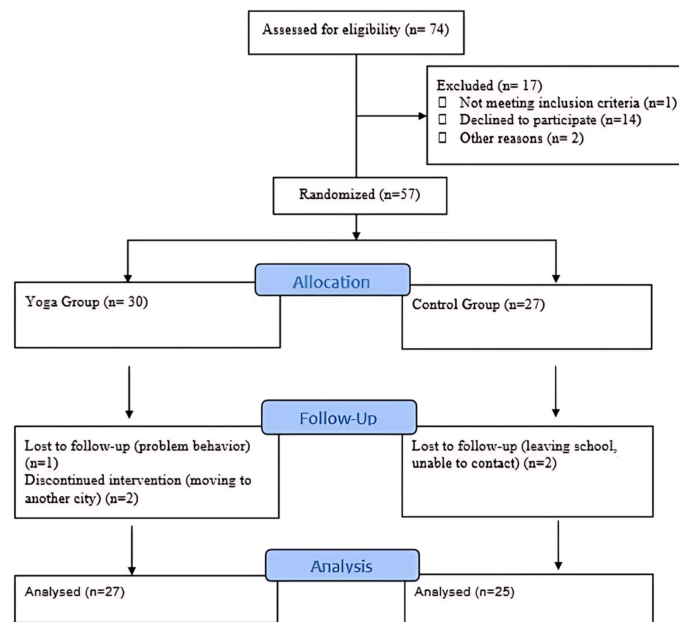
Figure 2. Study Flow Diagram

after-school support initiative is provided to students residing in Northern Cyprus. All families and children (n = 74) seeking services at the center were apprised of the study. Subsequently, the requisite sample size for achieving 95% power (1-β=0.95) at α=0.05 significance level was determined as 38 individuals in total. This encompassed 19 participants in both the experimental and control groups, calculated utilizing G*Power 3.1.9.2 software (11).Participants without any mental or physical impairment, and with no engagement in any exercise regimen over the preceding six months, were eligible for inclusion in the investigation. Exclusion criteria encompassed individuals who failed to attend 90 percent of the 10-week yoga training program. The study involved 57 children (mean age 10.82 ± 1.41 years, 21 males), who willingly agreed to partake and were allocated into two groups through computer-generated random numbers. An impartial research coordinator formulated the randomization sequence, with sequentially numbered cards enclosed in opaque envelopes dispatched to the yoga instructor.

Subsequently, three participants from the yoga group and two from the control group were excluded, culminating in the study's completion with 27 participants in the yoga group and 25 participants in the control group (Figure 2).

Throughout the research duration, participants received guidance to abstain from participating in any organized physical activities, excluding mandatory physical education as stipulated in their school curriculum.

Yoga Exercise Protocol

The yoga intervention was administered biweekly, with each session lasting 45 minutes, over a span of 10 weeks. The sessions were consistently led by the same certified yoga instructor. Each session encompassed a variety of distinct yoga postures, in addition to incorporating respiratory techniques and profound relaxation methods.

Commencing with a 5-minute warm-up, participants engaged in stretching exercises, including jogging, jumping, lateral body bending, and hip twisting. Subsequently, a 10-minute practice of 'suryanamaskar' was undertaken, comprising twelve sequential steps, encompassing three backbends and two forward bends. Notably, each step was executed with a synchronized respiratory pattern. Inhalation accompanied chest expansion during backward bending steps, while exhalation involved chest contraction during forward bending steps (Figure 3) (12).

Subsequently, a 20-minute session of 'asanas' was conducted, encompassing poses designed to enhance flexibility, balance, and strength. Fundamental yoga postures, spanning various difficulty levels, were carefully chosen for prone,

Figure 3. Surya Namaskar Circle

supine, sitting, and standing positions. The objective was to augment muscle length while concurrently fostering strength and endurance, as delineated in Table 1.

Following the 'asanas,' a 5-minute regimen of pranayama exercises ensued. Pranayama, a yogic respiratory technique, incorporated processes such as rapid diaphragmatic breathing, slow/deep breathing, and alternate nostril breathing. The comprehensive nature of pranayama encompassed four vital breathing components: inhalation, exhalation, internal breath retention, and external breath retention.

A 5-minute deep relaxation technique was employed to induce complete muscular relaxation, alleviating fatigue accumulated during the session. Participants reclined on their backs on the mat, maintaining normal breathing while voluntarily contracting and relaxing extremities as per the instructor's guidance.

Progressively, select poses were replaced with more demanding alternatives, with participants instructed to sustain them for extended durations. Despite the evolving challenges, a consistent algorithm was adhered to in each session. This protocol was devised based on the evidence gleaned from systematic reviews within the pertinent literature (8,9).

Assessment Tools
EUROFIT Test Battery
Formulated by the Council of the European Committee, the EUROFIT physical fitness examination serves as a widely employed test battery for assessing the fitness profiles of school-age children (13). The assessment comprises a range of health-related and skill-related fitness tests. Within the context of our investigation, the Flamingo left-leg balance (FLL) and Flamingo right-leg balance (FLR) tests were employed to evaluate balance, while the left-hand plate tapping (PTL) and right-hand plate tapping (PTR) tests gauged speed. Additionally, the Sit and Reach (SAR) test assessed flexibility, the standing broad jump (SBJ) test measured explosive muscle strength, and the left-hand grip strength (LHS) and right-hand grip strength (RHS) tests were utilized for static muscle strength evaluation. Muscular endurance was assessed through the Sit-ups (SUP) test and the Bend Arm Hang (BAH) test, the latter also serving as an indicator of muscular endurance and functional strength. The 10 x 5 meter shuttle run test was administered to evaluate running speed and agility, while the 20m shuttle run test (SHR) was conducted to assess cardiovascular endurance.

Anthropometric measurements involved determining participants' height using a non-elastic measuring tape (SECA brand), affixed to a wall. The Tanita MC-180MA III device facilitated weight analysis, and Body Mass Index (BMI) was calculated by dividing body weight (in kilograms) by the square of height (in meters).

Statistical Analysis
The statistical analysis employed the IBM SPSS Statistics 22 software. Given the non-normal

J Basic Clin Health Sci 2024; 8: 651-659

Bagkur M et al. The Effect of Yoga on the Fitness of Children

Table 1. Asanas (postures)

Asanas (postures)	
Standing postures	Padahastasana (Foot palm posture)
	Trikonasana (Triangle posture)
	Vrikshasana (Tree posture)
	Garudasana (Eagle posture)
	Veerbhadrasana (Warrior posture)
	Tadasana (Palm tree posture)
Sitting postures	Butterfly Pose (for warm up)
	Padmasana (Lotus posture)
	Sasankasana (Rabbit posture)
	Paschimottanasana (Back stretching posture)
	ArdhaMatsyendrasana (Half spinal twist)
	Gomukhasana (Cow face posture)
Prone postures	Bhujangasana (Cobra posture)
	Salabhasana (Grasshopper posture) Bhujangasana (Cobra posture)
	Dhanurasana (Bow posture)Salabhasana (Grasshopper posture)
Supine postures	Uttanapadasana (Raised legs posture),Dhanurasana (Bow posture)
Supine postures	Naukasana (Boat posture) Uttanapadasana (Raised legs posture),
	Sarvangasana (Shoulder stand posture) Naukasana (Boat posture) Uttanapadasana (Raised legs posture),
	Sarvangasana (Shoulder stand posture) Naukasana (Boat posture)
	Sarvangasana (Shoulder stand posture)

Table 2. Demographic and physical characteristics of the groups (yoga and control)

Variable	Yoga(n=27)	Control (n=25)	P
Gender (n, %)			
Female	17 (63 %)	14 (56 %)	0.613
Male	10 (37 %)	11(44 %)	
Age (years) (mean ± SD)	10.48 ±1.25	11.20±1.50	0.680
BMI (kg/m^2)	18.24±2.65	18.03±2.51	0.735

Values are presented as mean ± standard error or percentage. BMI: Body mass index, SD: Standard deviation, n: number of individuals, *p<0.05 statistical significance. kg: kilogram, m: meter

distribution of the data, nonparametric tests were employed for data analysis. The Wilcoxon signed rank test was utilized to compare the first and second measurement (M1 and M2) datasets within each group. The Mann-Whitney U Test was employed to assess differences between the two groups. A significance level of p < 0.05 was adopted.

RESULTS

Table 2 displays the gender, age, and BMI characteristics of the participants. No statistically significant differences were observed between the groups concerning demographic and physical attributes, indicating homogeneity in both intergroup and intragroup distributions.

Upon comparing the M1 and M2 values within the yoga group, noteworthy enhancements were evident in the FLL and FLR balance tests, PTL and PTR speed tests, SBJ, LHS muscle strength tests, SUP endurance test, SAR, and SHR tests.

Contrastingly, analysis of M1 and M2 data within the control group revealed a reduction in falls during the FLL test, an increase in sit-up performance in the SUP test, and elevated scores in the LHS and SAR tests. No statistically significant distinctions were found between the two groups in EUROFIT test results at baseline (M1). However, when comparing M2 results, the yoga group exhibited significant improvements in the FLR balance test, PTL and PTR speed tests, SUP endurance test, BAH functional strength test, 10 x 5 running speed and agility test,SAR flexibility test, and SHR cardiopulmonary endurance test.

In terms of muscle strength, no significant differences were observed in grip strength scores between the two groups. Nevertheless, a noteworthy improvement in SBJ scores was evident in the yoga group (Table 3).

DISCUSSION

This study sought to assess the impact of yoga training on the physical fitness of children. According to the findings, a 10-week regimen of yoga practice in children yielded significant positive effects on various components of physical fitness, including balance, cardiovascular and muscular endurance, coordination, and flexibility.

In our investigation, noteworthy enhancements were observed in the balance of the group undergoing yoga training. This is consistent with the findings of Berger and Stein, who, in their study involving 9- to 11-year-old children, reported an improvement in the single-leg stance test after a 12-week yoga intervention (14). Similarly, Donahoe-Fillmore and Grant noted significant improvements in balance test results among 26 healthy children aged 10-12 years following a yoga practice program (15). The heightened trainability of children, attributed to their lower performance levels, may contribute to their

increased adaptive capacity (16). The observed improvements in balance may be linked to the short-term enhancement of postural control and improved intramuscular and intermuscular coordination facilitated by the yoga exercises. These improvements may be attributed to the specific yoga poses incorporated into the training program, as most of these poses target static balance and core stability. An enhancement in the plate-tapping test, assessing extremity movement speed, was observed within the yoga group. Telles et al. reported statistically significant improvements in the plate-hitting test of the EUROFIT battery attributed to yoga in their study (17). Mohanty et al., in a study involving 83 visually

impaired children aged 9-16, noted positive advancements in the plate tapping test following yoga practice (18).

The augmentation in children's flexibility performance post-yoga aligns with prior research findings (15,19). In a study by Folleto et al. involving children aged 6-8, flexibility improved after 12 weeks of biweekly yoga practice compared to pre-intervention levels (20). Our study corroborates this trend, demonstrating increased flexibility values, with the poses Padahastasana and Paschimottanasana, emphasizing hamstring and spinal extensor muscle flexibility, contributing to observed improvements. Moreover, our study indicates that yoga can enhance

Table 3. Comparison of EUROFIT physical fitness measures of Yoga group and Control group

Variables	YOGA			CONTROL			
	Median	Mean	SD	Median	Mean	SD	P-1
FLL(M1)	12.00	10.77	5,17	9.00	8.48	4.22	0.108
FLL (M2)	7.00	6.92	5.21	8.00	7.40	4.20	0.451
	p-2	0.000		**p-2**	0.027		
FLR (M1)	10.00	10.59	5.29	8.00	8.40	3.66	0.079
FLR (M2)	6.00	6.37	4.99	8.00	9.16	4.32	0.023
	p-2	0.000		**p-2**	0.107		
PTL (M1)	19.00	19.18	3.61	18.00	18.28	3.98	0.514
PTL (M2)	10.00	11.81	4.68	17.00	17.16	3.90	0.000
	p-2	0.000		**p-2**	0.090		
PTR (M1)	20.00	18.55	4.29	17.00	17.72	3.52	0.491
PTR (M2)	10.00	10.88	3.43	17.00	16.96	3.76	0.000
	p-2	0.000		**p-2**	0.365		
SBJ (M1)	130.00	130.14	14.15	134.00	131.76	15.65	0.728
SBJ (M2)	146.00	140.92	15.39	134.00	131.36	17.62	0.037
	p-2	0.000		**p-2**	0.756		
LHS (M1)	12.50	13.05	3.77	15.00	14.64	3.03	0.071
LHS (M2)	13.60	14.13	3.34	16.00	20.53	3.82	0.193
	p-2	0.008		**p-2**	0.004		
RHS (M1)	13.50	14.35	3.51	16.30	15.63	3.26	0.128
RHS (M2)	14.30	14.67	3.55	16.00	15.49	3.04	0.420
	p-2	0.247		**p-2**	0.497		
SUP (M1)	15.00	14.00	3.78	13.00	12.84	4.52	0.079
SUP (M2)	18.00	17.70	4.05	13.00	16.64	4.29	0.000
	p-2	0.000		**p-2**	0.006		
BAH (M1)	1.80	2.17	2.72	1.57	2.40	2.62	0.805
BAH (M2)	10.00	10.18	3.59	6.00	6.96	3.55	0.013
	p-2	0.000		**p-2**	0.232		
10x5(M1)	36.31	37.58	4.16	38.48	38.12	6.35	0.552
10x5(M2)	24.27	25.84	6.63	36.69	36.23	7.94	0.000
	p-2	0.000		**p-2**	0.048		
SAR (M1)	7.00	8.40	3.86	9.00	9.12	2.81	0.203
SAR (M2)	13.00	13.18	3.59	9.00	9.96	3.55	0.000
	p-2	0.000		**p-2**	0.007		
SHR (M1)	18.90	18.86	0.90	19,40	19.58	2.06	0.084
SHR (M2)	20.40	20.15	1,30	18.90	19.63	2.12	0.011
	p-2	0.000		**p-2**	.0.237		

Flamingo left-leg balance (FLL), Flamingo right-leg balance (FLR), left-hand plate tapping test (PTL), right-hand plate tapping test (PTR), standing broad jump (SBJ), left-hand grip strength (LHS), right-hand grip strength (RHS), sit-ups (SUP), bend arm hang (BAH), 10 x 5 meter Shuttle Run (10X 5), sit and reach (SAR), and shuttle run (SHR); M1: first measurement; M2: second measurement. P1: Mann-Whitney test for comparison of two independent groups (yoga and control);P2: Wilcoxon test for comparison of two moments*p < 0.05, **p < 0.01 and ***p < 0.001

flexibility even with lower doses and in a shorter duration compared to exercise interventions outlined in the literature.

Concerning explosive muscle strength, a notable improvement in SBJ scores was evident in the yoga group versus the control group. The physiological mechanism underlying the enhanced SBJ performance may be linked to an improved stretch-shortening cycle rate, enhanced intramuscular and intermuscular coordination, and increased tendon elastic strength due to reduced muscle compression in children engaged in regular yoga exercises. Furthermore, asanas that mainly address core stabilization are also effective in improving SBJ scores (21).These results might be a result of an increase in lower extremity muscle strength through Vrikshasana, Trikonasana and Garudasana poses, in which body weight is transferred on legs.

Moreover, there was no statistically significant disparity observed between the two cohorts concerning handgrip strength as evaluated using a hand dynamometer. This lack of divergence could potentially be attributed to the absence of targeted movements specifically addressing the intrinsic muscles of the hand. Furthermore, to elicit an enhancement in handgrip strength, it becomes imperative to progressively augment both the resistance and repetition count within the training regimen. Comparable findings have been documented in extant literature, where studies have reported a non-significant augmentation in handgrip strength (17,22).

The advancement of Sit-Ups in 30 seconds within the yoga group aligns with existing literature (14). A plausible explanation for this progress lies in the efficacy of yoga poses such as UttanaPadasana, Naukasana, and Surya Namaskar, which are known to effectively fortify the abdominal musculature. Consistent with this outcome, Verma et al. investigated the impact of a 12-week yoga program on children, noting a substantial increase in abdominal muscle strength (23).

Significant enhancements were observed in the BAH test within the yoga group compared to the control group, a metric assessing upper body relative strength and endurance. In a study by Purohit et al., focusing on yoga's impact on physical fitness in adolescents, 72 healthy participants engaged in yoga sessions four times a week for three months, resulting in noteworthy improvements in the BAH test favoring the yoga group (18). Yoga postures, such as Bhujangasana and Surya Namaskar steps, involving hand-to-ground contact and utilization of upper body muscles for weight-bearing, likely contribute to improved BAH test performance by strengthening proximal arm muscles and enhancing stabilization.

The engagement in yoga practices exhibited a favorable impact on the outcomes of the 10 x 5-meter shuttle run test, assessing agility, speed, and directional change capabilities within the yoga group. While the control group demonstrated positive enhancements upon re-evaluation, the observed effects were more pronounced in favor of the yoga group. In contrast to Bal and Kaur's findings of positive effects on agility in male students practicing yoga, Phung et al.'s study reported no discernible impact on speed and agility (24,25). The observed enhancements in the 10 x 5-meter shuttle run test in our investigation are postulated to be linked to concurrent improvements in various parameters of physical fitness.

The authors posit that the incorporation of breathing exercises and Surya Namaskara contributed to an augmentation in aerobic capacity and cardiovascular endurance within the yoga group (26). The overall enhancement in whole-body endurance may be attributed to the 12-stage Surya Namaskara, encompassing static stretching and a deliberate dynamic component, placing optimal stress on the cardiorespiratory system, as documented by Bhutkar et al (27). Aligning with our study, D'souza and Avadhany implemented a three-month yoga regimen for healthy 7 to 9-year-old children in India, reporting notable improvements in aerobic capacity assessed through the 20m shuttle run test (20). Likewise, Satish et al. documented a significant elevation in VO2 max values, evaluated using the 20m shuttle run test, among children following two months of yoga training (28).

During preadolescence, numerous physiological systems relevant to sports undergo rapid changes due to growth and development. Consistent exercise routines during this phase facilitate the enhancement of motor skills in children. The 10-week, twice-weekly application of yoga exercises in our study positively impacted all biomotor abilities, akin to interventions addressing speed and modalities in various sports disciplines.

This study represents important results regarding the effects of yoga on physical fitness parameters during preadolescence. In the current literature, there are few studies examining the effect of yoga on all

physical fitness parameters. These are the strengths of the presented study. Interim evaluation during the study and long-term follow-up of the participants' effects of yoga after the study can increase the level of evidence. This limitation of the current study may guide future research.

In conclusion the study outcomes demonstrated that regular engagement in yoga training had a significant positive impact on balance, speed, flexibility, strength, and endurance among children. Yoga emerges as an easily applicable exercise modality that enhances children's preparedness for sports and ensures equitable access to sports activities that do not necessitate specialized equipment. Particularly in the preadolescent phase, it is believed that implementing yoga interventions can foster the development of specific motor skills foundational to various sports disciplines. Given that preadolescence represents a critical period for the maturation of capacities such as joint range of motion, flexibility, as well as neuromuscular-based strength and speed, yoga emerges as a supportive approach for the simultaneous and integrated enhancement of these capabilities. Consequently, we advocate for the incorporation of yoga into fieldwork initiatives alongside conventional sports activities or its integration into physical education classes by educators. This integration serves as an enjoyable and constructive physical activity, facilitating children in gaining a deeper understanding of their bodies and cultivating self-awareness.

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DO SODIUM GLUCOSE CO-TRANSPORTER-2 (SGLT-2) INHIBITORS AFFECT LOWER URINARY TRACT, SLEEP AND QUALITY OF LIFE IN PEOPLE WITH TYPE 2 DIABETES?

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ABSTRACT

Purpose: Sodium-glucose cotransporter-2 (SGLT-2) inhibitors are primarily preferred in patients with type 2 diabetes. The purpose of this paper was to elucidate the effects of SGLT-2 inhibitor use on patients' voiding habits, sleep, and quality of life.
Methods: Our study involved patients with type 2 diabetes who had an SGLT2 inhibitor added to their current treatment. The frequency of day-night urination, lower urinary tract symptoms, sleep and quality of life were assessed both prior to initiation of the treatment and during subsequent 1st and 3rd months.
Results: The study included 38 women and 34 men. At the third month after SGLT-2 inhibitor had been added, there was a significant decrease in HbA1c, triglyceride and microalbumin levels ($p<0.05$). There was no increase in voiding frequency (day/night) and there was no difference in the evaluation of lower urinary tract symptoms, but voiding volumes increased in uroflowmetry. When the short form-36 (SF-36) scale was examined, there was a significant improvement in physical function, one of the sub-parameters ($p = 0.01$). The factor affecting this most, was the improvement in HbA1c.
Conclusion: This paper suggests that SGLT-2 inhibitors don't increase voiding frequency and don't cause an increase in lower urinary tract symptoms.

Key Words: Sodium-glucose cotrasporter-2 inhibitor, lower urinary tract symptoms, nocturia, quality of life, type 2 diabetes.

INTRODUCTION

It is well established that the prevalence of type 2 diabetes is swiftly rising in both developed and developing nations, largely attributed to rapidly evolving lifestyles. Type 2 diabetes mellitus is widely recognised as the most common form of diabetes, accounting for over 90% of cases. (1). Patients with type 2 diabetes typically do not need insulin in the first

years after diagnosis and a considerable proportion may not require it during their lifetime. Many patients can be protected from the effects of hyperglycemia by diet, adequate physical activity, and oral antidiabetics (OAD). Sodium-glucose co-transporter 2 inhibitors (SGLT 2I), one of the oral antidiabetics, targets SGLT-2. SGLT-2 helps reabsorb 90% of filtered glucose in the proximal tubule of the kidney. SGLT 2 inhibitors contribute to the renal excretion of glucose and the reduction of elevated blood glucose in type 2 diabetic patients. Its main advantages are weight loss, low risk of hypoglycemia, and mild lowering effect on blood pressure (2). Studies showed that it slows the progression of kidney damage in diabetics with chronic kidney disease. Using SGLT2 inhibitors in patients with heart failure or those at high risk of heart failure can decrease the likelihood of hospitalization due to heart failure (3,4).

With the use of SGLT 2 inhibitors, an increase in urinary tract and genital infections due to glucosuria and dehydration due to diuresis can be observed (2). An increase in the frequency and amount of urine and an increase in nighttime urinary frequency (nocturia) may be expected in patients using the drug due to its diuretic activity. When the literature was examined, few studies investigated urinary complaints and evaluated the quality of life and sleep related to these complaints (5,6). Complaints such as urinary incontinence, frequent urination and nocturia are expected in diabetic patients. It remains unclear how taking a SGLT-2 inhibitor affects these patients' symptoms, sleep, and quality of life.

The objective of this study was to examine the impact of SGLT-2 inhibitor therapy on the day-night voiding habits of patients and to determine whether these changes have any effect on their quality of life and sleep.

MATERIALS AND METHODS
Study protocol
Ethical approval for the study was obtained from the Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date: 22.02.2023, Decision No: 2023/025-20). Among the type 2 diabetic patients who applied to the endocrinology outpatient clinic for routine check-ups and examination in 2021-2022, male and female patients over the age of 18 who had an SGLT-2 inhibitor added to their treatment were included in the study. Patient files were scanned retrospectively, demographic data, laboratory data, weight changes, day and night urination frequency,

urinary incontinence and the effects of these conditions on sleep and quality of life were investigated before treatment and in the 1st and 3rd months of treatment. Patients who reported any urinary symptoms were assessed with uroflowmetry and re-evaluated in the first and third month of treatment at the urology outpatient clinic. Type 1 DM, presence of active urinary tract infection (proven by urine culture), presence of genital infection, history of major surgery on the urinary system, active disease affecting the urinary system, presence of active cancer, patients with any diagnosed sleep problems, who used SGLT before and cases in which the inhibitor was stopped for any reason were not included in the study.

Laboratory Investigations
As part of routine check-ups, serum creatinine, GFR, hemoglobin A1c (HbA1c), triglyceride, LDL cholesterol, and urinary microalbumin values were recorded. Among the evaluated parameters, creatinine, LDL cholesterol, triglyceride, spot urine microalbumin, and creatinine were examined using the spectrophotometric method with a Beckman Coulter AU5800 biochemistry analyzer. GFR was calculated with the CKD-EPI GFR formula. HbA1c was studied using the HPLC method with a Tosoh G8 HPLC analyzer.

Uroflowmetry was performed using the Oruflow device with serial number ORF20210528-04 branded as 'Oruba' for patients with voiding problems and evaluated in the urology department.

Questionnaires
Lower urinary tract symptoms were evaluated with questionnaires (ICIQ-FLUTS/ MLUTS). Daytime sleepiness was examined with the Epworth sleepiness scale and quality of life with Quality of Life Scale-Short Form-36 (SF-36) scale. The effect of nocturia on the quality of life was determined by the NQOL scale. All parameters were evaluated at baseline, and in the first and third months of treatment.

Male lower urinary tract symptoms questionnaire (MLUTS) was applied to males, while FLUTS, which is a questionnaire about female lower urinary system symptoms, was completed by women. The first 5 questions on MLUTS inquire about voiding-related symptoms (V score), the next 6 questions ask about incontinence-related symptoms (I score), the next two questions ask daytime urination frequency (F score)

and nocturnal urination frequency (N score). Among the FLUTS scale components, the 'F score' relates to filling symptoms, the 'V score' asks voiding symptoms, and the 'I' score refers to incontinence symptoms. Uğurlucan and colleagues studied the reliability and validity of the Turkish version of the FLUTS scale in 2020 (7). Similarly, the reliability and validity of the Turkish version of the MLUTS scale were scrutinized by Mertoğlu et al. in 2016 (8).

The SF-36 was administered at the start of treatment as well as at the 1-month and 3-month treatment milestones. The reliability and validity of its Turkish version were established by Koçyiğit and colleagues in 1999 (9). The SF-36 scale is a self-assessment tool that evaluates eight sub-dimensions of health through 36 items. These items assess physical function, social functionality, role difficulties (physical and emotional), mental health, vitality, pain, and general evaluation of health. The form evaluates health on a scale of zero to 100, with a higher score indicating better health. The form provides an evaluation between zero and 100, and a higher score indicates a better level of health.

The NQOL about the effect of nocturnal urination (nocturia) on quality of life consists of 13 questions. The questionnaire was developed and validated by Abraham et al. (10). In the form, the questions are rated between 0 and 4, and the score is obtained by summing all the ratings. As the score increases, it indicates that the quality of life is badly affected by nocturia.

The Epworth sleepiness scale helps detect increased daytime sleepiness. Reliability and validity of the Turkish version was examined by İzci et al. in 2008 (11). It consists of 8 questions. The questions are scored as 0, 1, 2 or 3. While 0-5 points are defined as normal, 11 points and above indicate excessive daytime sleepiness.

Statistical Analysis
The statistical analyses were achieved with the SPSS software package, version 29. The normality of the distribution was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk tests, as well as by assessing skewness and kurtosis to check for distribution symmetry. For variables following a normal distribution, the mean and standard deviation (SD) are reported, whereas those not conforming to the normal distribution are described using the median and interquartile range (IQR). The analysis of categorical variables was executed using the Chi-

square and Fisher's exact tests. Comparisons of measurements at different time points were made using the Wilcoxon signed-rank test and the paired samples t-test. Operating a two-tailed test, a p-value below 0.05 was deemed to indicate statistical significance.

RESULTS
Baseline characteristics
Our study evaluated data from 82 patients. Four patients were removed from the study because they had undergone urinary surgery, while six more were excluded due to incomplete data. This study encompassed 72 participants in total, comprising 38 women and 34 men. The average age among these participants was 59.63 ± 8.19 years, and the mean duration of diabetes across the group was 10.28 ± 7.61 years. Among the patients, the most frequently observed comorbid conditions were hypertension (84.7%), hyperlipidemia (81.9%), and coronary artery disease (40.3%). When the antidiabetic treatments are examined, the most common oral treatments were metformin (88.9%) and DPP-4 (38.9%) inhibitors. While 8.3% of the patients were using basal insulin, 27.8% were using intensive insulin. SGLT-2 inhibitor therapy was discontinued due to

recurrent urinary -genital infections in 9 of 72 patients. During follow-up, documented urinary infections were detected in 8 (11%) patients and genital infections in 3 (4.2%) patients.

Metabolic control
Significant weight loss was observed in patients at the routine visit in the 3rd month of the treatment (p <0.001). There was a significant decrease in HbA1c, triglyceride level and microalbuminuria (p<0.001, p=0.005, p=0.003, respectively), while there was a significant increase in hematocrit values (p <0.001). The change in other laboratory tests was not statistically significant, LDL decreased slightly, and there was no significant change in creatinine (p>0.05). The metabolic values and the change in body weight of the patients in the 3rd month of treatment are summarized in Table 1.

Urine flow parameters - voiding frequency - infection
Patients with urinary complaints at the beginning of the treatment were evaluated in the urology

J Basic Clin Health Sci 2024; 8: 660-667

Gungor Semiz G et al. Sglt-2 inhibitors and lower urinary tract symptoms

Table 1. Metabolic control before and after treatment

	0 month	**3rd month**	**p**
Body weight	87.07±16.17	82.85±15.58	**<0.001***
HbA1c	8.15 (7.22-9.70)	7.50 (6.70-8.15)	**<0.001****
TG	146 (98-215)	135 (101.75-173.50)	**0.005****
LDL	98 (78-140)	96.20 (81.75-127.80)	0.700**
MAU	16.50 (9-52.50)	14 (6-44)	**0.003****
Creatinine	0.79 (0.66-0.94)	0.79 (0.66-0.96)	0.210**
GFR	96 (79.50-104.25)	95 (80-102)	0.200**
Hct	39.90 (37.4-42.75)	41.80 (39.02-44.57)	**<0.001****

Data are given as [median (25-75%)]. Data are given as mean ± standard deviation. *Paired samples T test. **Wilcoxon signed-rank test. **Abbreviations:** HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; TG, triglycerides; MAU, microalbumin; GFR, glomerular filtration rate; Hct, hematocrit.

Table 2. Comparison of uroflowmeter- urine flow parameters and day/night voiding frequency

	0 month (n=61)	**3rd month (n=44)**	**p***
Qmax (ml/sn)	15 (11-24.30)	18.50(14.6- 22)	0.090
Mean Velocity (mL/sn)	8.40 (4.95-15.4)	10 (7-14.60)	0.210
Voiding volume (ml)	209.0(178.5-381.0)	274.0 (205.5- 484.5)	**0.028***
PVR (ml)	40 (17.50-63.0)	35 (25-52.50)	0.960
Daytime urination frequency	6.79 ± 2.04	6.78 ± 2.10	0.957
	6 (6-7.75)	6 (6-8)	
Nighttime urination frequency	1.83 ± 1.06	1.77 ± 1.09	0.655
	2 (1-3)	2 (1-3)	

Data are given as [median (25-75%)], data are given as mean ± standard deviation. *Wilcoxon signed-rank test
Abbreviations: PVR: post voiding residue, Qmax: maximum flow rate

department and routine uroflowmetry measurements were examined. Urinary maximum flow rate, mean velocity and voiding volume increased at the 3rd month follow-ups. (Results with a voiding volume of 150 ml and above were evaluated.) The increase was statistically significant (p<0.05) when the voiding volume before treatment was compared with the voiding volume in the 3rd month. The post voiding residue (PVR) decreased (not statistically significant). The frequency of daytime and nighttime voiding was evaluated using a voiding diary at the beginning and end of the 3-month treatment period. No significant changes were observed in the frequency of daytime and nighttime voiding before and after treatment (p>0.05). Tables 2 shows uroflowmeter voiding dynamics and voiding frequencies according to voiding diaries.

The incidence of urinary tract infections was significantly higher in women than in men (p<0.01). All 8 patients with urinary infections were women. All 3 patients with genital infections were female too (Table 3).

Questionnaires

The NQOL was completed by patients at the start of treatment, as well as in the first and third months of treatment. No significant change was found for

patients when the initiation of treatment was compared with the 1st month and 3rd months.

The Epworth sleepiness scale detects increased daytime sleepiness, with a score of 11 and above indicating excessive daytime sleepiness. When the Epworth score was compared at 1 month and 3 months with the initiation of treatment (0-1st month; 0-3rd month; 1st-3rd months), no significant change was found (p>0.05).

MLUTs was completed by men, while women completed the FLUTS questionnaires. When the 1st month and 3rd month of treatment are compared in male patients, the frequency of daily voiding (MLUTS F score) increased (p<0.05). There was no significant

Table 3. Infection prevalence in men and women

Urinary Infection			
Gender	**No**	**Yes**	***p***
	n (%)	**n (%)**	
Female	30 (78.9)	8 (21.1)	**.006***
Male	34 (100.0)	0 (0.0)	
Genital infection			***p***
Gender	**No**	**Yes**	
	n (%)	**n (%)**	
Female	35 (92.1)	3 (7.9)	.242*
Male	34 (100.0)	0 (0.0)	

*Fisher exact test

Table 4. Epworth sleepiness scale, NQOL, MLUTS in men, FLUTS in women scores and month-by-month comparisons

	0 month	1st month	3rd month	p (0-3 m.)	p (1-3 m.)
Epworth	6 (2-10)	7 (2.75-13.25)	6 (3-10)	0.921	0.636
NQOL	10 (2-19)	7 (3-15.25)	10 (3-19)	0.557	0.082
MLUTS V	4 (2-9)	4 (2-7)	4 (2-8)	0.989	0.752
MLUTS I	2 (1-4)	1 (0-3)	2 (1-3)	0.059	0.546
MLUTS F	0.5 (0-1)	0 (0-1)	1 (0-1)	0.448	**0.046***
MLUTS N	2 (1-2)	2 (1-3)	2 (1-2.75)	1.000	0.414
FLUTS F	4 (2-6)	4 (3-6)	3.5 (2-7)	0.602	0.444
FLUTS V	1 (0-2)	2 (0-6)	0 (0-2)	0.642	0.072
FLUTS I	2 (1-6)	5 (0-7)	3.5 (1-7.25)	0.193	0.294

Data are shown as n (%) and [median (25-75%)]. *Wilcoxon signed-rank test.
Abbreviations: NQOL: Effect of nocturia on quality of life, MLUTS: Male lower urinary tract symptoms, FLUTS: Female lower urinary tract symptoms. MLUTS V: Voiding related symptoms, MLUTS I : Incontinence, MLUTS F : Daytime urination frequency, MLUTS N: Nocturnal urination frequency, FLUTS F : Filling symptoms, FLUTS V: Voiding related symptoms, FLUTS I: Incontinence, m: months

Table 5. Comparison of SF-36 symptom scores

SF-36	p=0-1 month	p=0-3 month	p=1-3 month
Physical function	0.204	0.203	**0.010***
Physical-role difficulties	0.485	0.799	0.161
Emotional-role difficulty	0.054	0.304	0.298
Energy	0.392	0.841	0.238
Mental health	0.914	0.239	0.851
Social function	0.288	0.924	0.583
Pain	0.359	0.096	0.129
General Health	0.773	0.244	0.345

*Wilcoxon signed rank test. **Abbreviations:** SF 36: Quality of life scale-short form.

change in lower urinary system symptom scores in female patients. In Table 4, month-by-month comparisons of NQOL, Epworth sleepiness scale and MLUTS-FLUTS scores are presented.

Evaluation of Life Quality
Patients completed the SF-36 questionnaire at the start of treatment, as well as in the first and third months. In our cases, physical function increased significantly in the 1st and 3rd month comparisons. No significant changes were detected for other sub-parameters. Table 5 shows the SF-36 symptom scores.

When multivariate linear regression analysis was performed, the parameter that most affected the significant change in SF-36- physical function was the decline in HbA1c.

If men and women are examined separately, there was an improvement in the mental health of women in the 3rd month of the treatment based on SF-36 mental health scores (p=0.03). HbA1c and body weight decreased significantly in both men and woman.

When patients with a history of coronary artery disease are examined (n=29), HbA1c decreased

significantly in this group and the group without coronary artery disease. There was significant weight reduction in both groups consistent with the whole group. According to the SF-36 scale, the group with coronary artery disease showed a significant improvement in physical function.

DISCUSSION
SGLT-2 inhibitors have been included in the treatment of diabetes in recent years. These drugs, which inhibit glucose reabsorption in the kidney proximal tubules and act as a glucosuric, are importance for their contribution to metabolic control, as well as their diuretic effect, providing weight loss, a reduction in blood pressure, and playing an active role in cardiovascular protection (12). An increase in the frequency and amount of urine and an increase in nocturia can be expected in patients using SGLT-2 inhibitors. Nocturia, which is one of the lower urinary tract symptoms, is described as waking from sleep to urinate at least once during the night. It causes sleep disturbances and decreased sleep quality and is related to an increased risk of loss of balance and falling, especially in the elderly group. Currently, diabetic and elderly patient groups may have lower

urinary system complaints. When the literature is examined, there are various studies evaluating urinary complaints (pollakiuria, nocturia, incontinence, urinary infection, etc.) associated with SGLT-2 inhibitors (5,13,14). There are few publications in the literature regarding the impact of SGLT-2 inhibitors on quality of life and sleep. (6,15). Adding SGLT-2 inhibitors to the current treatment did not increase the frequency of nocturia in our study. In parallel with our data, no significant changes were detected in the quality-of-life score and daytime sleepiness score related to nocturia. Significant decreases in HbA1c, triglyceride, spot urine microalbuminuria levels and body weight were identified. The health-Related Quality of Life - Physical Function score demonstrated significant improvement, with HbA1c being the most contributing parameter.

When the literature is examined, a study conducted in Japan in 2018 (5) recruited 55 patients. Their symptoms were recorded before treatment and one month after adding SGLT-2 inhibitors. The frequency of daytime voiding increased significantly after SGLT-2 treatment, and the frequency of nocturia did not change. In our study, similar to the results of this study, the daytime voiding frequency score increased significantly only in male patients when the 1st and 3rd months of treatment are compared (p<0.05). When all participants are evaluated, there was no significant change in the frequency of voiding both during the day and at night.

In a case series published in Italy in 2018, data for 50 diabetic male patients were reviewed retrospectively (12). After starting SGLT-2 inhibitor therapy, pollakiuria and nocturia were the most common lower urinary tract symptoms. Pollakiuria was statistically more frequent in the patient group without autonomic neuropathy than in the group with autonomic neuropathy. In addition, pollakiuria was stated to be the earliest finding after starting an SGLT-2 inhibitor. This study only evaluated male patients.

In our study, the urine maximum flow rate, mean velocity and voiding volume increased in uroflowmetry examinations of the patients (urinary volumes of 150 ml and above were evaluated). The increase in voiding volume was statistically significant (p<0.05). When voiding diaries were examined, no increase was observed in the frequency of daytime and nighttime voiding, and no significant change was observed in the quality-of-life scores due to nocturia. There was no significant change in Epworth scores

measuring daytime sleepiness, either (increased score represents increased daytime sleepiness). Our study is the first to evaluate the urinary flow dynamics of patients using uroflowmetry.

In 2021, a study evaluated the impact of SGLT-2 inhibitors on patients' quality of life, sleep quality, and anxiety levels. The study compared patients who received SGLT-2 inhibitor therapy in addition to other OADs with a group that only received other OADs and a healthy control group. After 3 months of treatment, the SGLT-2 group experienced a significant decrease in HbA1c and body mass index (BMI). However, the group receiving SGLT-2 inhibitors had a significantly higher frequency of genital-urinary tract infection, daytime voiding, nocturia, and incontinence. The SGLT-2 group showed a statistically significant improvement in the sub-parameters of the SF-36 quality of life scale, including physical function, pain, general health perception, emotional role difficulty, energy, and mental health perception scores. Energy and general health perception, which are sub-parameters of SF-36, and BMI had negative correlations. On the sleep scale, there was no difference between the groups (6).

Similar to the aforementioned study, a significant improvement in metabolic parameters and a significant decrease in body weight were found in our study. There was significant improvement in the SF36 score-physical function component (1st-3rd month comparison). The improvement in the SF-36 score was correlated with the decrease in HbA1c. In our study, contrary to the aforementioned study, there was no increase in the frequency of daytime and nighttime voiding, and there was no change in the daytime sleepiness scores of patients.

Individuals with diabetes generally experience a lower quality of life compared to those without chronic illnesses. However, managing blood glucose levels can improve the quality of life for those with type 2 diabetes (16). Few studies have evaluated the impact of SGLT-2 inhibitors on the quality of life of individuals with type 2 diabetes. In these studies, the effects of SGLT-2 inhibitors were found for weight loss, improvement in glycemic control and improvement in health-related quality of life scores (HR-QOL) with low incidence of hypoglycemia (6,15). In our study, quality of life scores were negatively correlated with HbA1c, which supports the literature. In addition to studies in the literature, there was a significant improvement in SF-36 physical function score in patients with coronary artery disease. This is an important finding

showing that the well-being of these patients increased. Another interesting finding is the improvement in the perception of SF-36 mental health in women.

During follow-up, after SGLT-2 inhibitor treatment, the drug was discontinued in 5 patients due to recurrent genital-urinary infections. Urinary infections were more common than genital infections. The frequency of documented urinary infections in women was significantly higher than in men ($p < 0.01$). Upon examination of previous studies, a meta-analysis of 56 randomized controlled studies revealed that SGLT-2 inhibitors significantly increased the risk of genital infections. However, no clear effect on urinary tract infections was observed (17).

Our study included more participants than other published studies, but lacking a control group is limiting the inability of the patients to properly record the amount of fluid taken and urine excreted while keeping a voiding diary is another limitation of our study. This study is the first to evaluate the urinary flow dynamics of patients using uroflowmetry.

Consequently, in this real-life study, SGLT-2 inhibitors improved quality of life by improving glycemic control. Although the amount of urine increases, it does not significantly change the frequency of urination (day or night). With SGLT-2 inhibitor treatment, no significant change was found in lower urinary tract symptoms and there was no change in daytime sleepiness or effect of nocturia on quality of life. But care should be taken in terms of genitourinary infections.

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CLINICAL CHARACTERISTICS OF CHILDREN AND ADOLESCENTS APPLYING FOR A SPECIAL NEEDS REPORT FOR CHILDREN

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ABSTRACT

Purpose: The aim of this study was to investigate the demographic and clinical characteristics of patients who applied for Special Needs Reports for Children (SNRFC).

