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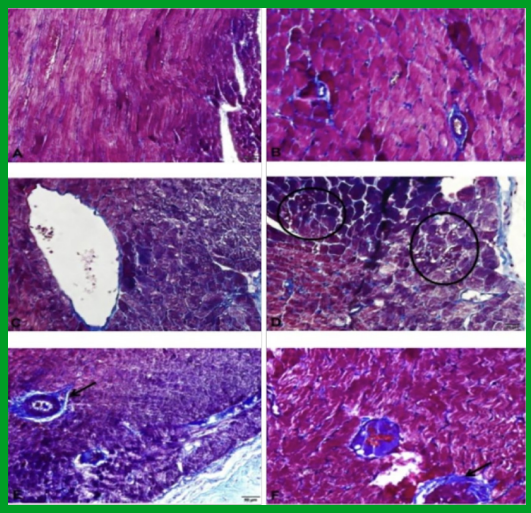

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Original Articles

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EDITORIAL

Dear Readers;

We are happy to meet you in the first issue of 2025. A new year offers a fresh start to take further steps in our scientific studies. The year 2024, which we left behind, was a year of significant success for our journal. With the devoted work of our editorial team and the contributions of our valuable authors, we managed to present you with a wide range of valuable articles in the scientific field.

This year, we will continue to present you with original and qualified studies from every field of health sciences. As of 2025, we will continue to support our young researchers in line with our goal of including a thesis or student study in each issue. We are very happy to encourage the work of these young minds who shape the future of science.

In this issue, we chose our cover image from the study titled **"The Effects of MicroRNAs on Cardiomyopathy in a Rat Model of Streptozotocin-Iiduced Diabetes Mellitus"**. In this study conducted by Doğan AŞ et al., it was stated that miRNAs played a role in diabetic cardiomyopathy, and the potential of using miRNAs as biomarkers in diagnosis and treatment was evaluated in a diabetes model induced by streptozotocin (STZ) in rats. It was emphasized that miR-200c-3p had diagnostic value and could be a biomarker candidate. Hematoxylene and eosin staining in control and diabetes samples for cardiomyocyte hypertrophy was shown in the cover image. In addition, other articles in this issue aimed to contribute to health sciences with a multidisciplinary approach. Some of the articles we have included are:

"BDNF levels and cognitive function in patients with Type 2 Diabetes Mellitus" by Sümbül Şekerci B et al.,

"*In vitro* Evaluation of Repair Bond Strength to Bulk-fill Composites Using Two Silane-Free Universal Adhesives with and without Silane Application" by Aksoy A et al.,

"Surgical and Non-sergic Treatments in Pleural Empyema" by Deniz S et al.,

"The Effects of Microseconds Electroporation on Pore Size, Viability and Mitochondrial Membrane Potential of Cervical Cancer Cells" by Gürsoy G et al.

Throughout 2025, we will continue to provide editorial support to improve the English language quality of your articles. In addition, we invite you, our valuable authors, to submit innovative and meticulously prepared studies with high ethical standards.

Finally, I would like to thank our new editors and referees who have joined us this year and will join us this year. The value that each of them adds to our journal is invaluable. Thanks to your devoted contributions, we will continue to increase our commitment to science and the quality of our work every day.

I hope that 2025 will be full of health, happiness and success for all our readers, writers and the scientific world. I hope to see you in the new issues...

Best regards,

Prof. Dr. Adem AKÇAKAYA
Editor in Chief

Original Article

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Bezmialem Science 2025;13(1):1-9

The Prechtl's General Movement Assessment, Hammersmith Infant Neurological Examination and Sensory Profile-2 in Prediction of Cerebral Palsy at Two Years of Age in High-risk Infants: A Retrospective Study

Yüksek Riskli Bebeklerde İki Yaşında Serebral Palsi Tahmininde Prechtl Genel Hareket Değerlendirmesi, Hammersmith Bebek Nörolojik Muayenesi ve Bebek Duyu Profili-2: Retrospektif Bir Çalışma

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ABSTRACT

Objective: The predictive values of the general movements assessment (GMA), the Hammersmith infant neurological examination (HINE), and the infant sensory profile-2 (ISP-2) were investigated for the diagnosis of cerebral palsy (CP) in high-risk infants at two years age.

Methods: Thirty-four high-risk infants with a mean gestational age of 33.1±4.2, who were followed up in the physiotherapy unit after discharge from the neonatal intensive care unit, were recruited. Prechtl's GMA results were collected for infants at 3rd month. The ISP-2 and HINE were used to evaluate neurological and sensory processing at 3rd month for once, respectively. A pediatric neurologist who was blinded to all testing made the diagnosis of CP at two years of age based on neuroimaging and clinical tests. The GMA, HINE, and ISP-2's predictive values were assessed seperately and sequentially (with two and three stage testings).

ÖZ

Amaç: Bu çalışmada yüksek riskli bebeklerde iki yaşında serebral palsi (SP) tanısı için genel hareket değerlendirmesi (GMA), Hammersmith infant nörolojik muayene (HINE) ve bebek duyusal profili-2'nin (ISP-2) prediktif değerleri araştırıldı.

Yöntemler: Yenidoğan yoğun bakım ünitesinden taburcu olduktan sonra fizyoterapi ünitesinde takip edilen, ortalama gebelik yaşı 33,1±4,2 olan 34 yüksek riskli bebek çalışmaya alındı. Bebekler için demografik veriler, doğum öncesi risk faktörleri ve üçüncü ayda Prechtl'in GMA sonuçları toplandı. Nörolojik muayene ve duyusal işlemeyi değerlendirmek için sırasıyla HINE ve ISP-2 anketi üçüncü ayda bir kez yapıldı. GMA, HINE ve ISP-2'nin prediktif değerleri sıralı (tek-iki-üç aşamalı) değerlendirildi.Tüm testlere kör olan bir pediatrik nörolog, nörogörüntüleme ve klinik testlere dayanarak iki yaşında SP tanısını koydu.