Material and Methods: 652 children and adolescents who applied to the medical board of a university hospital for SNRFC between April 2023 and May 2024 were included in the study. Demographic and clinical data were obtained by retrospective file review.

Results: The mean age of the patients was 8.65 ± 4.15 years. The most common diagnosis in the child and adolescent psychiatry area was specific learning disorder (39.2%). When comparing the psychiatric diagnoses of male and female participants, the prevalence of autism spectrum disorders and atypical autism was significantly higher in males than in females. When psychiatric diagnoses are evaluated according to age groups, autism spectrum disorder diagnosis was found to be significantly higher in cases younger than 6 years of age, and delayed milestone and specific learning disorder diagnoses were found to be significantly higher in cases aged 6 years and older.

Conclusion: It is thought that the results of the study will contribute to the clinical approach of physicians working in the field of child and adolescent mental health and diseases in the evaluation process of children who apply to the health board for SNRFC.

Keywords: Child and adolescent psychiatry, special needs, health board

INTRODUCTION

"Special needs" is a concept defined by the World Health Organization in three dimensions: "impairment", "disability" and "handicap". Impairment is defined as deficiency and abnormality in physical and psychological functions. Disability is defined as an activity limitation resulting from inadequate functionality, while handicap is characterized as a limitation in expected sociocultural roles based on age and gender due to impairment and disability (1). According to the United Nations Children's Fund (UNICEF) 2021 data, 240 million children worldwide have at least one disability (2). According to data from the Turkish Statistical Institute (TÜİK) in 2011, the prevalence of disability was 2.3% among individuals aged 3-9 years, 2.1% among those aged 10-14 years, and 2.3% among those aged 15-19 years (3). Physical, mental, or sensory disabilities adversely affect the academic and social lives of individuals, creating challenges in nearly every aspect of their daily experiences. These individuals require special support in numerous areas, particularly in education and social interactions, from an early age.

In Türkiye, the assessment of children's special needs is conducted in accordance with the "Regulation on Special Needs Assessment for

Children," published in the Official Gazette on February 20, 2019 (4). The SNRFC is developed based on evaluations by a board comprising at least six permanent physicians, including a specialist in child and adolescent mental health and diseases. During the preparation of this report, all cases presented to the health committee are evaluated by specialists in physical therapy and rehabilitation, ophthalmology, otorhinolaryngology, neurology (pediatrics), and child and adolescent mental health. Following the evaluation, the relevant specialists prepare a report, detailing the medical diagnosis, special needs level, and disability rate of each case, in accordance with the guidelines outlined in Annexes 1, 2, and 3 of the SNRFC regulation. In addition to managing the follow-up and treatment of patients, physicians specializing in child and adolescent mental health have responsibilities that include referring patients to special education programs, determining disability rates, and facilitating access to social and educational rights. Meeting the special education and social support needs of children with special needs promptly and adequately is a critical component of treatment, significantly enhancing therapeutic outcomes (5,6). Therefore, it is imperative that every child and adolescent psychiatry physician possesses comprehensive knowledge of the processes involved in identifying and addressing special needs.

This study aimed to examine the demographic and clinical characteristics of children and adolescents who applied for the SNRFC.

MATERIAL AND METHODS
Participants and Study design
652 children and adolescents aged 0-18 years, who applied to the Sivas Cumhuriyet University Faculty of Medicine, Department of Child and Adolescent Psychiatry between April 2023 and May 2024 to obtain a medical board report, were included into the current research. The sample size was calculated using G*Power (3.1.9.4). The minimum sample size, corresponding to a nominal significance level of $\alpha = 0.05$, a medium effect size of $d = 0.5$, and a power value of $1-\beta = 0.9$, was determined to be 587. After obtaining ethics committee approval, data on the patients' age, gender, reasons for admission, psychiatric diagnoses, additional diagnoses from other departments, and special needs levels were retrospectively extracted from patient files. Patients with unclear diagnostic evaluations, missing

psychiatric diagnoses, or incomplete sociodemographic and clinical information were excluded. A total of 667 patient files were accessed, with 15 patients excluded due to missing data.

The evaluation of the disability rates of the patients applying for a medical board report was conducted based on the "Regulation on Special Needs Assessment for Children," published in the Repeated Official Gazette on 20.02.2019 and numbered 30692 (4). Psychiatric evaluations of all children applying to the SNRFC health board committee were conducted by child and adolescent mental health physicians. Intelligence tests were administered to determine the intelligence levels of the cases. The Wechsler Intelligence Scale for Children-Revised (WISC-R) was used for children over 6 years old, whereas the developmental levels of children under 6 years old were assessed using the Ankara Developmental Screening Inventory.

Ethical Considerations
Ethics committee approval for the research was received from Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee (Date: 16.05.2024, Decision No: 2024/05-22).

Measures
Sociodemographic and Clinical Data Form
In this form prepared by the researcher, information such as the patient's age, gender, reason for application to the health board, psychiatric diagnosis, other medical diagnoses, level of special needs, and duration of the special needs report was collected.

Wechsler Intelligence Scale for Children-Revised (WISC-R)
The validity and reliability studies of the test in Turkish, originally developed by Wechsler in 1949, were conducted by Savaşır and Şahin (1995) (7). The test evaluates two primary domains, verbal and performance, with each domain consisting of 6 subtests, totaling 12 subtests. It is designed for administration to children and adolescents aged 6-16.

Ankara Developmental Screening Inventory
It is an assessment test developed by Savaşır et al. (1998) to determine the general developmental level of infants and children aged 0-6 years (8). Consisting of 154 items, the total scores of the test reflect the general developmental level. The AGTE has 4

Table 1. Diagnostic distribution and application patterns of cases in child and adolescent psychiatry and other medical areas

	SNRFC Diagnostic Area		Application Patterns		
			First report (N=234)	Objection (N=5)	Renovation (N=185)
	n	%	n (%)	n (%)	n (%)
Child and Adolescent Psychiatry Area (N=424)					
Delayed Milestone	132	31.1	50 (21.4)	1 (20)	81 (43.8)
Autism Spectrum Disorders	44	10.3	19 (8.1)	0 (0.0)	25 (13.5)
Atypical Autism	79	18.6	40 (17.1)	0 (0.0)	39 (21.1)
Specific Learning Disorder	166	39.2	122 (52.1)	4 (80)	40 (21.6)
Attention Deficit Hyperactivity Disorder	1	0.2	1 (0.4)	0 (0.0)	0 (0.0)
Bipolar Affective Disorder	2	0.5	2 (0.9)	0 (0.0)	0 (0.0)
Language-Speech-Communication Development Area (N=166)					
Other Developmental Disorders of Speech and Language	115	69.3			
Specific Speech Articulation Disorder	48	28.9			
Stuttering	3	1.8			
Other Medical Diagnosis Areas (N=153)					
Nervous System	7	4.6			
Endocrine System	104	67.9			
Hereditary-Congenital Diseases	11	7.2			
Hearing Function-Ear Nose, Throat	17	11.1			
Visual Function	4	2.7			
Movement Development	7	4.6			
Digestive System	3	1.9			

SNRFC: Special Needs Reports for Children

subtests: language-cognitive, fine motor, gross motor, social skills and self-care.

Statistical Analysis

All data were analyzed using Statistical Package for Social Science (SPSS) Windows version 24.0 software. Results of nominal data were reported as number (n) and percentage (%). Chi-square test was used to compare categorical data between independent groups. A p value <0.05 was accepted for statistical significance.

RESULTS

The mean age of the patients was 8.65 ± 4.15 years and 242 (37.1%) were female and 410 (62.9%) were male. Analysis of the types of applications to the health committee for the SNRFC revealed that 460 cases (70.6%) were "first report" applications, 187 cases (28.7%) were "report renewal" applications, and 5 cases (0.8%) were "objections" to previous SNRFC reports. Table 1 provides a detailed breakdown of the application types for cases diagnosed in the area of child and adolescent psychiatry. The reasons for application indicated that 513 cases (78.7%) sought "special education," 136 cases (20.9%) aimed for "social rights and tax reduction," and 3 cases (0.5%) were related to a "traffic accident."

In the "Regulation on the Evaluation of Children's Special Needs", psychiatric disorders in children and adolescents are defined under the headings "Child and Adolescent Psychiatry Area", and speech and language developmental disorders are defined under the headings "Language-Speech-Communication Development Area" (4). The findings were evaluated under similar subheadings.

Analysis of the diagnostic fields indicated that 327 cases (50.2%) had diagnoses solely in child and adolescent psychiatry, 221 cases (33.9%) had diagnoses in speech-language-communication and other medical fields, and 97 cases (15.0%) had diagnoses in both child and adolescent psychiatry and speech-language-communication. Furthermore, 6 cases (0.9%) had no medical issues. The most frequent psychiatric diagnosis in child and adolescent psychiatry was specific learning disorder [n=166 (39.2%)], whereas the most common non-psychiatric medical disorder was endocrine system diseases [n=104 (32.6%)] (Table 1).

Analyzing the distribution of diagnoses in the area of child and adolescent psychiatry according to the type of presentation, it was found that specific learning disorder was the most common diagnosis among patients applying to the health board for the SNRFC for the first time [n=234 (55.2%)], with 122 cases (52.1%). Among the 5 cases (1.2%) who applied for

Table 2. Special needs levels and disability ratios in SNRFC

Special need levels	n	%	Disability rate (%)
Have special needs (HSN)	286	43.9	20-39
Mild HSN	35	5.4	40-49
Moderate HSN	43	6.6	50-59
Very advanced HSN	11	1.7	70-79
Prominent HSN	2	0.3	80-89
Have special conditions needs (HSCN)	265	40.6	90-99
No special needs	10	1.5	-

Table 3. Comparison of diagnoses in child and adolescent psychiatry in area of gender

Child and Adolescent Psychiatry Area	Girl n (%)	Boy n (%)	p
Delayed Milestone	55 (36.2)	77 (28.3)	
Autism Spectrum Disorders	9 (5.9)	35 (12.9)	
Atypical Autism	13 (8.6)	66 (24.3)	
Specific Learning Disorder	73 (48.0)	93 (34.2)	0.000**
Attention Deficit Hyperactivity Disorder	1 (0.7)	0 (0.0)	
Bipolar Affective Disorder	1 (0.7)	1 (0.4)	

*p < 0.05, **p < 0.001; Chi-square test

Table 4. Comparison of diagnoses in child and adolescent psychiatry according to age groups

Child and Adolescent Psychiatry Area	Under 6 years old n (%)	6 years and older n (%)	p
Delayed Milestone	25 (29.8)	107 (31.5)	
Autism Spectrum Disorders	29 (34.5)	15 (4.4)	
Atypical Autism	30 (35.7)	49 (14.4)	
Specific Learning Disorder	0 (0.0)	166 (48.8)	0.000**
Attention Deficit Hyperactivity Disorder	0 (0.0)	1 (0.3)	
Bipolar Affective Disorder	0 (0.7)	2 (0.6)	

*p < 0.05, **p < 0.001; Chi-square test

an appeal, 1 case (20%) had a diagnosis of delayed milestone, and 4 cases (80%) had a diagnosis of specific learning disorder (Table 1).

The special needs levels of the cases were determined as specified in the SNRFC guideline (Annex 3). Analysis of the special needs levels revealed that the most frequent diagnosis was "have special needs (HSN)," with 286 cases (43.9%). The second most frequent diagnosis was "have special conditions needs (HSCN)" with 265 cases (40.6%).

Table 2 presents the special need levels of the cases along with their corresponding disability rates as outlined in the SNRFC guideline.

A statistically significant difference was observed between male and female patients in the field of child and adolescent psychiatry concerning psychiatric diagnoses (p < 0.001). Specifically, the prevalence of autism spectrum disorders and atypical autism was significantly higher in male patients compared to female patients (Table 3).

A significant difference was observed in psychiatric diagnoses when cases within the area of child and adolescent psychiatry were divided into two groups: those under 6 years of age and those 6 years and older (p < 0.001). Autism spectrum disorder diagnoses were significantly higher in patients under 6 years of age, while diagnoses of delayed milestones and specific learning disorders were significantly higher in patients aged 6 years and older (Table 4).

DISCUSSION

The current study examined the age, gender, application patterns, and child and adolescent psychiatry as well as other medical diagnoses of children and adolescents who applied for the SNRFC report at the health board of a university hospital, yielding significant findings.

The analysis revealed that the mean age of the children and adolescents applying for the SNRFC report was 8.65 ± 4.15 years, with a gender distribution of 242 (37.1%) females and 410 (62.9%) males. In a study conducted by Yıldız and Tarakçıoğlu (2020), it was determined that the average age of the cases applying for SNRFC was 8 ± 4 years old, 33.7% were female and 67.3% were male (9). Likewise, Kayhan and Öztürk (2020) found a mean age of 9.10±4.60 years, with 37.8% females and 62.2% males (10). The results of this study regarding age and gender distribution are consistent with these studies. The predominance of males in the sample, similar to other studies, may be attributed to the higher prevalence of certain psychiatric disorders requiring special needs, such as autism spectrum disorder and specific learning disorder, among males. In the current research, the analysis of the reasons for applying for the SNRFC evaluation revealed that the most common reason was special education, accounting for 78.7% of applications. The second most frequent reason was for social rights and tax reduction, comprising 20.9% of applications. Şahin et

al. (2014) similarly found that the predominant reason for applying for disability health board reports was special education, at 81.8% (11). Kayhan and Öztürk (2020) also reported comparable findings, with 52.4% of SNRFC applications being for obtaining a special education report (10). These findings are consistent with the literature. Since special education support is a fundamental requirement for many psychiatric disorders requiring special needs, such as delayed milestones, autism spectrum disorders, and specific learning disorders, it is expected that the most common reason for applying for SNRFC is special education.

The analysis of the diagnostic distribution of patients applying to the health board for SNRFC revealed that child and adolescent psychiatry was the most prevalent diagnostic area. Specifically, 327 (50.2%) patients received only psychiatric diagnoses, while 424 (65.0%) received both psychiatric and other medical diagnoses. These findings align with other studies conducted on children applying to the health committee for SNRFC (9-11). These results emphasize the need for child and adolescent psychiatry physicians to possess the necessary competencies in the evaluation process within their field.

The distribution of diagnoses in the area of child and adolescent psychiatry revealed that the most common diagnosis was specific learning disorder [n=166 (39.2%)], followed by delayed milestone [n=132 (31.1%)]. In a study by Yıldız and Tarakçıoğlu (2020), specific learning disorder was also found to be the most common diagnosis among patients applying for SNRFC, with a prevalence of 27% (9). However, other studies on the subject have identified delayed milestone as the most common diagnosis (10-13). This inconsistency may be attributed to the average age of the sample group, as specific learning disorder is most frequently diagnosed during the school-age period (14). In this study, the mean age of subjects was 8.6 years, a critical period for diagnosing specific learning disorder and initiating special education interventions.

The analysis revealed that the most common initial report among cases diagnosed in the area of child and adolescent psychiatry was specific learning disorder [n=234 (55.2%)]. Specifically, 52.1% of these initial report applications were for specific learning disorder. Additionally, 5 cases (1.2%) applied as objections, with 4 (80%) of these objecting cases having previously received a report diagnosing

specific learning disorder. Considering the average age of the cases, it is anticipated that specific learning disorder would be the most prevalent diagnosis at the initial presentation. Güller and Yaylacı (2021) reported an objection rate of 0.6%, whereas Temeltürek et al. (2023) found an objection rate of 2.1%. (12,13). These results suggest that patients may seek SNRFC not only to obtain a special needs report but also to contest previous diagnoses and reports.

Analysis of the special needs levels of the cases revealed that the most frequently reported level was "have special needs" (HSN), with 286 cases (43.9%). Terzioğlu et al. (2022) also identified HSN as the most commonly determined level of special need in patients presenting for SNRFC, at 40.3% (15). Similarly, Güller and Yaylacı (2021) found that the most frequent level of special need was HSN (48.2%) (12). These findings align with our study's results. The SNRFC classifies disability severity as "HSN," "Mild HSN", "Moderate HSN," "Very advanced HSN," "Prominent HSN," and "have special conditions needs (HSCN)". Severe disability is indicated by the classifications of very advanced HSN, prominent HSN, and HSCN. According to the SNRFC regulation, the disability rate for the diagnosis of specific learning disorder is categorized as HSN. The predominance of HSN in this study may be attributed to the high prevalence of specific learning disorder among the patients.

In this study, a comparative analysis of the diagnoses in the field of child and adolescent psychiatry revealed that the prevalence of autism spectrum disorder and atypical autism was significantly higher in males than in females. Consistent with our findings, Yıldız and Tarakçıoğlu (2020) and Temeltürek et al. (2023) also reported a significantly higher incidence of autism spectrum disorder in males compared to females (9,13). Autism spectrum disorder is a disorder that is more common in men (16). The results of the current study corroborate these findings.

An analysis of the diagnostic areas in child and adolescent psychiatry, stratified by age groups, revealed that diagnoses of autism spectrum disorder were significantly more prevalent in children under 6 years of age, whereas delayed milestone and specific learning disorder were significantly higher in cases aged 6 years and older. The mean age at diagnosis for autism spectrum disorder typically ranges between 38 and 120 months (17). In a meta-analysis

by van't Hof et al. (2021), the average age at diagnosis for autism spectrum disorder was reported to be 43 months (31-75 months) (18). Although the specific age at diagnosis was not determined in this study, the high number of cases diagnosed with autism spectrum disorder before the age of 6 years is likely related to these age-related diagnostic patterns. Similarly, specific learning disorder is typically diagnosed during school years, which explains the higher prevalence of this diagnosis in the 6 years and older age group (14). Delayed milestone diagnoses correspond to "delay in cognitive development" and "mental retardation" in the SNRFC. While mental retardation may manifest as developmental delay in early childhood, the definitive diagnosis is often made during school years (19). The elevated rate of delayed milestone diagnoses in the patients aged 6 years and older is likely attributable to the timing of diagnostic clarity.

Limitations
A significant limitation of this study is its retrospective design, which relies on previously recorded data. Additionally, the diagnostic evaluation of the cases was not conducted using a structured clinical interview, which is another notable limitation. Furthermore, the research data were obtained from cases that applied to the health board at a single center, which may limit the generalizability of the findings.

CONCLUSION
The findings of this study provide valuable insights into the clinical characteristics of cases that applied to the health committee for the SNRFC. Physicians specializing in child and adolescent mental health should be thoroughly knowledgeable about the SNRFC and adhere to current regulations when assessing the needs of children with special requirements in education, social rights, and other areas. It is thought that this study will contribute to the clinical approach of physicians in the field of child and adolescent mental health, particularly in evaluating children who apply to the health board for the SNRFC.

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PHYSICAL ACTIVITY IN EMERGENCY DEPARTMENT WORKERS: AN EXAMPLE OF INTENSIVE AND STRESSFUL WORK ENVIRONMENT

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ABSTRACT

Purpose: This study aimed to examine the use of graduated compression stockings in patients after surgery.
Methods: Employing a descriptive research design, the study was conducted on a sample of 173 postoperative patients using compression stockings in the surgical departments of a university hospital. The data, obtained through the sociodemographic and clinical characteristics form, and the evaluation form assessing the use and knowledge level of graduated compression stockings, were collected via face-to-face interviews and observation.
Results: Among all the patients, 80.9% did not elevate their feet while in bed before putting on stockings, and 72.3% did not wear stockings before getting out of bed. The researchers observed that 50.9% of the patients correctly positioned the stockings on the heel, while 79.2% had folds in the stockings they wore. Additionally, during the removal of stockings for observations, 59.5% of the patients exhibited redness in the corresponding area. The patients who received information from healthcare providers had used the stocking accurately, and there was less tissue damage (p<0.05).
Conclusion: Patients experience some problems during the use of stockings, such as incorrect usage and tissue damage; however, these problems are less observed in educated patients. Consequently, it is advisable to implement comprehensive and effective patient education programs on compression stocking usage to address these issues and improve patient outcomes.

Keywords: Graduated compression stockings, Nursing, Postoperative, Venous thromboembolism

INTRODUCTION

The importance of engaging in sports to enhance the performance of emergency department workers in a healthy manner is increasingly being recognized. Sports are a crucial factor for coping with stress and maintaining physical health. They enhance mental health, fitness, skeletal system strength, social connections, and self-confidence, improving overall quality of life (1).

The emergency department is a section or unit of a healthcare institution providing emergency medical services. It aims to save lives, improve health conditions, or provide appropriate referrals for situations requiring emergency medical intervention

J Basic Clin Health Sci 2024; 8: 675-682

Alkas B et al. An Example of Intensive and Stressful Work Environment

by ensuring rapid andı effective responses to emergencies. The emergency department operates 24/7 and is typically staffed with healthcare personnel from various specialties (2). Emergency department workers include emergency medicine specialists, emergency medicine assistants, nurses, medical technicians (paramedics), emergency medical technicians (EMTs), caregivers, cleaning staff, and other personnel (3). Additionally, emergency department staff actively use their musculoskeletal system when examining or intervening with patients. Engaging in sports helps improve muscle strength, flexibility, bone health, and posture. Furthermore, while isokinetic muscle strength and healthy joints facilitate sports participation, they also positively impact psychological and mental well-being, thereby enhancing overall quality of life (4).

Healthcare workers, especially in emergency departments, face high stress and intense workload (5).

Additionally, emergency department workers actively use their musculoskeletal system while examining or intervening with patients. Engaging in sports helps maintain muscle strength, flexibility, bone health, posture, and mental health. Emergency department workers are exposed to intense work environments, irregular sleep, stress, and irregular eating habits, all of which negatively impact the musculoskeletal system. This condition can cause neck, back, and waist pain, reducing life quality (6,7).

Healthcare workers, especially those in high-pressure and stressful environments like emergency departments, should prioritize physical health by maintaining a healthy lifestyle that includes regular exercise, healthy eating, and adequate sleeping habits. In stress management, sports can help recognize stress, identify its causes, and learn techniques to cope with it. These techniques may include regular exercise, healthy nutrition, maintaining a proper sleep schedule, creating a social support network, developing time management skills, practicing relaxation techniques, and engaging in stress-reducing activities (8).

The importance of sports in work performance lies in its ability to enhance focus, thereby positively impacting productivity. Regular exercise improves mood and team dynamics, helps cope with fatigue, and supports staying alert. Moreover, sports enhance communication and concentration, contributing to a reduction in workplace accidents. For these reasons, sports effectively improve work performance (9). In addition, the positive changes in physical and physiological parameters due to exercise and its impact on improving quality of life suggest that it can be used as an alternative to traditional training methods (walking, running, and gym activities) for those who do not have time to exercise (10).

In addition, emergency room workers may have to transport patients, perform CPR, and sometimes even run to reach patients for most of their working lives. For this reason, physical activity and fitness are very important for emergency room workers.

This study aims to investigate emergency department workers' attitudes and the influencing factors towards sports.

MATERIALS AND METHODS
Participants
This study is a cross-sectional survey conducted among emergency department workers at Gazi University Faculty of Medicine. The research was conducted on employees in the Gazi University Faculty of Medicine emergency department. Participate in the survey via Google Forms, of which 270 responded with complete answers.

Data Collection Tool
The data collection tool consists of two parts:
Personal Information Form: This section includes questions about participants' socio-demographic characteristics, such as age, gender, marital status, and length of employment, along with questions related to their professional lives. Attitude Towards Sports Scale (ATSS): Developed by Şentürk (2012) and validated for validity and reliability, the scale comprises a total of 25 items and three subscales: Interest in Sports (IS - 12 items), Incorporating Sports into Life (IL - 7 items), Active Sports Participation (ASP - 6 items),

For this study, the Cronbach's Alpha (α) values were calculated as follows: IS: 0.869, IL: 0.857, ASP: 0.793, Overall scale: 0.924

The scale uses a 5-point Likert scale (1- Strongly Disagree, 5- Strongly Agree). Higher scores define a more positive attitude towards sports (1). The survey form was created using the Google Forms platform and distributed to participants online.

Data Collection Process
Surveys were sent to participants via email between (08/07/2024 and 28/07/2024). Participants were informed about the aim of the study, and participation

J Basic Clin Health Sci 2024; 8: 675-682

Alkas B et al. An Example of Intensive and Stressful Work Environment

was voluntary. Adequate time was provided for participants to complete the survey, and the confidentiality of responses was ensured.

Statistical Analysis

Collected data were analyzed using SPSS 25.0 (Statistical Package for the Social Sciences). The skewness coefficient and Kolmogorov-Smirnov test were used to assess the normal distribution of research data. For data to be considered normally distributed, kurtosis and skewness values are expected to fall within the range of -2 to +2. Upon examining normality, it was found that scale scores exhibited a normal distribution. Socio-demographic characteristics of participants were presented as frequencies and percentages, while scale characteristics were calculated as mean, standard deviation, minimum, and maximum values. Cronbach's Alpha coefficient was calculated to assess the validity and reliability of responses to the survey. An independent samples t-test was used to examine differences between age, gender, marital status, and scale score averages. ANOVA was employed to analyze differences in scale score averages based on participants' educational level and job title variables. Post hoc tests, precisely the Least Significant Difference (LSD) test, were conducted to determine which variables showed significant results according to the ANOVA test. Results were considered statistically significant at $p < 0.05$ level.

Ethics Committee Approval

This study was approved by the Ethics Committee of Gazi University (Date: 09/07/2024, No:12). All participants provided informed consent electronically before participation. In this study, we have followed all the Helsinki guidelines at all stages for human studies and met the current ethical standards in Sport and Exercise Science.

RESULTS

The findings of our study, which aims to reveal the attitudes of emergency service workers towards sports and the influencing factors, are given below.

When examining the socio-demographic characteristics of the participants, 69.3% were aged 24 years or younger, 72.6% were female, 53.7% had an associate degree, 41.5% were intern paramedics, and 83.0% were single.

When examining Table 2, the participants had a

Table 1. Participants' socio-demographic characteristics

	n	%
Age		
≤24 years	187	69.3
≥25 years	83	30.7
Gender		
Male	74	27.4
Female	196	72.6
Education Level		
High School	23	8.5
Associate's Degree	145	53.7
Bachelor's Degree	70	25.9
Master's Degree	32	11.8
Occupation		
Intern paramedic	112	41.5
Paramedic	25	9.3
Intern nurse	9	3.3
Nurse	36	13.3
Doctor	40	14.8
Other	48	17.8
Marital Status		
Single	224	83.0
Married	46	17.0

mean age of 24.29 ± 7.59 years (minimum 18.00, maximum 59.00) and a mean total scale score of 80.19 ± 25.20 (minimum 25.00, maximum 125.00).

When examining Table 3, the gender variable shows significant differences in scale score averages. Male participants had significantly higher mean scores in total scale score (p=0.001), SY subscale score (p=0.043), and ASP subscale score (p=0.002) compared to female participants. Married participants had significantly higher mean scores in total score (p=0.008) and IS subscale score (p=0.030) compared to single participants.

When examining Table 4, education level creates significant differences in the mean total scale score and all subscales except ASP. Post hoc tests revealed that this difference stems from participants with postgraduate education having higher averages compared to those with high school and associate degree education.

Table 2. Some Characteristics of Age and Scale Scores

	Min	Max	Average	SD
Age (Years)	18.00	59.00	24.29	7.59
Total Scale Score	25.00	125.00	80.19	25.20
Interest in Sports Subscale	13.00	65.00	43.24	13.35
Integration of Sports into Life Subscale	7.00	35.00	22.59	7.69
Active Participation in Sports Subscale	5.00	25.00	14.35	5.80

Table 3. Comparison of Scale Total, Interest in Sports, Incorporating Sports into Life, and Active Sports Participation Scores Based on Participants' Age, Gender, and Marital Status (t-test)

Variables		Total Scale Score			IS			SL			ASP		
		x̄±SD	t	p*	x̄±SD	t	p*	x̄±SD	t	p*	x̄±SD	t	p*
Age	≤24 years	79.05±26.04	-1.115	0.26	42.82±13.50	-0.767	0.43	22.17±7.97	-1.366	0.17	14.05±5.86	-1.270	0.20
	≥25 years	82.75±23.12			44.18±13.02			23.55±6.95			15.02±5.64		
Gender	Male	83.93±29.94	1.502	0.00*	44.02±16.44	0.591	0.55	23.75±8.55	1.527	0.04	16.14±6.27	3.179	0.00*
	Female	78.78±23.08			42.94±12.01			22.15±7.31			13.67±5.47		
Marital Status	Single	79.44±25.27	-7.074	0.00*	42.87±13.34	-5.003	0.03*	22.38±7.79	-0.980	0.32	14.18±5.78	-1.055	0.29
	Married	83.82±24.78			45.04±13.36			23.60±7.15			15.17±5.89		

*p<0.05, t-test

DISCUSSION

Regular physical activity provides happiness and pleasure among individuals engaged in sports. It has been observed that it improves our overall health and quality of life (11). The importance of sports in coping with stress is unquestionable in contemporary times. Sports effectively alter individuals' monotonous lifestyles and perceptions. Engaging in sports promotes relaxation and stress relief, encouraging individuals to feel happy and prosperous and enjoy these emotions (12). Lee et al. (2012) Individuals who exercise regularly generally have higher job satisfaction and productivity. Yasukawa et al. (2006) It has been determined that sports are essential in professional duties other than health. (13).

In our sample, the proportion of female participants (72.6%) was higher compared to male participants. Similar trends are observed in other related studies in the literature. Özsarı et al. (2019) reported a higher participation rate among female participants (14), while Janssen et al. (2023) also found more female participants than male participants (15). We attribute the higher proportion of female participants in our study to the specific composition of our sample.

Additionally, we interpret the predominance of female participants in our study as reflective of the healthcare sector, where women are more prominently represented. In addition, almost two-thirds of the participants in the study were under the age of 24. This was due to the fact that the Paramedic profession was a new profession in our country. And we think that this is the reason why the majority of the participants are single.

When analyzing attitudes toward physical activity based on gender, it was observed that male participants achieved significantly higher mean scores in the total scale score, SY subscale score, and ASY subscale score compared to female participants. Similar findings were noted by Togo and Öztürk (2020), who also found that male participants had higher average scores across the total scale and all subscales (16). Moreover, Özsarı et al. (2022) reported higher total scale scores among male participants than females (14). Similarly, Gökdağ (2019) Compared to women, men received higher scores in both the scale total scores and subscales (17). In contrast to these studies, Konar and colleagues (2024) reported that women's success in

Table 4. Comparison of Scale and Subscale Scores by Participants' Education Level and Position (ANOVA Test)

	Total Scale Point				IS				SL				ASP		
	x̄±SD	F	p	Difference	x̄±SD	F	p	Difference	x̄±SD	F	p	Difference	x̄±SD	F	p
Educational Status															
1. High School	72.17±25.35				39.56±13.44				20.30±7.58				12.30±5.97		
2. Associate degree	78.05±25.46				42.01±13.37				21.92±7.82				14.13±5.77		
3. Bachelor's Degree	82.97±26.03				44.47±13.72				23.64±8.05				15.85±6.01		
4. Master's Degree	89.56±18.89	2.944	0.033	4>1.2	48.84±10.71	3.149	0.026	4>1.2	25.01±5.39	2.568	0.045	4>1.2	15.71±5.11	1.810	0.146
Occupation															
1. Intern Paramedic	77.83±25.75				42.16±13.42				21.80±7.97				13.87±5.83		
2. Paramedic	81.56±25.22				43.28±13.65				22.92±7.71				15.36±6.29		
3. Intern Nurse	78.01±34.23				42.44±17.60				21.55±9.92				14.01±7.14		
4. Nurse	85.38±25.14				45.94±13.11				24.22±7.71				15.22±6.37		
5. Doctor	87.67±21.37				47.32±11.93				24.37±6.33				15.97±4.98		
6. Other	75.25±23.91	2.148	0.048	5>1.3	40.47±13.02	1.627	0.153		21.77±7.52	1.143	0.338		13.01±5.18	1.637	0.150

*$p < 0.005$, ANOVA test

improving health-related quality of life and controlling body fat percentage increases their desire to engage in sports and exercise (18).

These outcomes may indicate that females perceive their attitudes towards sports less positively than males. In addition, although the average score for the interest in sports subscale was higher among male participants, it was not significant. In the study conducted by Varol et al. (2017), the average score for the interest in sports subscale was higher among male participants (19). Tomik et al. (2012) found in their study with 2,704 participants in Poland that male participants showed a higher interest in sports compared to female participants (20). The reason for the lack of a difference in the interest in sports subscale with respect to gender in this study might be due to the postponement of social activities like sports due to intense work conditions.

In our study, age did not significantly influence attitudes towards sports ($p > 0.05$). This finding supports the reported results by Togo and Öztürk (2020) and Özsarı et al. (2022) (16), (14). However, Gökdağ (2019) found significant differences in total scale and subscale scores between younger and older participants, with younger participants scoring higher (17). Gökdağ's study predominantly involved younger participants, who typically have more energy and are more physically active (17). This may encourage them to engage in regular physical activity. In this study, although the subscales of interest in sports, living with sports, and active participation in sports had higher averages in the older age group, no significant difference was found. Similarly, Varol et al. (2017) found that the age variable did not create a significant difference in the scale subdimensions (19). Tatar and Kuru's (2009) study, which examined factors affecting sports participation among women aged 20-40 who were either employed or unemployed, found that age was not a significant factor in sports participation among the women surveyed (21).

Research by Rullestad et al. (2021) indicates that sports are a popular social activity among young people, and engaging in sports with friends enhances their positive attitudes towards sports (22). However, in our study, we did not observe a significant difference between the participants according to age; there may be many reasons for this. In our opinion, intensive working conditions, lack of time for sports, and a sample with a similar age group may be the reasons for this situation.

The scale total score and IS subscale mean scores of married participants were significantly higher than those of single participants. This finding is consistent with the results reported by Wood et al. (2019), who found that married individuals are more inclined towards sports than singles (23). However, Togo and Öztürk (2020) did not find significant differences in total scale score and subscale scores between married and single individuals regarding marital status (16). We speculate that the more excellent family and childcare responsibilities among married individuals may limit the time they allocate to sports. Although the average scores for the sub-dimensions of sports lifestyle and active sports participation are higher among married participants, this result is not statistically significant. Sobal and Hanson (2010) found that married individuals participate less in active sports compared to singles, but they expend more energy on overall physical activity (such as household chores and leisure-time sports) (24). According to a study by Taniguchi and colleagues (2012), having a child under the age of 18 affects the relationship between marital status and physical activity. The study found that there was no change in attitudes towards sports between married and single participants, but participants with children under 18 showed a decrease in their attitudes towards sports (25).

In the Janssen et al. (2023) study, when the relationship between education and scale scores was evaluated, nurses scored higher than doctors (15). Highly educated individuals tend to be more aware of health issues and better understand the positive effects of regular exercise on health. This awareness leads them to embrace sports as part of their lifestyle and prioritize its importance (26). In the study by Togo and Öztürk (2020), no significant difference was observed in the subscale scores of SYTO according to education level. (16). However, Gümüşay (2020) determined that the level of education makes a significant difference on the Attitude Scale towards Sports. Mainly, healthcare professionals with a high school diploma showed a higher perception of attitude toward sports (27). Our study observed significant differences in the total scale score and all subscales except ASP based on education level and title variables. Postgraduate education participants scored higher than those with high school and associate degree education. We think individuals with higher education levels attach more importance to sports to improve their general health and quality of life. In our study, the sub-dimension of active sports participation did not show a significant difference according to the level of education variable. However, participants with a university education had higher average scores. Similarly, no significant difference was found between the sub-dimension of active sports participation and the participants' title variable. There may be several reasons why university education creates awareness of sports among emergency service workers. It may be thought that the age of the individuals during the education may have made it easier for them to perceive physical activity. Or it may be due to the fact that postgraduate education directs individuals to research. As individuals conduct research, they may have understood the importance of physical activity.

This study examined the attitudes of emergency service workers towards sports. In this context, this study revealed that the attitudes of emergency service workers towards sports are low. We believe that this study will create awareness among emergency service workers.

Limitations
The study's limitations include its execution solely within a single medical faculty and the restricted generalizability of findings to other healthcare institutions. There may also be differences across cities. Therefore, the results of the study may vary in different cities. This raises uncertainty about how the study's conclusions could be applied to other healthcare settings. Future research should examine the long-term effects of physical activity on work performance and the benefits of various sports. This approach could lead to a more comprehensive understanding of how physical activity impacts healthcare professionals' quality of life and job performance.

CONCLUSION
As a result, this study has identified significant demographic factors influencing the attitudes of emergency department staff toward sports. Younger age groups and higher levels of education mainly support positive attitudes towards sports. To promote physical activity within the healthcare sector, the following recommendations are suggested:
Enhancement of Corporate Support and Healthy Lifestyle Policies: Healthcare institutions should adopt and implement policies that promote healthy living. Work environments that encourage sports

J Basic Clin Health Sci 2024; 8: 675-682

Alkas B et al. An Example of Intensive and Stressful Work Environment

participation should be established. Organization of Education and Awareness Programs: Regular training sessions on the health benefits of sports should be provided to healthcare professionals, alongside awareness programs emphasizing the positive effects of sports on health and job performance. Integration of Sports into Health Programs, Sports activities should be integrated into health programs, with adequate employee support. For instance, healthcare workers should be given time and resources to engage in sports. These recommendations aim to enhance overall health and job performance by increasing physical activity among healthcare professionals. Morover, Sports materials, halls, showers, changing rooms, etc. hygienic spaces can be provided. Coach support can be provided through the Provincial Directorates of Youth and Sports for conscious sports. Pre-test and post-test measurements (such as fat percentage, circumference measurement) can be taken to ensure motivation of the participants. Dietician support can be provided in the hospital for conscious nutrition.

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EFFECTS OF TRUNK CONTROL, UPPER AND LOWER EXTREMITY FUNCTIONS ON FUNCTIONAL ACTIVITY, ACTIVITIES OF DAILY LIVING AND QUALITY OF LIFE IN PARKINSON'S DISEASE

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ABSTRACT

Purpose: To investigate the relationship between trunk control, upper and lower extremity function with functional activity, activities of daily living and quality of life in patients with Parkinson's Disease (PwPD).
Materials and Methods: The study was completed with 52 PwPD. Trunk control was evaluated with the Trunk Impairment Scale (TIS); upper extremity function with 9-Hole Peg Test (9-HPT) bilaterally; lower extremity function with 30-Second Sit-To-Stand Test (30STS); functional activity level with The Parkinson Activity Scale (PAS); activities of daily living with Barthel Index (BI); quality of life with The Parkinson's Disease Quality of Life Questionnaire (PDQ-39)
Results: 30STS and right-left 9-HPT results was correlated with PAS and PDQ-39, but not with BI in PwPD. All TIS scores except the coordination sub-parameter were found to be associated with PAS, all TIS scores except the static sitting balance sub-parameter were found to be associated with BI, and all TIS scores were found to be associated with PDQ-39 in PwPD (ρ between 0.293 and 0.689; $p<0.05$ for all).
Conclusion: Physical activity level and quality of life were associated with upper and lower extremity function and trunk control, whereas activities of daily living were only associated with trunk control in PwPD. These results suggest that physiotherapy and rehabilitation applications that will improve trunk control in addition to limb functions should be included in treatment protocols.

Keywords: Parkinson's Disease, trunk control, extremity function, activity level, daily living activity, quality of life

J Basic Clin Health Sci 2024; 8: 683-691

Ozkan T et al. Effects of Trunk Control and Extremity Functions

INTRODUCTION

In progressive Parkinson's Disease (PD), early motor problems such as tremor, rigidity, akinetic components and postural instability and late complications such as fluctuations, dyskinesias and behavioural disorders lead to serious deficiencies in all functional activities of patients (1). Tremor is the most common and most easily recognised finding of PD. It usually has unilateral onset. Although it is mostly seen in the hands, it may also occur in the feet, legs, arms, jaw, lips and tongue. In addition, postural and action tremor may also be observed, which adversely affects trunk control and balance. In addition to tremor, bradykinesia causes difficulty or decreased speed of finger, wrist, arm and leg movements. Bradykinesia primarily involves distal muscle groups, followed by proximal groups. Distal finger muscles are affected, resulting in difficulty in fine skills. Thus, activities such as cutting or holding food, writing, getting out of bed, dressing, bathing are affected, causing people to become dependent in their daily lives over time. Rigidity problem, which occurs in the later stages of the disease, is mostly seen in the wrist, but it also affects the neck, trunk, hip, shoulder and other joints. (2, 3). Postural instability, which is one of the motor problems of the disease, starts in the early-middle stages and increases in the following periods and adversely affects motor control (4). Motor symptoms and physical disabilities that also occur in PD limit the activities of daily living and affect the quality of life (5-7).

Functional activities of patients with PD (PwPD) are negatively affected due to rigidity, bradykinesia, loss of postural reflexes and postural instability. When the literature is analysed; it is known that upper and lower extremity function and trunk are affected activities of daily living, functional activity level and quality of life are decreased in PwPD (6-10). Choi et al. reported a positive correlation between manual dexterity and activities of daily living in PwPD (6). Stewart et al. reported that upper and lower extremity motor impairments were associated with quality of life and activities of daily living in idiopathic PD (7). The study's findings imply that manual dexterity has a significant role in predicting PwPD' physical performance in day-to-day activities. In a study of 101 PwPD, it was reported that 81% of the respondents reported difficulty in getting up from a chair and that lower extremity motor function may be affected (8). While the research has examined the impact of lower and upper extremity functions on daily living activities in PwPD, the trunk has not received enough attention. (9, 10). According to Verheyden et al., PwPD had considerably poorer scores on the trunk control scale overall score, static sitting balance subscale, and coordination subscale when compared to healthy controls (9). In the study conducted by Ünlüer et al. position sense of the trunk was evaluated in PwPD and as a result, it was shown that trunk position sense was associated with disease level, fear of falling, functional mobility, and balance (10). Although upper and lower extremity functions were analysed together or separately in these studies (6-10), the trunk remained in the background. It is important that the trunk is analysed and its effects on upper and lower extremity functions are considered together and what kind of contributions it may make to the functionality, independence and quality of life of the patients.

Nevertheless, studies on the effect of upper and lower limb function, especially the trunk, on functional activity level, quality of life and activities of daily living are limited. Masaki et al. examined the relationship between the masses and amounts of non-contractile intramuscular tissue in the trunk and lower extremity muscles and activities of daily living, mobility and balance ability and disease symptoms in PwPD (11). As a result of the study, it was reported that the characteristics of trunk and lower extremity muscles in PwPD may be critical in terms of activities of daily living, mobility and balance ability and disease symptoms. However, the use of easily applicable, accessible and cost-effective tests in our study will facilitate therapists in the evaluation of the performance of PwPD in the clinic. Examining the relationships between functional activity level, activities of daily living, and quality of life with trunk control, upper and lower extremity function in PwPD is the purpose of this research.

MATERIALS AND METHODS
Study design

This cross-sectional study was carried out at the Ankara City Hospital's Neurology Clinic between December 2023 and February 2024. The University Ethics Committee approved the study (Date: 23.11.2023 Decision No: 09-407) and the Declaration of Helsinki's tenets were adhered to in this investigation. Before beginning the study, each

participant completed a written informed consent form after learning about its goal.

Participants

Patients aged 40 years and older who had been diagnosed with PD by a neurologist using the Movement Disorder Society (MDS) diagnostic criteria and who were between stages 1-4 of the Hoehn & Yahr staging scale were included in the study. Patients with neurological diseases other than PD (MS, stroke, etc.) were not allowed to participate in the study. Every patients were medically evaluated in "on" periods (1-1.5 hours after taking the drugs).

Measurements

Age, height, weight, duration of the disease, body mass index, falling and freezing history were recorded. The following question's answer was used to determine the falling history: "Have you fallen in the last 6 months?" An unexpected event in which a person lands on the ground, a floor, or a lower level was defined as a fall (12). Patients with PD were classified as fallers if they reported one or more falls. Freezing of gait was defined by an episodic block to generating effective stepping despite the intention to walk and freezing history of the patients in the last 6 months was recorded (13). Disease stage was performed by a neurologist and other assessments were performed by a physiotherapist. Two-minute rest breaks were given during the evaluation. During the tests, the patient was stood next to the patient for safety.

Disease stage was evaluated with Hoehn & Yahr Scale (HYS). HYS analyses PD in 5 stages. Stage 1-2 is classified as mild, stage 3 as moderate and stage 4-5 as severe disability (14). The increase in the stage of the disease indicates that the disease progresses and its severity increases. Patients with stages 1-4 according to HYS were determined by the neurologist and referred to the physiotherapist for evaluation.

The Trunk Impairment Scale (TIS) was used to assess trunk control. There are 3 sections and 17 parameters in the scale. The sections of test are coordination, dynamic sitting balance, and static sitting balance. There is a minimum of 0 and a maximum of 23 points for the overall score. Higher score is considered to indicate better performance (9).

The 9-Hole Peg Test (9-HPT) was used to assess upper extremity function. 9-HPT is a hand dexterity test, was used to evaluate upper extremity function. There is a platform with nine holes and nine sticks in the test. In order to conduct the test, the platform is positioned immediately in front of the patients, and the holes are on the non-dominant hand side and the rods on the dominant hand side. The chance to practice before the test is offered to the persons following an explanation of the test guidelines. Patients are instructed to quickly place the rods on the board. Then, they are instructed to take out nine rods with the same hand, one at a time, and the time is noted. The test was repeated for the nondominant side with the same method (15). For every side, the test was repeated twice, and the mean time was noted in seconds.

Lower extremity function was assessed with the 30-Second Sit-To-Stand Test (30STS). During the test, patients were instructed to sit on a chair (approximately 48 cm high) with their feet on the floor and arms crossed in front of their chest (to prevent their arms from being pushed when coming to an upright standing position), and then quickly and safely stand up and sit down for 30 seconds. The assessor provides standardised instructions and demonstrates several repetitions of standing up without sitting down to avoid any misunderstanding; it is emphasised that patients should finish the standing position by bringing their hips and knees to full extension before starting to descend to sit down again. (16). The exact number of repetitions (i.e. standing up without sitting down) was recorded as the outcome.

Functional activity level was assessed with the Parkinson Activity Scale (PAS). PAS, a tool designed to evaluate functional activities in patients with PD, offers data regarding patient's transfer status. The scale includes subsections for things like gait akinesia, in-bed mobilization, and getting out of a chair. The scale consists of a total of 10 questions and each question is scored between 0-4. A higher score denotes better functional performance (17).

The Barthel Index (BI) was used to assess daily living activities (BI). The scale assesses the dependency and affectivity in activities of daily living and asks about feeding, bathing, using the toilet, bowel care, self-care, bladder care, dressing-dressing, transferring from the wheelchair, walking

J Basic Clin Health Sci 2024; 8: 683-691

Ozkan T. et al. Effects of Trunk Control and Extremity Functions

on smooth ground and being able to use a wheelchair. A score of 0–20 indicates complete dependence, 21–61 indicates highly dependence, 62–90 indicates moderate dependence, 91–99 indicates mildly dependence, and 100 indicates complete independence (18). The questions were answered by the patient and the score was recorded. The Parkinson's Disease Quality of Life Questionnaire (PDQ-39) was used to assess quality of life. The PDQ-39 is a self-report questionnaire that measures quality of life using a 5-point Likert scale. It evaluates mobility symptoms, emotional health, daily living activities, social support, cognitive function, and communication. The total score ranges from 0 to 100. A higher number denotes a lesser quality of life (19). PDQ-39 is valid and reliable for assessing quality of life in patients with PD (20, 21).

Statistical analysis

The software tool G*Power (Version 3.1.9.7, Franz Faul, Universität Kiel, Germany) was utilized to determine the necessary sample size for the research. Using the research data, which included 52 total sample numbers, the computation yielded the following results: a correlation value of -0.566 (coefficient of correlation between PAS and nondominant 9-HPT) and a power of 0.99 with a 5% margin of error (a=0.05) for the correlation analysis. The statistical program Statistical Package for Social Sciences (SPSS) version 20 was used to examine the data statistically. Using the Kolmogorov-Smirnov test, the normality of numerical variables was assessed. For numerical variables, we used the median (IQR25–75) and mean±standard deviation values; for categorical variables, we used the number and percentage values. For variables that had a normal distribution, Pearson correlation analysis was employed; for those that did not, Spearman correlation analysis was used. The very good correlation ranged from 0.81 to 1.00, good correlation from 0.61 to 0.80, moderate correlation from 0.41 to 0.60, fair correlation from 0.21 to 0.40, and poor correlation from 0.00 to 0.20, as determined by the correlation coefficient. Statistical significance level was accepted as p<0.05 (22).

RESULTS

Five PwPD who did not match the inclusion criteria were not included in the study, which involved screening fifty-seven patients.

Table 1 lists the demographics of PwPD, including age, height, body weight, body mass index, gender, and clinical parameters such as duration of illness, disease stage, and history of falling and freezing.

Table 2 displays the test findings for the patients' upper and lower extremity function, trunk control, physical activity level, activities of daily living, and quality of life.

30STS was moderately correlated with PAS and PDQ-39, but not with BI in PwPD (Table 3).

Right and left 9-HPT was moderately correlated with PAS and PDQ-39, but not with BI in PwPD (Table 3).

All TIS scores except the coordination sub-parameter were found to be associated with PAS between fair to good levels, all TIS scores except the static sitting balance sub-parameter were found to be associated with BI at fair level, and all TIS scores were found to be associated with PDQ-39 between fair to moderate levels in PwPD (Table 3).

DISCUSSION

We observed that physical activity level and quality of life were associated with upper and lower extremity function and trunk control, whereas activities of daily living were only associated with trunk control in PwPD in this study.

Amara et al. stated that physical activity level decreased with the progression of disease in PwPD. Higher self-reported physical activity levels are related to less advancement of motor symptoms, cognitive decline, daily living activities, depression, anxiety, and better sleep, according to exploratory analyses (5). According to research by Skidmore et al., PwPD who had more gait impairment and postural instability also had lower functional ambulation (23). Galperin et al. found that, as predicted, physical activity in daily life, dynamic balancing tests performed in a lab, subject characteristics, and demographics were related to the severity of motor symptoms in PwPD, accounting for about half of the variation in motor symptom severity (24). Wu et al. provided supporting evidence that advanced dynamic

balance is significantly associated with both physical activity participation and health-related quality of life in PwPD (25). These findings are noteworthy because they suggest that the level of physical activity may have an impact on trunk control, which is crucial for preserving balance. The PAS, which we used to assess the level of physical activity in our study, evaluates functions such as getting up from a chair, walking, turning, lying on the bed, turning in bed, getting out of bed and standing up (17). Upper and lower extremity function is the main factor in all these evaluated activities. Therefore, the relationship observed in our study is expected.

Table 1. Demographic and clinical characteristics of patients with PD

Age, years, *Mean±SD*		64±10
Height, m, *Mean±SD*		1.66±0.09
Body weight, kg, *Mean±SD*		78±12
BMI, kg/m^2 *, Mean±SD*		28.38±4.02
Gender, female/male, *n (%)*		16 (30.8) / 36 (69.2)
Disease stage (HYS), *Median (IQR25-75)*		1.5 (1-2)
Duration of illness, years, *Median (IQR25-75)*		6 (3-10)
Falling history, present/absent, *n (%)*		24 (46.2) / 28 (53.8)
Freezing history, present/absent, *n (%)*		18 (34.6) / 34 (65.4)
Disease stage (HYS), n (%)	1	32 (61.5)
	2	12 (23.1)
	3	6 (11.5)
	4	2 (3.8)

BMI: Body Mass Index; HYS: Hoehn and Yahr Scale; SD: Standard deviation; m: meter; kg: kilogram

Table 2. Lower and upper extremity functions, trunk control, physical activity level, activities of daily living and quality of life test results of patients with Parkinson's Disease

		Patients with Parkinson's Disease (n= 52) Median (IQR25-75)
30STS, second		11 (9-13)
9-HPT, second	Dominant	27.13 (23.82-33)
	Nondominant	30.16 (25.77-36.94)
TIS, score	Static seating balance	7 (3-7)
	Dynamic seating balance	6 (4-8)
	Coordination	4 (2-6)
	Total	15 (11-19)
PAS, score		34 (24-38)
Barthel Index, score		95 (88-100)
PDQ-39, score		27 (8-48)

30STS: 30-Second Sit-To-Stand Test; 9-HPT: 9-Hole Peg Test; TIS: Trunk Impairment Scale; PAS: Parkinson Activity Scale; PDQ-39: Parkinson's Disease Quality of Life Questionnaire

Table 3. The relationship of upper and lower extremity function and trunk control with functional activity, activities of daily living and quality of life in patients with Parkinson's disease

		PAS		Barthel Index		PDQ-39	
		ρ	p	ρ	p	ρ	p
30STS		0.452	**0.001**	0.257	0.066	-0.461	**0.001**
9-HPT	Dominant	-0.566	**<0.001**	-0.244	0.085	0.526	**<0.001**
	Nondominant	-0.495	**<0.001**	-0.222	0.113	0.471	**<0.001**
TIS	Static seating balance	0.330	**0.017**	0.223	0.111	-0.372	**0.007**
	Dynamic seating balance	0.689	**<0.001**	0.293	**0.035**	-0.502	**<0.001**
	Coordination	0.236	0.093	0.332	**0.016**	-0.472	**<0.001**
	Total	0.564	**<0.001**	0.355	**0.010**	-0.577	**<0.001**

p<0.05; 30STS: 30-Second Sit-To-Stand Test; 9-HPT: Nine Hole Peg Test; TIS: Trunk Impairment Scale; PAS: Parkinson Activity Scale; PDQ-39: Parkinson's Disease Quality of Life Questionnaire; *ρ:* Spearman's correlation coefficient

J Basic Clin Health Sci 2024; 8: 683-691

Ozkan T. et al. Effects of Trunk Control and Extremity Functions

To the best of our knowledge, however, this study is the first to demonstrate the connection between trunk control and level of physical activity. It is known that trunk control and trunk position sense are decreased in PwPD and these parameters are associated with disease severity, balance, functional mobility, and fear of falling (10, 26). It is known that the trunk is an important factor for both balance and mobility during functional activities. Our results suggest that decreased physical activity level may be multifactorial and may be affected by both upper and lower extremity function and trunk control.

Daily living activities include activities such as feeding, washing, self-care, dressing, personal hygiene and toileting, transferring, mobility, walking up and down stairs (18). Manual dexterity is a significant predictor of daily physical performance in PwPD, according to Choi et al (6). Stewart et al. reported that upper and lower extremity motor impairments were associated with daily living activities and quality of life in idiopathic PD (7). However, there was no discernible correlation between daily living activities and the function of the upper and lower extremities in our investigation. If we examine the results of the activities of daily living assessment of PwPD in our study, it is seen that the median value of the BI test results is 95. The results of activities of daily living show that PwPD are mildly dependent. In the early period, PwPD mostly have unilateral symptoms. As the disease progresses, symptoms are observed bilaterally and daily living activity limitations increase (27). We think that this may be the reason why limb functions are not related with activities of daily living. These results suggest that there may be more limitations in daily activities as the disease progresses. Our study's findings, however, point to a possible weak correlation between trunk control and everyday life activities. According to Ryan et al., there is a positive correlation between the degree of thoracic kyphosis and the inability to do daily tasks. (27). Studies have also shown that the flexion and/or lateral flexion posture of the trunk seen in PwPD negatively affects the balance and gait skills of the patients and causes them to experience difficulties in daily life (26, 28). In patients with mild to moderate PD, axial deficits relatedto gait abnormalities, postural instability, and trunk rigidity have been found to be highly associated with disability and poor health-related quality of life. Furthermore, it

has been discovered that there is a substantial correlation between the axial motor aspects of PD and physical inactivity, a decline in daily living capacity, and an increase in the dependence on daily living activities. (29-31). Our findings imply that, even in early-stage PwPD, trunk control may have an impact on the decline in daily living activities.

An essential function of the trunk is to lessen the forces brought on by motions that endanger the trunk's postural control system. As PD advances, patients frequently experience dopamine-resistant axial symptoms that impede trunk control and raise the risk of falls (29). Schrag et al. showed that disability, postural instability, cognitive impairment and depression had the greatest impact on quality of life scores in patients with akinetic rigid PD compared to those with tremor dominant disease, and the main reason for this was the deterioration of axial features (32). According to Muslimovic et al., self-report measures of mood state and axial impairment (gait difficulty and postural instability) are the primary predictors of low quality of life (31). Broderick et al. showed that impaired upper limb function is associated with reduced quality of life (33). Quality of life related to mobility and daily living activities correlated with low- and medium-complexity lower-extremity tasks and the complex upper-extremity test, respectively (34). These findings imply that motor difficulties significantly and adversely affect PwPD' quality of life. According to Wong-Yu et al., manual dexterity in PwPD was linked to self-perceived hand functions and health-related quality of life (35). Better quadriceps muscle performance has been related to improved quality of life, clinical status, and balance in PD, according to research by de Almeida Sá et al (36). According to Cano-de-la-Cuerda et al., PwPD' functional level (daily living activities) and quality of life are affected by axial motor deficits (30). In an another study, Cano-de-la-Cuerda et al. trunk flexion and extension movements were relate to axial extensors rigidity and functional mobility and trunk range of motion for trunk extension, rotations, and flexion were related to health related quality of life in those with milf to moderate PD (37). Ayvat et al. showed that trunk control was associated with quality of life in PwPD (26). All these results are consistent with the findings of our study. Considering that activities of daily living are associated with decreased trunk control, it is thought that trunk control has an indirect

effect on decreased quality of life by affecting activities of daily living as well as limb function.

In our study, the holistic approach to PD and the evaluation of both lower and upper extremity functions as well as the trunk, which is the central key point of the body, is a strong aspect of our study. In addition, there are some limitations to our study. The first limitation is that the Movement Disorders Society-Unified Parkinson's Disease Rating Scale, which is used to evaluate the severity of the disease in PwPD, was not used. Second, because the tests and scales were administered while PwPD were in the "on" state, the results of the tests and scales were not understood when PwPD were in the "off" state.

CONCLUSION

Our study provides evidence that physical activity level and quality of life were associated with upper and lower extremity function and trunk control, whereas activities of daily living were only associated with trunk control in PwPD. Therefore, it is thought that detailed evaluation of upper and lower extremity functions and trunk control and appropriate rehabilitation approaches may be important in improving physical activity level and quality of life, and that the decrease in trunk control may affect daily living activities in PwPD.

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J Basic Clin Health Sci 2024; 8: 683-691

Ozkan T. et al. Effects of Trunk Control and Extremity Functions

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A PAIRED-MATCH STUDY: EQUINE ATHLETES' NUTRITIONAL HABITS, ANTHROPOMETRIC MEASUREMENTS, AND POSTURES

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ABSTRACT

Background and Purpose: The Mediterranean diet-MeD provides beneficial nutrients that improve athletes' health. Posture is important for the performance of equine athletes-EA. This study aimed to determine adherence to the MeD, anthropometric measurements, and postures of EA.
Methods: There were two study groups, EA(n:121) and non-athletes(n:121). The Mediterranean Diet Quality Index, the Mediterranean Diet Adherence Screener, the Food Frequency Questionnaire, the New York Posture Analyses-NYPA, an electronic scale, and a non-stretch tape were used.
Results: EA's MeD-characterized food consumption (vegetables, fruits, fish) frequencies were more compatible with recommendations($p<0.05$). Whereas body height, body mass index-BMI, and waist circumference were not related to NYPA scores in non-athletes, negative correlations were found in EA($p<0.05$). 62.8% of riders had moderate and high adherence to the MeD, with children performing better than adults($p:0.003$). The prevalence of preobesity/obesity was higher among adult riders($p:0.008$). Professional adult riders had a lower health risk based on their waist circumferences than amateurs($p:0.036$).
Conclusion: Some anthropometric measurements showed a negative association with riders' postures. Although there was no statistical difference between the MeD adaptations and BMI classifications between study groups, the age group influenced these two variables in riders. Equestrian sports subgroups were also found to have an impact on athlete health.

Key-words: Equestrian sports; nutrition; posture; anthropometric measurements

INTRODUCTION

Horses have been a part of human life throughout history. They were one of the most important means of transportation and played an important role in wars and agriculture in the past. And today; their role in human life is more emotional and professional. There is an Olympic sports category called equestrian events. Show jumping is one of the three Olympic equestrian sports (1,2). The popularity of equestrian sports continues to increase. Equestrian sports are one of the rare sports types performed by two different types of living beings. Although the risk of accidents and injuries is potentially higher than in other sports, equestrian sports positively affect human physical and mental health (3). Performance success in equestrian sports depends not only on the

performance of horses but also on the rider's anthropometric measurements, muscle strength, nutritional behavior, etc (4,5). The body weight of equine athletes is one of the important anthropometric measurements that can influence performance (6). The Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine have published a position paper indicating that healthy eating habits and normal body composition have a very positive impact on athletic performance (7). As a balanced nutritional model, the Mediterranean diet can provide performance-relevant nutrients and dietary components, such as vitamins, minerals, and other antioxidants, for athletes. In addition, the use of the Mediterranean diet has gained worldwide acceptance (8). With this in mind, the present study aimed to investigate Mediterranean diet compliance, dietary habits, anthropometric measurements, and posture in licensed riders of the North Cyprus Equestrian Federation. All results were compared with those of a non-athlete group. This present study is the first to pursue this objective in the northern part of Cyprus and it is also one of the few studies in the literature on this topic.

MATERIALS AND METHODS
Participants
The study was conducted from July to December 2022 in the northern part of Cyprus. In the present study, there were two groups of participants, the equine athletes' group and the non-athletes. The equine athlete group consisted of licensed riders from the North Cyprus Equestrian Federation. The Federation is under the Ministry of National Education, Youth, and Sports of the northern part of Cyprus and there are nine riding clubs under the federation. There was a total of 152 registered (licensed) active riders (6-64 aged), who ride at least once a week, in these nine riding clubs. All riding clubs and riders were licensed in only show jumping. Due to the size of the population, the present study was not conducted with a random sample, but the entire population (N: 152) was invited to participate in the study. Participation in this study was voluntary and all adult participants and parents of child participants signed a consent form. Because some riders (n: 31) did not want to participate, the present study was conducted with 121 adult and child riders. This study was approved and registered by the Scientific Research Ethics Committee of Near East

University (Date: 30.06.2022, Decision No: 2022/104-1574).

Procedures
In this paired-match study, all nine riding clubs were visited a few times during this period and nutrition-related data were collected by dietitians, and posture-related data by a physiotherapist. The non-athlete group consisted of people who were not athletes in any type of sport. The non-athletes were selected by the researchers according to the equine athlete group and the 'individual matching/matched pair design' method was used (9). The inclusion criteria for both groups are shown in Figure 1. The data were collected in the face-to-face interviews conducted by researchers. The Mediterranean Diet Quality Index (KIDMED) was used by dietitians for children and the Mediterranean Diet Adherence Screener (MEDAS) for adults to determine dietary adherence across all age groups. Both questionnaires provided the same categories (low-moderate-high adaptation) as a result. The Food Frequency Questionnaire (FFQ) was used to determine all participants' food consumption frequencies. The New York Posture Analysis (NYPA) was used by the physiotherapist researcher to determine the postural status of the participants. Moreover, the dietitian researchers determined the anthropometric measurements of the participants, such as height, body weight, and waist circumference. All of the data collection material is shown as a summary in 1.

Mediterranean Diet Quality Index (KIDMED)
The KIDMED was developed by Serra-Majem et al. in English to assess children's adherence to the Mediterranean diet (10). Kaya and Temiz (2021) validated the questionnaire in Turkish (11). The KIDMED contains 12 questions compatible with the Mediterranean diet, and four incompatible questions for a total of 16 questions. Each positive response to the compatible questions yields +1 point, and adherence to incompatible questions yields -1 point. A score of ≥eight points indicates 'high' compliance with the Mediterranean diet, four-seven 'moderate', and ≤three 'low'(10).

Mediterranean Diet Adherence Screener (MEDAS)
Martínez-González et al. (2012) developed the MEDAS for assessing adherence to the Mediterranean diet in adults. This questionnaire includes 14 questions and each positive response to
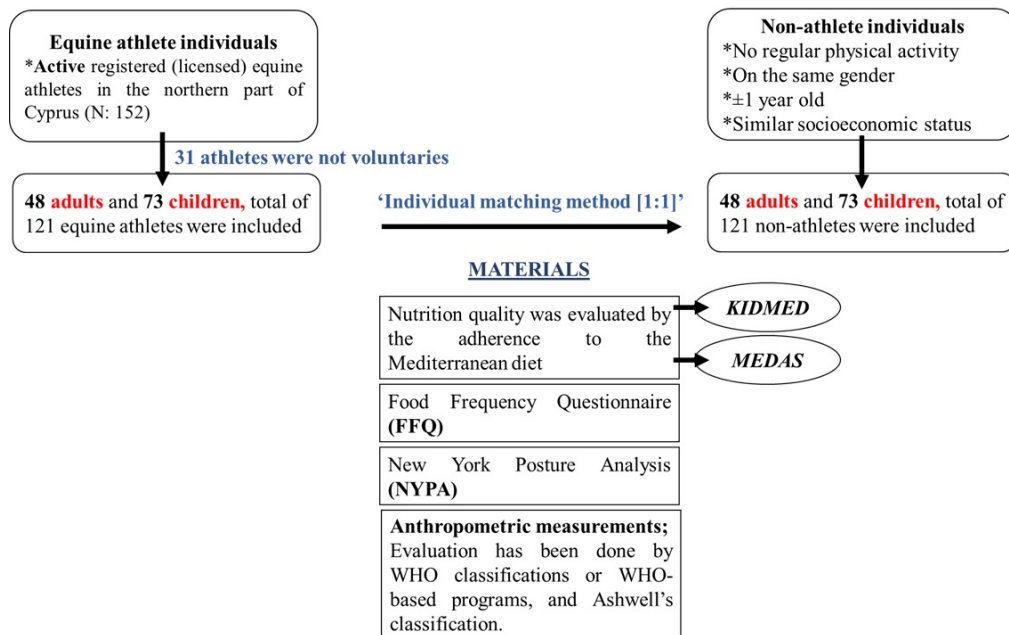
Figure 1. Study design, participant selection, and materials of data collection

all questions yields +1 point. If the participant scores ≥nine points, this indicates 'high', seven-eight 'moderate', and ≤six 'low' adherence to the Mediterranean diet (12). The Turkish validity-reliability study was done by Pehlivanoglu et al (13).

Food Frequency Questionnaire (FFQ)
The Likert-type FFQ was developed by the researchers and there were eight different response options for each food, such as 'every main meal', 'every day, 'one-two times/week', 'three-four times/week', 'five-six times/week', 'twice in a month', 'once in a month', and 'never'. The data from this section were used to assess the dietary habits of the participants according to the principles for optimal nutrition in the Dietary Guidelines for Turkey 2022 (14). According to this guideline, participants' consumption-related responses were categorized as 'recommended frequency' and 'not recommended frequency'.

New York Posture Analyses (NYPA)
The NYPA was published in 1958 and updated in 1992. This questionnaire assesses 13 different parts of the human body to determine the physical condition of various body segments. It is a visual scale and there are three different choices for each body segment, such as 'normal' (five points), 'moderately impaired' (three points), and 'severely impaired' (one point). ≥45 points of the NYPA total score indicate 'very good posture', 40-44 'good', 30-39 'moderate', 20-29 'poor', and ≤19 'very poor' (15,16).

Anthropometric Measurements
All measurements were performed correctly and in accordance with the methods. An electronic scale was used to measure the body weight of the participants (with 0.1 kg sensitivity). Furthermore, height and waist circumference were determined with a non-stretchable tape measure at the Frankfort plane. Body mass index (BMI) was calculated using the [(Body weight) ÷ (Body height)^2] formula. BMI values and waist circumferences of adult participants were evaluated according to the World Health Organization (WHO) classification (14), whereas BMI values of children were evaluated as percentiles using the WHO-Anthro plus package program. In addition, the ratio between waist circumference and height of the children was calculated and the Ashwell classification was used for evaluation. According to this classification, <0.4 signs as 'be careful', 0.4-0.5 'normal', 0.5-0.6 'think of action', and >0.6 'take action' to improve this ratio. As the ratio increases, the risk of childhood obesity increases (17).

Statistical Analysis
Data were analyzed by Statistical Package for the Social Sciences (SPSS) version 24.0. The mean (x̄), standard deviation (SD), and minimum and maximum

J Basic Clin Health Sci 2024; 8: 692-703

Dayi T et al. Horse riders' nutritional and physical features

Table 1. General backgrounds of participants and athletic characteristics of riders

General Backgrounds of Participants			
Age (year)			
Study Groups	**Mean±SD (Min-Max)**		
Equine athletes (n: 121)	20.43±11.88 (6.00-64.00)		
Non-athletes (n:121)	20.39±12.04 (7.00-65.00)		
	p_1: 0.664		
Gender			
	Equine athletes (n: 121)		**Non-athletes (n: 121)**
	n	**%**	**n**	**%**
Male	47	38.8	47	38.8
Female	74	61.2	74	61.2
Total	121	100.0	121	100.0
	p_2: 1.000			
Athletic Features of Riders			
Subcategory of equestrian sport			
	n	**%**	
Show jumping	82	67.8	
Flatwork	30	24.8	
Horseback safari	9	7.4	
Total	121	100.0	
Aim of the riders			
	n	**%**	
Professional (Competitive) riders	61	50.4	
Non-competitive riders	22	9.1	
Amateurs	38	31.4	
Total	121	100.0	
	Total training day/week Mean±SD (Min-Max)	**Total training duration (mnt)/training Mean±SD (Min-Max)**	
Professional riders (n: 61)	4.09±1.87 (1.00-7.00)	39.18±9.97 (15.00-60.00)	
Non-competitive riders (n: 22)	2.36±1.76 (1.00-7.00)	46.13±12.62 (20.00-60.00)	
Amateurs (n: 38)	2.50±1.65 (1.00-7.00)	38.82±14.65 (15.00-90.00)	
	p_3: **<0.001**	p_4: **0.049**	

p_1: Wilcoxon Signed Rank test; p_2: McNemar test; p_3: One-Way ANOVA, Post Hoc test Benferroni-Group comparisons: Competitive-Non-competitive p: <0.001; Competitive-Amateur p: <0.001; p_4: One-Way ANOVA, Post Hoc test LSD-Group comparisons: Non-competitive-Competitive p: 0.022; Non-competitive-Amateur p: 0.026; Bold **p-value** shows statistical significance (p<0.05); SD: Standard deviation

values were used to represent quantitative variables. In addition, the number (n) and percentage (%) were used for categorical variables. For evaluation of normality, the Kolmogorov-Smirnov and Levene's tests were used. Due to the data being collected by individual matching method, study groups were accepted as dependent. So the Wilcoxon Signed Rank test was used to observe the relationship between the two dependent groups (e.g. study groups; equine athletes vs non-athletes) for the quantitative variables. In addition, for independent subgroups (e.g. professional riders vs others in the equine athlete group, adult riders vs children riders, etc.), the Independent Sample t-test was used. For the categorical variables, the McNemar test was used for dependent groups.

On the other hand, the Pearson Chi-square test was used to compare the independent subgroups when the percentage of cells with an expected value lower than five is less than 20% of the total number of cells. Moreover, the One-Way ANOVA test was used to compare quantitative data between three independent subgroups. The LSD and Bonferroni's Post Hoc tests were used to determine which groups were related. In addition, the data were not normally distributed, so the Spearman correlation test was used for the correlations. If the p-value is less than 0.05, this indicates a statistically significant difference.

RESULTS
The general backgrounds of all participants as equine athletes and non-athletes, and the athletic characteristics of the riders are shown in Table 1. Due to the study design, there were no statistical differences between the age and gender of the groups (p>0.05). The majority (67.8%) of equine athletes were licensed show jumpers, half of whom were professional (competitive) athletes (50.4%). When comparing the total training days per week and the duration of training in horse rider subgroups, statistical differences were found (respectively p<0.001; p<0.05). According to the results of the Post Hoc test, the professional riders trained more than others (non-competitive riders and amateurs) in a week, and the non-competitive riders had a longer training duration per ride than other groups (p<0.05). When comparing body weight, BMI, waist circumference, and waist circumference to the body-height ratio (for children only), no statistical differences were found between the study groups. Although the difference was not statistically significant, 58.3% of adults in the non-athlete group had a high or very high health risk based on their waist circumference and this value was higher than adults in the equine athlete group (47.9%) (p>0.05). Furthermore, there was no difference in the Mediterranean diet adjustment status between groups in both adult and child participants. In addition,

Table 2. Anthropometric measurements of the participants and adherence to the Mediterranean diet (n: 242)

	Anthropometric Measurements								
	BMI (kg/m²)			Body weight (kg)			Waist circumference (cm)		
	Equine athletes	Non-athletes		Equine athletes	Non-athletes		Equine athletes	Non-athletes	
	Mean±SD (MinMax)		p₁	Mean±SD (Min-Max)		p₁	Mean±SD (Min-Max)		p₁
Children (n: 73)	20.25±3.75 (13.23-30.81)	20.25±4.47 (12.73-40.06)	0.575	50.40±14.69 (17.50-91.00)	50.58±17.61 (23.20-108.20)	0.531	67.96±11.49 (24.00-95.00)	69.14±12.88 (23.00-110.00)	0.380
Adults (n: 48)	25.08±4.36 (16.72-37.28)	24.98±4.90 (17.57-41.20)	0.272	74.05±14.58 (47.20-109.00)	72.14±16.03 (46.00-132.00)	0.056	83.57±13.55 (61.00-126.00)	87.06±15.94 (57.00-143.00)	0.582
Total (n: 121)	22.17±4.63 (13.23-37.28)	22.13±5.18 (12.73-41.20)	0.747	59.78±18.65 (17.50-109.00)	59.13±19.97 (23.20-132.00)	0.466	74.15±14.49 (24.00-126.00)	76.26±16.62 (23.00-143.00)	0.309

Waist circumference classification of adults (n: 96)					Waist circumference-body weight ratio classification of children (n: 146)				
	Equine athletes (n: 48)		Non-athletes (n: 48)			Equine athletes (n: 73)		Non-athletes (n: 73)	
	n	%	n	%		n	%	n	%
Low health risk•	25	52.1	20	41.7	Healthy▫	52	71.2	53	72.6
High or very high health risk••	23	47.9	28	58.3	Unhealthy▫▫	21	28.8	20	27.4
Total	48	100.0	48	100.0	Total	73	100.0	73	100.0
p₂	0.332					1.000			

Adherence to the Mediterranean Diet												
	All participants (n: 242)				Adults (n: 96)				Children (n: 146)			
	Equine athletes (n: 121)		Non-athletes (n: 121)		Equine athletes (n: 48)		Non-athletes (n: 48)		Equine athlete (n: 73)		Non-athletes (n: 73)	
	n	%	n	%	n	%	n	%	n	%	n	%
Low	45	37.2	51	42.1	27	56.3	25	52.1	18	24.7	26	35.6
Moderate	57	47.1	54	44.6	15	31.3	16	33.3	42	55.5	38	52.1
High	19	15.7	16	13.2	6	12.5	7	14.6	13	17.8	9	12.3
Total	121	100.0	121	100.0	48	100.0	48	100.0	73	100.0	73	100.0
p₂	0.298				0.833				0.211			

Comparison of participants' BMI classifications and MEDAS/KIDMED scores				
	Adults (MEDAS)		Children (KIDMED)	
	Equine athletes (n: 48)	Non-athletes (n: 48)	Equine athletes (n: 73)	Non-athletes (n: 73)
	Mean±SD (Min-Max)		Mean±SD (Min-Max)	
Healthy BMI value**	6.39±1.85 (3.00-11.00) (n: 28)	6.17±2.21 (2.00-11.00) (n: 28)	5.34±2.69 (0.00-11.00) (n: 47)	4.62±2.78 (0.00-11.00) (n: 48)
Non-healthy BMI values* and ***	6.30±2.49 (1.00-11.00) (n: 20)	6.45±1.73 (2.00-9.00) (n: 20)	5.65±1.99 (2.00-10.00) (n: 26)	5.00±2.38 (0.00-8.00) (n: 25)
p₃	0.883	0.650	0.606	0.569

p₁: Wilcoxon Signed Rank test; p₂ McNemar test; p₃: Independent Sample t test; •<80 cm for females; <94cm for males; ••≥80 cm for females; ≥94 cm for males; ▫: 0.4-0.5 ratio; ▫▫<0.4 or >0.5 ratio; *≤18.4 kg/m² for adults, 3rd-15th percentiles for children; **18.5-24.9 kg/m² for adults, 15th-87th percentiles for children; *** ≥25.0 kg/m² for adults, ≥87th percentile for children; BMI: Body Mass Index; MEDAS; Mediterranean Diet Adherence Screener; KIDMED: Mediterranean Diet Quality Index

no association was found between the BMI classification and mean MEDAS/KIDMED scores. All related results are shown in Table 2.

Table 3 shows the same data from the comparison of rider subgroups. According to these results, there was statistical significance between the BMI classifications of child and adult riders. The prevalence of preobesity and obesity was higher in adults than in children (p: 0.008). Although the results were not statistically significant, the prevalence was lower in the professional riders than in the others (p>0.05). Waist circumferences of adult riders were compared between the professional riders and others. 70.0% of adult professionals had a low health risk as measured by their waist circumference. For the other riders, this value was 39.3% (p: 0.036). In addition, no statistical difference was found between the body-to-height ratio of the child professionals and other riders (p>0.05). When the adaptation status of the Mediterranean diet was compared between

children and adult riders, the statistical differences showed that children riders adhered to the Mediterranean diet better than adults (p: 0.003). Furthermore, there was no statistical association between BMI classification and MEDAS/KIDMED scores in equine athletes.

The frequency of consumption of some foods by participants is shown in Table 4. The equine athletes had slightly more compatibility with the recommended consumption frequencies for milk, yogurt, all types of cheeses, processed meat products, chocolate, sugar, and fast foods. Conversely, the consumption frequencies of red meat, poultry, egg, legumes, and carbonated drinks were slightly more compatible with the recommendations in the non-athletes. However, all these differences were not statistically significant (p>0.05). Statistical significance was found for fish, vegetables, and fresh fruit. The frequency of consumption of equine athletes was found to be more consistent with the recommendations (p<0.05).

Table 3. Anthropometric measurements of riders and adherence to the Mediterranean diet (n: 121)

	Anthropometric Measurements			
	Classification of BMI (n: 121)			
	Child riders (n: 73)		**Adult riders (n: 48)**	
	n	**%**	**n**	**%**
Thin*	11	15.1	1	2.1
Normal**	48	65.8	28	58.3
Preobese or obese**	14	19.2	19	39.6
Total	73	100.0	48	100.0
	p_1: **0.008**			
	Professionals (n: 61)		**Others (n: 60)**	
	n	**%**	**n**	**%**
Thin*	7	11.5	5	8.3
Normal**	43	70.5	33	55.0
Preobese or obese**	11	18.0	22	36.7
Total	61	100.0	60	100.0
	p_1: 0.070			

Waist circumference classification of adult riders (n: 48)					Waist circumference-body weight ratio classification of child riders (n: 73)				
	Professionals (n: 20)		**Others (n: 28)**			**Professionals (n: 41)**		**Others (n: 32)**	
	n	**%**	**n**	**%**		**n**	**%**	**n**	**%**
Low health risk•	14	70.0	11	39.3	Healthy□	29	70.7	23	71.9
High or very high health risk••	6	30.0	17	60.7	Unhealthy□□	12	29.3	9	28.1
Total	20	100.0	28	100.0	Total	41	100.0	32	100.0
p_1	**0.036**					0.915			

Comparison of Mediterranean diet adaptations between child-adult riders (n: 121)				
	Child riders (n: 73)		**Adult riders (n: 48)**	
Adaptation status	**n**	**%**	**n**	**%**
Low	19	26.0	27	56.3
Moderate and High	54	74.0	21	43.7
Total	73	100.00	48	100.0
	p_1: **0.003**			

Comparison of riders' BMI classifications and MEDAS/KIDMED scores				
	Adults (MEDAS)		**Children (KIDMED)**	
	Professionals (n: 20)	**Others (n: 28)**	**Professionals (n: 41)**	**Others (n: 32)**
	Mean±SD (Min-Max)			
Healthy BMI value**	6.26±1.27 (5.00-9.00) (n: 15)	6.53±2.40 (3.00-11.00) (n: 13)	5.88±2.66 (0.00-11.00) (n: 27)	4.60±2.62 (1.00-11.00) (n: 20)
Non-healthy BMI values* and ***	5.60±1.67 (4.00-8.00) (n: 5)	6.53±2.72 (1.00-11.00) (n: 15)	5.50±2.37 (2.00-10.00) (n: 14)	5.83±1.52 (4.00-8.00) (n: 12)
p_2	0.361	0.996	0.649	0.150

p_1: Pearson Chi-square test; p_2: Independent Sample t test; *≤18.4 kg/m^2 for adults, 3rd-15th percentiles for children; **18.5-24.9 kg/m^2 for adults, 15th-87th percentiles for children; *** ≥25.0 kg/m^2 for adults, ≥87th percentile for children; •<80 cm for females; <94cm for males; ••≥80 cm for females; ≥94 cm for males; □: 0.4-0.5 ratio; □□<0.4 or >0.5 ratio; Bold **p-value** shows statistical significance (p<0.05); SD: Standard deviation; BMI: Body Mass Index; MEDAS; Mediterranean Diet Adherence Screener; KIDMED: Mediterranean Diet Quality Index

Almost all participants in both groups had very good postures according to NYPA [Equine athletes (99.2%); Non-athletes (96.7%)]. When comparing the NYPA totals, no statistical significance was found between professional and other riders in both the children and adult groups (p>0.05). On the other hand, there were negative correlations between age, body weight, BMI, waist circumference, and NYPA scores.

The correlations showed that when age, body weight, BMI, and waist circumference increased, the NYPA score of the riders decreased (p<0.05 for all).

Table 4. Food consumption frequencies of the participants. (n: 242)

Food items ▼	Compatible with recommended consumption frequencies				
	Equine athletes (n: 121)		Non-athletes (n: 121)		
	n	%	n	%	p
Milk	50	41.3	38	31.4	0.141
Yogurt	52	43.0	38	31.4	0.103
All types of cheese	62	51.2	56	46.3	0.504
Red meat	45	37.2	55	45.5	0.245
Poultries	36	29.8	38	31.4	0.885
Fish	60	49.6	28	23.1	**<0.001**
Proceed-red meat products	92	76.0	83	68.6	0.262
Egg	65	53.7	75	62.0	0.220
Legumes	92	76.0	99	81.8	0.324
Vegetables	61	50.4	43	35.3	**0.025**
Fresh fruits	62	51.2	45	37.2	**0.040**
Whole grain bread	22	18.2	22	18.2	1.000
Chocolate	61	50.4	58	47.9	0.795
Sugar	78	64.5	74	61.2	0.708
Fast foods	110	90.9	108	89.3	0.832
Carbonated beverages	74	61.2	81	66.9	0.427

p: McNemar test; Bold **p-value** shows statistical significance (p<0.05)

Furthermore, these correlations were found not to be statistically significant for the non-athletes. All results are shown in Table 5.

DISCUSSION

Athletic horses have similar physical characteristics and abilities thus anthropometric measurements of the riders are one of the most important factors that can influence athletic performance and success. However, Diedhiou et al. (2019) reported that there was no relationship between BMI and athletic performance in show jumpers. Furthermore, BMI values were similar in show jumpers and dressage riders (18). In addition to regular physical activity, genetic factors, physiology, and dietary habits can also influence anthropometric measures in humans (19). Although the BMI values for the equine athlete and non-athlete groups were similar, there was a non-significant difference between the BMI classifications of professional riders and other riders in this study. Table 3 shows that the BMI values of professional riders were more consistent with the recommendations of WHO than those of the other riders (p>0.05). On the other hand, when comparing the BMI classifications of the riders with the age groups, statistical significance was found and the BMI values of the child riders were more consistent with the recommendations than adults (p: 0.008).

This could be related to the fact that children adhere better to the Mediterranean diet (Table 3, p: 0.003). In addition to BMI, no statistical differences were found in body weight and waist circumference between the two study groups (Table 2). However, 70% of adult professional riders had a low health risk according to the classification of their waist circumference by WHO. This proportion was almost twice as high as for the other riders (p: 0.036; Table 3). This could be explained by professionals having a higher weekly training frequency than others (p<0.001). The duration and frequency of training are among the effective factors in anthropometric measurements and can therefore potentially affect the health of athletes. In one study, the positive effects of regular and intensive strength training on body weight, BMI, and waist circumference were observed (20).