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ABSTRACT

Results: The mean birth weight of the infants was 1993.7±889.3 grams (g), the 3rd month HINE and ISP-2 total score averages were 56.9±9.3 and 53.8±13, respectively. In order to diagnose CP, it was important to consider the predictive values of the GMA (absent FMs) (sensitivity: 100%; specificity: 96.15%, p<0.001; 95% confidence interval: 80.36-99.9), HINE (sensitivity: 87.5%; specificity: 80.77%; p=0.004), and the combination of both GMA and HINE (sensitivity: 87.5%; specificity: 100%; p<0.001). The combination of ISP-2, GMA, and HINE was significant in identifying CP diagnosis (sensitivity: 62.5%, specificity: 100%, p=0.002) despite the ISP-2's unremarkable predictive performance (sensitivity: 75%, specificity: 61.54%, p=0.123).

Conlusion: Early follow-up of high-risk of infants may enable early diagnosis of CP and referral to early intervention by clinical application and combination of GMA and HINE.

Keywords: General movement assessment, Hammersmith infant neurological examination, infant sensory profile-2, cerebral palsy, high risk infant

ÖZ

Bulgular: Bebeklerin ortalama doğum ağırlığı 1993,7±889,3 gram (gr), 3. ay ve sırasıyla HINE ve ISP-2 toplam puan ortalamaları 56,9±9.3 ve 53,8±13 idi. SP tanısı koymak için GMA (FM olmaması) (sensitivite: %100; spesifite: %96,15, p<0,001; %95 güven aralığı: 80,36-99,9), HINE'nin (sensitivite: 87,5 spesifite: %80,77; p=0,004) ya da GMA ile HINE kombinasyonunun (sensitivite: %87,5; spesifite: %100; p<0,001) prediktif değerlerinin yüksek olduğu, ISP-2, GMA ve HINE kombinasyonunun sensitivite: %62,5, spesifite: %100, p=0,002), ISP-2'nin tek kullanıldığında dikkat çeken prediktif performansına rağmen sensitivite: %75, spesifite: %61,54, p=0,123) SP tanısını belirlemede anlamlı olduğu bulundu.

Sonuç: Yüksek riskli bebeklerin takibiyle SP'nin erken tanısının; klinik uygulama, GMA ve HINE kombinasyonuyla yapılması erken müdahaleye yönlendirmeye olanak sağlayabilir.

Anahtar Kelimeler: Genel hareket değerlendirmesi, Hammersmith bebek nörolojik muayenesi, bebek duyu profili-2, serebral palsi, yüksek riskli bebek

Introduction

High-risk infants may encounter many problems in terms of neuromotor, cognitive, sensory and psychosocial developments (1-4). Cerebral palsy (CP), which is known as a disease with neurosensorimotor impairment, can also be observed in high-risk infants (5,6). The possibility to access early intervention to improve neurosensorimotor functions is provided by the early diagnosis of CP (5,7,8). Around the world, 1 in 10 newborns are born preterm each year, and those who are born very preterm (VPT) have the highest rates of neurodevelopmental disabilities (8). Up to 50% of infants with VPT experience mild to severe motor impairments such as CP (9,10).

Clinical diagnosis of CP can delay until 2 years of age (5,11,12). The guidelines state that high-risk of infants should be evaluated before the age of five months utilizing magnetic resonance imaging (MRI) neuroimaging, Prechtl's general movement assessment (GMA) for motor assessment, and the Hammersmith infant neurological examination (HINE) for neurological examination (5,11,12). Absence of fidgety movements (FMs) according to Prechtl's GMA in early infancy and total score of HINE <57 at 3 months have high predictive value in the diagnosis of CP (5,6,13-15). Early intervention is crucial for newborns who are at high risk for neurological or sensory abnormalities. By encouraging the empowerment of the family, early intervention can promote neuroplasticity while promoting the development of the motor and sensory systems (11). It is advised to use the GMA, HINE at high risk of infants to diagnose neurodevelopmental abnormalities (5,6,11,14-16). With high specificity and sensitivity, neurodevelopmental problems can be early predicted by these assesments (11). But there is not enough study which shows the relationship between the predictive power of the sensory processing disorders with neurodevelopmental disorders like CP. Kara et al. (17) stated sensory processing was linked to motor development in preterm infants at 1-4 months of corrected age (CA), using the infant sensory profile-2 (ISP-2).

The ISP-2 gives professionals a tool to record children's sensory processing patterns starting at early age, and Dunn has indicated that ISP-2 provides information as to whether a follow-up is necessary (18).

High risk of infants encounter many sensory stimuli in the neonatal intensive care unit (NICU), which are not present in the uterus, such as extremely noise, shiny lights, and painful medical applications. Infants' physiological reactions are impacted during this crucial time for brain development, and this can have a severe impact on their motor, neurological, and sensory developments, which can result in sensory processing problems (17). In particular, preterm newborns may experience sensory processing difficulties in addition to developmental disorders (17,19). However, studies on early spontaneous movements, neurological examination, and sensory processing are scarce (17,19).

Sensory processing is the neurological regulation of the stimulus coming with vestibular, proprioceptive, kinesthetic, visual, auditory, tactile, oral, olfactory inputs, and responding with appropriate reactions and behaviors (20). These processes show adequate development with natural stimuli from infancy. The infant's normal sensory and motor development may suffer as a result of decreased spontaneous movements for any reason and excessive sensory stimulation in the NICU. This can affect developing brain and the natural development of sensory systems. Studies revealed a significant difference in the sensory profiles of preterm infants compared to the terms (21,22). Looking at the long-term impact, children with sensory processing problems show weakness in fine and gross motor skills, and delays and losses in balance and coordination. Weak sensory processing in the preschool period can also lead to problems with distraction,

advocacy, language, and visuospatial skills (23). Various academic success problems, emotional difficulties, and poor interaction skills with peers can be seen at school age (24).

There are few studies evaluating the connection between motor development in newborns from the neonatal period and sensory processing factors (17,19,21). These infants at risk of biological vulnerability and neurological injury require neurodevelopmental follow-up after discharge. The purpose of this study was to assess the accuracy of GMA, HINE, and ISP-2, both alone and in combination, in predicting the diagnosis of CP in high-risk infants at two years of age.

Methods

Study Design

A retrospective methodological study.