Nutrition is one of the most important determinants of athletic performance (21). As the healthiest dietary model, the Mediterranean diet is important for reducing the risk of chronic diseases due to its content of nutrients. In addition, the Mediterranean diet can potentially reduce exercise-related inflammation and oxidative stress thereby supporting athletic performance (21,22). Bifilco et al. (2019) have shown that the Mediterranean diet can provide the required nutrient intake and adherence to this nutritional model can reduce the need for supplements (23). Bitler et al. (2018) conducted a study with 526 licensed adult riders and observed normal BMI values, healthy lifestyles, and nutritional behaviors (24). Martínez-Rodríguez et al. (2021) reported that there was a positive association between the Mediterranean diet adaptation of the handball players and their body weight (25).

Table 5. Posture analysis of the riders and the related factors (n: 121)

NYPA score	All riders (n: 121)		Child riders (n: 73)		Adult riders (n: 48)	
	Professionals (n: 61)	Others (n: 60)	Professionals (n: 41)	Others (n: 32)	Professionals (n: 20)	Others (n: 28)
Mean±SD	58.96±4.95	59.11±5.03	60.21±3.76	59.65±4.56	56.40±6.11	58.50±5.54
Min-Max	41.00-65.00	47.00-65.00	51.00-65.00	47.00-65.00	41.00-63.00	47.00-65.00
p_1	0.870		0.565		0.221	
Correlation between age, anthropometric measurements, and NYPA score in riders (n: 121)						
	Age		Body weight		BMI		Waist circumference	
	r	p_3	r	p_3	r	p_3	r	p_3
NYPA score	-0.212	**0.019**	-0.220	**0.015**	-0.254	**0.005**	-0.228	**0.012**
Correlation between age, anthropometric measurements, and NYPA score in the non-athletes (n: 121)						
	Age		Body weight		BMI		Waist circumference	
	r	p_3	r	p_3	r	p_3	r	p_3
NYPA score	-0.153	0.093	-0.169	0.064	-0.158	0.084	-0.176	0.054

p_1: Independent Sample t-test; p_2: Spearman's correlation test; ⧧⧧: Correlation is significant at the 0.01 level; ⧧: Correlation is significant at the 0.05 level; SD: Standard deviation; NYPA: New Yok Posture Analyses; BMI: Body Mass Index; Bold p-value shows statistical significance (p<0.05)

J Basic Clin Health Sci 2024; 8: 692-703

Dayi T et al. Horse riders' nutritional and physical features

However Table 3 shows that the association between Mediterranean diet adaptation and BMI values was not significant in the presented study. Although some studies aimed to evaluate Mediterranean diet adherence in athletes, no data on horse riders were found in the current literature. The study of 2037 cyclists and 2000 triathlon athletes observed that these branched athletes adapted to the Mediterranean diet. It was also found that the mean MEDAS scores of the cyclists were higher than those of the others (26). Mayolas-Pi et al. (2017) conducted a study with three groups: amateur cyclists, indoor cycling instructors, and sedentary adults. The lowest adaptation to the Mediterranean diet was found in sedentary adults. It was also found that the adaptation status of amateur cyclists was better than that of indoor cycling instructors (27). Another study showed that most elite female athletes (58.33%) had poor compliance with the Mediterranean diet (28). On the other hand, Alacid et al. (2014) observed that the adaptation status of female athletes to the Mediterranean diet was more than moderate, and more than half of the female participants had high adherence. Furthermore, similar to our results, no differences were found between the Mediterranean diet adherence and BMI values in this study (29). Although there were no statistically significant results when comparing the two study groups for Mediterranean diet adherence, more than half of the riders had moderate and high adaptation status (62.8%) (Table 2). In addition, child riders were more compliant with the Mediterranean diet than adults. This difference was statistically significant (Table 3). The result may be related to nutritional care needs in childhood. Parents with higher socioeconomic status may have well nutritional knowledge that can influence children's dietary behaviors. Dayi et al. (2021) observed on the island of Cyprus that children's mean KIDMED scores and dietary diversity were related to the mothers' educational level and dietary behavior (30). Consumption of olive oil, vegetables, fruits, nuts, lentils, and whole grains is high; consumption of fish, red wine, and dairy products is moderate, and poultry, red meat, and processed red meat products are rare in the Mediterranean diet (31). When the frequency of food consumption was compared between study groups, it was found that the frequency of fish, vegetable, and fruit consumption was more in line with the recommendations in the equine athlete group (p<0.05). Although there was no statistical

association between the groups' Mediterranean diet adjustments, higher adherence to some food consumption frequencies was observed for the Mediterranean diet food components in the equine athlete group (Table 2; Table 4).

The postural profile has important implications for general health, well-being, aging, and also athletic performance. Growth and development speed, sedentary lifestyle, etc. are related to postural defects. Physical activity provides muscle strength and physically active people spend less time in a sitting position than sedentary people (32). However, some sports can be crucial for postural development, especially in adolescents (33). Jurjiu and Pantea (2018) conducted a study with male athletes from different sports such as basketball, volleyball, and football. When they assessed the postures of the athletes based on their stance positions, they reported that athletes from each sport had negative postural changes and the type of changes depended on the sport (34). In this study, 99.2% of equine athletes had very good posture according to NYPA assessments. And, almost all participants from the non-athletes group also had good posture. One study underlined that the postures of the riders, especially during training, are very important for the movement of the horse and thus for athletic performance (35). Hobbs et al. (2014) collected posture data using 3-D motion capture technology from 134 adult dressage riders. The result of the study was that competitive dressage riders were at higher risk for developing asymmetry (36). As shown in Table 5, there were no statistically significant differences between the average NYPA scores of professional and other riders, both for riders overall and for children and adult riders. From this, it can be said that the purpose of riding in the present study was not found to be effective on the postures of the athletes. However, negative statistically significant correlations were found between athletes' age, body weight, BMI, waist circumference, and NYPA scores while there were no important correlations for non-athletes in the present study (Table 5). That is when age, body weight, BMI, and waist circumference increase, the NYPA scores of the riders decrease and this shows that postural health deteriorates. One study found similar results in children and observed a negative correlation between age, body weight, BMI scores, and New York Posture Rating Chart scores (37). In addition, some studies in the current literature show that weight gain has a negative impact on posture (38, 39). Although there

are not enough studies on the determinants of postural conditions of athletes, a study by Andreeva et al. in many sports such as shooting, boxing, tennis, football, running, etc. (except horse riding) found that age is one of the determinants of posture, and postural stability deteriorates as athletes' age (40).

Strengthens and Limitations

Generally, when the authors compared results with other published similar articles, the method of the presented study (individual matching/matched pair design) strengthens the evidence level of results. In addition, 3-D motion capture technology could be better for determination of participants' postures. Also, participants' detailed body analyses by Body Impedance Analysis (BIA) could be better. The presented study can be a guideline for future studies that will aim to use these materials.

CONCLUSION

The present study aimed to determine the effects of equine sports on athletes' Mediterranean diet compliance, anthropometric measurements, and posture. No statistically significant effect on the Mediterranean diet adaptation status was observed for both equestrian sports and sub-branches. However, the adaptation of children riders was significantly better than that of adults.

On the other hand, although the Mediterranean diet adaptation status was similar for both study groups, the frequency of food intake characterized by the Mediterranean diet in equine athletes was more in line with the principles of optimal nutrition. In addition, the prevalence of preobesity and obesity was lower in child riders than in adult riders. And, when the waist circumferences of adult riders were assessed, the adult competitive riders were found to have a lower health risk than the other adult riders. The lower health risk might be related to competitive riders' higher training frequencies. Some anthropometric measurements were found to be risky for postural health in equine athletes. When their height, BMI, and waist circumference increased their NYPA values decreased, while this situation was not observed in the non-athlete group. Equine athletes' posture has an important role in the performance of both riders and horses. Thus, this situation may affect their athletic performance. In the current literature, there are only a limited number of studies on the general health status of riders, their lifestyle behavior, and also their posture. Therefore, this study is essential

and sheds light on the unknown areas of the sports, physiotherapy, and nutritional sciences. However, there is still a need for more studies on this topic with similar objectives to increase the level of evidence of the existing results.

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INVESTIGATION OF CANCER STEM CELL SURFACE MARKERS IN THE TUMOR TISSUES OF PATIENTS WHO HAD LIVER TRANSPLANTATION DUE TO HEPATOCELLULAR CANCER AND EVALUATION OF THE EFFECT OF THESE MARKERS ON PROGNOSIS

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ABSTRACT

Background and purpose: To investigate the relevance between cancer stem cell(CSC) markers and tumor progression in hepatocellular carcinoma(HCC).

Methods: Data of patients who underwent liver transplantation(LT) for HCC between February 1998 and September 2018 were collected. Patients over 18 years of age were included. Immunohistochemical staining were performed in paraffin blocks of liver explants containing HCC in terms of CSC markers, CD13, CD44, CD47, CD90 and EpCAM. Follow-up period, cancer recurrence, disease-free and overall survival were investigated.

Results: There were 71 patients who met the inclusion criteria. Optimal evaluation conditions were not met for CD13 and CD90 staining. Disease recurrence was found to be more frequent in CD 44+ cases (p=0.008). Disease-free survival was significantly longer in CD44- group(160.2 vs 103.0 months, p=0.043). Overall survival was significantly shorter in CD44+ cases(171.7 vs 107.8 months, p=0.018). No statistically difference was found between CD47+/- or EpCAM+/- groups in terms of recurrence (p=0.27, p=0.24). There was no significant difference in disease-free and overall survival in CD47+/- or EpCAM+/- cases, respectively (CD47+/-; p=0.82, p=0.90, EpCAM; p=0.76, p=0.69).

Conclusion: Positive CD44markers in HCC is associated with a more aggressive course of disease. Targeted therapies for CD44antigens of CSCs may prevent disease recurrence and increase survival.

Keywords: Hepatocellular cancer, cancer stem cell, tumor markers, liver transplantatio

INTRODUCTION

Hepatocellular cancer (HCC) is the 3rd most common cause of death worldwide (1). The most important reason for this is that it is resistant to treatment and it relapses easily. Local recurrences and metastases that occur in a short period of time lead to the death of patients due to the lack of effective treatment option. Currently, the most effective treatment modalities for HCC are liver resection with tumor-free surgical margins or liver transplantation (LT) in selected cases (2,3). Liver transplantation provides the chance to eliminate both HCC and the underlying chronic liver disease (3,4). Different criteria have been defined for selection of patients with HCC as liver transplant recipients (4). Although Milan and University of California San Francisco (UCSF) criteria are frequently used, some liver transplant centers may also use other criteria. Recurrence rate of HCC after liver transplantation is reported to be around 20-25% worldwide (5). Unfortunately, there is still no effective treatment for prevention and treatment of recurrence. Systemic chemotherapeutic agents are inadequate. Therefore, solutions to prevent and treat HCC recurrence after surgical resection or LT are needed.

In recent years, importance of cancer stem cells (CSCs) in tumor biology has been revealed (6,7). This topic has been widely investigated and studies have discovered many specific CSC surface markers for certain tumors. Determining spesific CSCs markers promises to develop target therapies for tumors (1,6,7). As a result of current studies, the main CSCs surface markers of HCC are: CD 133, CD44, CD47, CD13, CD24, OV6, CD90 and EpCAM (epithelial cell adhesion molecule) (8-10). Experimental and clinical researches for targeted treatment strategies regarding defined markers continue worldwide. However, most of the studies are cell line based and there are few studies using human tissues.

The aim of this study was to investigate the presence of CSCs markers in the liver tissues of patients that underwent LT for HCC and to determine the relationship between these markers and disease prognosis.

MATERIAL AND METHODS

The study was designed as a cross-sectional study. Institutional ethics committee approval was obtained. Cases who underwent LT for HCC between February 1998 and September 2018 in our Hospital were analysed. Patients over 18 years of age and whose data could be obtained reliably were included.

Paraffin blocks of liver explants containing HCC from the patients included in the study were obtained. For immunohistochemical staining of CSCs surface markers, CD13, CD44, CD47, CD90 and EpCAM specific monoclonal antibodies were used. These

antibodies and their brands/models were CD90 (Santa Cruz Biotechnology, SCBT, sc-5316), CD44 (Santa Cruz Biotechnology, SCBT, sc-9960), CD13 (Santa Cruz Biotechnology, SCBT, sc-13536), CD47 (Thermo Fisher Scientific, Invitrogen B6H12), EpCAM (Thermo Fisher Scientific, Invitrogen MA512604). Staining patterns were scored and recorded. Data of age, gender, type of liver transplantation (living/cadaveric), follow-up period, cancer recurrence, disease-free and overall survival were recorded.

The values were entered into a Microsoft Office Excel 2020 (Microsoft Corp., Redmond, Washington, USA) database. The records underwent an extensive data editing process to check for inconsistencies between data fields. After validation, error-free data were entered into the master file. Data were imported into IBM SPSS statistics 23.0 (SPSS, Chicago, Illinois, USA) for analysis. Descriptive statistical methods (mean, median, standard deviation), univariate and multivariate analyses and survival analyses were performed.

In univariate analysis, t-test or Mann-Whitney U test was used for univariate analysis according to the compliance with normal distribution in the comparison of the data obtained by measurement. Chi-square test was used to compare the data obtained by counting. Chi-square test or Fisher's exact test was used in the analysis of census and/or categorical data. According to these results, $p<0.05$ was considered significant. "Kaplan-Meier" method was used for survival analysis and "log-rank" test was used for comparison of groups.

RESULTS

There were 71 patients who met the inclusion criteria. After exclusion of 5 patients with early mortality (first 90 days), 54 (81%) males/12 (18%) females, total 66 patients were analysed. The mean age of the patients was 55.95±7.4 years (37-68) and the median age was 55 years. Thirty-five (53.7%) of the patients received LT from living and 31 (46.9%) from cadaveric donors. The mean follow-up period was 107.46±65.82 (3.22-231.36) months. HCC recurrence occurred in 14(21%) patients. The sites of recurrence were lung, bone, liver and skin. The median time to recurrence was 25.17±21.48 (2.10-58.35) months. The median disease-free survival was 145.81±11.92 and the median overall survival was 145.81±11.58 months.

Paraffin blocks were evaluated immunohistochemically for the presence of HCC stem cell markers CD13, CD44, CD47, CD90 and EpCAM. Despite many attempts, optimal immunohistochemical evaluation could not be achived for CD13 and CD90. Therefore these two markers could not be evaluated. Assesment of other 3 markers accomplished successfully.
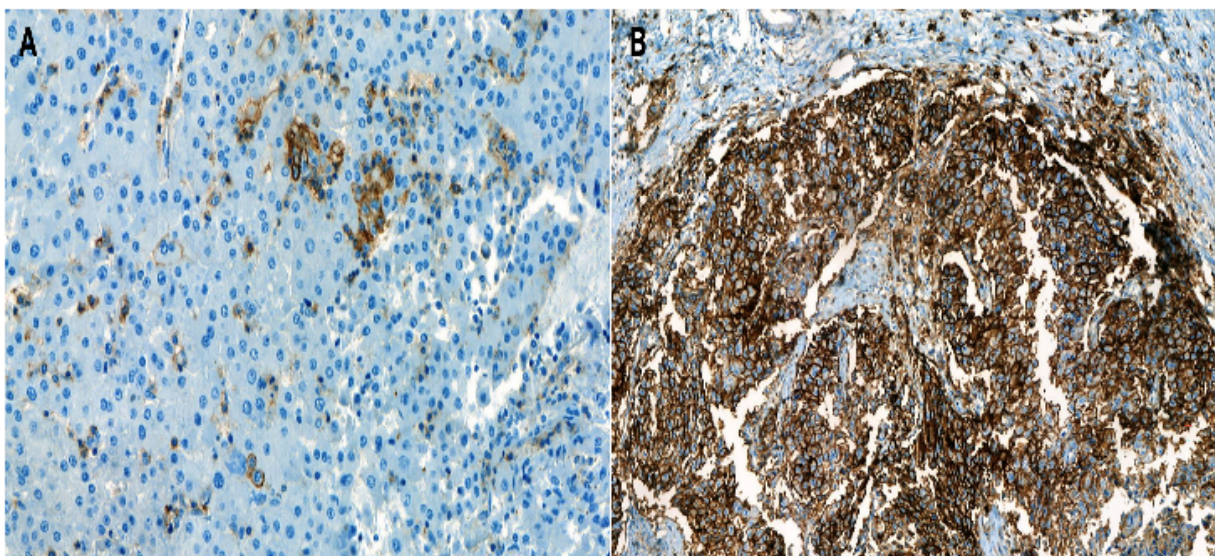
Figure 1. A. Hepatocellular cancer case of without recurrence after LT. Low density of CD44+ cells (brown staining) in explanted liver (x20 magnification. **B.** Case with HCC recurrence after LT. Dramatically dense CD44+ cells (brown staining) are seen in (x10 magnification).

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Egeli T et al. Exploring cancer stem cell markers in tumors

CD44 was positive in 20(38%) of 52 patients without recurrence and 11(78%) of 14 patients with recurrence. Disease recurrence was found to be statistically more frequent in CD 44 + cases (p=0.008). A much more intense staining pattern was detected in the 11 CD44+ patients with recurrence compared to CD44+ patients without recurrence (Figure 1A, B). In Kaplan-Meier survival analysis, according to the log-rank test, disease-free survival was significantly longer in CD44- group when compare CD44+ (160.2 vs 103.0 months, p=0.043) (Figure 2). Similarly, overall survival was significantly shorter in CD44+ cases (171.7 vs 107.8 months, p=0.018) (Figure 3).

CD 47 was positive in 5 (9%) of 52 patients without recurrence and 2 (14%) of 14 patients with recurrence. EpCAM was positive in 6 (11%) of patients without recurrence and 2 (14%) of patients with recurrence. No statistically significant difference was found between CD47+/- or EpCAM+/- groups in terms of recurrence (p=0.27, p=0.24). No significant difference in staining pattern was found between either the CD47 +/- or EpCAM +/- groups. There was no significant difference in disease-free survival and overall survival in CD47+/- or EpCAM+/- cases, respectively (CD47+/-; p=0.82, p=0.90, EpCAM; p=0.76, p=0.69).

DISCUSSION

Hepatocellular cancer is the 7th most common cancer worldwide and the 3rd most common disease responsible for cancer-related deaths (8). Surgical resection or LT, combined with chemotherapy are the most effective strategies for cure (2,3). Despite developments in treatment modalities, HCC still has a high recurrence rate and mortality (11-13). Thus, the search for new therapy modalities for better treatment continues.

Since the first successful liver transplant was performed by Starzl in 1963, it became widely practiced worldwide acute or chronic liver failure and HCC in selected cases constitute the most common indications for LT (5). The factors affecting survival after LT are mainly vascular complications graft rejection, infection and disease recurrence in patients with HCC. Egeli et al. reported

that, long-term survival in patients who underwent LT for HCC was significantly lower compared to other etiologies due to early disease recurrence (14). Similarly, studies conducted to determine the prognosis after LT have shown that HCC recurrence
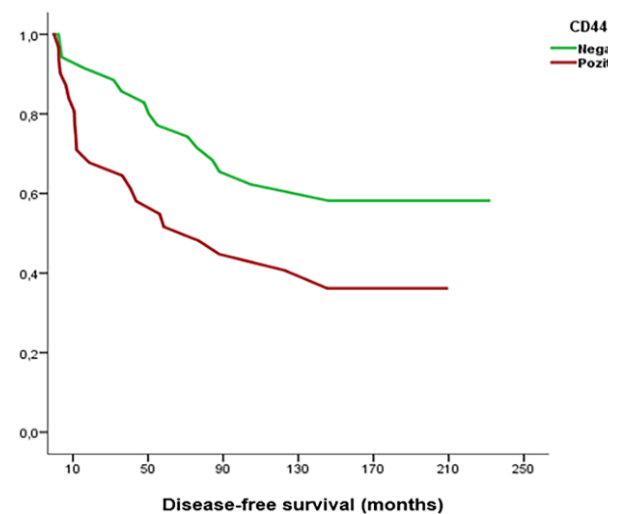
Figure 2. Disease-free survival curves in CD44-/+ cases. Disease-free survival is significantly shorter in CD44+ cases (p=0.043)
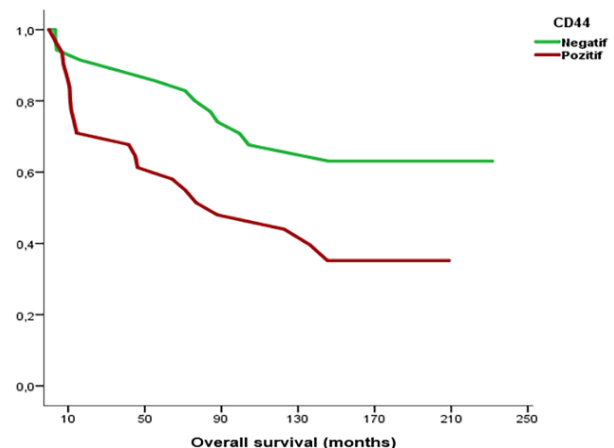
Figure 3. Overall survival curves in CD44-/+ cases. Overall survival was significantly decreased in CD44+ cases (p=0.018).

has a negative effect on survival (15,16). Many studies have revealed the mechanism of recurrence of HCC or to understand the development of de novo or recurrent malignancy in transplanted patients and important results have been obtained (17,18). However, new findings are needed to prevent recurrence and provide effective treatment.

The CSC theory in cancer development has been known for about 40 years (9). According to this theory, a group of stem cell-like cells with the characteristics of self-renewal, change and proliferation are responsible for the development and progression of cancer (8,9). It has been suggested that CSCs that are resistant to chemotherapy and other treatment

modalities and lead to local recurrence and metastasis. Therefore, it is thought that effective treatment of the disease and prevention of recurrences may be possible by developing therapies targeting CSCs.

In order to target CSCs, important studies have been carried out in recent years to identify cell surface markers specific to these cells and significant progress has been made. The main HCC CSC surface markers identified as a result of current studies are: CD133, CD44, CD47, CD13, CD24, OV6, CD90 and EpCAM (1,9,10). Thanks to these developments, targeted treatment strategies for cells carrying these markers have become more promising. Some new HCC CSC surface markers have also been identified in recent studies (13). Experimental and clinical studies regarding this topic continue intensively (10,11).

In this study, HCC recurrence was found to be statistically significantly higher in CD44+ CSCs after LT. In addition, disease-free and overall survival periods were found to be statistically significantly shorter in CD44+ patients. Consistent with this result, Rozeik et al reported in HCC, increased CD133 and CD44 expression corresponded to higher grade, thus indicating poorer prognosis (1). In accordance with our opinion, they advocated the expression profiles of several CSCs markers may enhance understanding of HCC prognosis, metastasis and relapse. That may facilitate development of novel therapeutic agents targeting and/or preventing HCC. In addition, some novel studies showed higher recurrence rate and shorter disease free survival in CD44+ HCC patients (19,20). On the other hand, no significant difference was found associated with CD47 or EpCAM positivity in terms of recurrence or survival.

There were some limiting factors about this study. COVID-19 pandemic, occurred during the course of the research and it negatively affected our study in many ways. Another important limitation was despite all efforts not being able to study on CD13 and CD90 markers. Thus we could not have information about these markers. Retrospective design of the study and limited number of patients may be considered as other handicaps.

In conclusion, this study demonstrated that HCC with high CD44+ CSCs, is associated with a more aggressive course of the disease. This result suggest that targeted therapies for CD44 surface marker in the treatment of HCC may prevent disease recurrence and increase survival. We consider in near future , comprehensive studies including novel HCC CSC markers will provide the opportunity to development of efficacious target therapies for HCC.

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COMPARISON OF THE EFFECTS OF A SIX-WEEK PHYSIOTHERAPY PROGRAM ON TEMPOROMANDIBULAR DISORDER PATIENTS WITH AND WITHOUT POSTERIOR EDENTULISM: A QUASI-EXPERIMENTAL STUDY

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ABSTRACT

Purpose: The impact of posterior edentulism on the management of temporomandibular disorder remains unclear, with a paucity of studies examining the influence of posterior edentulism on symptoms. The objective of this study is to investigate the relationship between posterior edentulism and bruxism, tinnitus, and depression in temporomandibular disorder patients and to compare treatment outcomes in two groups with and without posterior edentulism.

Material and Methods: In a quasi-experimental controlled trial, 26 patients with temporomandibular disorder participated in a six-week intervention, including manual therapy, massage, postural exercises and kinesiotaping. The patients were divided into 13 in the posterior edentulous group and 13 in the full dentate group. The participants attended 12 physiotherapy sessions, twice a week, over six weeks. Outcome measures, including bruxism, tinnitus, joint clicking, Beck Depression Inventory, and cervical range of motion, were evaluated at baseline and the end of the six weeks.

Results: The six-week physiotherapy program significantly improved bruxism, tinnitus, cervical range of motion, and depression ($p<0.05$). The posterior edentulous group showed significantly greater improvement in cervical range of motion compared to the full dentate group ($p<0.05$).

Conclusion: Physiotherapy effectively reduces temporomandibular disorder symptoms and highlights the importance of considering posterior edentulism in comprehensive temporomandibular disorder management.

Keywords: Bruxism, depression, temporomandibular disorder, tinnitus, physiotherapy

INTRODUCTION

Temporomandibular disorder (TMD) is a clinical condition that refers to functional disorders associated with the temporomandibular joint (TMJ) and surrounding tissues, such as pain and restricted mandibular movement. Various factors, including trauma, malocclusion, fibrocartilage disc dysfunction, muscle hyperfunction, and psychosocial factors, may influence the formation of TMD (1). Posterior edentulism can cause damage the TMJ, such as resorption of the articular eminence and disc displacement. This results in a loss of occlusal support, which is a critical factor contributing to degenerative changes in the TMJ (2). Symptoms of dysfunction become more common as the duration and extent of edentulism, the number of missing teeth and quadrants involved increase (3). Clinical symptoms may include TMJ pain, joint noise, ear pain, bruxism, tinnitus, restricted mandibular movement, and headaches (4). Fallahi HR et al. (5) showed that partial edentulism is an important etiological factor for TMJ disorders. Therefore, it is recommended to replace the lost teeth and create a stable occlusion (6).

Bruxism is a parafunction characterized by excessive and abnormal activation of the mandibular muscles, resulting in clenching or grinding of the teeth (7). Bruxism is often associated with anxiety, malocclusion, sleep disorders, stress and the side effects of certain medications. It may cause excessive stress and pressure on the TMJ and surrounding tissues due to overloading of the mandibular muscles, leading to pain, fullness, and tinnitus in the ear (7). Continuous clenching may cause damage to the articular disc, resulting in TMD. Studies have reported a positive correlation between TMD pain and self-reported bruxism diagnosis. The occurrence of self-reported parafunctional habits has also been identified as a significant predictor of TMD onset (8,9).

Tinnitus is frequently associated with TMD. Trigger points in the masseter, lateral and medial pterygoids, sternocleidomastoid (SCM), upper trapezius, and scalene muscles can cause tinnitus (10). In a study by Buergers et al. (11) to investigate the relationship between tinnitus and TMD, tinnitus was eight times higher in patients with TMD. Still, improvements in tinnitus symptoms were reported due to splinting and physiotherapy.

Psychosocial stress factors are believed to play a role in the development of TMD-related pain, particularly muscle pain experienced during chewing. Individuals afflicted with TMD exhibit elevated levels of anxiety, stress, somatic awareness, pain catastrophising, depression and kinesiophobia when compared to control groups (12,13). Stress may lead to parafunctional activities such as clenching and grinding.

The existing literature indicates that the optimal treatment programme and dosage for TMD have yet to be determined. Due to the complexity of TMD, a multidisciplinary approach among healthcare professionals is necessary. TMD patients commonly experience symptoms such as headaches, neck and shoulder pain, and postural abnormalities. Physiotherapy can help manage these symptoms by improving the strength and flexibility of relevant muscles.

This study aims to examine the relationship between posterior edentulism and bruxism, tinnitus, and depression in patients with TMD and to compare the treatment outcomes between two groups with and without posterior edentulism. We hypothesize that there will be differences in the response to treatment between posterior edentulous and full dentate TMD patients.

MATERIALS AND METHODS
Study design
The study was a quasi-experimental controlled trial conducted at Dokuz Eylul University. The study was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013), and the research was approved by the Dokuz Eylul University, Non-Interventional Clinical Research Ethics Committee with protocol number GOA-7337 (Date: 29.06.2022, Decision No: 2022/22-02).

Participants
The study participants were selected from patients diagnosed with myofascial TMD. The inclusion criteria were individuals aged between 18 and 70 years who were diagnosed with myofascial TMD according to the Research Diagnostic Criteria for TMD, experienced TMJ pain lasting for at least three months, complained of headache and neck pain, and had not used intraoral appliances.

Exclusion criteria for participants were history of neck or jaw fractures, trauma, diagnosis of fibromyalgia, degenerative changes, rheumatic or neurological problems, TMJ disc displacement, postoperative conditions or burns involving the cervical or

Table 1. Comparison of initial values of Full Dentate Group and Posterior Edentulous Group

Outcomes		Full Dentate Group N=13 X±SD Med(Min-Maks)	Posterior Edentulous Group N=13 X±SD Med(Min-Maks)	Between-group
Bruxism		1.54±0.52	1.46±0.78	*t*:0.30; *p*:0.77
Tinnitus		n:5	n:8	*X²*:1.38; *p*:0.23
TMJ clicking		1.38±1.45	0.85±1.21	*t*:1.03; *p*:0.31
Cervical range of motion				
Lateral flexion	**R**	31.32±7.00	30.02±9.11	*t*:0.41; *p*:0.69
	L	30.56±9.12	32.59±7.33	*t*:-0.63; *p*:0.54
Rotation	**R**	69.92±7.89	69.73±9.27	*t*:0.06; *p*:0.95
	L	74.67(46.70-86.50)	71.20(46.17-86.00)	*z*:0.90; *p*:0.38
Flexion		56.73±8.92	52.94±11.03	*t*:0.96; *p*:0.34
Extension		63.74±16.13	65.35±7.89	*t*:-0.32; *p*:0.75
Beck Depression Inventory		12.08±9.14	16.62±9.35	*t*:-1.25; *p*:0.22

L: left; n: number of people; p: p value; R: right; SD: Standard deviation; t: Independent t-test; TMJ: Temporomandibular Joint; X^2: Ki kare test; z: Mann-Whitney U Test

temporomandibular region, previous orofacial treatment within the last 12 months, use of analgesics or muscle relaxants within 8 hours before physiotherapy, hypermobility of cervical movements, acute infections, any systemic disorder, or osteoporosis.

The objective of the study was clearly delineated, and written informed consent was obtained from all participants.

Study procedure

Patients diagnosed with TMD were classified into two groups based on the presence of posterior tooth contact loss: the full dentate group (FDG) and the posterior edentulous group (PEG). The study involved 26 patients with TMD, with 13 in the PEG and 13 in the FDG. The same programme was applied to all patients.

Outcome measures

All measurements were performed by the same physiotherapist at baseline and after six weeks. The study's primary outcome measure was bruxism, while secondary outcome measures included tinnitus, TMJ clicking, depression, and cervical range of motion (ROM).

Bruxism, tinnitus, and TMJ clicking: Participants self-reported their bruxism and tinnitus. Bruxism was recorded as one point if observed exclusively during the daytime or exclusively at night, and as two points if observed both during the daytime and at night. A score of 1 was assigned to participants who reported experiencing tinnitus, whereas a score of 0 was assigned to those who did not report any complaints

of tinnitus. The clicking sound of the TMJ was evaluated using a scoring system that ranged from 0 to 4. This assessment considered both the right and left sides of the joint, as well as the opening and closing movements of the mouth. One point was assigned for each condition, resulting in a total possible score of 4.

Psychological situation: The patients' mental well-being was assessed using the Beck Depression Scale, a self-assessment scale developed by Beck (14), and its Turkish validity and reliability were established by Hisli (15) in 1989. The scale comprises 21 questions, each scored from 0 to 3. The findings indicate that a score of 0-9 corresponds to minimal depression, 10-16 to mild depression, 17-29 to moderate depression, and 30-63 to severe depression (11,12).

Cervical ROM: The study measured the patients' active neck flexion, active extension, active lateral flexion in both directions, and active rotation in both directions. Recent research has shown that smartphone apps can replace universal goniometers (16). The Clinometer smartphone application was used to measure cervical joint movements. This application has been shown to have excellent validity and reliability in measuring the lower cervical ROM in patients with chronic cervical pain (17). Each measurement was conducted three times, and the mean value of these repetitions was documented.

Intervention

All patients received the same 12 physiotherapy sessions twice a week for six weeks. The physiotherapy programme applied myofascial

Table 2. Baseline and follow-up results for bruxism, tinnitus, click sound and Beck Depression Inventory of each group

Outcomes		Baseline X±SD	Follow-up X±SD	Within-group[†]	Between-group[‡]
Bruxism	FDG	1.54±0.52	1.08±0.49	*MH:2.45 p:0.01*	*t:1.68; p:0.11*
	PEG	1.46±0.78	0.77±0.44	*MH:2.71 p:0.01*	
Tinnitus	FDG	n:5	n: 4	*McNemar p:0.98*	*X²:2.22; p:0.13*
	PEG	n: 8	n: 1	*McNemar p:0.03**	
TMJ clicking	FDG	1.38±1.45	1.54±1.81	*MH:-0.29 p: 0.77*	*t:1.79; p:0.09*
	PEG	0.85±1.21	0.54±0.88	*MH:1.07 p:0.29*	
Beck Depression Inventory	FDG	12.08±9.14	8.31±6.12	*t:2.65 p:0.02**	*z:1.59 p:0.12*
	PEG	16.62±9.35	8.31±10.18	*t:3.37 p:0.01**	

[†] t: dependent t-test; z: independent t-test; [‡] t: independent sample t test ; X^2: Fisher's Excat test; FDG: Full Dentate Group; MH: Marginal Homogeneity test; ; n: number of people; PEG: Posterior Edentulous Group; p, p value; SD: standard deviation; *: $p<0.05$.

releases, massage, trigger point compression, suboccipital release, stretching, and posture exercises to the SCM, scalene, trapezius, and pectoral muscles to relax the cervical region. Kinesiotape was applied once a week to the SCM and upper trapezius. The sessions lasted approximately one hour. Physiotherapy aimed to relax the cervical spine, strengthen weak muscles, and correct posture to reduce strain on the TMJ and masticatory muscles.

Data processing and statistical analysis
The sample size was estimated using G*Power Software (version 3.1.9.7) A margin of error (α) of 0.05, 90% power (β), and an effect size (Cohen's d) of 1.02, derived from a similar study, were used to determine the sample size (18). This resulted in 13 participants in each group, comprising 26 participants. The confidence interval was set at 95%. All data were recorded and analyzed using the SPSS (Statistical Package for Social Sciences) for the Windows 22 programme. To determine the normality of the distribution, we used the Shapiro-Wilk test, kurtosis and skewness values, and a histogram graph. Two independent groups were compared using the independent sample t-test and Mann-Whitney U test. The difference between two related numerical variables was analyzed using the paired sample t-test and Wilcoxon test. Fisher's exact test was used to examine relationships between independent categorical variables, and McNemar and marginal homogeneity tests examined relationships

between dependent categorical variables. A significance level of 0.05 was used to interpret the obtained values.

RESULTS
The study assessed 37 patients with TMD, of whom 29 met the inclusion criteria. Three participants withdrew from the study for personal reasons: two from the FDG and one from the PEG. Therefore, 26 participants were included in the final analysis, of whom 88.5% (n:23) were female, and 11.5% (n:3) were male. Regarding educational level, 30.8% (n:8) had completed high school, 53.8% (n:14) had an associate's or bachelor's degree, and 15.4% (n:4) had a doctorate. Of the participants, 73.1% (n:19) were non-smokers, while 26.9% (n:7) had a history of smoking. Any participant did not report alcohol consumption. The mean age of the patients was 38.7 years. In the FDG, the mean age was 36.46 (±14.03) years, whereas in the PEG, it was 41.00 (±14.27) years. The mean body mass index (BMI) was 23.73±5.22 in the FDG and 24.52±4.98 in the PEG. In addition, the mean symptom duration (in years) was 4.78±5.67 in the FDG and 4.45±3.26 in the PEG. No statistically significant differences in age, BMI, and symptom duration were observed between the groups (p>0.05).

Bruxism
There was no statistically significant difference between the groups in bruxism before and after the

Table 3. Comparison of cervical range of motion before and after the treatment.

Outcome measures		Groups	Pre-treatment X±SD Med(Min-Maks)	Post-treatment X±SD Med(Min-Maks)	Within group[†]	Between groups [‡]
Lateral flexion	R	Full Dentate	31.32±7.00	39.21±6.49	*t:*-7.04 *p:0.00**	*t:*-2.39; *p:0.03**
		Posterior Edentolus	30.02±9.11	43.28±7.81	*t:*-6.79 *p:0.00**	
	L	Full Dentate	30.56±9.12	42.35±6.56	*t:*-5.60 *p:0.00**	*t:*-0.55; *p:0.59*
		Posterior Edentolus	32.59±7.33	45.83±6.48	*t:*-8.43 *p:0.00**	
Rotation	R	Full Dentate	69.92±7.89	77.84±9.38	*t:*-6.56 *p:0.00**	*t:0.36; p:0.72*
		Posterior Edentolus	69.73±9.27	76.95±7.59	*t:*-4.81 *p:0.00**	
	L	Full Dentate	74.67(46.70-86.50)	81.53(63.53-96.37)	*z:*-2.97 *p:0.00**	*t:*-1.14; *p:0.27*
		Posterior Edentolus	71.20(46.17-86.00)	83.73(62.87-87.67)	*z:*-2.90 *p:0.00**	
Flexion		Full Dentate	56.73±8.92	59.08±5.64	*t:*-0.87 *p:0.40*	*t:1.07; p:0.30*
		Posterior Edentolus	52.94±11.03	59.82±8.53	*t:*-2.11 *p:0.04**	
Extension		Full Dentate	63.74±16.13	76.05±8.33	*t:*-4.13 *p:0.00**	*t:1.31; p:0.20*
		Posterior Edentolus	65.35±7.89	72.82±8.68	*t:*-3.41 *p:0.01**	

[†] t: Dependent sample t test ; z: Wilcoxon test, [‡] t: Independent sample t test; SD: standard deviation; p: p value; *: p<0.05.

treatment (p>0.05, Table I). Both groups showed a significant within-group improvement in bruxism after treatment (p<0.05, Table 2).

Tinnitus
At baseline, there was no significant difference in tinnitus values between the two groups. (p>0.05, Table I). Although no statistically significant difference was observed in intra-group changes in the FDG (p>0.05), a statistically significant intra-group difference was observed in the PEG (p<0.05, Table 2). It was determined that all participants who experienced tinnitus before treatment no longer experienced it after treatment.

TMJ clicking
There was no statistically significant difference in any measurements within or between groups for TMJ clicking (p>0.05, Table 2).

Cervical ROM
Both groups exhibited statistically significant differences in the pre-treatment and post-treatment outcomes for the measurements of lateral flexion, rotation, and extension ROM (p<0.05). In particular,

there was a statistically significant improvement in flexion in the PEG (p<0.05), whereas the FDG did not exhibit a statistically significant change (p>0.05, Table 3). Additionally, the PDG demonstrated a statistically significant improvement in right lateral flexion following the intervention (p<0.05).

Beck Depression Inventory
Similarly, both groups showed a statistically significant difference in Beck Depression Inventory scores before and after treatment (p<0.05, Table 2). The data indicates that there is no statistically significant difference between the two groups (p>0.05).

DISCUSSION
The study involved 26 participants, with a mean age of 38.7 years. There were no significant differences in age, BMI, or symptom duration between the groups. Following a six-week physiotherapy programme comprising manual therapy, taping and exercise, both groups showed significant improvements in bruxism, Beck Depression Inventory and cervical ROM scores following treatment, while there were no significant differences within-group scores in TMJ clicking. The

J Basic Clin Health Sci 2024; 8: 710-718

Narin-Aral S et al. Temporomandibular Disorder: Bruxism, Tinnitus, Depression

PEG demonstrated a more significant improvement in cervical flexion and right lateral flexion compared to the FDG. Additionally, tinnitus resolved for all participants in the PEG who had experienced it before treatment.

The study sample consisted of 88.5% (n:23) females and 11.5% (n:3) males with a mean age of 38.7 years. According to the literature, the prevalence of TMD is reported to be twice as high in women as in men and to peak between the ages of 20 and 40 years. Therefore, our sample in this study is consistent with the literature.

The relationship between bruxism and posterior edentulism has been extensively studied. However, further research is still required. While some studies suggest an association between clenching and grinding and posterior tooth loss (19), others indicate the opposite. According to a Japanese survey of sleep bruxism and tooth loss due to aging, bruxism increases with age, regardless of tooth loss (20). Similarly, in our study, there was no significant difference between TDG and PDG when comparing bruxism values. Quintero et al. (21) reported a decrease in bruxism complaints after a 10-week postural awareness training to correct head posture in children experiencing sleep bruxism. Following our 6-week posture exercises, specifically designed to correct head and upper body posture, there was an improvement in bruxism complaints in both groups of our patients. The improvement in bruxism may be attributed to a combination of factors, including muscle relaxation, stress reduction, improved posture, implementation of relaxation techniques, and potentially enhancing stress management skills during treatment.

Tinnitus commonly co-occurs with TMD. A systematic review and meta-analysis examining the relationship between tinnitus prevalence and TMD reported that the majority of tinnitus in patients with TMD ranged from 35.8% to 60.7%. In comparison, this rate was 9.7% to 26.0% in the group without TMD (22). Systematic reviews conducted in 2019 supported this opinion and emphasized that symptoms of TMD should be considered in patients complaining of tinnitus (23,24). We also investigated the presence of tinnitus in the participants and found that 50% of our sample size reported experiencing it. Trigger points in the masticatory or neck muscles, such as the SCM (10), can cause tinnitus. The masseter muscle can remain contracted for extended periods due to parafunctional habits, stress, and occlusal changes,

forming trigger points. We believe that posterior edentulism can lead to a deterioration in occlusion, causing the mandible to be positioned posteriorly. This can result in the head of the condyle putting pressure on the meatus acousticus, which can lead to an overload of the masseter muscles and an increase in tinnitus. Despite this hypothesis, our study did not reveal a significant difference in tinnitus between the PDG and TDG prior to treatment. A statistically significant improvement in tinnitus symptoms was observed on PDG after our six-week treatment period. The reduction in tinnitus is due to the positive effect of exercise and manual therapy on the posterior position of the mandible, relieving overloaded muscles and correcting head posture.