Participants

We conducted a retrospective study on high-risk infants who were delivered in different hospitals in Gaziantep, Türkiye between 2016 and 2020 and were referred to the Lokman Hekim University Faculty of Health Sciences Department of Physiotherapy and Rehabilitation for post discharge follow-up. All parents signed written informed consent.

All clinical data, including demographic characteristics, prenatal, and postnatal history were obtained from medical records. The inclusion criteria were as follows; perinatal asphyxia, stage 3 hypoxic ischemic encephalopathy (HIE), intracranial hemorrhage (ICH) (level 2, 3, or 4), periventricular hemorrhage (PVH), cystic periventricular leukomalacia (PVL), kernicterus, bronchopulmonary dysplasia (BPD), respiratory distress syndrome (RDS), long-term oxygen support (7 days), >24 hours on mechanical ventilator (MV) support, retinopathy of prematurity (ROP), neonatal sepsis, 5th minute Apgar Score <3, necrotizing enterocolitis (NEC), preterm/multiple births <1500 g, and gestational age (GA) <32 weeks. Infants with genetic, metabolic, or congenital disorders were not included.

GA at birth, date of birth, sex, HINE, GMA, and ISP-2 scores at 3 months, as well as 2-year outcome, which comprised typical development or CP diagnosis, were the minimum necessary data for inclusion. The evaluation of 60 records resulted in the inclusion of 34 infants (14 girls and 20 males) (Figure 1). The GMA, HINE, and ISP-2's predictive values were assessed seperately and, sequentially (with two and three stage testingstwo-stage). testing and three stage testing. The Non-interventional Clinical Research Ethics Committee of Lokman Hekim University approved the study (approval no: 2022/95, date: 31.05.2022). Also the study was conducted in accordance with the Helsinki Declaration. Clinical Trial study registry identifier is NCT05217199.

Outcome measures

General Movement Assessment

We obtained the results of GMA of fidgety periods using Prechtl's method. The Prechtl's method is a valid and reliable

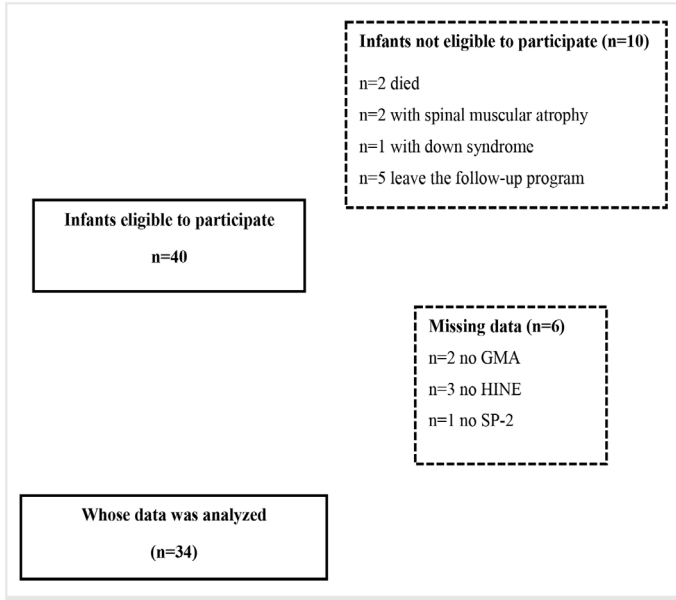

Figure 1. Flow diagram of study participants

GMA: General movement analysis, HINE: Hammersmith Infant neurological examination, SP-2: Sensory profile

video observation that uses gestalt perception to examine video recordings of spontaneous movements and reveal information about the functional integrity of the developing nervous system. Especially, GMA based on FMs, which typically occur between 3-5 months post-term age, is the most sensitive and specific indicator for later disabilities. Normal FMs are described as modest amplitude circular movements of the neck, trunk, and extremities moving at moderate speeds in all directions with varied acceleration. FMs are divided into three categories: normal, absent, and aberrant FMs (6,13,16). In present study, the 3-min-long video recordings taken during the fidgety period were evaluated by two certified experienced pediatric physiotherapists (B.E and H.A.) in basic and advanced GMA, who were blinded to the participants' clinical histories and neurological conditions at 3rd months. These physiotherapists evaluated each patient separately. They scored differently, but all of the scorings had interobserver agreement. When more than 1 record of GMA classification was available, the one closest to 12 weeks post-term was retrieved.

Hammersmith Infant Neurological Examination

The HINE is a reliable tool for the clinical neurological examination of newborns between the ages of 2 and 24 months. HINE has 26 items that test 26 various aspects of neurological functions, such as reflexes, posture, motions, and cranial nerve functions. Each item is scored between 0 and 3, and the individual scores are summed to create the global score. The overall score can vary from 0 to 78. To predict CP, international guidelines suggest a cut-off score of 57 at three months of age (5,14,25). The clinical records of infants were used to gather the HINE scores for this study, which were completed by pediatric

physiotherapists with at least 10 years of expertise in the field. HINE was scored at 3rd months once.

Infant Sensory Profile-2 (ISP-2)

The ISP-2, which is a parent-reported questionnaire, describes the sensory processing skills of infants from zero to six months, is a component of the sensory profile-2. It contains 25 items that assess different aspects of sensory processing. Each item is given a score between one and five, and the aggregate of the individual scores yields the raw score. It is a reliable and valid tool for sensory processing disorders of infants (18,23,24,26). Based on norm reference values, raw scores between 41 and 61 were classified as typical sensory development, while other scores were classified as atypical in the present study. The ISP-2 was scored at 3rd months once.

Diagnosis of CP

Based on neuroimaging and clinical evaluations at 2 years of age, a pediatric neurologist who was blinded to all assessments diagnosed CP. The neuroimaging scans were classified into one of seven primary patterns of abnormality as defined and described by Ashwal et al.'s (27) study. The classification categories were normal, periventricular white matter injury (PWMI), diffuse encephalopathy, focal ischaemic or haemorrhagic lesions, brain malformations, infection, and miscellaneous or unclassifiable lesions.