Studies in the literature have shown that physiotherapy can reduce TMJ clicking (25). In contrast, the results of our study did not demonstrate a statistically significant difference in clicking sounds following the completion of the treatment period. The lack of change in the clicking sound can be attributed to the absence of interventions targeting the masticatory muscles and joint stabilization in our physiotherapy programme. Instead, the interventions focused on the neck and back regions without including exercises to regulate mandibular and joint movements or to promote joint stabilization.

Impaired chewing quality, dysfunctional muscles, and posture changes in TMD patients may restrict movement in the cervical joints. Several studies have shown that cervical ROM is lower in patients with TMD than in healthy individuals (26–28). Limitations were observed in the cervical ROM of the participants in our study, but no differences were found between the groups. Piekartz and Haul reported a significant increase in cervical ROM in patients with headache and TMD who received cervical and orofacial manual therapy (29). In parallel with this study, we observed a significant increase in both groups' lateral flexion, rotation, and extension values after treatment. Although the flexion angle significantly increased PDG, this was not the case in TDG. The absence of significant improvement in the FDG may be attributed to an insufficient treatment programme or the symptoms having a more complex etiology.

Furthermore, the FDG might have better proprioception, leading to a more precise perception of muscle flexibility limits and preventing excessive strain during measurements. In addition, the improvement in right lateral flexion after treatment was found to be different between the groups. The

difference in right lateral flexion values between the groups is our decision not to sub-group based on right or left TMJ involvement and our belief that there may be different tension levels in the trapezius muscles between the groups.

Several studies in the literature have shown that mental state disorders increase the prevalence of TMD (12,30). There is a bidirectional causal relationship between psychosocial factors and TMD. Resende et al. (31) reported that psychological problems not only increase the prevalence of TMD but may also exacerbate symptoms. Patients with TMD may experience limitations such as difficulty chewing, inability to open the mouth sufficiently, pain in the jaw, joint noises, and inability to lie on the chin, which can lead to psychological problems such as stress, high anxiety, and depression (32). Our study is consistent with the literature, as both groups showed mild symptoms of depression at baseline. At the end of the physiotherapy, all patients had significant improvement, and the severity of depression was minimized. We attribute the improvement in depression to patients' increased ROM and functionality, reduced pain, and improved quality of life. A study conducted on TMD patients with somatic tinnitus showed a significant reduction in depressive symptoms in patients who received exercise training and manual therapy (33). This study supports our findings that manual therapy and exercise training can improve the psychosocial status of TMD patients.

The results suggest improvements in certain outcomes for both treatment groups, but the differences between the groups were not statistically significant for the variables examined. Further research with extended treatment durations and longer follow-up periods may be needed to better understand the effectiveness of the interventions.

Limitations

The study has some limitations. There were more female patients than male patients in the sample. Additionally, monitoring the daily home exercises that the patients had to perform was challenging, and the patients were not categorized based on the involvement of their right or left TMJ.

CONCLUSION

In conclusion, this study emphasizes the intricate relationship between posterior edentulism and TMD symptoms, highlighting the need for further

investigation into its impact on treatment outcomes. The results reveal the key role of physiotherapy in effectively managing TMD symptoms and emphasize the importance of a multidisciplinary approach involving dentists and physiotherapists in addressing posterior edentulism within comprehensive treatment strategies.

In addition, the study brings to light the potential psychological effects, such as depression and anxiety, associated with posterior edentulism and TMD, suggesting that mental health support should be integrated into treatment plans. It is recommended that future research prioritise longitudinal studies in order to gain a deeper understanding of the long-term effects of various treatment modalities on TMD symptoms in patients with posterior edentulism. Furthermore, future studies should explore more specific treatment designs, such as grouping participants based on the number of missing teeth or comparing different age ranges, as age may influence outcomes. Additionally, different treatment modalities, including exercises and manual therapy more specifically targeting the jaw, could also be investigated to provide clearer guidance for advancing research and improving clinical outcomes.

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ACONITINE IMPEDES CELL MOTILITY IN MDA-MB-231 BREAST CANCER CELLS

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ABSTRACT

Purpose: Aconitine, a potent alkaloid from Aconitum plants, has shown promising anticancer properties. The aim of the study is to investigate the effects of aconitine on lateral migration, and matrix metalloproteinase (MMP) activity in MDA-MB-231 triple-negative breast cancer cells.
Material and Methods: A WST-1 viability assay was conducted to determine the effect of aconitine on the viability of MDA-MB-231 cells. Following treatment with non-cytotoxic doses of aconitine, lateral migration was evaluated through wound healing assays. Additionally, gelatin zymography was conducted to analyze MMP-2 and MMP-9 activity and secretion levels.
Results: Aconitine concentrations up to 200 μM did not significantly affect cell viability for up to 72 hours, whereas higher doses (400-600 μM) reduced viability in a time-dependent manner. Aconitine at 200 μM showed a trend towards decreased lateral motility, with a significant reduction at 9 hours post-treatment. Gelatin zymography revealed no alterations in MMP-2 and MMP-9 activity or secretion levels following aconitine treatment.
Conclusion: Aconitine demonstrates limited efficacy in modulating the migratory capacity of MDA-MB-231 cells and does not affect gelatinase activity. Further investigation into underlying mechanisms is necessary, potentially leading to novel therapeutic strategies for triple-negative breast cancer.

Keywords: aconitine, cell motility, matrix metalloproteinases, triple negative breast cancer.

INTRODUCTION

According to the GLOBOCAN 2022 report, breast cancer is the most commonly diagnosed cancer among women globally, with approximately 2.3 million new cases, accounting for 11.6% of all cancer diagnoses. It is also the leading cause of cancer-related death among women, resulting in an estimated 665,000 deaths worldwide, which represents 15.4% of all cancer deaths in females. Higher incidence rates are observed in transitioned countries than those in transitioning countries; however, transitioned countries have lower mortality rates, partly due to better detection practices (1). Despite advancements in therapy options and early detection, breast cancer continues to be a significant public health challenge. Particularly triple-negative breast cancers are of critical importance due to their aggressive nature and limited treatment options, making targeted research and the development of potential therapeutic strategies essential.

Aconitine, a dominant alkaloid found in the Aconitum plants, has been widely studied for its potent

J Basic Clin Health Sci 2024; 8: 719-725

Keleş D et al. Aconitine and Breast Cancer Cell Motility

pharmacological and toxicological properties. Despite its narrow therapeutic index, aconitine is utilized in traditional Chinese medicine for various conditions such as joint pain, gastroenteritis, and rheumatoid arthritis due to its antipyretic, analgesic, and anti-inflammatory activities (2). Recent research has increasingly focused on its potential applications and risks in cancer therapy. Studies have demonstrated that aconitine exhibits promising anticancer properties by inhibiting cell proliferation, inducing apoptosis, and disrupting cancer cell signaling pathways (3). Despite its potential benefits, the high toxicity of aconitine poses significant challenges for its therapeutic application. The narrow therapeutic window and severe cardiotoxic effects necessitate careful consideration and rigorous research to harness its anticancer potential safely (2).

Matrix metalloproteinases (MMPs) are a family of calcium- and zinc-dependent endopeptidases that play a crucial role in turnover of extracellular matrix (ECM) remodeling and degradation. These enzymes are capable of cleaving various ECM components, including collagens, elastin, and proteoglycans. Moreover, MMPs can activate growth factors and cytokines, further supporting tumor growth and progression (4). They also promote tumorigenesis including inflammation, epithelial-mesenchymal transition, angiogenesis, cell migration and metastasis (4–7). Notably, gelatinases, MMP-2 and MMP-9 have been implicated in the degradation of type IV collagen, a major component of the basement membrane, thereby promoting tumor cell invasion (4,5). These findings highlight the potential of MMP inhibitors as promising anti-metastatic therapeutic agents in cancer treatment.

The aim of this study was to explore whether aconitine affects the lateral migration of MDA-MB-231 breast cancer cells and its role in modulating the secretion and activity of MMP-2 and MMP-9. This study seeks to enhance our understanding of aconitine's potential as a therapeutic agent in targeting metastasis-related processes in aggressive breast cancer phenotype.

MATERIAL AND METHODS
Ethical Considerations
Ethical approval was obtained from the Health Sciences Research Ethics Committee of Izmir University of Economics, Izmir, Turkey (Date: 12.08.2024, No: B.30.2.İEÜSB.0.05.05-20-317).

Cell Culture
Triple-negative human breast cancer cell line MDA-MB-231 (obtained from ATCC) was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 4 mmol/L L-glutamine and 5% fetal bovine serum (FBS) (Gibco) but devoid of phenol red and antibiotics. These cells were maintained at 37°C in a humidified incubator with a 5% CO_2 atmosphere. The media were changed every 2-3 days, and when the cells reached 70-80% confluency, they were detached using 0.25% Trypsin-EDTA (Gibco) for subculturing or experimentation.

Pharmacology
Aconitine (purchased from Sigma-Aldrich) was initially prepared as a 13 mM primary stock solution in ethanol (EtOH) and stored at -20°C until use. An intermediate stock solution of 2 mM aconitine was prepared by diluting the primary stock solution with the culture medium. The ethanol concentration corresponding to the highest dose of aconitine used in the experiment was utilized as the solvent control. During the experiments, the culture media were refreshed every 24 hours, regardless of whether or not they contained the added agent.

Cell Viability
WST-1 colorimetric assay was performed in order to assess the effect of aconitine on cell viability. In brief, 1×10^4 MDA-MB-231 cells were seeded into a 96-well plate and incubated for 24 hours for adherence. Cells were subsequently treated with varying concentrations of aconitine (50µM - 100µM - 200µM - 400µM - 600µM) and corresponding solvent control (4.6 % EtOH) or left untreated for 24, 48 or 72 h. At each time point, 10 µL of WST-1 reagent (Roche Diagnostics, Basel, Switzerland) was added to each well, and the plates were incubated for an additional 2 hours at 37°C in a humidified atmosphere with 5% CO_2. Absorbance was measured at 450 nm, with background correction at 620 nm. The percentage of cell viability was calculated using the formula: Cel viability (%) = A treated cells/A untreated control × 100 (where A is absorbance).

Lateral Motility
The motility behavior of MDA-MB-231 cells was evaluated with wound healing assay as described previously (8). Briefly, 3×10^5 cells were plated in a 24-well plate and the next day pre-treated with aconitine

J Basic Clin Health Sci 2024; 8: 719-725

Keleş D et al. Aconitine and Breast Cancer Cell Motility

(200 µM), solvent control (0.77 % EtOH) or left untreated for 24h. A wound across the cell monolayer was created with a sterile 200µl micropipette tip. Following the wound creation, the cells were washed with phosphate-buffered saline (PBS) to remove any debris, and then the media with 1% FBS with agent or solvent was added to the wells. The migrated cell area was monitored at specified time intervals (0, 6, 9 and 24 h) using a microscope, and images were captured over time for the wound closure analysis. Wound widths were measured using Image J program MRI wound healing tool and lateral motility was calculated using the following equation:

Motility index, MoI (%) = [1- (wound width at given time/initial wound width)] x100.

Gelatin Zymography
MDA-MB-231 cells were treated with 200 µM aconitine or solvent control (0.77 % EtOH) in complete medium for 48 hours. Following this treatment, the cells were transferred to serum-reduced (1% FBS) media containing either the agent or the solvent for an additional 24 hours. After the incubation period, the media were collected, and loaded together with a molecular weight marker (PageRuler Pre-stained Protein Ladder, Thermo Fisher Scientific, USA) into 1 mg/mL gelatin copolymerized SDS-PAGE gels, which were then electrophoresed at 4 °C. The zymogram gels were then washed with 2.5% Triton X-100, and subsequently incubated in a development buffer composed of 50 mM Tris-base, 50 mM NaCl, 1 mM CaCl$_2$, and 0.05% Brij 35 (pH 7.6) at 37 °C for 48 hours. The gels were stained using Coomassie Blue R-250 solution and destained in a solution of 40% methanol and 10% acetic acid. The band locations for MMP-2 and MMP-9 in the gel were validated using recombinant MMP-2 and MMP-9 proteins, as previously reported (8). Gel images were captured using the UVP gel documentation system (UVP Ltd., UK), and band densities were analyzed with the UVP Bioimaging system utilizing LabWorks 8.20 Image Acquisition Software (UVP Ltd., UK). The levels of gelatinolytic activity were expressed as protease activity calculated using the formula: [area (mm²) x optical density / µg protein].

Statistical Analysis
Data analyses were conducted using Graph Pad Prism 10.2.3 software (Graph Pad Inc, US). Data were represented as means ± standard deviations (SD). We performed the experiments in duplicate (technical replicates) and calculated the mean of each technical replicates. The mean value from each independent replicate was then used in our final statistical analysis. Consequently, our statistical calculations were based on the three mean values obtained from each independent replicate, rather than pooling all technical replicates together. First, the normality of the data distribution was confirmed with the Shapiro–Wilk test. Next, statistical significance was assessed with either two-way ANOVA, or, for unequal group sizes, the mixed-effect analysis. Tukey test or Dunnett's tests were utilized for the multiple group comparisons. Statistical significance was defined as follows: *p < 0.05; **p < 0.01

RESULTS
The effect of aconitine on MDA-MB-231 cell viability
We initially investigated the impact of varying concentrations of aconitine on the viability of MDA-MB-231 cells. The data revealed that after 24 hours, 400 µM aconitine treated-MDA-MB-231 cells exhibited a notable but not statistically significant reduction in viability (12.9% reduction) compared to the solvent control group (p > 0.05; n = 3; Figure 1). As the treatment duration increased to 48 and 72 hours, further declines in viability were observed in response to aconitine concentrations. In this context, significant decreases in viability were observed at 400 µM aconitine after both 48 h (31.9 % reduction, p < 0.05; n = 3) and 72 h (50.5 % reduction, p < 0.05; n = 2), as well as at 600 µM aconitine after 48 h (46.1 % reduction, p < 0.01; n = 3) and 72 h (76 % reduction, p < 0.05; n = 2) of exposure (Figure 1). Notably, treatment with aconitine at concentrations of up to 200 µM for periods up to 72 hours did not significantly affect the viability of MDA-MB-231 cells (p > 0.05; n = 4; Figure 1). Consequently, aconitine treatments were restricted to non-cytotoxic doses of 200 µM administered for a maximum of 72 hours, for further analysis.

Aconitine decreases MDA-MB-231 cell motility in time dependent manner
Next, we conducted a wound-healing lateral migration assay to evaluate the effect of 200 µM aconitine on the motility of MDA-MB-231 cells. As reported previously (8), we again confirmed the high motility capacity of MDA-MB-231 cells, as evidenced by an increase in the motility index (MoI) of the untreated
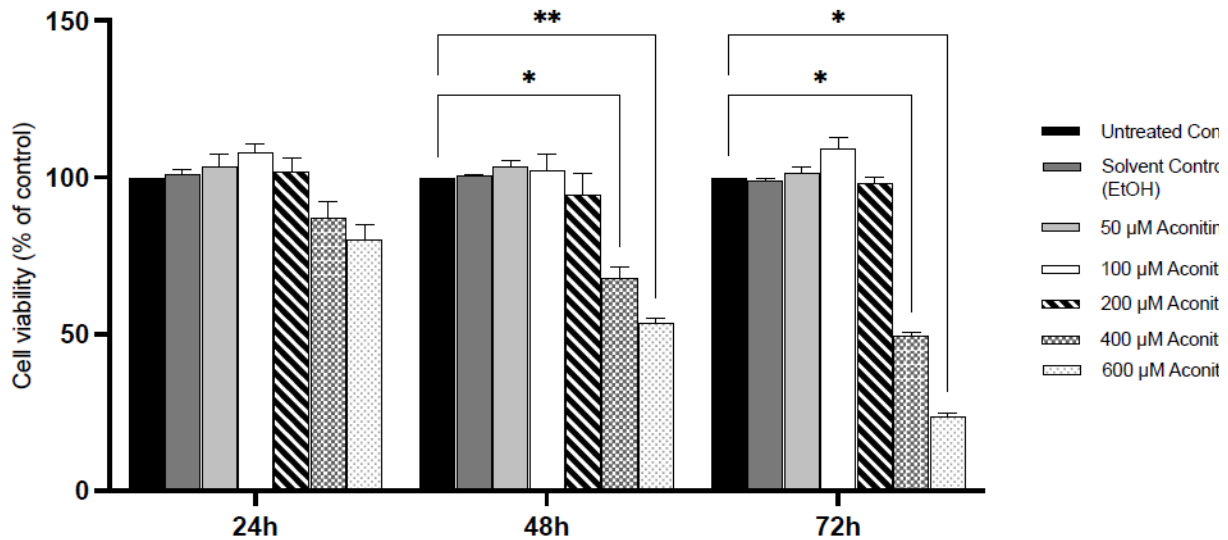
J Basic Clin Health Sci 2024; 8: 719-725

Keleş D et al. Aconitine and Breast Cancer Cell Motility

Figure 1. Effect of aconitine on MDA-MB-231 cell viability. MDA-MB-231 cells were treated with varying concentrations of aconitine (50μM - 600μM) or solvent control (4.6% EtOH) for 24, 48, and 72 hours. Cell viability was assessed using the WST-1 assay. Each bar presented as mean ± SD. *$p < 0.05$, **$p < 0.01$ compared to solvent control

control group over time, as follows: 33% at 6 h, 52% at 9 h, and 99% at 24 h (n = 3; Figure 2a and b). The solvent control (0.77% v/v EtOH) showed no significant effect on cell motility ($p > 0.05$; n = 3; Figure 2A and B).

Importantly, treatment with aconitine (200 μM) showed a trend towards decreased lateral motility, with MoI values of 25% (n = 3) at 6 h, 36% (n = 2) at 9 h, and 95% (n = 3) at 24 h, compared to the control groups (Figure 2A and B). Although this trend was not statistically significant overall ($p > 0.05$), the MoI value at 9 h was significantly decreased in aconitine-treated cells compared to the untreated control group (n = 2; $p < 0.05$; Figure 2A and B). These results suggest that aconitine slightly inhibits the lateral motility of MDA-MB-231 cells in a time-dependent manner.

Aconitine did not alter the activity and secretion levels of MMP-2 and MMP-9 in MDA-MB-231 cells

We also performed the gelatin zymography to investigate whether aconitine affects gelatinase activity and secretion in MDA-MB-231 cells. Gel analysis revealed that treatment with non-cytotoxic doses of aconitine (200 μM) did not alter the activity and secretion levels of either MMP-2 or MMP-9 (Figure 3A and B). This suggests that the inhibitory effect of aconitine on cellular motility may occur through an MMP-independent mechanism. However, this conclusion is based on a single biological repeat, and therefore, further replications are needed to confirm our result.

DISCUSSION

Triple-negative breast cancer (TNBC) presents significant therapeutic challenges due to the absence of hormone receptors, highlighting an urgent need for novel therapeutic strategies. The use of MDA-MB-231 cells, a well-established model for aggressive TNBC, can contribute insights into potential treatments. This study focused on the anti-migratory effects of aconitine, a potent alkaloid derived from the Aconitum plant, on the highly metastatic and aggressive TNBC MDA-MB-231 cell line. Our key findings are: i) Up to 200 μM of aconitine had no effect on MDA-MB-231 cell viability, ii) Aconitine exhibited a slight inhibitory effect on cellular motility, though overall results were not statistically significant, and iii) Non-cytotoxic doses of aconitine did not alter gelatinase secretion and activity in MDA-MB-231 cells. These suggest that aconitine may have only limited efficacy in modulating pathways associated with TNBC metastasis.

Aconitine has been characterized as a proarrhythmic agent that can open tetrodotoxin-sensitive Na+ channels, leading to persistent activation, a continuous influx of Na+, prolong the action potential and inducing cardiac arrhythmias (9-11). However, recent studies indicate that aconitine may exert effects beyond Na+ channel activation. Studies have demonstrated the aconitine and its derivatives can inhibit tumor growth by affecting various signaling pathways and inducing apoptosis in multiple cancer
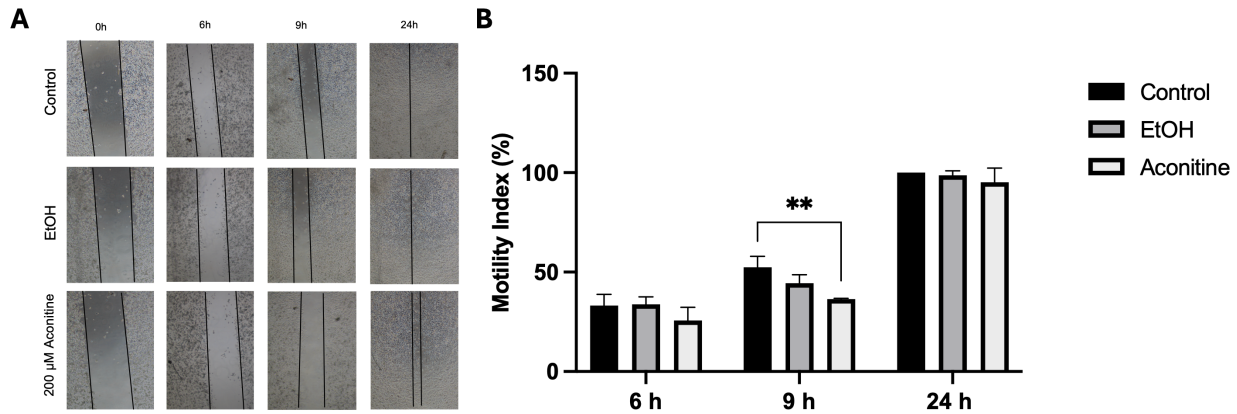
Figure 2. Impact of aconitine on lateral motility of MDA-MB-231 cells. A) Representative images of wound healing assay at 0, 6, 9, and 24 hours post-wounding (4x magnification). Cells were treated with 200 µM aconitine, solvent control (0.77% v/v EtOH), or left untreated. B) Lateral motility expressed as percentage of Motility Index (MoI%). Data are presented as mean ± SD. **$p < 0.01$ compared to untreated control
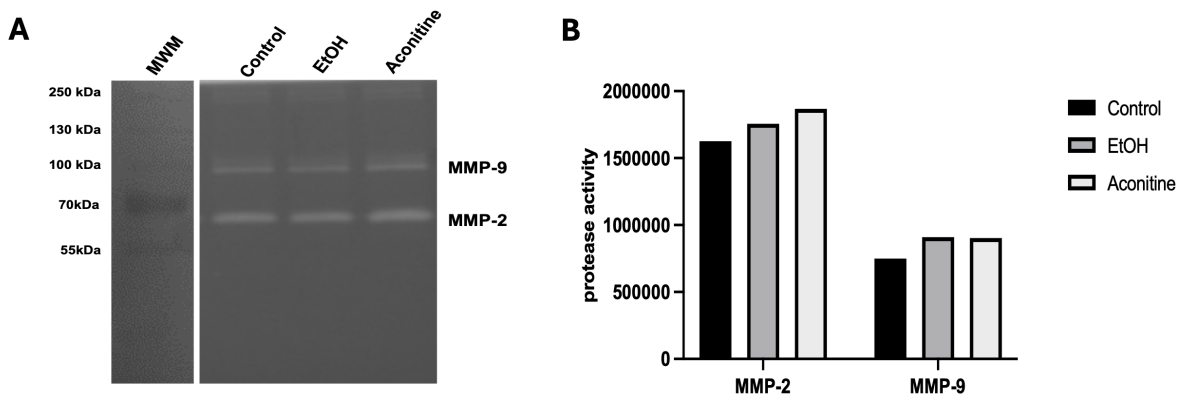
Figure 3. Effect of aconitine on MMP-2 and MMP-9 activity in MDA-MB-231 cells. A) Representative zymogram image showing gelatinase activity in conditioned media from MDA-MB-231 cells treated with 200 µM aconitine or solvent control (0.77% EtOH) for 72 hours. B) Quantification of band intensities is shown. Data are from a single biological repeat. MWM: Molecular Weight Marker

types, including pancreatic cancer (12), melanoma (13), and hepatocellular carcinoma (14).

Despite these findings, relatively few studies have reported on the effects of aconitine on cancer cell migration and invasion (15-19). For instance, aconitine has been reported to inhibit migration in MHCC97 hepatoma carcinoma cells (15). Conversely, another study revealed that aconitine increased the motility of MAT-LyLU prostate cancer cells by 15%, but had no effect on AT-2 prostate cancer cells (16). Meanwhile, Feng et al. showed that the aconitine derivative, hypaconitine, reversed induced TGF-β1 induced epithelial-mesenchymal transition (EMT) and suppressed migration and invasion in A549 lung cancer cells (17). Guo et al.

found that co-treatment of aconitine with osthole and psoralen inhibited cancer cell invasion, primarily by modifying the TGF-β/Smad signaling pathway and decreasing the levels of NF-κB and RANK expression in MDA-MB-231BO breast cancer cells metastatic to bone (18). Notably, in A2780 ovarian cancer cells, aconitine decreased protein expression levels of MMP-2 and MMP-9 and cellular migration in dose dependent manner (19). Our investigation into the effect of aconitine on migration and gelatinases in TNBC cells uncovered a slight inhibition of lateral migration in MDA-MB-231 cells without a corresponding effect on the extracellular activities of MMP-2 and MMP-9.

Limitations

While our study provides valuable insights into the effects of aconitine on MDA-MB-231 cells, it is limited by insufficient replication of viability, motility and gelatin zymography assays, as well as the lack of invasion assays. These limitations necessitate further investigation to confirm the reliability of these results and clarify the potential therapeutic application of aconitine for TNBC. Additionally, since the effect of aconitine on cell viability was observed only at high doses (600 μM), we used an ethanol concentration of 4.6%, corresponding to this dose, even though it is relatively high. However, for the main experiments (zymography and motility), we applied ethanol concentrations (0.77 %) within the recommended range of 0.1-1%.

CONCLUSION

Overall, our findings suggest that aconitine may have limited efficacy in modulating the migratory capacity of the MDA-MB-231 TNBC cells, and does not affect the gelatinase activity. Future investigations should focus on four areas: i) exploring the molecular mechanisms underlying the effects of aconitine on migratory pathways, including the role of protease regulators, ii) conducting invasion assays to fully understand aconitine's impacts on metastatic behavior, iii) examining potential synergistic effects of aconitine with other anti-cancer compounds, and iv) conducting in vivo studies to elucidate the anti-metastatic potential of aconitine in a more complex physiological context.

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EFFECT OF COLLAGEN-COATING VARIATIONS ON THE MORPHOLOGY AND VIABILITY OF HUMAN VASCULAR SMOOTH MUSCLE CELLS

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ABSTRACT

Purpose: Collagen is a critical extracellular matrix (ECM) component that significantly influences cellular behaviors such as adhesion, migration, and proliferation. Optimizing collagen coating protocols is essential for developing accurate in vitro models, particularly for studying vascular smooth muscle cells (HVSMCs). The aim of this study was to optimize collagen coating protocols for in vitro models using HVSMCs by assessing cell morphology, adhesion potential, and viability under various collagen concentrations and incubation conditions.

Methods: HVSMCs were cultured on surfaces coated with different concentrations of Type 1 Rat Tail Collagen with different cell number (as 10^4 cells/well and 20^4 cells/well). The cells were incubated at various temperatures (4°C, 25°C, and 37°C). Morphological analysis was performed using phase-contrast microscopy to observe the alignment and phenotype of the cells. Cell adhesion was assessed using DAPI staining, and cell viability was evaluated using the Presto Blue assay after 96 hours of incubation.

Results: Collagen coating significantly influenced HVSMC behavior. The cells transitioned to a contractile phenotype, evidenced by tight, parallel bundle alignment, which is critical for maintaining vascular tone. Enhanced cell adhesion was observed in specific collagen-coated groups across different temperatures, particularly in the F, G, and H groups. Additionally, collagen coating did not significantly increase cell proliferation, making it suitable for in vitro vascular models. Optimal results were observed in groups seeded with 10^4 cells and incubated at 25°C and 37°C.

Conclusion: The study highlights the importance of optimizing extracellular matrix components like collagen in developing functional in vitro models. The identified optimal conditions for collagen coating will be valuable for future vascular modeling studies, providing a reliable foundation for in vitro research.

Keywords: Collagen-coating, in vitro vascular model, human vascular smooth muscle cells, morphology, viability

INTRODUCTION

Although diseases related to abnormal vascular systems generally exhibit common pathological features, they are highly heterogeneous, and each disease has its own unique characteristics. This necessitates the development of disease-specific vascular models (1). In this context, developing models specific to each disease is crucial for a better understanding of the pathogenesis and response to treatment. In modern medical research, it is essential to study the three-dimensional structures, mechanical properties, and biochemical microenvironments of tissues to understand how they form, function, become pathological, and behave as part of a living organism (1,2)

Traditional two-dimensional (2D) cell cultures are often unable to adequately replicate the complex dynamics of the in vivo cellular microenvironment, and therefore, they fail to fully sustain the differentiated functions that cells exhibit in their natural surroundings (4). In 2D cultures, cells may lose their natural morphology and fail to adequately replicate cell-cell or cell-extracellular matrix (ECM) interactions. These limitations hinder the accurate investigation of biological processes and disease mechanisms (5,6).

To overcome these shortcomings, three-dimensional (3D) cell cultures have been developed, which have the potential to better mimic the characteristics that cells exhibit in vivo. 3D culture systems can more accurately reflect important biological processes such as cell morphology, differentiation state, polarity, proliferation rate, gene expression, and genomic profiles by considering critical cell-cell and cell-ECM interactions. Cells grown in 3D cultures can adapt to the new microenvironment through changes in genetic and transcriptional activities, as well as in protein levels (5,6,7).

In this context, designing a three-dimensional human vascular system in vitro is of great importance for accurately mimicking vascular structures and functions (8). Such a model could significantly contribute to a better understanding of disease-specific vascular structures and processes, the development of new therapeutic strategies, and the advancement of personalized medicine approaches. The aim of this study is to optimize the conditions that best support the physiological state of smooth muscle cells as a prerequisite for transitioning to a three-dimensional vascular model. In the development of

three-dimensional (3D) cell culture models, accurate assessment of cell morphology and viability is critically important. Cell morphology reflects how well cells replicate their natural structures in vivo and whether biological processes are being correctly executed. Maintaining the correct phenotypic characteristics of cells, especially for vascular smooth muscle cells, is essential for preserving their functionality and accurately modeling diseases (5,8). Moreover, monitoring cell viability is a fundamental criterion for evaluating the biological suitability and sustainability of the developed model. Ensuring that cells proliferate healthily but do not grow uncontrollably is necessary for disease models to accurately mimic biological reality under in vitro conditions. Therefore, optimizing basic parameters such as cell morphology and viability before beginning 3D modeling efforts should be prioritized to enhance the success and validity of the model (9,10). For this reason, in the first phase of our study, we focused on the morphology and viability of smooth muscle cells to examine how these cells behave under physiological conditions and to what extent they meet the requirements of the 3D vascular model to be developed. Culturing cells under appropriate conditions is a critical step for maintaining their in vivo functions. The foundational data obtained will serve as a guide for the design and optimization of 3D structures that will be used in later stages of developing disease-specific models. In this way, it is aimed to preserve the correct phenotypic characteristics of the cells and contribute to the creation of models capable of mimicking the pathological processes of diseases. Ultimately, these studies will facilitate the development of more effective and specific therapeutic strategies for diseases.

MATERIAL AND METHODS
Cell Culture

In this study, primary human venous smooth muscle cells (CellBiologics, Cat No: H-6086) were cultured in vitro. The cells were maintained in a 5% CO_2 atmosphere at 37°C in an incubator, using vascular cell basal medium (ATCC, PCS-100-030) supplemented with a vascular smooth muscle cell growth kit (ATCC, PCS-100-042) and 1% Penicillin/Streptomycin. The cells were cultured in 75 cm² flasks.

Collagen Coating Methods

In this study, various coating protocols were developed using eight different collagen concentrations. The protocols were optimized by adjusting collagen concentrations and different culture medium conditions, and the most suitable coating method was determined. Type 1 Rat Tail Collagen (Corning 354236) was used for all groups and diluted in vascular cell basal medium and smooth muscle cell growth kit with 1% Penicillin/Streptomycin. The groups were prepared to contain different amounts of collagen per cm²; specifically, 0.2 µg (A), 2 µg (B), 5 µg (C), 10 µg (D), 156 µg (E), 42 µg (F), 93.75 µg (G), and 250 µg (H). Wells without collagen coating were used as the control group. The 96-well plates were prepared with 100 µl/well at the specified concentrations and incubated at +4°C, 25°C, and 37°C. Groups were formed using different cell densities of 10,000 cells/well and 20,000 cells/well to examine cell-cell interactions (11,12,13).

Morphological Observation

The 96-well plate surfaces were coated with the determined collagen coating protocols and allowed to dry. Human venous smooth muscle cells (HVSMC) were seeded at 10,000 (10k) and 20,000 (20k) cells per well for each group. The cells were incubated for 96 hours to assess cell morphology. After the incubation period, cell morphology was observed using phase-contrast microscopy.

DAPI Staining

To observe the attachment potential of cells to collagen-coated surfaces, DAPI staining was performed following a 2-hour incubation after applying the collagen coating protocols. The cells were washed twice with cold 1x PBS and fixed with 4% paraformaldehyde solution at room temperature for 10 minutes. The fixed cells were stained with DAPI (4',6-diamidino-2-phenylindole) diluted at a 1:1000 ratio (14).

Cell Viability

Following the application of the collagen coating protocols, conditioned media were collected from the wells after 96 hours of incubation and transferred to new 96-well plates. Cell viability was assessed using the Presto Blue reagent (15).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Categorical variables were analyzed using the chi-square test and Fisher's exact test. Continuous variables were analyzed using the t-test under the assumption of normal distribution or the Mann-Whitney U test if the distribution was not normal. Statistical significance was accepted as $p \leq 0.05$. The significance levels were defined as follows: Ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$). All data are presented as mean ± standard deviation (mean ± SD).

RESULTS

Morphological Observations and Contraction of Vascular Smooth Muscle Cells

Morphological observations revealed that vascular smooth muscle cells (VSMCs) transitioned to a contractile phenotype in the collagen-coated groups (Figure 1). The alignment of cells into tight and parallel bundles is indicative of a contractile phenotype, suggesting that the collagen-coated groups are morphologically suitable for in vitro applications. The transition to a contractile phenotype reflects the proper functioning of actin-myosin interactions and the associated cellular signaling pathways within the cytoskeleton of VSMCs. Maintaining this phenotype indicates that the biological conditions in the in vitro environment are appropriately sustained, allowing for healthy interactions with the extracellular matrix.

Adhesion of Smooth Muscle Cells

The aim of this study was to determine the most effective method for enhancing the adhesion of smooth muscle cells. After 2 hours of incubation, cells were immediately fixed. Compared to control groups at different temperatures, groups that provided superior cell adhesion at all three temperatures were identified (Figure 2). In particular, the F, G, and H groups showed high success in cell adhesion. These findings highlight the biocompatibility of the collagen
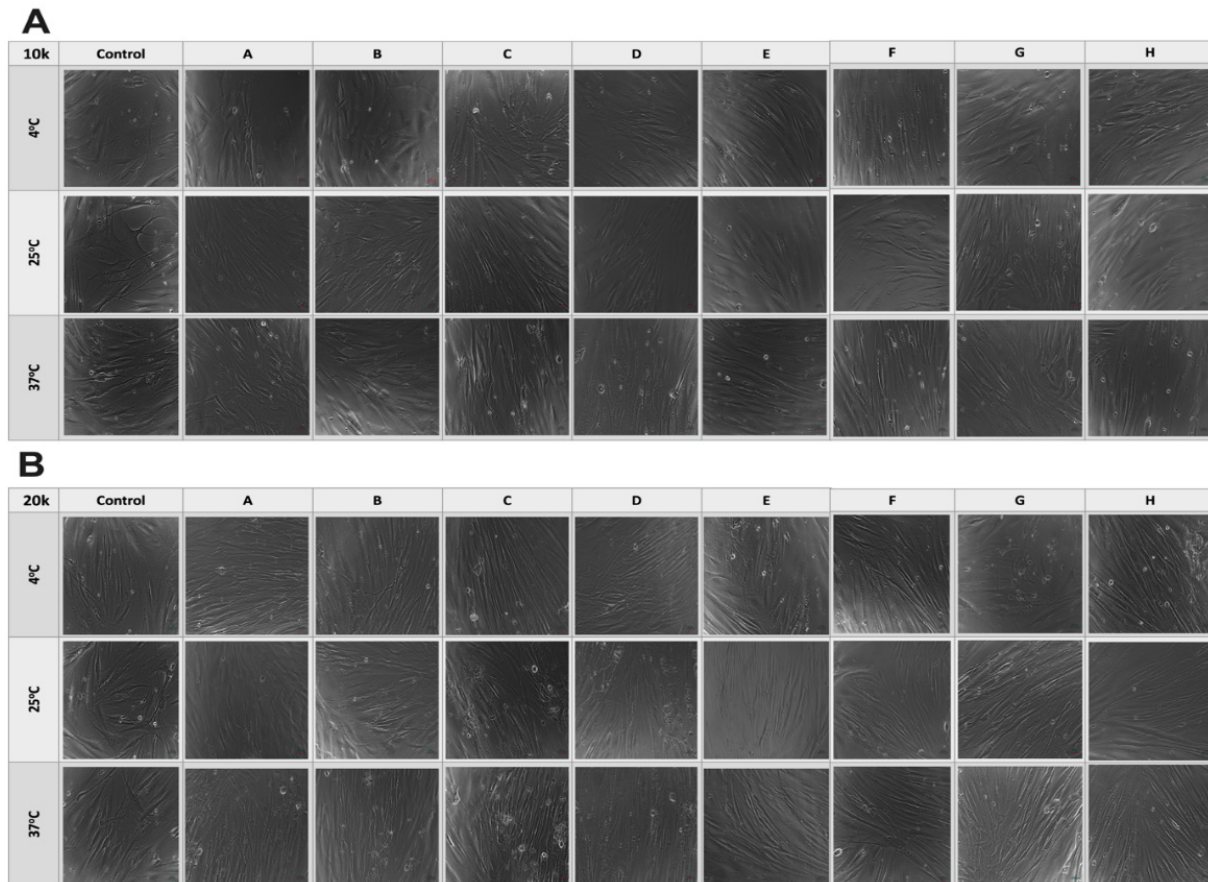
Figure 1. Morphological analysis of human primary vein smooth muscle cells (HVSMCs) on different collagen-coated surfaces. HVSMCs were cultured on plates coated with various collagen dilutions (A, B, C, D, E, F, G, and H) and visualized after 96 hours using phase-contrast microscopy. The images demonstrate successful collagen coating, as evidenced by tightly packed parallel bundles.

coating methods used and their positive impact on cell behavior.

Proliferation of Smooth Muscle Cells

To analyze the proliferation of smooth muscle cells, they were incubated for 96 hours, which is sufficient for a full cell cycle. In vascular pathologies, smooth muscle cells typically transition from a contractile phenotype to a synthetic one, leading to increased proliferation. In this study, it was crucial to select the collagen coating methods that did not promote excessive proliferation.

Among the groups seeded with 10k cells, no significant increase in cell viability was observed compared to the control group at 4°C, 25°C, and 37°C. These results suggest that this model is appropriate for in vitro modeling of vascular pathologies, as it does not promote excessive cell proliferation and accurately mimics physiological conditions (Figure 3). However, a statistically significant effect of collagen coating on cell proliferation was observed in the group of 10k cells

incubated at 4°C and in all temperature groups seeded with 20k cells. Given that the aim of the study is to develop a healthy vascular model and that cell proliferation can indicate endothelial damage, it is preferable to select experimental conditions that do not affect cell proliferation. Since the groups seeded with 20k cells are completely excluded from consideration, it appears more appropriate to use 10k cells for cell seeding. As proliferation differences were observed in the 4°C group of 10k cells, the 25°C and 37°C conditions are deemed more suitable.

DISCUSSION

In our study, we aimed to optimize collagen coating protocols for in vitro vascular models by considering the morphology, adhesion potential, and viability of HVSMCs. The findings of this study demonstrated significant differences in cell behavior depending on the collagen concentration and incubation conditions, highlighting the importance of protocol optimization in the biocompatibility of biological materials.
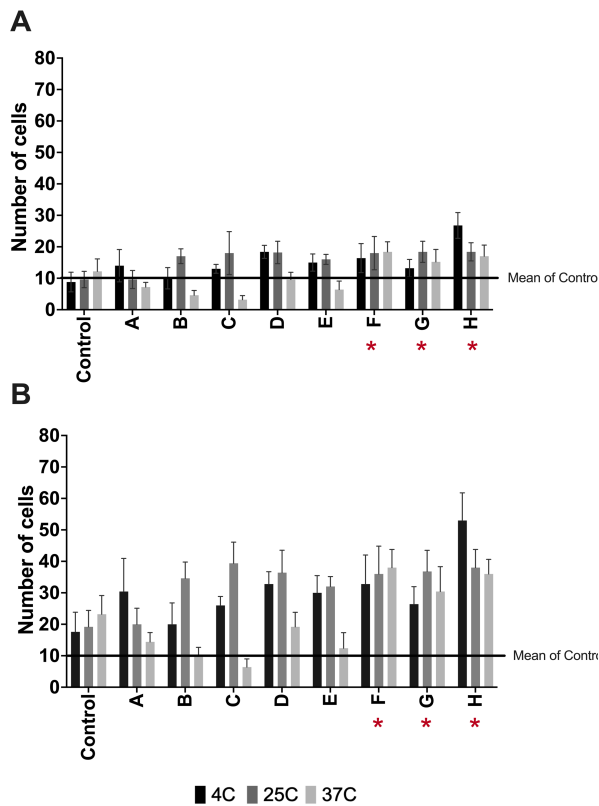
Figure 2. Adhesion efficiency of HVSMCs on collagen-coated surfaces at different temperatures. The number of adhered HVSMCs was quantified for each collagen coating method (A, B, C, D, E, F, G, and H) at 4°C, 25°C, and 37°C. DAPI staining was used to visualize the nuclei of adhered cells. The data indicate that F, G, and H coatings provided superior adhesion across all tested temperatures.