Statistical analysis

The GMA, HINE, and ISP-2 evaluations were dichotomized; for GMA, FMs (normal) or absent FMs (aberrant); for HINE, normal (scores ≥57) or abnormal (scores ≤57); and for ISP-2; typical (scores between 41 and 61) or atypical (scores ≤41 or ≥61). The accuracy of the assessment tools in accurately identifying those with and without CP was examined using receiver operating characteristics (ROC) analyses. Sensitivity, specificity, overall accuracy, area under the curve, and 95% confidence intervals were calculated. The ROC analysis was conducted with dependent (outcome) variable as typical development versus CP and independent (predictor) variables as GMA (normal versus aberrant), HINE (normal versus abnormal), and ISP-2 (typical versus atypical). Statistical significance was set as $p<0.05$. All statistical analyses were conducted with SPSS Statistics version 26 (IBM Inc, Chicago, IL, USA).

Results

The clinical and demographic characteristics of the infants were summarized in Table 1. The current study included 34 high-risk infants with a mean GA of 33.1±4.2 weeks. At age two in CA, eight infants (or 23.5%) had CP. In contrast to the 25 newborns who showed normal FMs at post-term age of 12 weeks, 8 infants had no (absent) FMs, according to the GMA data. At 12 weeks post-term, the mean HINE and ISP-2 scores were 56.9 and 53.8, respectively. According to the diagnostic, CP was present in all of the newborns (n=8) without FMs. Seven (58.3%) of the 12 infants with HINE scores below the threshold of 57 and six (33.3%) of the 18 children with impaired sensory processing had CP (Table 2).

Table 3 displays the GMA, HINE, and ISP-2's predictive values assessed seperately and, sequentially (with two and three stage testings two-stage), testing and three stage testing, seperately, sequential (two-stage) testing and three stage testing. In order to diagnose CP, it was important to consider the predictive abilities of the GMA (without FMs) (sensitivity=100%, specificity=96.15%, p=0.001), HINE (sensitivity=87.5%, specificity=80.77%, p=0.004), and the combination of both GMA and HINE (sensitivity=87.5%, specificity=100%, p=0.001). The combination of ISP-2, GMA, and HINE was significant in identifying CP diagnosis (sensitivity=62.5%, specificity=100%, p=0.002) despite the ISP-2's unremarkable predictive performance (sensitivity=75%, specificity=61.54%, p=0.123) (Figure 2, Table 3).

Table 1. Clinical and demographic characteristics (n=34)

Age (month), mean (SD)	**2.56 (0.16)**
Male, n (%)	20 (58.8)
Female, n (%)	14 (41.2)
Term birth, n (%)	8 (22.8)
Gestational age (weeks), mean (SD)	33.1 (4.2)
Birth weight (g), mean (SD)	1993.7 (889.3)
Preterm birth, n (%)	25 (73.5)
Multiple birth (yes), n (%)	13 (38.2)
NICU duration (d), mean (SD)	41.1 (26.4)
Perinatal pathologies	**n (%)**
PVL	4 (12.12)
Asphyxia	6 (18.18)
RDS	10 (30.3)
CLD	4 (12.12)
Pneumonia	1 (3.03)
Hyperbilirubinemia	3 (9.09)
Sepsis	3 (9.09)
PDA	1 (3.03)
SGA	1 (3.03)
Clinical characteristics	
Absent fidgety, n (%)	9 (26.5)
HINE total score at 3 months, mean (SD)	56.9 (9.3)
HINE scores <57 at 3 months, n (%)	12 (35.3)
ISP-2 total score, mean (SD)	53.8 (13.0)
ISP-2 total score 41 to 61, n (%)	16 (47.1)
CP diagnosis, n (%)	8 (23.5)

g: Gram, d: Day, NICU: Neonatal intensive care unit, PVL: Periventricular Leukomalacia, RDS: Respiratory distress syndrome, CLD: Chronic lung disease, SGA: Small for gestational age, PDA: Patent ductus arteriosus, HINE: Hammersmith infant neurological examination, CP: Cerebral palsy, ISP-2: Infant sensory profile-2, SD: Standard deviation

Table 2. GMA, HINE, ISP-2, and two-year outcome results of infants

Case	Fidgety (+: Normal and -: absent)	HINE (+: normal and -: abnormal)	ISP-2 (+: typical and -: atypical)	CP diagnosis
1	+	+	+	No
2	+	-	-	No
3	+	+	+	No
4	+	+	+	No
5	+	+	+	No
6	+	+	+	No
7	-	-	-	Yes
8	+	+	-	No
9	+	+	-	No
10	+	+	-	No
11	-	-	-	Yes
12	-	-	-	Yes
13	+	+	+	No
14	+	+	-	No
15	+	+	+	No
16	+	+	+	No
17	+	-	+	No
18	-	-	-	Yes
19	+	+	-	No
20	-	+	-	Yes
21	+	-	-	No
22	+	-	+	No
23	+	+	+	No
24	+	+	+	No
25	+	+	-	No
26	-	-	+	Yes
27	+	+	-	No
28	-	+	+	No
29	+	-	+	No
30	+	+	+	No
31	+	+	-	No
32	-	-	+	Yes
33	-	-	-	Yes
34	+	+	+	No

GMA: General movement analysis, HINE: Hammersmith infant neurological examination, CP: Cerebral palsy, ISP-2: Infant sensory profile-2

Discussion

This study revealed that high risk infants had abnormal early spontaneous movements, divergency in neurological examination, and atypical sensory processing in the first three months of life. To the best of our knowledge, this is the first study to investigate the relationship between GMA and sensory processing in the first few months of life using GMA, HINE, and ISP-2. In addition, this study is the first study to determine the predictabilityof CP at two years of age with the use of these three tests in the early period. This study showed that the specificity of GMA, which is crucial for identifying children without CP, was improved when GMA and HINE were combined. ISP-2 was added to the combination of GMA and HINE, but this did not increase predictive power. But, the use of the ISP-2 test in this study enabled the detection of early sensory disorders in high risk infants. In the current study, it was determined that the clinical implementation of GMA, HINE, and ISP-2 together in the early period of follow-up of high risk of infants might enable early detection of the risks of neurodevelopmental and sensory processing disorders and refer them for early intervention. There was debate regarding earlier research that looked at sensory processing and motor development before the age of one (17,21,28).

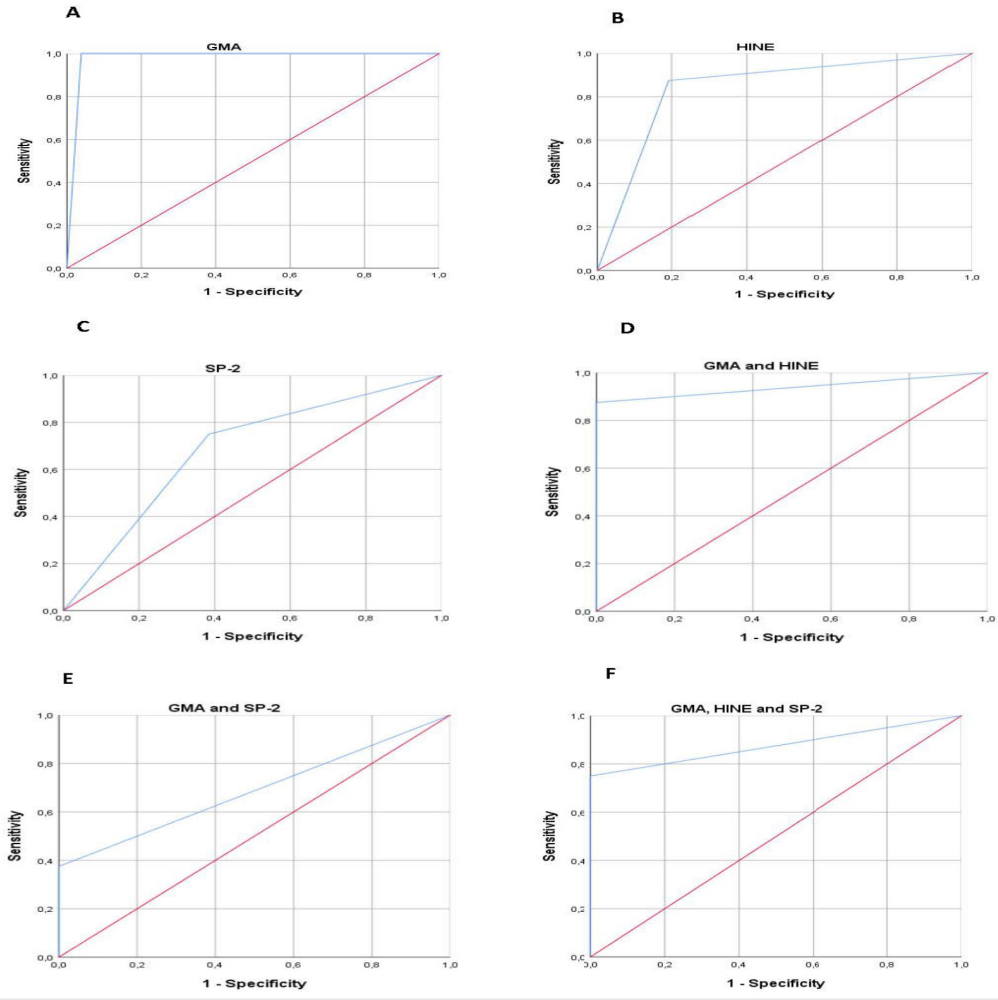

Figure 2. The ROC curves for each assessment tool individually and in combination

GMA: General movement analysis, HINE: Hammersmith Infant neurological examination, SP-2: Sensory profile, ROC: Receiver operating characteristics

Table 3. Predictive accuracy of the GMA, HINE, and SP-2 for CP diagnosis

Predictor of CP	Sensitivity % (CI 95%)	Specificity % (CI 95%)	Accuracy % (CI 95%)	AUC (CI 95%)	p-value
GMA (Absent FMs)	100 (63.06-100)	96.15 (80.36-99.9)	97.06 (84.67-99.93)	0.981 (0.936-1,000)	**<0.001**
HINE (Abnormal)	87.5 (47.35-99.68)	80.77 (60.65-93.45)	82.35 (65.47-93.24)	0.841 (0.679-1,000)	**0.004**
ISP-2 (Atypical)	75 (34.91-96.81)	61.54 (40.57-79.77)	64.71 (46.49-80.25)	0.683 (0.473-0.892)	0.123
GMA and HINE	87.5 (47.35-99.68)	100 (86.77-100)	97.06 (84.67-99.93)	0.938 (0.801-1,000)	**<0.001**
GMA and ISP-2	75 (34.91-96.81)	100 (86.77-100)	94.12 (80.32-99.28)	0.688 (0.444-0.931)	0.113
GMA and HINE and ISP-2	62.5 (24.46-91.48)	100 (86.77-100)	91.18 (76.32-98.14)	0.875 (0.691-1,000)	**0.002**

GMA: General movement analysis, HINE: Hammersmith Infant neurological examination, CP: Cerebral palsy, ISP-2: Infant sensory profile-2, CP: Cerebral palsy, CI: Confidence interval, AUC: Area under the curve

The GMA (absent FMs) had the highest CP prediction accuracy with high sensitivity and specificity in the current study. Although the combination of the tools did not improve the overall accuracy of GMA, it did improve the specificity of the GMA. Also, infants with atypical sensory processing and abnormal HINE score in the third month were diagnosed as having CP at two years CA with lower sensitivity and specificity more than GMA.

In the previous studies, it was stated that neonatal MRI, GMA and HINE had the best predictive values of CP diagnosis in the corrected 5th months of life (5,11,12,25). According to Novak et al.'s (5) study, term MRI performed before to the corrected 5th month had a sensitivity rate of 86% to 89% for diagnosing neurodevelopmental problems like CP. GMA is another method that is frequently used to predict CP. According to Prechtl et al. (29), the assessment of FMs could predict neurological outcome with 96% specificity and 95% sensitivity (11,16). According to Kwong et al. (15), GMA during the fidgety period had 97% sensitivity and 89% specificity for prediction of CP diagnostic. Similarly, according to Morgan et al. (11), FMs' sensitivity and specificity ratings for detecting neurodevelopmental problems in infants at risk were 95% and 97%, respectively. In the current study, the GMA (absent FMs) had the highest prediction accuracy with 95% sensitivity and 96% specificity. In this respect, our results were similar to these studies. GMA requires basic and advanced training and is also based on visual perception. When detecting neurodevelopmental diseases like CP, it has a strong predictive power. However, there are few clinics in our country, which limits the possibility of early diagnosis and, thus, early intervention. Both recent research and the most recent analysis indicate that GMA usage in our nation should increase. We believe that appropriate rules should be created and that GMA usage in our nation should be increased.