Figure 3. Cell viability of HVSMCs on different collagen-coated surfaces. HVSMCs were seeded at 10k and 20k cells per well on collagen-coated plates and incubated for 96 hours. Cell viability was assessed using the Presto Blue assay, and the results were normalized to control (uncoated) conditions. The analysis revealed that F, G, and H coatings maintained HVSMC viability, particularly at 4°C, 25°C, and 37°C, without promoting excessive proliferation.

Collagen is an abundant fibrous protein that constitutes the majority of the extracellular matrix (ECM) in all animals. This biological polymer is the main component of connective tissues, accounting for more than 30% of total protein in mammals. Generally, collagen provides structural integrity to all tissues, including blood vessels, skin, and bones. It is well established that collagen possesses biological properties that significantly influence cellular adhesion, migration, and proliferation (16, 17,18).

In this study, morphological observations revealed that vascular smooth muscle cells (HVSMCs) cultured on collagen-coated surfaces transitioned distinctly to a contractile phenotype (Figure 1). The alignment of cells into tight and parallel bundles is indicative of a contractile phenotype, which is critical for maintaining vascular tone and function in vivo. The contractile phenotype is characterized by the

upregulation of contractile proteins such as α-smooth muscle actin (α-SMA) and smooth muscle myosin heavy chain (SM-MHC), which are necessary for actin-myosin interactions and cellular contractility (19).

The ability of HVSMCs to maintain this phenotype in vitro indicates that collagen coating not only provides an appropriate extracellular matrix for cell adhesion but also supports the biochemical signals necessary for the effective functioning of the contractile mechanism. This observation is consistent with previous studies that have shown the critical role of ECM composition in HVSMC phenotype (20,21). Additionally, the contractile phenotype observed and maintained in the collagen-coated groups suggests that the cellular signaling pathways remain intact and functional (22). These findings indicate that collagen

J Basic Clin Health Sci 2024; 8: 726-733

Bayrak O et al. Collagen-Coating Impact on Vascular Cells

coating protocols are crucial for developing in vitro vascular models that aim to mimic the physiological conditions of blood vessels.

Our results showed that groups F, G, and H exhibited significantly higher cell adhesion compared to control groups across different incubation temperatures (Figure 2). These findings suggest that the collagen concentrations used in these groups enhanced the biocompatibility of the surfaces, positively impacting cell behavior. The observed effect on cell adhesion in these groups is likely due to the ECM properties provided by collagen. Previous studies have reported that the biochemical composition and structural properties of ECM proteins, such as collagen, are critical for cell adhesion, migration, and survival (23,24). The increased adhesion observed in groups F, G, and H suggests that collagen coating not only facilitates initial cell adhesion but also supports integrin-mediated signaling pathways necessary for stable adhesion (6,25). The consistent cell adhesion observed at different temperatures indicates that the bioactive properties of collagen coatings are maintained despite environmental changes, aligning with studies on physiological compatibility under various conditions (26).

In our study, HVSMCs were incubated for 96 hours with collagen coating to analyze cell proliferation. This duration is sufficient not only for the completion of a cell cycle but also for observing the transition from a contractile phenotype to a synthetic phenotype, which is associated with vascular pathologies (19). Selecting a collagen coating concentration that does not increase cell proliferation is important for the potential to mimic physiological conditions in vitro. It was observed that collagen coating had a statistically significant effect on cell proliferation in the group seeded with 10k cells and incubated at 4°C, as well as in all groups seeded with 20k cells. This suggests that cell density and specific temperatures can alter cell behavior. This finding supports the idea that these variables can affect cell proliferation and phenotypic stability in *in vitro* models.

The lack of significant increase in cell viability compared to the control group in groups incubated at 4°C, 25°C, and 37°C indicates that these coating methods do not trigger cell proliferation. This suggests that this model is suitable for in vitro vascular pathology modeling. Additionally, although no statistically significant changes were observed, detailed examination of cell viability compared to the control group (where cell viability was considered 100%) revealed that the G group at 25°C with a viability rate of 100.1% and the F group at 37°C with a viability rate of 99.7% were selected as the most applicable groups (Figure 3).

However, a statistically significant effect of collagen coating on cell proliferation was observed in the 4°C group seeded with 10k cells and in all temperature groups seeded with 20k cells. Given that the goal of the study is to develop a healthy vascular model and that cell proliferation can be an indicator of endothelial damage, experimental groups that do not affect cell proliferation were selected as the most appropriate conditions. Since the groups seeded with 20k cells were completely excluded, it appears more appropriate to use 10k cells for seeding. Furthermore, since proliferation differences were observed in the 4°C group of 10k cells, the 25°C and 37°C conditions are deemed more suitable.

In both temperature conditions, groups F (42 µg), G (93.75 µg), and H (250 µg) represented different collagen concentrations. To determine the optimal temperature, proliferation data among the F-G-H groups were compared, and given the lack of differences between the groups, the 37°C condition was deemed more appropriate. Among the F-G-H groups, the F group was preferred due to the lower collagen concentration. Therefore, for the subsequent studies required for vascular modeling in our project, it was determined that experiments would continue with 10k cell seeding and 42 µg collagen coating at 37°C.

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THE IMPACT OF THE RECREATIONAL FLOW EXPERIENCE ON THE PERCEPTION OF WELLNESS AMONG INDIVIDUALS ENGAGED IN EXTREME SPORTS

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ABSTRACT

Purpose: The purpose of this research is to determine the impact of recreational flow experience on perceived wellness among extreme sports participants.

Material and Methods: Employing a quantitative research method, a correlational survey model was utilized in this study. The research group consists of 532 extreme sports participants, including 397 men and 135 women. Data were analyzed using SPSS 24.0, with Pearson correlation and multiple linear regression used to examine the relationship and impact of flow experience on perceived wellness.

Results: The findings revealed that anxiety and boredom during the activity were negatively correlated with perceived wellness, while acquaintance and flow were positively correlated. Regression analysis showed no significant impact of boredom on wellness. Acquaintance positively affected psychological, emotional, physical, and intellectual wellness, while anxiety had a negative effect on all dimensions of wellness. Additionally, recreational flow experience had a positive impact on all dimensions of perceived wellness.

Conclusion: Consequently, it was determined that anxiety during extreme sports negatively affected perceived wellness, while the attitude towards acquaintance during the activity and recreational flow experience positively influenced perceived wellness. Recommendations for practitioners include organizing trainers and informing recreation specialists and coaches to establish a balance between challenge and skill in order to facilitate the flow experience.

Keywords: recreational flow experience, perceived wellness, extreme sports

INTRODUCTION

In contemporary times, individuals seeking to transcend their limits and pursue diverse experiences are increasingly turning to extreme sports as a form of recreation. Activities such as rock climbing on steep slopes, paragliding from high peaks, and scuba diving to explore the underwater world offer intense experiences that are difficult to encounter in everyday life. Extreme sports, which hold a significant place in the tourism and recreation industry, have gained increasing popularity in recent years. Extreme athletes are often characterized as unconventional individuals in search of excitement and action (1). These individuals aim to feel good by experiencing different sensations during their activities. Humans are beings who strive to understand and make sense

of themselves and their lives, continually seeking ways to live happily, healthily, and peacefully. Scientists have long studied the steps leading to a happy and peaceful life. Well-being and optimal happiness depend on avoiding negative stimuli and feeling good psychologically, physically, emotionally, socially, spiritually, and intellectually (2). People pursue various emotions and experiences and try to distance themselves from negative stimuli in their lives to achieve their desired state of well-being.

Extreme sports inherently involve various risks and dangers, including the risk of death. Despite these dangers and risks, athletes participate in extreme sports, accepting all risks in their quest for meaning, excitement, and different experiences. In this context, it can be said that individuals engaging in extreme sports aim to experience unique states, such as flow (3). The physical and mental challenges inherent in extreme sports provide opportunities for experiencing recreational flow. Individuals who experience flow are fully immersed in their activities, distancing themselves from negative emotions such as worry, anxiety, and stress (4). Therefore, it can be suggested that the recreational flow experience during extreme sports contributes to increased perceived levels of well-being. The psychological mechanism of distancing from negative emotions such as anxiety, worry, and stress during the flow experience is based on several key factors. Firstly, the flow experience allows individuals to fully concentrate their attention on the activity. This concentration enables more efficient use of mental resources and facilitates a detachment from external concerns. Additionally, during flow, individuals gain confidence in overcoming challenges (20). This confidence enhances intrinsic motivation and strengthens their ability to cope with stressful situations. The flow state increases individuals' sense of control, helping them elevate their performance to a higher level. Finally, the flow experience provides individuals with a sense of achievement and satisfaction, thereby enhancing overall happiness and well-being. These processes help individuals become more resilient to negative emotions. Therefore, experiencing flow has a positive effect on psychological well-being (22).

Literature review reveals that recreational flow experience holds a significant place in positive psychology literature. Furthermore, it can be said that recreational flow experience has positive effects on well-being. When examining the conducted studies, it

is known that flow experience is associated with optimal performance, positive subjective experience, positive mood, happiness, subjective well-being, and good mood, as well as life satisfaction, satisfaction, quality of life, and socialization, and it has positive effects on well-being (5-9,10,11). Additionally, flow experience is positively related to sports, exercise, and exceptional performance (12-14). In studies focused on extreme sports, it is often observed that attention is given to a single discipline, such as surfers and mountain climbers. However, it is necessary to include different extreme athletes in the research for a comprehensive examination of the topic (5, 16).

Particularly, there is a need for studies that comprehensively cover different extreme sports branches and all dimensions of well-being. Additionally, in the conducted studies, flow experience has been predominantly examined in terms of quality of life, psychological and subjective well-being, motivation, self-efficacy, or positive mood (5,6,15,17-19). The conducted research often considers the psychological well-being effects of flow. However, the state of well-being is formed not only psychologically but also integrated with other dimensions of well-being.

Csikszentmihalyi defines the flow experience as a holistic state of being and optimal experience that individuals experience when they fully concentrate on the task at hand, giving all their attention and focus (20). In this experience, individuals feel a sense of unity in their mental and emotional state and completely immerse themselves in the activity. Flow experience refers to the experience gained by an individual who is aware of their own performance, completely focusing on overcoming challenges, tasks, or actions, and achieving harmony with control and performance (21). For example, when an extreme athlete experiences flow, environmental factors and various thoughts in the mind are filtered out, concentration on the activity is at its highest level, and a sense of pleasure and happiness emerges (22). This situation has a positive effect on the individual's mental and physical health and contributes to an increase in perceived wellness.

Perceived wellness refers to individuals' perceptions of being healthy and happy psychologically, emotionally, physically, socially, spiritually, and intellectually (23). The perceived wellness model consists of six dimensions, including physical, spiritual, psychological, social-emotional, and

intellectual dimensions. This model is defined as a coherent and balanced lifestyle conducive to development, taking into account the outlines of integrated systems (2,24,25). Considering the operation of the perceived wellness model, it is believed that experiencing flow during activity will increase perceived wellness. Recreational flow experience is a subjective and positive state resembling an optimal experience that occurs when an individual's mind is completely focused on the current activity without realizing how time passes and shutting down non-functional thoughts (7). Extreme sports, being adrenaline-fueled and risky activities, intensify the experience of recreational flow in these activities. During recreational flow experience, individuals find opportunities to use their skills and abilities according to the difficulty of the activity. This situation helps to increase their self-confidence, feel better about themselves, become motivated towards the activity, enhance their self-esteem, distance themselves from negative emotions such as stress and anxiety, release endorphins, the happiness hormone in the brain, and strengthen their perceptions of wellness (26). Additionally, it is known that the flow experience not only creates positive emotions but also blocks negative emotions (10). In light of this information, it can be said that experiencing recreational flow during extreme sports contributes to increasing levels of perceived wellness. The aim of this research is to determine the impact of recreational flow experience on perceived wellness among extreme sports participants. In this context, the research seeks answers to the following questions:

I.   Is there a significant relationship between recreational flow experience and perceived wellness among extreme sports participants?
II. Does recreational flow experience have a significant effect on perceived wellness among extreme sports participants?

The originality of the study is demonstrated by the fact that it involves extreme sports participants from various branches such as air, land, and water, which constitute a challenging sample to reach, and by considering all dimensions of perceived wellness in the assessment of recreational flow experience. Additionally, no previous study has been encountered that utilized a three-channel flow experience model and perceived wellness model, as employed in this research. Therefore, understanding the impact of

recreational flow experience on perceived wellness among individuals engaged in extreme sports is important in contributing to theoretical understanding in the fields of sports recreation and sports psychology.

MATERIALS AND METHODS
In this study, a correlational survey model was used, designed using quantitative research methods. Survey models are a research approach aimed at identifying a situation that has occurred or is still ongoing in its existing form. The correlational survey model is a research model that aims to determine the presence, direction, and level of co-variation between two or more variables (27,28).

The model discussed by Csikszentmihalyi, addresses the anxiety that arises when the difficulty level of an activity exceeds an individual's skill and the boredom that occurs when the difficulty level is below the individual's skill. According to the three-channel flow model, the balance between skills and challenges results in the experience of flow and the transition into the flow channel. As shown below, Figure 1 presents the three-channel flow experience model (20).
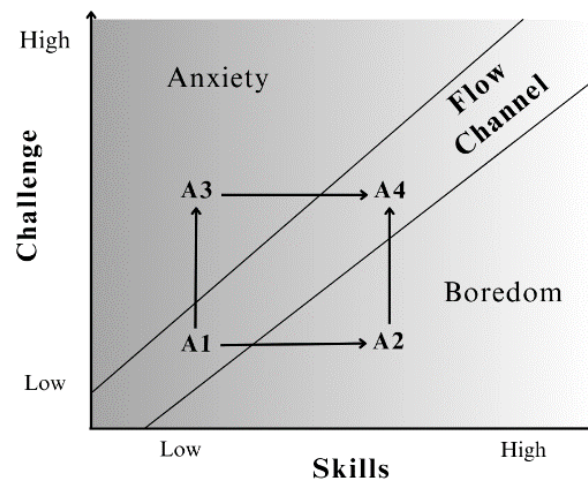

Figure 1. Three-Channel Flow Experience Model

The perceived well-being model is more concerned with how individuals perceive their own health from a personal perspective rather than providing a direct definition of health. In this context, the six dimensions within the perceived well-being model interact with each other to form a holistic structure. As illustrated in Figure 2 below, the components of perceived well-being are shown (2).
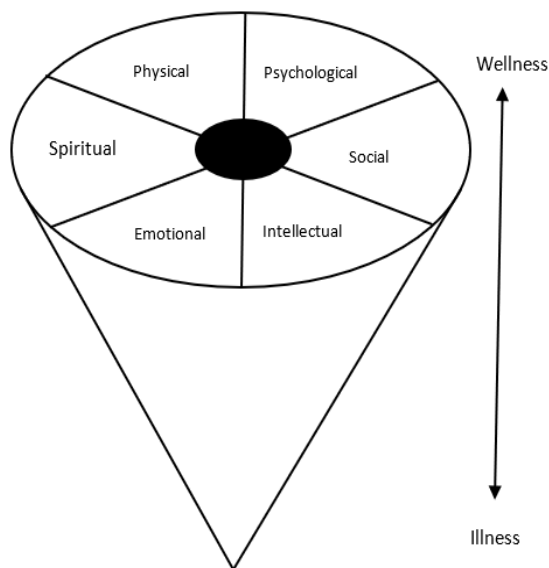
Figure 2. The Perceived Wellness Model

As shown in Figure 2, the upper part of the perceived well-being model represents well-being. As the perception of well-being increases, the upper section of the wheel representing well-being expands, while the narrowing lower part of the model represents illness and a decline in the perception of well-being. Understanding the impact of recreational flow experiences on the perception of well-being among individuals engaged in extreme sports is important for contributing to theoretical insights in the fields of sports recreation and sports psychology. In line with this information, the research has been designed within the framework of these models to determine the effect of recreational flow experiences on the perception of well-being among extreme athletes.

Research Sample

The research sample consisted of individuals engaged in extreme sports in Turkey. In selecting the research sample, the convenience sampling technique, which is a type of non-probability sampling based on voluntariness, was employed. In this context, the research sample consisted of a total of 532 participants, including 397 males (74.6%) and 135 females (25.4%). The participants included in the study were individuals who engage in at least one of the extreme sports branches in the air, land, and water, and who are interested in extreme sports at a

recreational, semi-professional, competitive, or extreme sports instructor level.

Even though the main population constituting the universe in the literature is 10 million, it is stated that for many studies, a sample size of 384 participants, with a confidence level of 95% and a margin of error of 5%, is sufficient to collect data with high reliability. That is, if the elements in the population exceed ten million, it is considered appropriate to collect data with a high level of reliability with a minimum required sample size of 384 participants (29-31). In this study, the sample of 532 extreme athletes was deemed suitable for generalizing to athletes in Turkey, as it exceeds the minimum of 384 participants indicated in scientific research literatüre (29-31), thus supporting the representativeness of the sample for the population. The exact number of recreational, semi-professional, competitive, and extreme sports instructors in Turkey is not precisely known. Although the total number of extreme sports athletes in Turkey is unclear, it is estimated to be under 10 million. Based on this, a minimum sample size of 384 participants was deemed appropriate for the study. A final sample of 532 extreme athletes was reached by including as many participants as possible.

Descriptive information regarding the participants' "age, gender, marital status, education level" is provided in Table 1 below.

The descriptive information regarding the participants' "area where extreme sports are practiced, years of experience in extreme sports, frequency of engaging in extreme sports, introduction and interest in extreme sports" is presented in Table 2.

Data Collection Instruments

The data collection instruments for this research consist of three sections. In the first section, a "Personal Information Form" prepared by the researcher was used. In the second section, the "Alpak Flow Scale (AFS)" was utilized, and in the third section, the "Perceived Wellness Scale (PWS)" was employed.

Personal information form

The first section of the questionnaire, which served as the data collection tool, included a researcher-developed personal information form consisting of a total of 11 statements aimed at determining the socio-demographic characteristics of the participants.

Table 1. Variables related to participants' personal information.

Variables	Groups	Frequency (N)	Percent (%)
Age	Under 18 years old	57	10,7
	19-25	139	26,1
	26-35	147	27,6
	36-45	127	23,9
	46	62	11,7
Gender	Male	397	74,6
	Female	135	25,4
Marital Status	Married	147	27,6
	Single	385	72,4
Educational Status	High school graduate	164	30,8
	Collage graduate	268	50,4
	Postgraduate	101	18,8
Total		532	100

Table 2. Various variables related to participants' engagement in extreme sports.

Variables	Groups	Frequency (N)	Percent (%)
Area of Extreme Sports Participation	Air	111	20,9
	Land	262	49,2
	Water	159	29,9
Years of Experience in Extreme Sports	Less than 1 year	63	11,8
	Between 2-4 years	144	27,1
	Between 5-7	102	19,2
	Between 8-10	80	15
	11 years and above	143	26,9
Frequency of Engaging in Extreme Sports	At least once every 3 days	183	34,4
	At least once a week	145	27,3
	At least once a month	116	21,8
	At least once every 6 months	88	16,5
Introduction to Extreme Sports	Through friends	189	35,5
	Through social media	93	17,5
	By observing live	250	47
Interest in Extreme Sports	At a recreational level	222	41,7
	Semi-professional	142	26,7
	Competitor	89	16,7
	Extreme sports instructor	79	14,8
Total		532	100

These statements covered information such as "age, gender, marital status, education level, extreme sports branch, years of interest in extreme sports, frequency of engaging in extreme sports, introduction to extreme sports, and interest in extreme sports." The personal information form was designed by researchers to closely examine the personal characteristics of extreme athletes.

Alpak flow scale

The "Alpak Flow Scale (AFS)" developed by (32) was used to measure the flow experience of individuals interested in extreme sports. The primary reason for selecting the Alpak Flow Scale is its role as a multidimensional measurement tool that effectively represents the three-channel flow model in the literature. This scale not only captures the complexity of flow experiences but also aligns well with our study's focus on wellness. Furthermore, it is believed that the factors within the scale can comprehensively explain all parameters of perceived well-being in relation to flow and wellness. These attributes underscore the scale's suitability for achieving the objectives of our research, enabling us to explore the intricate relationships between flow experiences and overall well-being among extreme athletes. The items in the scale were ranked according to a 5-point Likert scale (1=Strongly disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly agree). The scale consists of four dimensions and twenty items. The dimensions of the scale are "encounter, boredom, anxiety, and flow." The overall Cronbach's alpha reliability coefficient (α) of the scale was calculated as .807. When examining internal consistency in terms of sub-dimensions, it is observed that the Cronbach's alpha coefficient was α=.65 for the encounter sub-dimension, α=.78 for the boredom sub-dimension, α=.87 for the anxiety sub-dimension, and α=.79 for the flow sub-dimension (32).

Perceived wellness scale

The levels of wellness perception of individuals were examined in the research. In this context, the Perceived Wellness Scale (PWS), developed by (1) and adapted into Turkish by (33) through reliability and validity studies, was used. The primary reason for choosing the Perceived Wellness Scale is its comprehensive coverage of all wellness components. Unlike scales that measure only one aspect of wellness, this scale assesses six fundamental components, providing a holistic view of well-being.

This multidimensional approach is particularly important in the context of extreme sports, where various factors can influence athletes' overall wellness. Additionally, the Perceived Wellness Scale is considered a crucial measurement tool for determining the well-being of extreme athletes, as it captures the complex interplay between physical, emotional, and social dimensions of wellness. By utilizing this scale, our study aims to gain deeper insights into how flow experiences impact the overall wellness of extreme sports participants. Perceived wellness scale consists of six dimensions, each comprising six items. The dimensions of the scale are "physical, psychological, emotional, intellectual, social, and spiritual wellness perception." The PWS is a 6-point Likert-type scale (1=Strongly disagree, 2=Disagree, 3=Partially disagree, 4=Partially agree, 5=Agree, 6=Strongly agree).

Of the 36 items in the scale, 21 are positive and 15 contain negative expressions. Negative statements were reverse-coded during the analysis process. An increase in the average scores obtained from the scale indicates an increase in wellness perception. The Cronbach's alpha reliability coefficient of the scale was found as α=.81 in the initial application by (34) and α=.83 in the second application. The internal consistency coefficient of the PWS was determined as α=.91 in the study conducted by (1).

Data Collection Method

Permissions regarding the use of measurement instruments for the research were obtained by contacting the responsible authors via email. Subsequently, The Ethics Committee of the Sakarya University of Applied Sciences Rectorate decided that the study is ethically appropriate (Date: 10.03.2023, Deciison no: 29/10). To collect the data, a questionnaire was designed using Google Forms. The research data were obtained using the online survey technique. Consent was obtained from participants for their voluntary participation in the online survey. Virtual groups consisting of extreme sports enthusiasts were considered as an important source to achieve the desired sample size in the research. In this regard, relevant community members were directed to the online survey form through social media and messaging platforms such as Instagram, Facebook, Telegram, and WhatsApp. Additionally, the online survey form was sent to various official institutions related to extreme sports (Ministry of Youth and Sports, Sports Federations,

etc.) and sports clubs. The survey for this study was distributed to individuals interested in extreme sports, social media groups, and relevant sports federations to reach an appropriate sample that could represent the population. It is assumed that participants answered the questions in the survey form completely, sincerely, and objectively. During the data collection process, only extreme athletes who have participated in at least one of the following sports—such as aerial sports including cliff diving, sky surfing, sky diving/freediving, hang gliding (delta), bungee jumping, and paragliding; land sports like mountaineering and rock climbing, enduro/extreme motocross and rally, parkour/freerunning, BMX and mountain biking, snowboarding/snowsurfing, and aggressive inline skating and skateboarding; and water sports including wakeboarding, windsurfing, kitesurfing, wakesurfing, freediving and scuba diving, as well as rafting and canoeing—were included as recreational, semi-professional, competitive, or instructors. Additionally, it was required that the extreme athletes included in the study have engaged in extreme sports for at least one year and have reached a certain level of proficiency. Additionally, the data collection process was conducted with attention to the principles of voluntariness. Furthermore, 57 individuals aged 18 were included in the study, and written consent forms were obtained from the relevant participants during the data collection process.

Data Analysis

To prevent potential problems arising from the data collection instruments and alleviate reader concerns, a pilot test was conducted. The pilot study, applied to 30 extreme sports enthusiasts, revealed no formal or semantic issues in the questionnaire form.

For the statistical analysis of the data obtained in the research, SPSS (Statistical Package for the Social Sciences) 24.0 program was utilized. Descriptive statistics such as "frequency, percentage, mean, and standard deviation" were employed to determine the demographic characteristics of the participants. Cronbach's Alpha internal consistency coefficients were calculated to determine the reliability of the scales used in the research.

The normality test was applied, and skewness and kurtosis values were examined to confirm that the data obtained from the research exhibited normal distribution. Therefore, parametric tests were employed for statistical analysis. Pearson Correlation analysis was used to determine the relationship

Table 3. Reliability analyses of the study variables.

Scale Name	Dimensions	Number of Items	Cronbach Alpha (α)
Alpak Flow Scale	Encounter	5	,791
	Boredom	5	,666
	Axiety	5	,851
	Flow	5	,828
	AFS Total	20	,814
Perceived Wellness Scale	Psychological	6	,709
	Emotional	6	,729
	Social	6	,757
	Physical	6	,729
	Spiritual	6	,768
	Intellectual	6	,514
	PWS TOTAL	36	,890

Table 4. Results of the normality analysis for the scales used in the research.

Dimensions	N	X̄	Ss	Kurtosis	Skewness
Encounter	532	4,76	,32	,884	-1,246
Boredom	532	2,48	,80	-,041	507
Anxiety	532	2,10	,94	-,206	717
Flow	532	4,78	,38	1,024	-1,425
AFS Total	532	4,25	,45	,151	-,655
Psychological	532	4,24	,77	,042	-,016
Emotional	532	4,56	,88	-,510	-,277
Social	532	4,48	,91	-,296	-,368
Physical	532	4,41	,90	-,075	-,324
Spiritual	532	4,43	,92	-,100	-,479
Intellectual	532	4,64	,72	-,442	-,165
PWS Total	532	3,91	,39	,751	,589

between recreational flow experience and wellness perception, while Multiple Linear Regression analysis was utilized to determine the levels of influence of AFS sub-dimensions on PWS sub-dimensions.

Reliability analysis results for the scales used in the research

Table 3 below presents the reliability analysis results for the Alpak Flow Scale (AFS) and the Perceived Wellness Scale (PWS).

The Cronbach Alpha (α) values of the total score averages for the scales used indicate that the Alpak Flow Scale (α: 0.814) is highly reliable, and the Perceived Wellness Scale (α: 0.890) is also highly reliable. The high reliability level of the scales used in the research means that the measurement tools consistently and reliably measure the variable of interest (34).

Results of normality analysis for the scales used in the research

As seen in Table 4 below, the results of the normality analysis for the Alpak Flow Scale and the Perceived Wellness Scale used in the research are presented.

According to Table 4, when the kurtosis and skewness values of the scores for the Alpak Flow Scale and the Perceived Wellness Scale are examined, it is determined that the data obtained in the research fall within the range of "-/+ 1.5" values and exhibit a normal distribution. Therefore, it has been determined that the use of parametric tests in the analyses is appropriate (35).

RESULTS

This section presents the research findings obtained from the statistical analyses conducted within the scope of the research.

Pearson correlation analysis on the relationship between recreational flow experience and perceived wellness

The Pearson correlation analysis results between the sub-dimensions of the Alpak Flow Experience Scale and the sub-dimensions of the Perceived Wellness Scale for extreme sports athletes are presented in Table 5.

According to Table 5, a positive and significant relationship was found between the dimensions of acquaintance and flow and all subdimensions of well-being. Additionally, the anxiety subdimension was negatively related to all subdimensions of well-being, while the boredom subdimension had no significant relationship with physical well-being but showed a negative significant relationship with the other subdimensions of well-being.

The acquaintance dimension was positively and weakly correlated with psychological well-being (r=0.260, p<0.01), emotional well-being (r=0.269, p<0.01), social well-being (r=0.075, p<0.05), physical well-being (r=0.233, p<0.01), spiritual well-being (r=0.209, p<0.01), and moderately correlated with intellectual well-being (r=0.311, p<0.01).

The boredom dimension was negatively and weakly correlated with psychological well-being (r=-0.143, p<0.01), emotional well-being (r=-0.171, p<0.01), social well-being (r=-0.134, p<0.01), and spiritual well-being (r=-0.138, p<0.01), but showed no

Table 5. Results of the analysis regarding the relationship between AFS subscales and PWS subscales.

		Psychological Wellness	Emotional Wellness	Social Wellness	Physical Wellness	Spiritual Wellness	Intellectual Wellness
Encounter	r	,260**	,269**	,075*	,233**	,209**	,331**
	p	,000	,000	,042	,000	,000	,000
Boredom	r	-,143**	-,171**	-,134**	-,051	-,138**	-,129**
	p	,000	,000	,001	,120	,001	,001
Anxiety	r	-,231**	-,429**	-,235**	-,153**	-,302**	-,199**
	p	,000	,000	,000	,000	,000	,000
Flow	r	,286**	,277**	,175**	,283**	,263**	,356**
	p	,000	,000	,000	,000	,000	,000

**statistically significant at p<0.01 *statistically significant at p<0.05

Table 6. Results of the analysis on the impact of flow experience on psychological wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	,235	,076	,146	3,098		,002**
Boredom	-,049	,042	-,052	-1,177	,123	,240
Anxiety	-,124	,036	-,152	-3,431		,001**
Flow	,266	,073	,174	3,646		,000**

Dependent Variable: Psychological Wellness; R: ,360; R^2: ,130; F: 19,679; p: ,000; Durbin-Watson: 1,786; Method: Enter

Table 7. Analysis results on the impact of flow experience on emotional wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	,270	,081	,146	3,324		,001**
Boredom	-,006	,045	-,005	-,132	,231	,895
Anxiety	-,350	,039	-,377	-9,076		,000**
Flow	,217	,078	,124	2,779		,006**

Dependent Variable: Emotional Wellness; R: ,486; R^2: ,237; F: 40,813; p: ,000; Durbin-Watson: 2,059; Method: Enter

significant relationship with physical well-being. It was also negatively correlated with intellectual well-being (r=-0.129, p<0.01).

The anxiety dimension was negatively correlated with psychological well-being (r=-0.231, p<0.05), emotional well-being (r=-0.429, p<0.01), social well-being (r=-0.235, p<0.01), physical well-being (r=-0.153, p<0.01), spiritual well-being (r=-0.302, p<0.01), and intellectual well-being (r=-0.199, p<0.01).

The flow dimension was positively and weakly correlated with psychological well-being (r=0.286, p<0.01), emotional well-being (r=0.277, p<0.01), social well-being (r=0.175, p<0.01), physical well-

being (r=0.283, p<0.01), spiritual well-being (r=0.263, p<0.01), and moderately correlated with intellectual well-being (r=0.356, p<0.01).

Multiple linear regression analysis on the effect of recreational flow experience on perceived wellness

In the study, the sub-dimensions of the Alpak Flow Scale, including encounter, boredom, anxiety, and flow, were determined as dependent variables, while the psychological, emotional, social, physical, spiritual, and intellectual dimensions of the Perceived Wellness Scale were determined as independent variables. The results of the multiple linear regression analysis conducted on the effect of flow experience on psychological wellness are presented in Table 6 below.

According to Table 6, it is observed that the model developed for the effect of extreme athletes' flow experience on perceived psychological wellness is statistically significant (F=19.679; p<0.01). According to the model, 12.3% (R^2_{adj}=0.123) of the participants' perceived psychological wellness levels (dependent variable) are explained by their flow experiences (independent variable). When examined in terms of sub-dimensions, it was determined that the encounter dimension (β=0.146; t=3.098; p=0.002), anxiety dimension (β=-0.152; t=-3.431; p=0.001), and flow dimension (β=0.174; t=3.646; p=0.000) scores have a significant effect on perceived psychological wellness. However, it was found that the boredom dimension (β=-0.052; t=-1.117; p=0.240) does not statistically significantly affect perceived psychological wellness.

According to Table 7, it has been determined that the model developed for the effect of extreme athletes' flow experience on perceived emotional wellness is statistically significant (F=40.813; p<0.01). According to the model, 23.1% (R^2_{adj}=0.231) of the participants'

J Basic Clin Health Sci 2024; 8: 734-749

Dilmac E et al. Recreational flow and wellness in extreme sports

Table 8. Analysis results on the impact of flow experience on social wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	-,057	,093	-,030	-,610		,542
Boredom	-,050	,051	-,044	-,971	,067	,332
Anxiety	-,188	,044	-,194	-4,238		,000**
Flow	,259	,090	,142	2,883		,004**

Dependent Variable: Social Wellness; R: ,272; R²: ,074 F: 10,534; p: ,000; Durbin-Watson: 1,750; Method: Enter

Table 9. Analysis results on the impact of flow experience on physical wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	,218	,090	,115	2,415		,016*
Boredom	,027	,050	,025	,551	,093	,582
Anxiety	-,94	,043	-,099	-2,202		,028*
Flow	,372	,087	,208	4,298		,000**

Dependent Variable: Physical Wellness; R: ,316; R²: ,100; F: 14,585; p: ,000; Durbin-Watson: 2,054; Method: Enter

Table 10. Analysis results on the impact of flow experience on spiritual wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	,181	,098	,086	1,837		,067
Boredom	-,021	,054	-,017	-,381	,132	,703
Anxiety	-,262	,047	-,247	-5,601		,000**
Flow	,332	,095	,166	3,505		,000**

Dependent Variable: Spiritual Wellness; R: ,372 ; R²: ,139; F: 21,221; p: ,000; Durbin-Watson: 1,805; Method: Enter

Table 11. Analysis results on the impact of flow experience on ıntellectual wellness perception.

Independent Variables	B	Standard Error	Beta (β)	t	R^2_{adj}	p
Encounter	,298	,069	,197	4,309		,000**
Boredom	-,039	,038	-,044	-1,027	,167	,305
Anxiety	-,078	,033	-,102	-2,364		,018*
Flow	,330	,067	,230	4,959		,000**

Dependent Variable: Intellectual Wellness; R: ,416; R²: ,173; F: 27,608; p: ,000; Durbin-Watson: 1,858; Method: Enter

perceived emotional wellness levels are explained by their flow experiences. When examined in terms of sub-dimensions, it was found that the encounter dimension (β=0.146; t=3.324; p=0.001), anxiety dimension (β=-0.337; t=-9.076; p=0.000), and flow dimension (β=0.124; t=2.779; p=0.006) have a significant effect on perceived emotional wellness.

However, it was found that the boredom dimension (β=-0.005; t=-0.132; p=0.895) does not have a significant effect on perceived emotional wellness. In Table 8, it is observed that the model developed for the effect of extreme athletes' flow experience on perceived social wellness is statistically significant (F=10.534; p<0.01). According to the model, 6.7% (R²adj=0.067) of the participants' perceived social wellness levels are explained by their flow experiences. When examined in terms of sub-dimensions, it was found that there is no statistically significant effect of the encounter dimension (β=-0.030; t=-610; p=0.542) and the boredom dimension (β=-0.44; t=-0.971; p=0.322) on perceived social wellness. However, it was found that the anxiety dimension (β=-0.194; t=-4.238; p=0.000) and the flow dimension (β=0.142; t=2.883; p=0.004) have a significant effect on perceived social wellness.

According to Table 9, it is seen that the model developed for the effect of extreme athletes' flow experience on perceived physical wellness is statistically significant (F=14.585; p<0.01). According to the model, 9.3% (R²adj=0.093) of the participants' perceived physical wellness is explained by their flow experience. The dimensions that have a significant effect on physical wellness are determined to be the encounter dimension (β=0.115; t=2.415; p=0.016), the anxiety dimension (β=-0.099; t=-2.202; p=0.028), and the flow dimension (β=0.208; t=4.298; p=0.000). It was found that the boredom dimension (β=0.025; t=551; p=0.582) does not have a statistically significant effect on perceived physical wellness.

According to Table 10, it is observed that the model developed for the effect of extreme athletes' flow experience on perceived spiritual wellness is statistically significant (F=21.221; p<0.01). According to the model, 13.2% (R²adj=0.132) of the participants' perceived spiritual wellness is explained by their flow experience. The dimensions that have a significant effect on spiritual wellness are determined to be the anxiety (β= -0.247; t=-5.601; p=0.000) and flow dimensions (β=0.166; t=3.505; p=0.000). It was found that the encounter dimension (β=0.086; t=1837; p=0.067) and the boredom dimension (β=-0.017; t=-381; p=0.703) do not have a statistically significant effect on perceived spiritual wellness.

Table 11 indicates that the model developed for the effect of extreme athletes' flow experience on perceived intellectual wellness is statistically significant (F=27.608; p<0.01). Upon examining Table 11, it is determined that 16.7% (R²adj=0.167)

of the participants' perceived intellectual wellness is explained by their flow experience. The dimensions that have a significant effect on intellectual wellness perception are found to be encounter (β= 0.197; t=4.309; p=0.000), anxiety (β=-0.102; t=-2.364; p=0.018), and flow dimensions (β=0.230; t=4.959; p=0.000). It is concluded that the boredom dimension (β=-0.044; t=-1.027; p=0.305) does not have a significant effect on perceived intellectual wellness perception.

DISCUSSION

"The aim of this study is to determine the effect of the recreational flow experience on the well-being perception of extreme athletes. Within the scope of the study, the hypotheses 'H1: There is a significant relationship between the recreational flow experience and the perception of well-being in extreme athletes' and 'The recreational flow experience has a significant impact on the perception of well-being in extreme athletes' were tested and confirmed.

Focusing on well-being, which has not been previously emphasized in research involving a specific group such as extreme athletes, is thought to contribute to the theoretical literature within the framework of flow theory. Additionally, the research results highlight the importance of recreation and recreational flow experiences as a means of renewal for maintaining "well-being and balance" in terms of the triad of mind, body, and spirit. Therefore, the study's support for and validation of flow theory, along with the testing of the relationship between perceived well-being and flow theory within a specific group of extreme athletes, underscores the significance and unique value of this research.

Based on the regression analysis conducted, it was concluded that 12.3% of the perceived psychological wellness, 23.1% of emotional wellness, 6.7% of social wellness, 9% of physical wellness, 13.2% of spiritual wellness, and 16.7% of intellectual wellness perceptions were explained by the Alpak Flow Experience Scale. These results indicate the significant role of recreational flow experience in explaining wellness perception. While no research specifically investigating the impact of flow experience on perceived wellness was found in the literature, the findings of this study can be paralleled with studies focusing on different dimensions of wellness (4- 6,15,16).

The Pearson correlation analysis results between the Alpak Flow Experience Scale dimensions and the Perceived Wellness Scale dimensions revealed a significant positive relationship between the encounter dimension and psychological, emotional, social, physical, spiritual, and intellectual wellness dimensions. Within this framework, it was determined that extreme athletes' attitudes toward acquaintance with the activity had a significant effect on psychological, emotional, physical, and intellectual wellness perceptions.

The stage of acquaintance with the activity is a process where both physical and mental perceptions are active. It is known that feelings and states such as happiness, self-confidence, and excitement are associated with an individual's perceptions of themselves and the activity. Therefore, it can be said that positive emotions and states experienced during participation in the activity will increase psychological and emotional wellness perceptions perceived by the individual. Csikszentmihalyi, in his studies on flow experience mentioned that states such as happiness and motivation during the participation in the activity positively affect wellness, especially psychological well-being (36).

Research supports the idea that there is a positive relationship between activity-related positive attitudes and wellness perceptions, and as positive attitudes toward the activity increase, wellness also increases (6,37-42). Studies indicating that positive feelings during the activity process positively affect wellness support the findings of this study (43,44).

Brymer et al. (2009) concluded from their study with rock climbers that extreme sports create a sense of closeness between athletes and nature, can increase respect and sensitivity to nature, and can support environmental sustainability (17). This study emphasizes that extreme sports enhance perceived intellectual skills and make athletes more conscious of the natural environment, which supports the findings of this research. Akyol and İmamoğlu (2019) stated in their study that highly motivated students experience flow more frequently and at higher levels (45). Based on this study, it can be said that there is a positive relationship between motivation and flow experience during the acquaintance with the activity, and high motivation can increase flow experience. Dietrich (2004) in his study on flow experience from a cognitive and neuroscience perspective mentioned that flow experience activates different areas of the brain such as the prefrontal cortex, temporal lobe, parietal lobe, and cerebellum (46). These activations are believed to affect mental processes such as

attention, learning, memory, and motor control, and support factors for the occurrence of flow experience. It is also stated that neurochemicals such as dopamine and norepinephrine play an important role in flow experience. These neurochemicals are thought to increase the activation of reward centers in the brain, thereby increasing motivation and strengthening the factors that provide flow experience. These findings support the idea that extreme athletes' experiences of flow during the acquaintance with the activity, resulting in perceptions of time dilation and rapid flow of time towards the activity, have effects on perceived wellness.

Regression analysis results of the study indicate that while wellness perception is explained by recreational flow experience, it is also important to note the existence of other contributing factors such as happiness level, health status, stress level, received social support, job satisfaction, personal development, social relationships, and the presence of purpose in life. In this context, perceived wellness can be said to be explained in different ways according to individual, social, and environmental factors.

Although no significant relationship was found between the dimension of boredom and physical wellness, a significant negative relationship was identified between psychological, emotional, social, spiritual, and intellectual wellness dimensions. Previous studies have shown that the level of boredom during free time is negatively associated with life satisfaction, psychological resilience, self-esteem, perceived social competence, and mood states. Additionally, studies have consistently shown a negative relationship between boredom and wellness dimensions such as psychological well-being. The findings of our research parallel these studies, indicating a negative relationship between boredom and various dimensions of wellness.