Neurological examination is important in infants with high risk of CP. It is shown that HINE cut-off scores in the first year of life can predict the first signs of CP. Romeo et al. (25) showed that there were various neuromotor development problems investigated by HINE in infants with high risk of CP in the first years of life (30). Novak et al. (5) and Romeo et al. (25) stated that cut-off scores of HINE (scores less than 57) were predictive for CP in the 3rd months of life. Similar to these studies, we found that the infants having the HINE total scores <57 in the 3rd months were diagnosed as having CP in the CA of two years. HINE (total score <57) had high predictive accuracy with 87% sensitivity and 80% specificity similar to these studies. These results show that the use of HINE for early diagnosis is necessary in the follow-up units from an early age should be expanded in the diagnosis of early CP. Turkish version of HINE is available and should be used more in the clinics for different health professionals in Türkiye (31).

Sensory processing disorders are seen frequently in high risk infants (17,19,21,22,28). Inappropriate sensory stimulation in NICU may cause sensory processing difficulties causing developmental delays. According to a neurobehavioral evaluation of preterm newborns, according to Smith et al. (32), increased stressors like medical procedures or stimulation in the NICU cause the frontal and parietal brain lobes to shrink and cause abnormal motor behavior. In preterm newborns, Chorna et al. (33) discovered that aberrant sensory sensitivity throughout the first year was linked to inferior developmental outcomes at two years of age. Additionally, Eeles et al. (34) demonstrated a relationship between sensory processing and developmental milestones in preterm children at age two. Using the ISP-2, Kara et al. (17) revealed that sensory processing was related to motor development in preterm infants at 1 and 4 months of corrected age. Similar to these studies, ISP-2 scale revealed atypical sensory processing disorders in high risk infants, but it didn't have predictive accuracy for CP diagnosis. Therefore, ISP-2 should be used in the follow-up clinics, or NICU for detection of sensory disorders. The use of the combination of GMA, HINE and ISP-2 doesn't provide early diagnosis of CP but can guide rehabilitation at early ages. The triple combination of these tests did not have high predictive accuracy, sensitivity and specificity for CP diagnosis. But we suggest that not only assessing motor development, but also sensory processing may be considered necessary as a predictor of developmental abnormality.

Owing to alterations in this process during the first few months of a preterm infant's life owing to the NICU environment, it is believed that rates of sensory processing issues may be higher in preterm newborns. According to Chorna et al. (33), the rate of sensory processing issues was 82% in newborns with GA30 weeks, 60% according to Celik et al. (21) and 73.33% according to Cabral et al. (28) in children born <37 GA, and 46.6% according to Bart et al. (35) in late preterm infants. Similar to these studies, 73% of infants were preterm in this study. But atypical sensory processing difficulties were observed in all preterm and term infants. These results suggest that motor development may be related to atypical sensory development in preterm infants.

Study Limitations

Potential limitations of this study were the use of the version of ISP-2 parent questionnaire. Although parents' characteristics were thought to play a role in their answers, it was reported that less time was spent in the assessment, and daily life activities could be questioned (33). In addition to sensory evaluation with a family questionnaire, further studies are needed in which the infants between 7-18 months are directly evaluated and followed up with tests such as the test of sensory functions in infants. Also, this study was conducted with high risk infants. So, the results of this study can not be generalized to all preterm infants. Another limitation of this study was that some data of the parents, such as educational level and employment status, were missed.

Conclusion

The best method for predicting CP at age two in high risk infants is to interpret GMA and to combine GMA and HINE assessments when they are young. Additionally, the addition of GMA to HINE enhances GMA's specificity, which is crucial

for CP prediction. Early infant follow-up units may be able to identify infants who are at risk for sensory processing problems and refer them for early intervention programs thanks to the clinical implementation of ISP-2.

Written informed consent was obtained from all parents of all infants who were included in this study.

Ethics

Ethics Committee Approval: The Non-interventional Clinical Research Ethics Committee of Lokman Hekim University approved the study (approval no: 2022/95, date: 31.05.2022). Also the study was conducted in accordance with the Helsinki Declaration.

Informed Consent: Written informed consent was obtained from all parents of all infants who were included in this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.U.S., Concept: H.A., H.İ.Ç., Y.U.S., N.E., B.E., Design: H.A., H.İ.Ç., Y.U.S., N.E., B.E., Data Collection or Processing: H.A., H.İ.Ç., Analysis or Interpretation: H.A., H.İ.Ç., Y.U.S., N.E., B.E., Literature Search: H.A., H.İ.Ç., Writing: H.A., H.İ.Ç.

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Original Article

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Perceived Burden and Healthy Lifestyle Behavior in Family Caregivers of the Persons with Disabilities in Bukhara

Buhara'da Engelli Bireylere Bakım Veren Ailelerde Algılanan Yük ve Sağlıklı Yaşam Biçimi Davranışları

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ABSTRACT

Objective: Family caregiving is a common tradition to Uzbekistan. The responsibilities of caring for a person with disabilities may cause caregivers to develop risky health behaviors and become a burden for them. The aim of this study was to determine the relationship between perceived caregiver burden and healthy lifestyle behavior in family members caring for young persons living with disabilities under the age of 24 years.

Methods: The sociodemographic characteristics of the participants were recorded. The number of children in the family, duration of care period, disability group of the one who is cared, duration and type of disability, age and gender of the young person were asked. The Zarit Burden Interview and the Health Promoting Lifestyle Profile II (HPLP II) were used.

Results: Out of 155 caregivers, 89.8% were women and 10.2% were men. There was no correlation between burden and HPLP II in general (r=0.048, p=0.551). A positive correlation was found between burden and nutrition, one of the sub-dimensions of the HPLP II (r=0.038; p=0.016), whereas negative correlations were found between burden and health responsibility and stress management sub-dimensions (r=-0.170, p=0.034; r=-0.184, p=0.022).

Conclusion: Perceived burden did not affect healthy lifestyle behavior in general. This may be due to the positive meaning that Uzbeks attach to caregiving. This study is the first in Uzbekistan concerning the effect of caregiving to young persons with disabilities on family members. The results show that family caregivers might require additional support for stress management and health responsibility.

Keywords: Burden, disability, family, caregiver, lifestyle

ÖZ

Amaç: Aile bakımı Özbekistan'da yaygın bir gelenektir. Engelli bir bireyin bakımına ilişkin sorumluluklar, bakım verenlerin riskli sağlık davranışları geliştirmelerine neden olabilmekte ve onlar için yük haline gelebilmektedir. Bu çalışmanın amacı, 24 yaş altı engelli gençlere bakım veren aile bireylerinde algılanan bakım verme yükü ile sağlıklı yaşam biçim davranışları arasındaki ilişkiyi belirlemekti.

Yöntemler: Katılımcıların sosyo-demografik özellikleri kaydedildi. Ailedeki çocuk sayısı, bakım süresi, bakım verilenin engel grubu, engel süresi ve türü, yaşı ve cinsiyeti sorgulandı. Zarit Bakım Verme Yükü Ölçeği ve Sağlıklı Yaşam Biçim Davranışları Ölçeği II (SYBDÖ II) kullanıldı.

Bulgular: Yüz elli beş bakımverenin %89,8'i kadın ve %10,2'si erkekti. Bakım verme yükü ile SYBDÖ II arasında genel olarak bir ilişki bulunmamıştır (r=0,048, p=0,551). Bakım verme yükü ile SYBDÖ II'nin alt boyutlarından beslenme arasında pozitif bir ilişki (r=0,038; p=0,016), yük ile sağlık sorumluluğu ve stres yönetimi alt boyutları arasında negatif bir ilişki bulunmuştur (r=-0,170, p=0,034; r=-0,184, p=0,022).

Sonuç: Algılanan bakım yükü genel olarak sağlıklı yaşam tarzı davranışını etkilememiştir. Bu durum Özbeklerin bakım vermeye yükledikleri olumlu anlamdan kaynaklanıyor olabilir. Bu çalışma, engelli gençlere bakım vermenin aile üyeleri üzerindeki etkisine ilişkin Özbekistan'da yapılan ilk çalışmadır. Sonuçlar, ailede bakım veren bireylerin stres yönetimi ve sağlık sorumluluğu için ek desteğe ihtiyaç duyabileceğini göstermektedir.

Anahtar Kelimeler: Yük, engellilik, aile, bakım veren, yaşam tarzı

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Introduction

Parents of children with disabilities have multidimensional responsibilities and experience various financial, social and/or psychological problems (1). Family caregiving is a tradition common to Uzbekistan and many low-and middle-income countries where formal long-term care services are not available or accessible. Family members may be well-placed to understand the health issues (2). Having a disabled child is a situation that significantly changes parents' daily life, as they have limited time and social interaction for other activities, including work, leisure, and personal care (3). During the coronavirus disease-2019 (COVID-19) pandemic, it remained unclear how family caregivers were adapting to the changes. Maintaining a healthy lifestyle may be more difficult, as they need to spend more time to maintain the level of care. Children with disabilities are dependent on their parents with their needs (4). The requirements of family caregiving are often overwhelming (5). Although taking care of children is a normal role for parents, it becomes more difficult as the child's functional dependencies increase (2). Family members caring for a person with a disability are more likely to have chronic diseases such as asthma, arthritis, chronic bronchitis, hypertension, and to engage in unhealthy behaviors such as smoking and irregular sleep (1).

The caregiver is a person who regularly looks after an individual in need. The caregiver burden is defined as an individual's perception of the negative effects they experience from the stress and responsibilities of caregiving (6). These perceptions are dynamic and changing, and may negatively affect the caregiver physically, psychologically, emotionally, socially and economically because of the negative emotions and experiences (7). Having an imbalance between care demands and care support during the caregiving process, may cause stress and burden to emerge (8). The evaluation of perceived caregiver burden is valuable because family members can be aware of how they are affected from the situation (8). For example, in Mochari-Greenberger and Mosca's (9) study, caregivers stated that having a healthy diet and undertaking physical activity were less frequent. The most commonly cited caregiver burdens included changes in personal plans, time demands, and sleep disturbance. Qualitative and quantitative assessment methods for caregiver burden may enable the development and delivery of support interventions that meet the needs of caregivers and help reduce their burden (6). Studies have shown that the health status of caregivers has an effect on their caregiving, and caregivers with poor health have greater caregiving burden (3,10). It was suggested that support, including meeting the needs of caregivers and increasing their quality of life, might play an important role in their health improvement (11,12).

Studies on the factors affecting caregiver burden are diverse and vary in population, so investigation of caregiver burden in societies with different characteristics and the relationship with health has been recommended (2,6,13). There are no studies from Uzbekistan concerning the effect of caregiving to disabled children on family members. Furthermore, there is no study examining the relationship between caregiving burden and healthy lifestyle behavior in caregivers of children with disabilities. Therefore, the aim of this study was to evaluate the relationship between caregiver burden and healthy lifestyle behavior in family members caring for the young people living with disabilities in Bukhara, Uzbekistan.

Methods

This descriptive and cross-sectional study was carried out as field research in a collaboration with the students, when one of the authors was on duty as a guest lecturer in the Division of Physiotherapy and Rehabilitation at Bukhara Ibn-i Sina Vocational School of University of Health Sciences in Uzbekistan. The data were collected between the 1st and the 28th of December, 2021. By using census sampling method, it was attempted to contact all individuals caring for a young person with disability living in Bukhara, Uzbekistan. Consenting individuals who were able to read and write and who were caring for a young person below 24 years were recruited for the study. Caregivers caring for persons with disability older than 24 years of age were excluded.