The research findings also reveal that when the level of challenge in the activity falls below the individual's skills, resulting boredom does not significantly impact wellness perception. Boredom during an activity usually arises due to its monotony, repetitiveness, or low level of challenge. Wellness perception generally refers to an individual feeling happy, satisfied, balanced, and good about themselves. Therefore, it can be said that there is a negative relationship between boredom during the activity and wellness perception. However, the study found that the level of

boredom did not have a significant effect on perceived wellness. This may be because the feeling of boredom during the activity is temporary and may not be significant enough to affect wellness perception. The lack of impact of perceived wellness on boredom may be due to the transient nature of boredom in extreme sports and the rapid transition from the boredom channel to the acquaintance or flow channels. It is thought that boredom may not occur as frequently in extreme sports as in other passive recreational activities. The lack of influence of perceived wellness on boredom may also be due to factors such as the impact of motivation and flow experience on wellness perception, or other (physical, social, psychological, environmental, and cultural) factors not tested in this research.

According to the research findings, the anxiety state that occurs when the level of challenge in the activity exceeds the individual's skills is negatively associated with psychological, emotional, social, physical, spiritual, and intellectual wellness dimensions. Additionally, it was found that the anxiety dimension had a significant negative effect on all dimensions of perceived wellness. These findings suggest that as anxiety levels increase, perceived wellness decreases, and the anxiety state experienced by extreme athletes during the activity negatively affects wellness perception.

The anxiety dimension in the AFS involves negative attitudes toward the activity. As individuals encounter new things and the level of difficulty increases during the activity, if their skills are insufficient, they may consider quitting the activity, not wanting to participate, feeling like everyone is watching them, and experiencing anxiety. In some sports activities like extreme sports, situations may arise where the perceived difficulty exceeds the skills, leading to anxiety. Increased anxiety levels may lead to doubt in performance and exposure to negative stimuli mentally. Particularly in competitive sports or excessive exercise, anxiety can increase, leading to a decrease in wellness perception. Therefore, it is important for individuals to recognize their own limits and choose activities in which they feel comfortable while engaging in sports. It is expected that when faced with difficulty in the activity, the individual will challenge the activity and balance the perceived difficulty with their skills. In this case, the individual's self-confidence increases, they move away from the anxiety state, and gain motivation. Otherwise, if the individual's ability falls below the perceived difficulty

level, they enter the anxiety channel, although this situation may not always result in negative outcomes. For example, Brymer and Schweitzer (2013) suggest that perceived anxiety in extreme sports can enhance the quality of the experience (26). Additionally, it is expected that for recreational flow experience not to occur, the level of performance anxiety should be balanced rather than absent (47,48). Ultimately, for recreational flow experience to occur and for individuals to derive pleasure, enjoyment, and happiness from the activity, it is expected that there will be sufficient levels of performance anxiety (4,20). Furthermore, research has found a negative relationship between flow experience and anxiety levels (4,49).

Situational anxiety arises depending on environmental conditions during extreme sports. Negative attitudes and thoughts toward one's own performance create cognitive anxiety. Along with these anxiety states, the individual's heart rate accelerates, shallow breathing, tense muscles, sweating, distraction, and inattention occur, leading to physical anxiety. Physical wellness perception is negatively affected by bodily anxiety. The continuation of anxiety turns the situation into chronic stress, and the athlete begins to view events with a negative approach. This leads to dissatisfaction and unhappiness in the athlete and eventually leads to burnout. Therefore, it can be said that anxiety affects emotional, psychological, and spiritual wellness perception negatively. In light of this information, it can be said that the findings of the study are in parallel with the literature and provide support for studies conducted in the field of positive psychology. According to the research findings, there is a significant positive relationship between the flow dimension and psychological, emotional, social, physical, spiritual, and intellectual wellness dimensions. Furthermore, it was found that recreational flow experience positively influenced all dimensions of perceived wellness. The flow dimension of the AFS includes experiencing emotions and situations such as returning to the activity with developing skills, being satisfied with performance, experiencing pleasure, joy, happiness, and motivation during the activity. It was determined that extreme athletes who experience recreational flow during the activity also experience increased levels of wellness perception.

Ayhan (2022) found in their study that experiencing recreational flow during recreational activities is positively correlated with the recreational benefit dimensions of physical, psychological, and social benefits, and that recreational flow experience has a positive effect on leisure satisfaction (50). Kim and Lee (2008) conducted a study on individuals participating in sports recreational activities and found that recreational flow experience contributes positively to individuals' recreational benefits, allowing them to enjoy activities more and feel more satisfied (8). In a study conducted by Jang (2016) on ski athletes, it was found that recreational flow experience has a positive effect on recreational benefits and life satisfaction (7). Other studies conducted on individuals interested in aviation sports, swimmers, and paragliders have also found that recreational flow experience has a positive effect on recreational benefits (8-11). These findings are consistent with our study results.

The findings of the research parallel the results of studies conducted within the scope of positive psychology. For example, (16) suggested in their study on mountaineers that the flow experience has a positive effect on their happiness levels. Cheng and Lu (2015) found in their study on surfers that the flow experience has a positive effect on wellness perception (5). Sahoo and Sahuu (2009) concluded in their study that the flow experience has a positive effect on happiness and wellness (15). Additionally, a study by Mountinho et al. (2019) found that the flow experience has a positive impact on subjective well-being among students (51). Moreover, in conjunction with the study by Mountinho et al. (2019), a study by Lynch and Troy (2009) identified a positive relationship between flow experience and well-being (15, 51). Similar results were reported in studies by Asakawa (2004) and Carpentier (2012) (52-53). Likewise, a study by Cathcart, McGregor, and Groundwater (2014) concluded that the levels of "mindfulness" and "flow" in elite athletes are positively related to the athlete's performance and well-being (54). It was also found that an increase in mindfulness levels corresponds to an increase in flow levels. These findings align with those of Kee and Wang (2008), which were based on university students (55). In light of all this information, it can be seen that the findings of our research regarding the positive effect of the flow dimension on perceived wellness are consistent with research in the field. Furthermore, the fact that this conclusion is reached with the increasingly popular extreme sports community

highlights the importance of extreme sports in increasing wellness perception.

CONCLUSION

This study was conducted to determine the impact of recreational flow experiences of extreme sports participants on their wellness perceptions. The research findings indicate that the anxiety and boredom experienced by individuals during extreme sports activities are negatively correlated with wellness perceptions, while the acquaintance and flow dimensions are positively correlated with wellness perceptions. In other words, as negative experiences during the activity increase, wellness perception decreases. Additionally, it was found that the boredom dimension does not have a significant effect on perceived wellness dimensions, while the acquaintance dimension has a positive effect on psychological, emotional, physical, and intellectual wellness perception. The anxiety dimension has a negative effect on all dimensions of perceived wellness, while the flow dimension has a positive effect on all dimensions of perceived wellness. In conclusion, it was determined that the anxiety experienced during extreme sports negatively affects wellness perception, while the attitude during the acquaintance with the activity and the recreational flow experience positively influence wellness perception.

In extreme sports, the emotions and attitudes during acquaintance with the activity, as well as the recreational flow experience that occurs when one is fully immersed in the activity without being aware of the passage of time, can have positive effects on all dimensions of individuals' wellness perceptions. Therefore, the feeling of losing oneself in the flow of time during the activity, which allows individuals to escape from the negative stimuli of daily life, can positively affect various parameters of well-being.

Limitations & Future Research

Like many scientific studies, this research has several limitations. The limitations of the study are discussed below:

This research is limited to individuals engaged in extreme sports in Turkey. While this suggests that the research group may represent the universe, it also restricts the generalizability of the findings to the broader population. For example, individuals participating in extreme sports in different countries or

cultural contexts may have varying opinions and experiences.

The data needed for the research were obtained between July 20 and November 23, 2022. This specific time frame means that external factors (such as seasonal changes or local events) during this period have not been considered in terms of their potential impact on participants' opinions. Additionally, the validity of the findings may be questioned when compared to studies conducted outside this time frame.

The research is limited to the opinions of participants and the scale items used. This may lead to participants thinking only within a specific framework, thus restricting the possibility of reaching a broader perspective. Other important factors outside the participants' expressed opinions may also exist.

It is assumed that the individuals participating in the study easily and accurately understood the statements in the survey form and provided objective and sincere answers. However, the level of understanding of the survey questions by participants and the accuracy of their responses can affect the reliability of the study's findings. Factors such as participants' motivation, willingness to complete the survey, and social pressures may significantly influence the validity of the obtained data.

These limitations should be carefully considered when interpreting the findings of the study and generalizing the results.

Recommendations for practitioners

- Training sessions should be organized for instructors, recreational experts, and coaches to ensure the correct balance of challenge and skill during the activity, aiming to facilitate the occurrence of flow experiences.
- Providing educational programs and counseling services for individuals participating in recreational activities to help them manage their flow experience.

Recommendation for future research

- Future research could consider variables such as recreational benefits, intrinsic motivation, thrill-seeking, fear, and risk perception instead of perceived wellness in extreme sports participants.
- Additionally, examining other variables that contribute to perceived wellness and identifying

mediator and moderator variables in explaining perceived wellness could be recommended.

- This study was limited to individuals involved in extreme sports. Future research could focus on individuals engaged in different sports, from various cultures, and participating in different recreational activities.
- While this study adopted quantitative research methods, future research could explore the experience of flow and wellness perception using different measurement methods or conduct qualitative studies to determine why athletes may not experience flow.

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IS THE OCCURRENCE OF GESTATIONAL DIABETES MELLITUS IN PREGNANCIES HIGHER FOLLOWING IN VITRO FERTILIZATION TREATMENT? WHY? A RETROSPECTIVE COHORT STUDY

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In accordance with the decision (Decision No: 4), made at the meeting of the Scientific Research and Publication Ethics Committee of Dokuz Eylül University Health Sciences on 03 April 2023, an ethical review had been initiated pursuant to Article 9 of The Council of Higher Education (YÖK) Publication Ethics Directive for the article titled "Is the Occurrence of Gestational Diabetes Mellitus in Pregnancies Higher Following in Vitro Fertilization Treatment? Why? A Retrospective Cohort Study," published in the Journal of Basic and Clinical Health Sciences with the citation details '2023; 7: 94-102. https://doi.org/10.30621/jbachs.1084860'. Upon completion of the review, the Committee decreed that no ethical violation was found in the article at its meeting held on 25 April 2024 (Decision No: 2). In light of this final decision, the previously retracted article has been republished in our journal with the new citation details '2024; 8: XX-XX. https://doi.org/10.30621/jbachs.1543485'.

ABSTRACT
Purpose: This study aim to determine the occurrence of gestational diabetes mellitus (GDM) in pregnancies after IVF treatment and to evaluate the factors that reduce this risk.
Material and Methods: This retrospective cohort study was conducted using the medical records of pregnant women who conceived following IVF at the in-vitro fertilization center between 2002–2019. The data were obtained from medical records and phone interviews. Univariate and multivariate logistic regression analyses were performed.
Results: The incidence of GDM was found to be 16.7%. The regression model indicated that the risk of GDM was 4.57 times higher in the age group 36–40 at conception during the IVF cycle than the age group 31–35 (95% CI = 1.18–17.73, p = .028). Furthermore, women who conceived after the second IVF trial had a risk of GDM 3.464 times higher than those that conceived after their first IVF trial (95% CI = 1.07–11.23, p= .038).
Conclusion: As age and number of IVF trials increase in infertile women, the risk of GDM increases after IVF treatment.
Keywords: Gestational diabetes mellitus, in vitro fertilization, risk factors

INTRODUCTION

The lack of consensus on screening standards in countries across the world causes problems in comparing the prevalence of gestational diabetes mellitus (GDM) (1). However, it is reported that 21.1 million pregnant women worldwide are affected by hyperglycemia and 80.3% of them have GDM (2). Although the reported prevalence rates in Turkey (7.7%) are less compared to the rest of the world (in Europe 19%, in South-East Asia 24%), they have been increasing over the years (3). Therefore, Turkey is considered a priority country in the world regarding the prevention of diabetes. These rates for GDM, which cause serious health problems for both mothers and babies in the world and in Turkey, are alarming (4).

From the evaluation of the increasing rates, it is understood that the most important risk factors for occurrence of GDM are family history, advanced maternal age, obesity (5,6). It is also known that the hormones used to treat infertility can lead to disorders in lipid, carbohydrate, and protein metabolism and cause diabetes post IVF (7-16). The fact that the use of IVF has increased up to 4.1% in recent years in our country (17) indicates that it presents a significant risk for GDM.

In a study conducted by Ashrafi et al. (2014) evaluating GDM risk, it was reported that the incidence of GDM was higher in women who became pregnant after IVF treatment compared to those who were pregnant without it. Similar studies also found a high risk of GDM after IVF (13,14). However, it was also observed that many factors that exist before pregnancy also affect the occurrence of complications in pregnancies through IVF (11,18). Hormones used in IVF affect women's conception processes as well as pregnancy and post-natal processes. This study therefore aims to determine the development of GDM in women who conceived after IVF and identifies the factors that can improve this risk.

MATERIAL AND METHODS
Study Design

This study designed as the retrospective cohort study.

Setting and Sample

The population of the retrospective cohort study comprised women who had undergone IVF treatment in the in-vitro fertilization center of at Dokuz Eylul University Hospital in Izmir and had conceived between 2002–2019. The sample size was not calculated since it was intended to reach the entire population. The study inclusion criteria were the following: BMI level between 18 and 25 kg/m2, conception after IVF, and having a single fetus. Confusing factors such as the following were excluded from this study: BMI over 25 kg/m^2, polycystic ovary syndrome (PCOS), and multiple pregnancy conditions developed after treatment, maternal age of 40 years and over, having glucose intolerance or using a hypoglycemic drug, GDM history, stillbirth history, missed abortion history, given birth to a baby with a weight of 4000 grams and above (macrosomia), chronic diseases (hypertension, cardiovascular diseases, untreated thyroid disease, liver diseases, kidney diseases, autoimmune diseases, connective tissue diseases, among others) and a history of corticosteroid treatment. All women receiving the IVF treatment at this center used an equal dose of Human Chorionic Gonadotropin (HCG) injection during the ovulation period and vaginal progesterone treatment after embryo transfer. In total, 621 women conceived and gave birth in the mentioned timeframe. However, 369 women were not included in the study because they met at least one of the exclusion criteria. Therefore, a total of 252 pregnant women were included. Furthermore, 83 women could not be reached probably due to change in phone numbers over time and 43 registered women did not answer the phone calls; so they were excluded from the sample. Additionally, 18 women refused to participate in the research. Ultimately, a total of 108 women among the 252 could be reached. The post-hoc statistical power analysis was used to calculate the sample size. Using the G-power 3.1.9.4 program, the sample size was indicated to have 95% sufficient statistical power with 108 women (Effect size = 0.15; Alpha err prob = .05).

Tools

It was used that the using tools prepared in line with the literature review (7,8,10,13,14).

Phone Interview Form: The questions in this form were directly asked to women during telephone interviews. The number of gravidas, parity, birth week, and age at conception during IVF cycle comprised obstetric information included in the form. All participants reported that fasting glucose was measured in first trimester. Moreover, those diagnosed with GDM reported the use of the same

standard screening at 24–28 weeks of gestation by using a 75 mg oral glucose challenge test (OGTT) with abnormal blood sugar (> 92 mg/dl fasting values, > 180 mg/dl 1 hour after the start of a meal or > 153 mg/dl 2 hours after the start of a meal) during measurement (19). Finally, it was asked whether GDM was observed during their pregnancy.

Clinical Patient Medical Records: The questions in the section on fertility treatment were obtained from the medical records in the clinic by checking names, last names, and ID numbers of the women included in the study. Information such as the cause of infertility, type of medication used for ovulation induction, total dose of medication, and status of progesterone use after embryo transfer were taken from the "Clinical Patient Medical Records".

Data Collection
This study was conducted between May-July 2019. Initially, a list of women who gave birth after IVF was created from medical records and they were called by phone in order. Interviews were held with corporate phone tools and lasted an average of 15 minutes. During the interviews, brief information about the research was provided and verbal consent was obtained. Women who satisfied the inclusion criteria and who agreed to participate in the research were interviewed. Besides the response from participants, GDM diagnosis was confirmed by checking patient information from the clinical database. The information about medications used for ovulation induction and after embryo transfer for supporting the luteal phase was obtained from the patient's medical records in the clinic; these were evaluated in detail.

Statistical Analysis
The data were analyzed using the IBM SPSS Statistics for Windows, Version 22.0. Descriptive statistics for some infertility and obstetric characteristics were presented as mean ± standard deviation ($\bar{x}$ ± SD) or number (percent). First, the logistic regression model was created by taking GDM diagnosis as the dependent variable and the obstetric and infertility treatment characteristics as independent variables. Second, independent variables with statistical significance ($p < .05$) in the model were included in the multiple bivariate logistic regression model. The Backward Wald method was used to determine the variables that would remain in the final model. Furthermore, odds ratio (OR) and 95% confidence intervals (95% CI) were estimated.

Additionally, we accepted $p <0.05$ as statistically significant.

Ethical Consideration
The study, approved by the non-invasive Research Committee of Dokuz Eylul University ((Date: 17.04.2019, Decision no: 2019/10–23)).

RESULTS
The Incidence of Gestational Diabetes Mellitus After IVF
The incidence of GDM in pregnancy after In Vitro Fertilization was determined as 16.7% (Table 1).

Table 1 The Incidence of Gestational Diabetes Mellitus in Pregnancy after In Vitro Fertilization (n=108)

GDM status developed in women	n	%
Yes	18	16.7
No	90	83.3

Abbreviation:GDM = Gestational Diabetes Mellitus.

Obstetric and Fertility Treatment Characteristics of Participants
The age at conception during IVF cycle was classified according to change in fertility status with age in the literature (19) as 20–30 years (n = 44, 40.7%), 31–35 years (n = 44, 40.7%) and 36–40 years (n = 20, 18.5%).

This study found that GDM occurrence rate in women who had their first IVF trial was 55.6%. Regarding the ovulation induction medications, three types of medical treatment were applied: the recombinant Follicle Stimulating Hormone (rFSH) (n = 76, 70.4%), Human Menopause Hormone (hMG) (n = 18, 16.7%), or rFSH and hMG combination (n = 14, 13.0%) (Table 2).

Factors Affecting Gestational Diabetes Mellitus Incidence After IVF
The factors affecting the incidence of Gestational Diabetes Mellitus after IVF were evaluated using the univariate logistic regression analysis (Table 3).

The variables age at conception during IVF trial and number of IVF trials, which were found important with univariate logistic regression analysis, were included

in the multivariate bivariate logistic regression analysis as well. Both variables included in the final model were found significant. Observing the final model, the risk of GDM in the age group 36–40 at conception during the IVF trial was found 4.566 times higher than the age group 31–35 (OR = 4.57, 95% CI = 1.18–17.73, p = .028). Additionally, women who conceived after second IVF trial had a risk of GDM 3.46 times higher than those who conceived after their first IVF trial (OR = 3.46, 95% CI = 1.07–11.23, p = .038) (Table 4).

DISCUSSION
The Incidence of Gestational Diabetes Mellitus After IVF
Different pregnancy complications after IVF are common. In a meta-analysis study, it was found that women who had conceived through the assisted

Table 2. Obstetric and Fertility Treatment Characteristics of Women Conceived after In Vitro Fertilization

	GDM group (n=18) n (%)	Non-GDM group (n=90) n (%)	Total (n=108) n (%)
Age at conception during IVF cycle			
20-30 age	6 (33.3)	38 (42.2)	44 (40.7)
31-35 age	5 (27.8)	39 (43.3)	44 (40.7)
36-40 age	7 (38.9)	13 (14.4)	20 (18.5)
Gravida			
Primigravida	13 (72.2)	74 (82.2)	87 (80.6)
Multigravida	5 (27.8)	16 (17.8)	21 (19.4)
Parity			
Primiparity	18 (100)	82 (91.1)	100 (92.6)
Multiparity	0 (0)	8 (8.9)	18 (7.4)
The infertility factor			
Female	5 (27.8)	21 (23.3)	26 (24.1)
Male	9 (50.0)	35 (38.9)	44 (40.7)
Female and Male	0 (0)	1 (1.1)	1 (.9)
Unexplained	4 (22.2)	33 (36.7)	37 (34.3)
Female infertility factor			
Anovulation	1 (5.6)	8 (8.9)	9 (8.3)
Endometrial factors	1 (5.6)	6 (6.7)	6 (5.6)
DOR	2 (11.1)	2 (2.2)	6 (5.6)
Tubal factor	1 (5.6)	6 (6.7)	6 (5.6)
Number of IVF trials			
1	10 (55.6)	71 (78.9)	81 (75.0)
2	7 (38.9)	15 (16.7)	22 (20.4)
3	1 (5.6)	4 (4.4)	5 (4.6)
Ovulation induction drugs			
rFSH	11 (61.1)	65 (72.2)	76 (70.4)
HMG	3 (16.7)	15 (16.7)	18 (16.7)
rFSH + HMG	4 (22.2)	10 (11.1)	14 (13.0)
Vaginal progesterone for luteal phase support			
Yes	11 (61.1)	60 (66.7)	71 (65.7)
No	7 (38.9)	30 (33.3)	37 (34.3)
	x̄±SD	**x̄±SD**	**x̄±SD**
Drug starting dose (unit kg / day)	245.13 ± 144.83	260.55 ± 103.70	257.98 ± 110.95
Drug total dose (unit kg / day)	2966.66 ± 2249.28	2763.63±1256.81	2797.47 ± 1457.20
Drug use time (days)	11 ± 2.56	18 ± 10.52	10.60 ± 1.92

Abbreviations: DOR = Diminished Ovarian Reserve; GDM = Gestational Diabetes Mellitus; HMG = Human Menopause Hormone; rFSH = Recombinant Follicle Stimulating Hormone.

Table 3. Variables Predictive of The Occurrence of Gestational Diabetes Mellitus in Pregnancies after In Vitro Fertilization: Univariate Logistic Regression Analysis

	β	S.E.	Wald Statistics	p	OR	95% CI Lower	95% CI Upper
Age at conception during IVF cycle							
31-35 age	Ref						
20-30 age	.21	0.65	0.10	.748	1.23	0.35	4.38
36-40 age	1.44	0.67	1.62	.032*	4.20	1.14	15.54
Gravida							
Primigravida	Ref						
Multigravida	.58	0.59	0.94	.332	1.78	0.56	5.70
Parity							
Primiparity	Ref						
Multiparity	-.11	0.57	0.04	.851	0.90	0.29	2.78
The infertility factor							
Unexplained	Ref						
Female	.68	0.73	0.86	.353	1.96	0.47	8.16
Male	.75	0.65	1.35	.246	2.12	0.60	7.56
Female and Male	-	-	-	-	-	-	-
Number of IVF trials							
1	Ref						
2	1.20	0.57	4.44	.035*	3.31	1.09	10.10
3	.57	1.17	0.24	.623	1.78	0.18	17.51
Induction drugs							
rFSH	Ref						
HMG	.17	0.71	0.06	.814	1.18	0.29	4.77
rFSH + HMG	.86	0.68	1.62	.203	2.36	0.63	8.88
Vaginal progesterone for luteal phase support							
Yes	Ref						
No	.24	0.53	0.21	.651	1.27	0.45	3.62
Drug starting dose	-.00	0.00	0.29	.589	0.99	0.99	1.00
Drug total dose	.00	0.00	0.29	.589	1.00	1.00	1.00
Drug use time	.12	0.13	0.91	.339	1.13	0.88	1.44

Table 4. Variables Predictive of The Occurrence of Gestational Diabetes Mellitus in Pregnancies after In Vitro Fertilization: Multivariate Bivariate Logistic Regression Analysis*

	β	S.E.	Wald Statistics	p	OR	95% CI Lower	95% CI Upper
Constant	-2.51	0.56	20.02	.001**	0.09		
Age at conception during IVF cycle							
31-35 age	Ref						
20-30 age	.40	0.67	0.35	.555	1.49	0.40	5.51
36-40 age	1.52	0.69	4.81	.028***	4.57	1.18	17.73
Number of IVF cycles							
1	Ref						
2	1.20	0.60	4.29	.038***	3.46	1.07	11.23
3	.57	1.21	0.22	.636	1.77	0.17	18.81

*Multivariate bivariate logistic regression analysis results (Variables included in the model: Age at conception during IVF cycle, number of IVF cycles); **p < .001, ***p < .05.

reproductive technology (ART) method and who had a single fetus, experienced complications such as preeclampsia, GDM, placenta previa, ablatio placentae, antepartum hemorrhage, polyhydramnios, and oligohydramnios (14). In studies conducted at different times, the common complication was GDM, and it was determined that the risk increased by 1.99 times in pregnancies after ART (11-15,21). However, in our study, potential confounding factors were controlled with exclusion criteria to eliminate situations that are generally seen as risk factors in GDM.

In the current retrospective cohort study, it was found that GDM developed in 16.7% of pregnant women after undergoing infertility treatment. However, according to this result, the incidence of GDM in women who conceived after IVF were quite higher than our national GDM incidence (7.7%) (3) and this has a meaningful clinical importance. Similarly, in other studies that evaluated the pregnancy outcomes in Canada (11.2%) and Singapore (30.3%), it was stated that a high rate of GDM were developed after infertility treatment (9,15). The meta-analysis studies conducted in recent years showed that GDM is affected by the use of ART (16,22). In a meta-analysis conducted by Mohammadi et al., It was shown that those who became pregnant with ART developed 1.51 times more GDM than those who conceived spontaneously (16). The different medical ovulation induction protocols used in the ART procedure may have influenced the increased likelihood of GDM. Moreover, it is known that progesterone affects blood sugar by increasing insulin resistance (12). Therefore, the use of progesterone for luteal phase support in all ART cycles as well as in the first trimester of pregnancy might be a possible explanation for the increased risk of GDM.

Affecting Factors of Gestational Diabetes Mellitus Incidence After IVF

In the current study, data analysis based on a univariate logistic regression and multiple bivariate logistic regression model provides us a comprehensive understanding of the relationships underlying all potential determinants of the occurrence of GDM. The risk of GDM in women at age group 36–40 during conception with IVF cycle was 4.57 times higher than the age group 31–35. This finding indicates that age at conception during IVF trial plays an important role for GDM risk. Similarly, previous research studies have demonstrated that as

the age increases in infertility treatment, the rate of GDM complications in pregnancy may increase (12,23-25). In a study conducted by Moaddab et al. (2017) evaluating the GDM incidence in women who had conceived through the ART method, it was found the highest risk group was women between 45 and 50 years of age with the incidence of GDM being 13.9% (24). Changes in women's metabolic activities should be considered as age increases. It is known that increased risks in advanced-age pregnancies will continue in the pregnancies through ART as well, and certain factors such as medication used and stress can increase the risks in the process.

However, the number of gravida and parity of women receiving IVF treatment did not affect the incidence of GDM. Similar to the current research results, Wang et al. (2013) stated that gravida and parity numbers were not effective in terms of GDM risk in women who had conceived through ART. The stress experienced due infertility negatively affects the fertility of women regardless of the cause behind the infertility (26,27). Moreover, social pressure for having a child that is common in Turkish culture can increase this stress. A successful treatment process resulting in childbirth reassures women that at least this burden is removed. It is thought that this might reduce the risk of GDM brought about by IVF.

The cause of infertility and type of female factor did not have an impact on the risk of occurrence of GDM in women who conceived through IVF. Similarly, in a retrospective evaluation of two groups with and without GDM who had conceived through the ART method, any difference between the groups in terms of causes of infertility was not found (23). In a study evaluating the incidence of pregnancy complications in ART group, it was stated that PCOS (12.8%) being the cause of infertility caused insulin resistance (28). In our study, risk factors in GDM that increase insulin resistance were not included to eliminate potential confounding factors and represent the influence of IVF treatment. Therefore, factors that are accepted as the cause of infertility but that do not cause insulin resistance do not influence the increase in the incidence of GDM.

The number of IVF trials explained a significant proportion of GDM occurrence rate in women. The women who conceived after the second IVF trial had higher GDM incidence than those who conceived after their first IVF trial. Factors such as undergoing infertility treatment many times, repeatedly entering an unknown process and experiencing failures can

increase the stress level of women. This may be associated with the increase in blood glucose level (29). In addition to these situations, as the number of trials increase, the ovulation induction and the duration of exposure to these drugs also increase. Therefore, it is necessary to assess and inform women about GDM risk, encourage them to share their experiences, and provide information to clarify any uncertainty.

In the current study, it was determined that induction drugs, drug starting dose, total dose, and use duration of medication for ovulation induction during treatment was not statistically different in groups with and without GDM. Similarly, in different studies, it was found that the drugs used in IVF treatment (gonadotropin hormone agonist, hCG) had no effect on insulin resistance and lipid metabolism (30) and did not increase the incidence of GDM (26,31). However, contrary to these studies, in a prospective population-based cohort study performed with 3126 pregnant women who conceived after ART, it was found that as the drug dose used in the infertility treatment increased, risk of development of GDM increased (28). This difference might be due to the sampling size and feature and the proportion of women with a history of PCOS (12.9%). In our results, although the drug dose did not affect the development of GDM, a higher risk of GDM in women who had a second IVF trial was found compared to those who conceived after the first IVF trial; this may be associated with more exposure to induction drugs.

Another finding of the current study was that using vaginal progesterone after embryo transfer was also not an influential factor in the development of GDM. In the study conducted by Zipori et al. (2018), vaginal progesterone used daily for preventing abortion caused changes in the glucose tolerance test, but there was no significant effect on GDM diagnosis (32). In another study supporting Kouhkan et al. (2018), it was determined that the administration of injectable progesterone (ART) increased the risk of GDM approximately twofold compared to vaginal progesterone in single pregnant women following ART (23). In the country where the current study was conducted, while vaginal progesterone is routinely administered to every woman after ART, in spontaneous pregnancies, it is used only by women who have a risk of abortion. The result of this study regarding the effect of progesterone on GDM risk may be explained by the combination of routine practice and a lower risk of vaginal progesterone in the literature compared to intramuscular progesterone.

Our study has some limitations. The major limitation is the lack of a control group. GDM risk factors were determined as exclusion criteria at the beginning of the study. And, all women received progesterone therapy. Therefore, a control group could not be formed during the IVF treatment process. The second limitation was that although the number of people receiving treatment in the institution was high within the selected time period, a significant number of women could not be reached, and the data were accessed via the telephone registered in the institution. This may be due to the lengthy treatment process of some women and their contact information changing over time. Therefore, the sample size was limited. Moreover, the external validity of our results needs to be confirmed as the study was conducted in a single IVF center. Therefore, it is recommended that studies be conducted in other clinics with similar IVF procedures. Finally, as this study used a retrospective design, we cannot ensure that all information related to the past was recorded or remembered properly by the women. Therefore, the results of the current study should be validated with a prospective cohort and multicenter research.

CONCLUSION

The results of current research indicate that the risk of GDM after IVF treatment increases with age. Therefore, women who become pregnant with IVF at 35 years or above should be evaluated more carefully in terms of GDM and should undergo a controlled antenatal process. Furthermore, the increase in the risk of GDM as the number of IVF trials increased was found important in terms of IVF trail management. Women should be encouraged to share their feelings and thoughts to reduce their stress at the beginning of every IVF treatment cycle. Fertility and obstetrics nurses and doctors can play a vital role in early risk evaluation and can encourage healthy diet and physical activity to prevent excessive weight gain and development of GDM during pregnancy. In this manner, it is believed that healthy pregnancies and babies can contribute to the health system in the country and decrease health expenditures. In further studies, GDM prevalence and risk factors should be compared with pregnant women who conceived spontaneously and after IVF in a similar age group.

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A STUDY ON THE SYSTEMIC INFLAMMATORY INDEX (SII) AND RELATED FACTORS IN PATIENTS WITH MALIGNANT OTITIS EXTERNA

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ABSTRACT

Purpose: This study aims to investigate the relationship between systemic inflammatory index and various clinical and microbiological factors in patients with malignant otitis externa (MOE).

Materials and Methods: In this retrospective study, patients diagnosed with MOE and treated between January 2017 and March 2023 were examined. The data include clinical evaluations, laboratory tests, and imaging results. All patients underwent computed tomography of the temporal bone, and MOE was staged according to Tengku's radiological classification .SII was calculated using neutrophil, platelet, and lymphocyte counts.

Results: 11 patients were examined retrospectively, including 8 males (72.7%) and 3 females (27.3%). The mean age of the patients was 72.3±7.5 years. Comorbidities such as Diabetes Mellitus (DM) and Chronic Kidney Disease (CKD) were associated with higher SII values. CT phases and cranial nerve involvement were also related to high SII values. Microbiological analyses showed that pathogens such as Pseudomonas aeruginosa and Candida tropicalis were associated with high SII values.

Conclusion: SII is an important biomarker in evaluating the prognosis and treatment response in MOE. Early diagnosis, a multidisciplinary approach, and personalized treatment strategies are critical in managing MOE. Further studies involving larger patient groups will contribute to validating these relationships and improving treatment processes.

Keywords: Malignant otitis externa, systemic inflammatory index, diabetes mellitus, cranial nerve involvement, skull base osteomyelitis, CT phases.

INTRODUCTION

Malignant otitis externa (MOE) is an aggressive osteomyelitis that typically begins in the external auditory canal and subsequently spreads to the base of the skull. This disease is most commonly observed in elderly and diabetic patients and is associated with significant morbidity and mortality. MOE occurs more frequently in high-risk patients, particularly in immunocompromised individuals and can lead to serious complications if left untreated (1,2,3).

The diagnosis and management of MOE require the use of broad-spectrum antibiotics and antifungal agents, surgical debridement, and long-term follow-up. However, early diagnosis and appropriate treatment strategies are of great importance in the management of MOE (4,5). High-resolution computed tomography (HRCT) plays a crucial role in the diagnosis and staging of MOE, allowing for the evaluation of the extent and severity of the disease (6,7).

The Systemic Inflammatory Index (SII) is a biomarker used to assess the inflammatory response and is calculated using neutrophil, platelet, and lymphocyte counts (8). SII is particularly useful in determining the severity of infections and inflammatory diseases and can serve as an important indicator in assessing the prognosis of serious infections such as MOE (8,9,10). Chronic infections and inflammation can increase the systemic inflammatory response, leading to various complications. MOE is particularly common in diabetic patients, where the severity of the inflammatory response may affect the progression of the disease and the response to treatment. Diabetes imposes stress on the immune system, reducing the body's resistance to infections and increasing the inflammatory response (1,2,3). Elevated levels of inflammatory markers may indicate the severity and extent of the disease, which can guide the determination of treatment strategies. Specifically, SII can be used as an objective measure of the inflammatory response, aiding in the assessment of the prognosis and treatment response in MOE patients (8,9,10).

The treatment of MOE involves the use of broad-spectrum antibiotics, antifungal agents, and surgical interventions when necessary. Serious complications such as cranial nerve involvement can occur in patients with MOE, affecting the severity and prognosis of the disease. Patients with cranial nerve involvement require more aggressive treatment and close monitoring (11,12, 13).

The prevalence and severity of MOE are important factors that determine the prognosis and response to treatment. CT phases are used to assess the extent and severity of MOE, and these phases reflect the degree of inflammatory response and the stage of the disease. Higher CT phases may be associated with higher SII values, which can serve as a guide in determining the prognosis of the disease. (5,6,7,8,14,15,16)

Elevated SII values indicate the severity of the inflammatory response and the extent of the disease, which can guide the determination of treatment strategies. In this study, the relationship between comorbidities, CT phases, cranial nerve involvement, HbA1c levels, and microbiological findings with SII in patients diagnosed with MOE was investigated. Evaluating these relationships may contribute to the identification of more effective treatment strategies in the management of MOE.

760

MATERIALS AND METHODS
In this retrospective study, patients diagnosed with MOE and treated between January 2017 and March 2023 were examined. The data include clinical evaluations, laboratory tests, and imaging results. All patients underwent high-resolution computed tomography (HRCT) of the temporal bone, and MOE was staged according to Tengku's radiological classification (6).

Definition of CT Phases
According to Tengku's radiological classification, MOE is examined in five phases:
- Phase I: Inflammation is limited to the soft tissue in the external ear canal, with no bone involvement.
- Phase II: Inflammation extends beyond the soft tissue and is limited to the mastoid with bone involvement.
- Phase III: Inflammation progresses medially to involve the petrous temporal bone or the temporomandibular joint, sometimes involving the parapharyngeal soft tissue.
- Phase IV: Inflammation progresses medially to involve the nasopharynx, sometimes with abscess formation.
- Phase V: Inflammation extends to the contralateral ear base or the contralateral skull base.

Systemic Inflammatory Index (SII)
The systemic inflammatory index (SII) is a relatively new parameter that has been shown to increase in inflammatory diseases. SII uses three blood cell subtypes (neutrophils, lymphocytes, and platelets) that reflect the balance between inflammation and immune response. SII was calculated using the following formula: SII = (platelet count × neutrophil count) / lymphocyte count (8,9,10).

Statistical analyses
All statistical analyses were conducted using SPSS 15.0 software. The normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. For continuous variables following a normal distribution, data were expressed as mean ± standard deviation (SD) and analyzed using a t-test. For non-parametric data, values were presented as median (minimum-maximum), and the Mann-Whitney U test was applied. A p-value of <0.05 was considered statistically significant.

Ethical approval for the study was received from Izmir Katip Celebi University Non-Invasive Clinical Research Ethics Committee (Date: 21.03.2024, Decision No:0143).

RESULTS

A total of 11 patients diagnosed with malignant otitis externa (MOE) were included in this retrospective study. The cohort consisted of 8 males (72.7%) and 3 females (27.3%), with a mean age of 72.3±7.5 years. Comorbidities were prevalent in the patient population, with Diabetes Mellitus (DM) identified in 10 patients (90.9%), Hypertension (HT) in 6 patients (54.5%), and Chronic Kidney Disease (CKD) in 3 patients (27.3%).

Cranial nerve involvement was observed in 5 patients (45.4%), with the 7th cranial nerve affected in 4 patients (36.6%), and both the 10th and 7th cranial nerves affected in 1 patient (9.1%).

Microbiological analysis revealed Pseudomonas aeruginosa in 4 patients (36.4%), Enterobacter cloacae in 1 patient (9.1%), Candida tropicalis in 1 patient (9.1%), skin flora in 3 patients (27.3%), and Corynebacterium spp. in 1 patient (9.1%).

When classified according to Tengku's radiological classification, the distribution of CT phases was as follows: Phase 1 in 2 patients (18.2%), Phase 2 in 3 patients (27.3%), Phase 3 in 3 patients (27.3%), Phase 4 in 1 patient (9.1%), and Phase 5 in 2 patients (18.2%). The demographic features and clinical findings of the patients are given Table 1.

The mean Systemic Inflammatory Index (SII) value was 1337.19±1006.954, with the mean HbA1c level at 8.08±2.2, and the mean CT phase at 2.9±1.4. These findings suggest a significant association between higher SII values and advanced CT phases, as well as comorbid conditions such as DM and CKD. Various comorbid conditions and their effects on SII values were examined. Comorbidities such as Chronic Kidney Disease (CKD), Diabetes Mellitus (DM), and Hypertension (HT) were associated with higher SII values. CKD was found to have the highest average SII value among the comorbidities. Comorbidities and SII values are given Figure 1. The relationship between CT phases and SII values was examined. Phase 1 had the lowest SII value, while phase 4 had the highest SII value. The chi-square test results showed no statistically significant relationship

Table 1. The demographic features and clinical findings of the patients

Patient	Age	Gender	HbA1c	Comorbidities	Cranial Nerve Involvement	Microbiology	CT Phases	SII	Treatment
1	71	M	9.11	DM	7	Pseudomonas a.	Phase 3	1232.89	Meropenem, Metronidazole
2	78	M	8.20	DM, HT, CRF	None	Enterobacter cloacae	Phase 2	2001.95	Ceftazidime, Metronidazole
3	62	M	10.50	DM, HT, CRF, CHF	None	Pseudomonas a.	Phase 2	1058.14	Cefepime
4	62	F	8.20	DM, HT, CRF, CAD	7	Pseudomonas a.	Phase 5	1713.32	Imipenem, Amikacin, Piperacillin Tazobactam
5	76	M	12.00	DM	10,7	Candida tropicalis	Phase 5	1923.27	Meropenem, Linezolid, Caspofungin
6	62	M	8.70	DM	None	None	Phase 1	563.18	Ciprofloxacin
7	84	M	5.90	DM, CHF, COPD, Parkinson,	None	Skin flora	Phase 2	229.93	Piperaslin Tazobactam, Ciprofloxacin
8	78	M	6.60	HT	7	Skin flora	Phase 1	109.13	Meropenem, Teicoplanin, Ciprofloxacin
9	75	M	8.80	DM, HT,CAD	7	Skin flora	Phase 3	759.85	Meropenem, Metronidazole
10	83	F	6.50	DM, HT, AF	None	Pseudomonas a.	Phase 3	1020.70	Meropenem
11	72	F	4.80	HT, DM	None	Corynbacterium spp.	Phase 4	3331.25	Meropenem, Teicoplanin

J Basic Clin Health Sci 2024; 8: 759-764

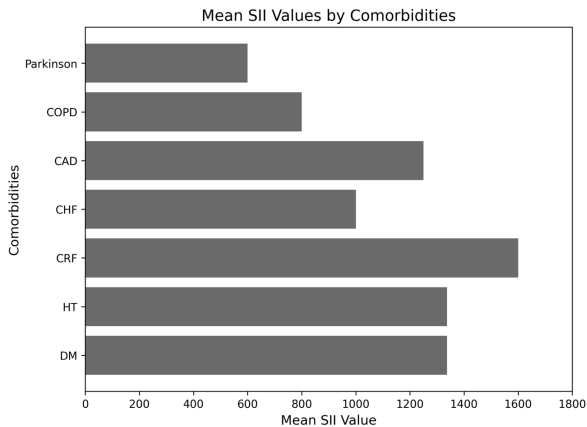Bayrak AF et al. Systemic Inflammatory Index in Malignant Otitis Externa

Figure 1. Comorbidities and systemic inflammatory index (SII) values. Comorbid diseases accompanying malignant otitis externa and SII values are shown. (SII: Systemic Inflammatory Index, CKD: Chronic Kidney Disease, DM: Diabetes Mellitus, HT: Hypertension, CRF: chronic Renal Failure, CHF: Congestive Heart Failure, CAD: Coronary Artery Disease, COPD: Chronic Obstructive Pulmonary Disease)
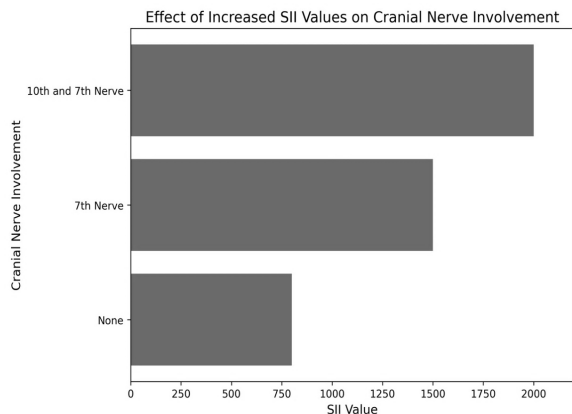
Figure 2. Cranial nerve involvement and systemic inflammatory index (SII) values (SII: Systemic Inflammatory Index)

between CT phases and SII categories (p > 0.05). However, higher CT phases were generally associated with higher SII values.

The relationship between increased SII values and cranial nerve involvement was evaluated. The mean and median SII values of patients with cranial nerve involvement were higher than those without. However, the t-test results showed that this difference was not statistically significant (p > 0.05). Cranial nerve involvement and SII values are given Figure 2. The correlation between HbA1c values and SII was examined. The correlation coefficient was indicating a very weak negative correlation between HbA1c and

SII, suggesting that HbA1c values do not have a significant effect on SII (r =-0.053, p > 0.05).

The relationship between microbiological findings, CT phases, and SII values was analyzed. Pseudomonas aeruginosa was the most isolated microorganism and was associated with high SII values. Additionally, microorganisms such as Corynebacterium spp. and Candida tropicalis were also associated with high SII values.

DISCUSSION

Comorbidities such as Chronic Kidney Disease (CKD), Diabetes Mellitus (DM), and Hypertension (HT) may lead to an increased inflammatory response and consequently higher SII values (1). These patients require more careful monitoring and treatment approaches. Particularly, DM is a common condition in MOE patients due to its effects on inflammation and immune response and the inflammatory response is generally more severe in diabetic patients, making close monitoring crucial in MOE management. While HbA1c levels reflect the overall health status and effectiveness of diabetes management, the direct impact on the inflammatory response may be more complex (2,12).

The findings demonstrate that SII is an important biomarker in MOE patients, and various clinical and microbiological factors can affect SII. The impact of comorbidities on SII may influence the severity of the inflammatory response. Association of high SII values with CT phases and cranial nerve involvement may provide information on the progression and severity of the disease. This highlights the need for careful interpretation when using inflammatory biomarkers in clinical assessments.

The higher SII values in patients with cranial nerve involvement highlight the impact of the inflammatory response on the central nervous system. Early diagnosis and regular monitoring are critical in managing these patients. Cranial nerve involvement is considered a serious complication of MOE, and patients in this condition require more aggressive treatment (17,18). In this study, the SII values of the patients were measured when they were first examined. Repeat measurements can be made with blood counts performed during the clinical follow-up of the patient and can provide useful information in terms of prognosis.

The association of higher CT phases with higher SII values provides information on the extent and severity of the disease. In our study, observing the

highest SII values in phase 4 indicates a severe inflammatory response at this stage. This finding is based on Kamalden et al.'s Tengku's radiological classification in MOE (6). CT phases play a crucial role in determining the clinical course and treatment strategies of the disease. Similarly, Yiğider et al. proposed a classification system based on clinical and radiological findings in monitoring MOE disease (19). Özer et al. also proposed a classification based on MRI that could help clinicians predict prognosis (14).

Microorganisms such as Pseudomonas aeruginosa and Candida tropicalis were associated with high SII values. These pathogens can increase the severity of the disease and complicate the treatment process. Microbiological examinations are critical in determining the etiology of the infection and guiding appropriate antibiotic treatment (4). In the treatment of MOE, broad-spectrum antibiotics and antifungal agents should be used to effectively control pathogens (15). Orji et al. noted that some pathogens might develop resistance to antibiotic treatment, which could lead to misleading inflammatory markers (16).

The relationships between SII and other clinical parameters should also be evaluated, and more research should be conducted on this subject. For example, the treatment duration, comparison of CT phases at the end of treatment, and their relationship with SII values. Additionally, the impact of different treatment methods and drug regimens on SII values should be investigated. These findings can contribute to the development of more personalized treatment strategies in managing MOE.

A multidisciplinary approach is crucial in managing MOE. Close collaboration between otolaryngologists, neurologists, and infectious disease specialists should be ensured. Additionally, patients should be regularly monitored, and early detection of cranial nerve involvement symptoms is necessary. New treatment methods and protocols should be researched and developed for these patients.

Our small number of patients may be the reason why our results were not statistically significant. This situation constitutes the limitation of our study.

CONCLUSION

In this study, the relationships between systemic inflammatory index (SII) values and various clinical and microbiological factors in patients with malignant otitis externa (MOE) were examined. Although some of the findings were not statistically significant, notable trends were observed demonstrate that comorbidities, especially diseases such as Diabetes Mellitus (DM) and Chronic Kidney Disease (CKD), significantly affect SII values. CT phases and cranial nerve involvement were also associated with high SII values. SII can be used as an important biomarker in evaluating the prognosis and response to treatment in MOE. Further studies involving larger patient groups will contribute to validating these relationships and improving treatment processes.

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COMBINED INHIBITION OF THE FAK-Rho-ROCK SIGNALING CASCADE, ONE OF THE IMPORTANT PLAYERS IN MECHANOTRANSDUCTION, IN COLORECTAL CANCERS

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ABSTRACT

Purpose: Colorectal cancer (CRC) is one of the most common cancer types globally, with a high mortality rate. The FAK-Rho-ROCK successive signaling cascade promotes growth, migration and invasion of cancer cells. Focal adhesions are major sites of interactions between extracellular mechanical environments and intracellular biochemical signaling molecules/cytoskeleton and therefore focal adhesion proteins have been proposed to play important roles in mechanotransduction. This study aims to evaluate the effects of combination treatments with Focal Adhesion Kinase (FAK), Rho-ROCK, and YAP/TAZ inhibitors on the proliferative and epithelial-mesenchymal transition (EMT)-related metastatic characteristics of colorectal cancer cells.
Material and Methods: In vitro experiments were performed using the HCT-116 colon cancer cell line. The effects of Y-15 (FAK inhibitor), ROCK inhibitor-2, and YAP/TAZ inhibitor-2, either applied alone or in combination, on cell proliferation were analyzed using the WST-1 cell viability assay. Epithelial-mesenchymal transition (EMT) markers, E-cadherin and N-cadherin, were evaluated via immunofluorescence staining, and fluorescent intensity was analyzed using ImageJ software.
Results: Y-15, when applied alone or in combination with other inhibitors, significantly reduced cell proliferation ($p \leq 0.005$). Moreover, the combination of Y-15 and ROCK inhibitor-2 increased E-cadherin levels while decreasing N-cadherin levels ($p \leq 0.0159$, $p \leq 0.0286$). While the effect of YAP/TAZ inhibitor-2 alone was limited, specific effects were observed in combination treatments.
Conclusion: This study demonstrates the potential of FAK-Rho-ROCK pathway inhibitors in the treatment of colorectal cancer. The ability of Y-15, in particular, to inhibit cell viability/proliferation and metastatic processes suggests that combination strategies targeting these pathways could contribute to the development of new therapeutic approaches for CRC.

Keywords: Colorectal cancer, Focal Adhesion Kinase, Rho-ROCK, YAP/TAZ

INTRODUCTION
Colorectal cancer (CRC) ranks third among cancer types worldwide and stands out as a significant cause of mortality (1,2,3). Due to its impact on health and the burden it places on healthcare costs, new treatment approaches for CRC are constantly being

explored. In recent years, mechanobiology has played a crucial role in the progression of CRC, and the knowledge gained in this field has paved the way for new therapeutic targets (4).

The main components of the cytoskeleton, including microtubules, microfilaments, and intermediate filaments, play critical roles in regulating the viscoelastic properties of cells. These structures are associated with actin/myosin complexes, which are fundamental in determining cell stiffness. Cell stiffness influences key processes such as cancer cell proliferation, differentiation, motility, and adhesion. The Rho-ROCK signaling pathway also contributes significantly to these processes by regulating actomyosin contractility (5). Focal Adhesion Kinase (FAK) emerges as a protein that regulates various biological processes related to cancer cell growth and metastasis. FAK also interacts with Rho family proteins to regulate the actin cytoskeleton, managing cancer cell survival, proliferation, migration, and invasion (6). Focal adhesions play a critical role in linking extracellular mechanical signals with intracellular biochemical signals, with FAK proteins playing a key role in mechanotransduction. The impact of mechanotransduction on cancer progression has garnered attention in recent years, and the inhibition of these processes has become a key target in developing new therapeutic strategies (7,8).

In the current literature, studies have shown that Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitors are used separately in different cancer types, but there is no study investigating their combined use in colorectal cancer. The results of studies in cancer types such as pancreatic and breast cancers suggest that Y-15 is effective both alone and in combination with other inhibitors (9,10). Additionally, research has shown that ROCK inhibitors play a role in preventing metastasis (11,12).

In this study, the effects of the combination of FAK, Rho-ROCK, and YAP/TAZ inhibitors on cell viability/proliferation and epithelial-mesenchymal transition (EMT)-related metastatic characteristics were evaluated in colorectal cancer cells. Cell proliferation was analyzed using the WST-1 assay, and the EMT process was evaluated by immunofluorescence staining of E-cadherin and N-cadherin biomarkers. The results reveal the potential effects of pathway-specific inhibitor combinations on the proliferative and metastatic characteristics of cells associated with cell stiffness.

MATERIAL AND METHODS
Cell Culture and Inhibitor Application
In this study, the HCT-116 colon cancer cell line (ATCC® CCL-247) was used to model colorectal cancer under in vitro conditions. The HCT-116 cell line was isolated from the primary colon cancer of an adult male patient at Dukes D stage and carries a mutation in codon 13 of the RAS proto-oncogene. The cell line was cultured in media recommended by the suppliers, supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin, and maintained in an atmosphere of 5% CO_2 at 37°C. For cell preservation, a freezing medium containing 90% FBS and 10% DMSO was used, and the cells were stored at -80°C.

Y-15 (FAK Inhibitor 14) (MedChemExpress, Cat no: HY-12444), ROCK inhibitor-2 (MedChemExpress, Cat no: HY-119937), and YAP/TAZ inhibitor-2 (MedChemExpress, Cat no: HY-147322) were prepared in solutions according to their stock concentrations. Y-15 was dissolved in PBS and stored at -80°C, while ROCK inhibitor-2 and YAP/TAZ inhibitor-2 were dissolved in DMSO and stored under appropriate conditions.

HCT-116 cells were seeded into 96-well plates at a volume of 100 μl. The cells were incubated at 37°C in 5% CO_2 to allow for proper adherence and morphology. Subsequently, different concentrations of Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitor-2 were applied to the cells.

*This study, conducted as part of a master's thesis, received ethical approval from the Dokuz Eylül University Non-Interventional Research Ethics Committee (Date: 20.07.2022, Decision no: 2022/23-09).

Cell Viability Assay (WST-1)
The WST-1 viability assay was used to evaluate the effects of the drugs on cell viability. HCT-116 cells were seeded into 96-well plates at a density of 10^4 cells/100 μl, and drug doses were applied after 24 hours of incubation. After 48 hours of drug treatment, the WST-1 reagent was added, and absorbance values were measured spectrophotometrically at 440 nm.

Immunofluorescence Staining
To evaluate the effects of the Y-15 and ROCK inhibitor-2 combinations on epithelial-mesenchymal transition (EMT), the immunofluorescence staining
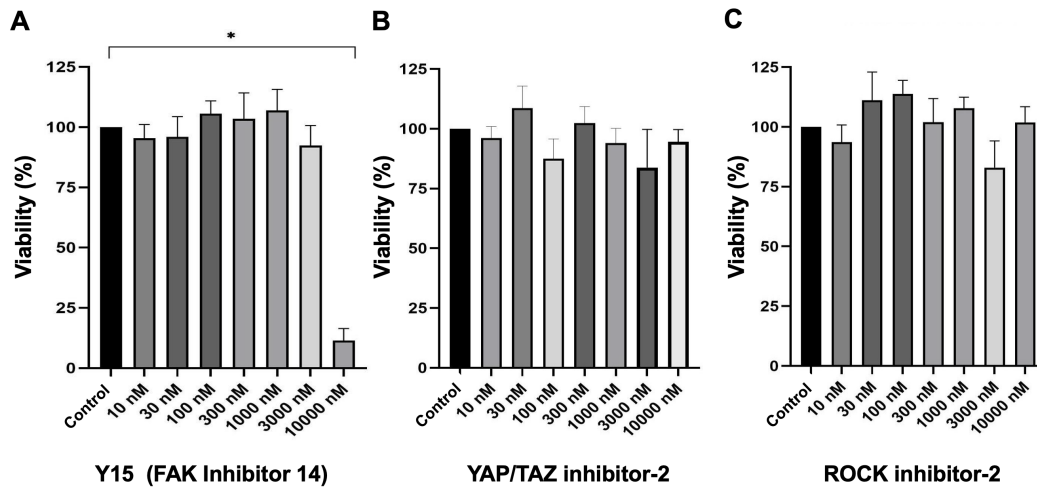
Figure 1. A) The effect of Y-15. B) YAP/TAZ inhibitor-2. and C) ROCK inhibitor-2 on cell viability. (* indicates p≤0.05)
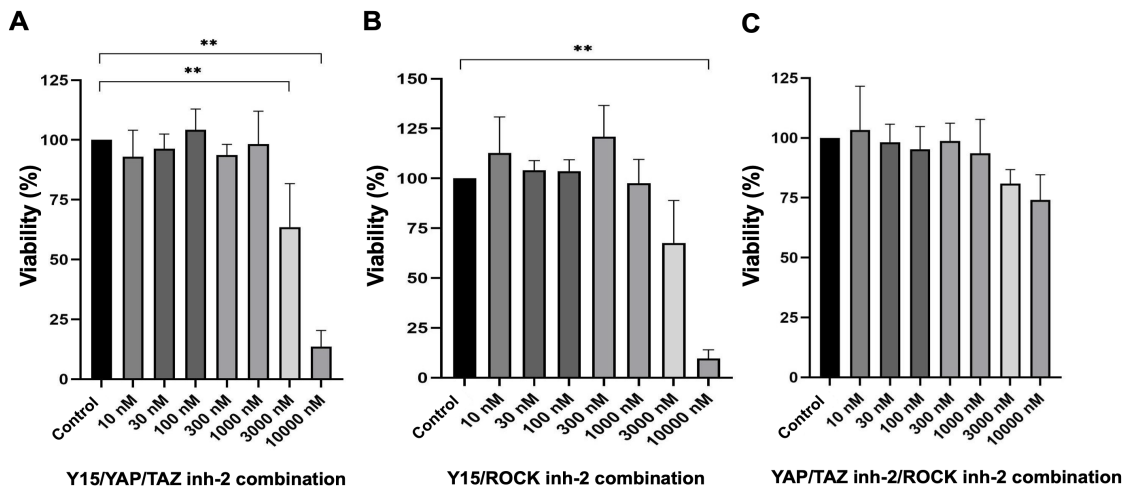

Figure 2. The effect of dual combinations on cell viability. A) Combination of Y-15 and YAP/TAZ inhibitor-2. B) Combination of Y-15 and ROCK inhibitor-2. C) Combination of YAP/TAZ inhibitor-2 and ROCK inhibitor-2 (**p≤ 0.002)
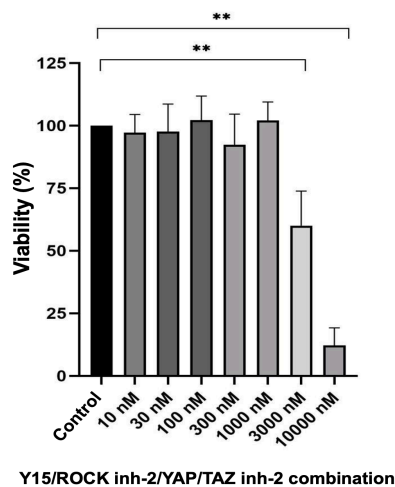

Figure 3. The effect of Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitor-2 drug combinations on cell viability (**p≤0.002)

method was used. E-cadherin and N-cadherin biomarkers were stained with Alexa Fluor secondary antibodies, and cells were counterstained with Hoechst 33342 for nuclear staining. The cells were then visualized using a confocal microscope. Fluorescence intensity changes in EMT biomarkers were analyzed using ImageJ software.

Statistical Analysis

Data analysis was performed using GraphPad Prism software. Variables were expressed as mean ± standard deviation and percentages. All experiments were performed with at least three replicates. Statistical significance was considered at *p≤0.005 and **p≤0.002.
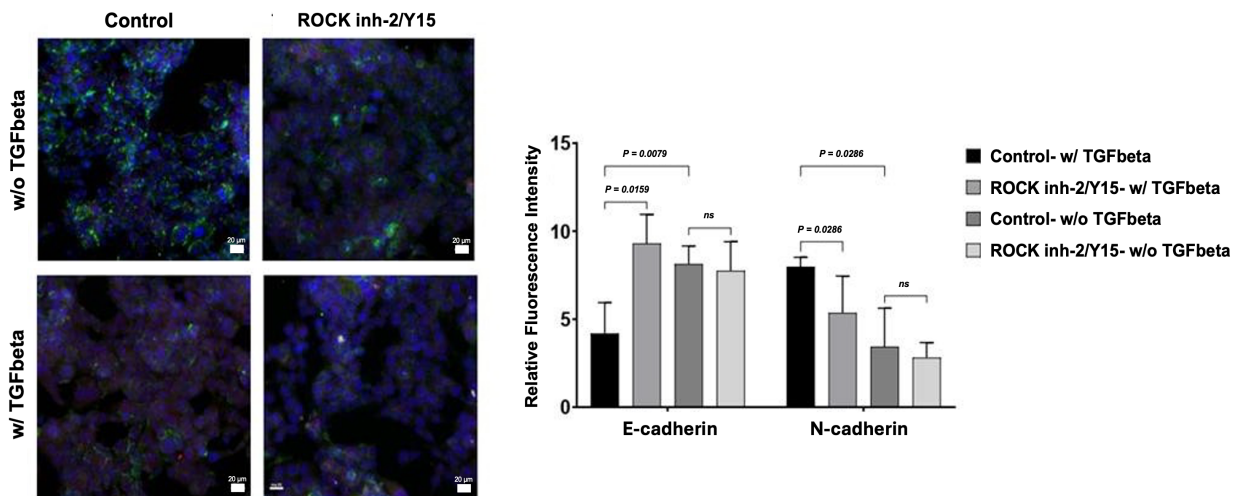
Figure 4. The effect of ROCK inhibitor-2/Y-15 inhibitor on epithelial-mesenchymal transition in TGF-β induced and non-induced colorectal cancer HCT-116 cells (Scale bar = 20 μm)

RESULTS

Effects of Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitor-2 on Cell Viability

Y-15 inhibitor was applied to HCT-116 cells at concentrations ranging from 10 to 10000 nM. According to the cell viability assay, cell viability was significantly reduced at the 10000 nM dose (p≤0.005). However, YAP/TAZ inhibitor-2 and ROCK inhibitor-2 did not have a statistically significant effect on cell viability (Figure 1).

Effects of Dual Drug Combinations on Cell Viability

The combination of Y-15 and YAP/TAZ inhibitor-2 significantly reduced cell viability at 3000 and 10000 nM doses in HCT-116 cells (p≤0.005). Similarly, a significant reduction in cell viability was observed with the Y-15 and ROCK inhibitor-2 combination at the 10000 nM dose (p≤0.002) (Figure 2). However, the combination of YAP/TAZ inhibitor-2 and ROCK inhibitor-2 did not show a statistically significant effect on cell viability (Figure 2).

Effects of Triple Drug Combinations on Cell Viability

The triple combination of Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitor-2 significantly reduced cell viability at 3000 and 10000 nM doses in HCT-116 cells (p≤0.002). The effect of this combination is attributed to the strong inhibitory properties of Y-15 (Figure 3).

Effects of the Y-15 and ROCK inhibitor-2 Combination on EMT

In the process of Epithelial-Mesenchymal Transition (EMT), HCT-116 cells induced by TGF-β showed a decrease in E-cadherin levels and an increase in N-cadherin levels. In the induced cells treated with the Y-15 and ROCK inhibitor-2 combination, a significant increase in E-cadherin levels (p=0.0159) and a significant decrease in N-cadherin levels (p=0.0286) were observed. These findings suggest that the treatment combination is particularly effective on cells undergoing the metastatic process (Figure 4).

DISCUSSION

Research on tumor biology related to cell mechanics has shown that FAK-targeted agents are typically used alone or in dual combinations. In our study, the effects of Y-15, ROCK inhibitor-2, and YAP/TAZ inhibitors on colorectal cancer cell lines were examined. Studies on the role of FAK in processes such as extracellular matrix (ECM) remodeling, immune cell filtration, and epithelial-mesenchymal transition (EMT) have demonstrated the critical role of the FAK pathway in cancer progression (13,14). These findings are consistent with our results, which showed that the Y-15 inhibitor significantly reduced cell viability when used alone.

In addition to FAK inhibition, the effects of the ROCK pathway on cancer cell proliferation and metastasis are also significant. In our study, ROCK inhibitor-2, when used either alone or in combination, did not show a significant effect on cell viability. However, the

literature suggests that ROCK inhibitors, when combined with chemotherapy and immunotherapies, enhance efficacy in cancer treatment (15). Furthermore, studies by Nam et al. have shown that ROCK inhibition can increase the efficacy of doxorubicin, reducing tumor burden (16). In contrast, in our study, ROCK inhibition alone did not have a significant effect on colorectal cancer cells.

The YAP/TAZ pathway is associated with the mechanics and responses of cancer cells to their microenvironment. In our study, YAP/TAZ inhibitor-2 alone did not significantly affect cell viability, but a limited reduction in cell proliferation was observed when combined with other inhibitors. The literature indicates that YAP/TAZ inhibitors, when used in combination with SWI/SNF complex agents, can affect mechanotransduction processes in cancer cells (12).

In our study, it was found that combining Y-15 with other inhibitors further reduced cell stiffness and proliferative properties. Specifically, Y-15 stands out as a promising agent in colorectal cancer treatment by inhibiting cancer cell survival and metastasis through FAK inhibition (17, 18). Studies by Golubovskaya et al. have also shown that Y-15 blocks tumor growth through FAK inhibition in various cancer types, including colon, pancreatic, and breast cancers (18).

CONCLUSION

In conclusion, this study demonstrated that the inhibition of the FAK-Rho-ROCK signaling cascade suppresses proliferation and metastasis in colorectal cancer cells. Given the effects of cell stiffness and mechanics on cancer progression, combination therapies using inhibitors of this pathway provide opportunities for developing new therapeutic strategies. As highlighted in the literature, considering the contribution of matrix stiffness to cancer progression and its relationship with resistance to anti-VEGF therapy, agents such as FAK and YAP inhibitors may be more effective in combination treatments (19).

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Conflict of interests: The authors declare no conflict of interest.

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AI-ASSISTED SURVIVAL PREDICTION IN COLORECTAL CANCER: A CLINICAL DECISION SUPPORT TOOL

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ABSTRACT

Purpose: This study was planned to determine the problems and affecting factors that children encounter Purpose: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Accurate survival prediction is crucial for advanced-stage patients to optimize treatment strategies and improve clinical outcomes. This study aimed to develop an artificial intelligence-assisted clinical decision support system (CDSS) for survival prediction in CRC patients using clinical and genomic data from the Cancer Genome Atlas Colon Adenocarcinoma Collection (TCGA-COAD) dataset.
Methods: Machine learning algorithms, including C4.5 Decision Tree, Support Vector Machines (SVM), Random Forest, and Naive Bayes, were employed to create survival prediction models. Clinical parameters and genomic data from key pathways, such as glycolysis/gluconeogenesis and mTORC1, were integrated into the models. The models were evaluated based on accuracy and performance.
Results: The Random Forest algorithm achieved the highest accuracy (82.3%) when only clinical parameters were used. When clinical data were combined with gene expression data, the model's accuracy increased further. The resulting models were incorporated into a user-friendly web interface, SurvCOCA, for clinical use.
Conclusions: This study demonstrates the potential of AI-based tools to improve prognosis predictions in CRC patients. Further research is needed, with larger datasets and additional machine learning algorithms, to enhance clinical decision-making and optimize treatment strategies.

Key words: Colorectal cancer, survival prediction**,** artificial intelligence, **c**linical decision support system, machine learning

INTRODUCTION

Colorectal cancer (CRC) is one of the most common types of cancer worldwide in both men and women and ranks third in cancer-related mortality (1). The multifactorial nature of CRC indicates that genetic, environmental, and lifestyle factors play roles in the development of the disease (2). Early diagnosis and

treatment of CRC are critical for improving survival rates. However, the effectiveness of standard treatment approaches, such as surgical interventions, chemotherapy, and radiotherapy, largely depends on the stage of the disease and the biological characteristics of the tumor (3).

In cancer treatment, survival predictions have emerged as an important component in clinical decision-making processes. However, the accuracy of these predictions is often limited by traditional methods based on available clinical data. At this point, advances in genetics and molecular biology offer a powerful tool for more accurately predicting patient survival times. Specifically, technologies such as gene expression profiling allow for the identification of molecular subtypes of tumors and provide insights into how these subtypes affect survival (4, 5).

In recent years, the use of artificial intelligence (AI) and machine learning (ML) technologies in healthcare has increased, offering significant opportunities for integrating large datasets into clinical decision-making processes. ML algorithms can combine various clinical and genomic data to predict disease prognoses more accurately and personalized (6). In this context, integrating clinical and genomic data to improve survival predictions in heterogeneous tumors like CRC can contribute to identifying more suitable treatment options for patients (7).

The aim of this study is to develop a clinical decision support system (CDSS) that can predict survival outcomes in CRC patients using clinical and genomic data from the Cancer Genome Atlas Colon Adenocarcinoma Collection (TCGA-COAD) dataset (8). Survival prediction models were created using machine learning algorithms such as C4.5 Decision Tree, Support Vector Machines (SVM), Random Forest, and Naive Bayes, and the accuracy of these models was evaluated. Additionally, these models were integrated into a user-friendly web interface called SurvCOCA, making it a tool that can be used in clinical applications. The developed CDSS aims to provide accurate and reliable results for predicting survival in colon cancer, thereby contributing to clinical decision-making processes.

METHODS
Data Source and Study Material
This study, conducted as a master's thesis, was approved by the Non-Interventional Research Ethics Committee of Dokuz Eylul University (Date:

23.11.2020, Number: 2020/28-29). For this study, clinical and genomic gene expression data of colorectal cancer (CRC) patients were obtained from the Cancer Genome Atlas Colon Adenocarcinoma Collection (TCGA-COAD) project (8), accessed through the Genomic Data Commons (GDC) Data Portal (9). The clinical data included variables such as gender, race, tumor stage, T stage, N stage, M stage, previous cancer diagnosis, tissue or organ origin, body mass index (BMI), and primary diagnosis. Genomic data focused on pathways like glycolysis/gluconeogenesis, glycan degradation, pantothenate and CoA biosynthesis, apoptosis, mTORC1 signaling pathways, and the genes active in these pathways (ADH1C, AKR1A1, BCAT1, CAPN2, CASP2, MAN2B2, PFKM, TCEA1, TOMM40).

Data Collection and Processing
Data from the TCGA-COAD project was downloaded and analyzed using the TCGAbiolinks package in the R programming language (10-12). TCGAbiolinks is an R/Bioconductor package used for querying, downloading, and analyzing cancer data stored in GDC (11,13).

The following steps were applied to the downloaded data:

1. Normalization and Filtration: The data were cleaned to remove noise. Gene expression data were normalized by selecting the primary solid tumor subtype, and samples containing multiple data points were filtered out.

2. Right and Left Colon Information: To show the gene expression changes between right and left colon patients, gene expression analysis was performed, fold change was calculated, and p-values were adjusted using the False Discovery Rate (FDR) method.

Statistical Analyses
The statistical analysis of clinical data was performed using SPSS 24.0 (SPSS Inc., Armonk, NY, USA) (14). The following tests were applied: Mann-Whitney U test for comparing two independent groups, Kruskal-Wallis test for comparing two or more groups, Fisher's exact test for comparing categorical data.

The effects of factors on survival were evaluated using Kaplan-Meier and Log Rank (Mantel-Cox) tests. Parameters that were significant in the univariate analysis were further evaluated with

multivariate Cox Regression Analysis. A significance level of $p < 0.05$ was accepted.

Gene Enrichment Analysis

Gene enrichment analysis was performed for genes that showed significant changes in gene expression data. For this process, the Enrichr package was used (15-17), and the gene lists were subjected to enrichment analysis in the MSigDB (Molecular Signatures Database) (18-20) and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases (21-23). The significant pathways and genes were then used as features in machine learning algorithms.

Machine Learning Algorithms

Machine learning algorithms were used to predict survival outcomes. These algorithms included C4.5 Decision Tree, Naive Bayes, Random Forest, and Support Vector Machines (SVM).

- *C4.5 Decision Tree:* This algorithm is a machine learning method that creates decision trees to classify data. Decision trees divide the data, making a decision at each node, and ultimately classify it. C4.5 is one of the most popular decision tree algorithms, often preferred for its ease of interpretation (24, 25).
- *Support Vector Machines (SVM):* SVM is a powerful algorithm used for solving classification problems. SVM finds the best hyperplane that separates the data between two classes. It is particularly effective in high-dimensional data and is widely used for classifying complex diseases like cancer (24, 26).
- *Random Forest:* This is an ensemble learning method where multiple decision trees work together. Random Forest analyzes the data with several decision trees and combines their results to offer more accurate and generalizable predictions, making it a suitable option for healthcare data analysis (24, 27).
- *Naive Bayes:* Naive Bayes is a probabilistic classification algorithm. It makes predictions based on the likelihoods of each class. Despite its simplicity, it produces effective results in areas such as text classification and disease diagnosis (24).

The performance of the algorithms was evaluated through the following steps:

- Model Training: Training sets were defined for each algorithm, and models were trained.

- Ten-Fold Cross-Validation: The ten-fold cross-validation method was used to separate the training and test data.
- Performance Evaluation: Metrics such as accuracy, sensitivity, and specificity of the algorithms were calculated, and a confusion matrix was created for each model.

Clinical Decision Support Tool: SurvCOCA

The developed machine learning algorithms were integrated into a web-based clinical decision support system using the R-Shiny package. This system, named SurvCOCA, provides an interface for users to make survival predictions. SurvCOCA runs on TCGA-COAD data and is accessible via the shinyapps.io platform (7,28).

RESULTS
Analysis of Clinical Data

This study included 454 patients from the "COAD" (Colon adenocarcinoma) project, which is part of the National Cancer Institute's Cancer Genome Atlas Program (TCGA), accessible via the GDC Data Portal. The demographic characteristics and clinical parameters of these patients, along with survival analyses, are presented below.

Of the patients, 47.14% (n: 214) were female, and 52.86% (n: 240) were male. Among the 283 patients whose race was known, 74.91% (n: 212) were White, 20.85% (n: 59) were African American, 3.89% (n: 11) were Asian, and 0.35% (n: 1) were classified as Native American or Alaska Native.

Among the 232 patients for whom body mass index (BMI) data were available, 33.19% (n: 77) had a normal BMI, while 66.81% (n: 155) had a high BMI.

A total of 86.56% (n: 393) of the patients had no prior cancer diagnosis, while 13.44% (n: 61) had a previous cancer diagnosis.

Of the 443 patients with available tumor staging data, 16.93% (n: 75) were classified as Stage 1, 39.73% (n: 176) as Stage 2, 28.89% (n: 128) as Stage 3, and 14.45% (n: 64) as Stage 4.

Kaplan-Meier Survival Analysis Results

Kaplan-Meier survival analyses were conducted based on variables such as gender, body mass index, prior cancer diagnosis, primary diagnosis, resection or biopsy site, tumor stage, T stage, N stage, and M stage.

Table 1. Gene enrichment analysis (KEGG)

KEGG	P value	Pathway	Matching Genes
1	0,001	Glycolysis/Gluconeogenesis	ADH1C; AKR1A1; PFKM (3/68)
2	0,044	Other glycan degradation	MAN2B2 (1/18)
3	0,046	Biosynthesis of pantothenate and CoA	BCAT1 (1/19)
4	0,049	Apoptosis	CAPN2; CASP2 (2/143)

Table 2. Gene enrichment analysis (KEGG) (Cancer Hallmark)

Hallmark	P value	Pathway	Matching Genes
1	0,014	mTORC1 pathway	TOMM40; TCEA1; BCAT1 (3/200)

Table 3. Performance results of clinical parameters

Evaluation / Algorithms	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)	Accuracy (ACC)	AUC
C4.5 Decision Tree	%96,6	%28,8	%83,5	%69,7	%82,3	%67,8
Support Vector Machines	%93,0	%16,3	%80,5	%38,2	%76,7	%54,6
Random Forest	%83,9	%45,0	%85,0	%42,9	%75,7	%72,2
Naive Bayes	%88,3	%41,3	%84,8	%48,5	%78,3	%73,7

Table 4. Common performance results of genes in the glycolysis-gluconeogenesis pathway with clinical parameters

Evaluation / Algorithms	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)	Accuracy (ACC)	AUC
C4.5 Decision Tree	%92,6	%28,8	%82,9	%51,1	%79,1	%66,2
Support Vector Machines	%93,0	%17,5	%80,8	%40,0	%77,0	%55,2
Random Forest	%91,6	%31,3	%83,2	%50,0	%78,8	%73,8
Naive Bayes	%88,6	%36,3	%83,8	%46,0	%77,5	%69,9

- Survival by Gender: No significant difference in survival was found between genders (Log Rank test: 0.334, p: 0.563).
- -Survival by Body Mass Index: No significant difference in survival was observed based on BMI (Log Rank test: 2.555, p: 0.110).
- Survival by Prior Cancer Diagnosis: No significant difference in survival was found between patients with and without a prior cancer diagnosis (Log Rank test: 1.164, p: 0.281).
- Survival by Tumor Stage: A significant difference in survival was found based on tumor stage (Log Rank test: 57.061, p: 0.000). Patients in Stages 1, 2, and 3 had higher survival rates compared to Stage 4 patients.

Analysis of Gene Expression Data
Gene enrichment analyses conducted in the KEGG and MSigDB databases revealed that specific genes play a significant role in survival prediction.

- KEGG Database: Genes associated with glycolysis/gluconeogenesis pathways, such as ADH1C, AKR1A1, and PFKM, showed significant matches.
- MSigDB Cancer Hallmark Gene Set: Genes involved in the mTORC1 signaling pathway, including TOMM40, TCEA1, and BCAT1, were found to be important.

Machine Learning Algorithm Results
Model evaluations using various machine learning algorithms showed that the Random Forest algorithm achieved the highest accuracy when clinical and genetic data were used together.

- Performance Results of Clinical Parameters: The Random Forest algorithm showed the
- best performance with an accuracy rate of 82.3% when evaluated using clinical parameters.
- Performance Results of Clinical Parameters Combined with Glycolysis-Gluconeogenesis Pathway Genes: When clinical parameters were combined with gene expression data, the accuracy increased to 79.1%.

Survival Prediction Clinical Decision Support Tool: SurvCOCA

The web interface of the developed SurvCOCA tool was created using the Shiny platform. This open-access tool integrates clinical and genetic data to predict the survival outcomes of colorectal cancer patients.
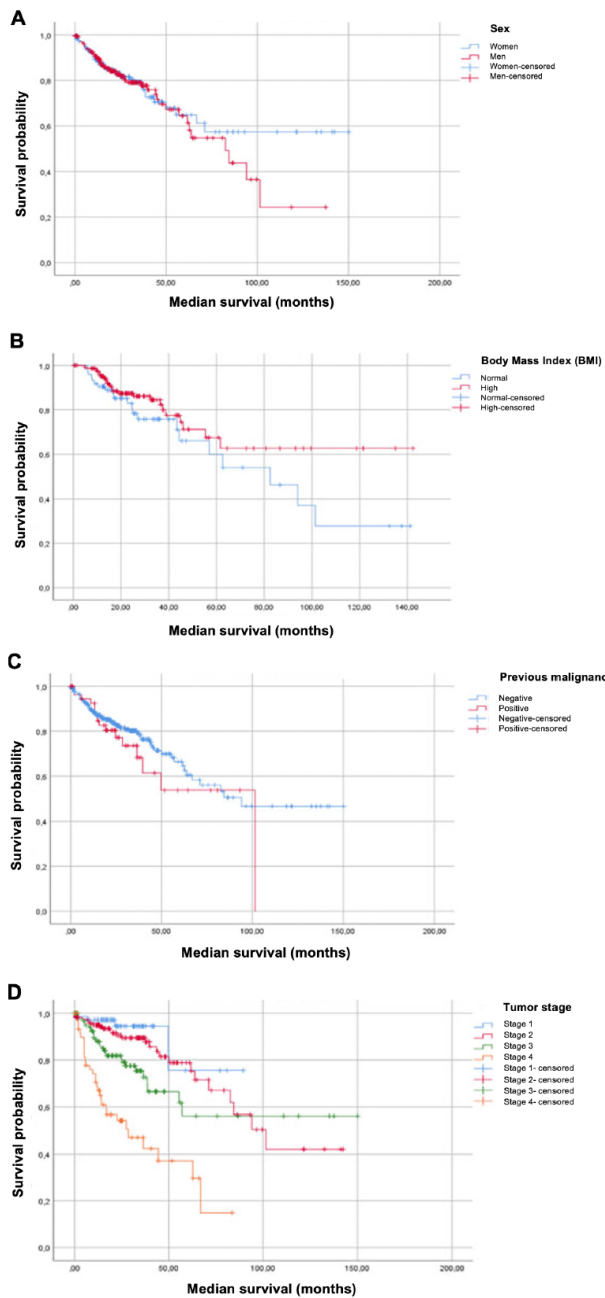
DISCUSSION

This study aimed to evaluate the capacity of an artificial intelligence-assisted clinical decision support system (CDSS) to predict survival in colorectal cancer patients using machine learning techniques. Particularly, given that cancer treatments can cause side effects that may negatively impact the quality of life of patients, survival prediction is of great importance, especially for advanced-stage (Stage 4) cancer patients. It is known that physicians often tend to be more optimistic in their survival estimates compared to actual outcomes, highlighting the need for accurate prognostic models (29,30). Kaplan-Meier survival analyses in our study revealed that many demographic and clinical factors examined did not have a significant impact on survival. Factors such as
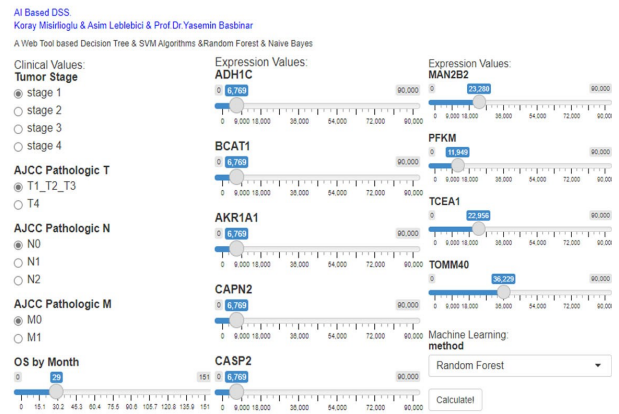
Figure 2. Clinical decision support tool for predicting survival: SurvCOCA Shiny web interface parameter setting screen

Figure 1. Survival analysis. A) Survival by gender. B) Survival by body mass index. C) Survival by previous cancer diagnosis. D) Survival by tumor stage.
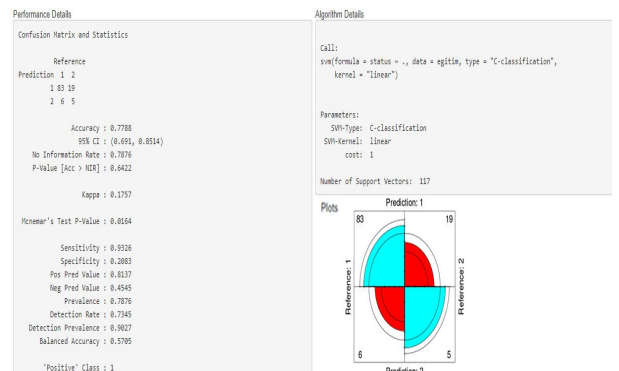
Figure 3. Clinical decision support tool for predicting survival: SurvCOCA Shiny web interface decision output screen

gender, body mass index, prior cancer diagnosis, and primary diagnosis showed no significant difference in survival (Figure 1). However, factors like tumor stage had a significant effect on survival, demonstrating that survival decreases markedly in the more advanced stages of cancer (Figure 1).

Cancer signaling pathways regulate critical biological processes involved in tumor progression and drug resistance. Gene enrichment analyses conducted in this study identified that glycolysis/gluconeogenesis, apoptosis, and mTORC1 signaling pathways play a crucial role in survival prediction (31). Specifically, the expression data of genes such as ADH1C, AKR1A1, PFKM, MAN2B2, BCAT1, CAPN2, CASP2, TOMM40, and TCEA1 showed significant associations with survival (Table 1 and Table 2). In this study, four different machine learning algorithms (C4.5 Decision Tree, Support Vector Machines, Random Forest, and Naive Bayes) were evaluated for their use in survival prediction by utilizing clinical and genomic data. The Random Forest algorithm achieved the highest accuracy, particularly in models where all data were combined (Table 3 and Table 4). As supported by the literature, this result demonstrates that more data leads to better machine learning performance (3).

A key contribution of this study is the integration of survival prediction models developed using machine learning algorithms into a clinical decision support system called SurvCOCA, which features a web interface. This system allows users to make colorectal cancer survival predictions by offering different algorithm options. It is emphasized that such clinical decision support systems should be used more frequently in prognosis and survival predictions and that further research in this area is necessary (31).

CONCLUSION

In conclusion, this study demonstrates the potential of an AI-based clinical decision support system developed for survival prediction in colorectal cancer patients. For further improvement, it is recommended to work with larger datasets and evaluate a broader range of machine learning algorithms. Additionally, enhancing the user interface of SurvCOCA and presenting survival predictions as percentage probabilities could increase the system's acceptance in clinical practice.

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