Pre-study sample size was calculated. Sample size was calculated by using G*power 3.0 software (14). The current study required a sample size of 138 participants to provide 95% power to detect a difference at a 0.05 significance level, with a medium effect size (d=0.3) and for correlation (15). Considering the possibility of data loss, the sample size was increased by 10%, resulting in a total of 152 participants A census sampling method was used. Clinical Trial study registry identifier is NCT05351528.

Measures

The researchers identified the families with the information they received from the demarch/reeve of the towns. Data collection was carried out with the students under a researcher's supervision through home visits by making an appointment with the family. After obtaining informed consent from the participants, they were asked to fill in a study-specific personal details form. Data items included were the caregiver's age, height, weight, employment status, income status, marital status, relationship, educational level, place of residence, disease history, and family history. Additionally, information about the caregiver's smoking status, regular exercise habit, and sports habits were obtained. The form also included questions regarding how many children were in the family, the duration of the care period, and the disability group of the young person who was cared for, disability duration and the type of disability, as well as the young person's age and gender.

The Zarit Burden Interview was used because it was used widely to study caregiving burden experienced when caring for patients. It was developed in 1980 by Zarit, Reever, and Bach-Peterson to evaluate caregiver stress (16). The scale, filled by the caregivers themselves or by the researcher, consists of 22 statements that determine the effect of caregiving on the individual's life. The scale has a Likert-type rating, ranging from 0 to 4 as "never,

rarely, sometimes, quite often, almost always", respectively. A minimum of 0 and a maximum of 88 points can be scored from the scale and a high score indicates a high level of distress. The items in the scale are generally related to the social and emotional domains (16). In studies, internal consistency coefficients of the scale were 0.87 and 0.94; and test-retest reliability was 0.71. The Turkish validity and reliability were confirmed and reported by İnci and Erdem (17).

The Health Promoting Lifestyle Profile II (HPLP II) was developed by Walker et al. (18) and was revised in 1996 (19). The scale consists of 52 items in six sub-dimensions. These are health responsibility (items 3, 9, 15, 21, 27, 33, 39, 45, 51.), physical activity (items 4, 10, 16, 22, 28, 34, 40, 46.), nutrition (items 2,8, 14, 20, 26, 32, 38, 44, 50.), spiritual development (items 6, 12, 18, 24, 30, 36, 42, 48, 52.), interpersonal relations (items 1, 7, 13, 19, 25, 31, 37, 43, 49.), and stress management (items 5, 11, 17, 23, 29, 35, 41, 47.). The scale is scored as "never (1)- regularly (4)". The lowest score for the whole scale is 52 and the highest score is 208. The higher the score, the higher the participants' healthy lifestyle behavior. The alpha reliability coefficient for the total scale is 0.922; alpha coefficients for the subscales range from 0.702 to 0.904. The Turkish validity and reliability were confirmed and reported by Bahar et al. (20).

Ethical Consideration

Ethical approval was obtained from University of Health Sciences Türkiye, Hamidiye Faculty of Health Sciences Scientific Research Ethics Committee (approval number: 21/720, date: 26.11.2021). The study was conducted in accordance with the Declaration of Helsinki. Participants were informed that the information they provided would be kept confidential if the results of the research were published. Those who agreed to participate in the study read and signed the written informed consent form.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), version 25, was used for statistical analysis (IBM Corp, Armonk, NY, USA). The histogram, Kurtosis and Skewness values and Kolmogorov-Smirnov test were used to evaluate whether the data showed normal distribution. If the Kurtosis and Skewness values were greater than twice the standard deviation and Kolmogorov-Smirnov test value was greater than 0.05 were considered in favor of normal distribution (21). Data were presented as mean and standard deviation (SD) or median and interquartile range (IQR) while frequencies were reported as numbers (n) and percentages (%). Pearson and Spearman correlation analyses were used to assess the perceived caregiver burden and health promoting life style scores. Statistical significance level was accepted as $p<0.05$.

Results

Initially 196 individuals were planned to be included in this study approached, as it was planned to include all caregivers in Bukhara who met the criteria. The evaluation forms of 41

Table 1. Sociodemographic characteristics of the caregivers

	Min-max	Mean ± SD
Age (year)	19-69	39.53±11.96
Height (cm)	145-190	166.21±8.358
Weight (kg)	10-98	67.73±12.64
	Median	IQR
Number of children	2	2-3
Duration of care (month)	100	48-120
	Number (n)	**Proportion (%)**
Gender		
Women	139	89.8
Men	16	10.2
Relationship with young person with disability		
Mother	118	75.9
Father	12	7.7
Grandmother	2	1.3
Grandfather	6	3.9
Stepmother	1	0.6
Brother	2	1.2
Elder sister	6	3.9
Aunt	6	3.9
Uncle	2	1.3
Education level		
Primary school	32	20.6
High school	51	32.9
University	42	27.1
Employment status		
Working	58	37.4
Not working	97	62.6
Marital status		
Single	13	9
Married	142	91
Place of residence		
Village	67	43.2
Town	31	20
City center	57	36.8
Income status		
≤100 USD/month	30	19.4
100-200 USD/month	113	72.9
≥200 USD/month	12	7.7
Smoking		
No	147	94.8
Yes	8	5.2
Regular exercise/sports habits		
No	104	67.1
Yes	51	32.9

Table 1. Continued

	Min-max	Mean ± SD
Disease history		
No known features	97	62.6
Hypertension	16	10.3
Cardiovascular system disease	4	4.5
Diabetes	9	5.8
Musculoskeletal disease	6	3.9
Other	20	12.3
Family history		
No known features	126	81.3
Hypertension	4	2.6
Cardiovascular system disease	5	3.2
Diabetes	9	5.8
Musculoskeletal disease	2	1.2
Other	9	5.6
Total	**155**	**100**

Min: Minimum, Max: Maximum, SD: Standard deviation, IQR: Interquartile range

participants were excluded due to certain deficiencies. Therefore, the final study population included 155 caregivers. The response rate of the participants in this study was 79.1%. The mean age of the caregivers was 39.5±1.97 years and the mean duration of period of care giving theyprovided was 100 (48-120) months. Other socio-demographic characteristics of caregivers are shown in Table 1. The mean age of the young people with disabilities was 11.8±6.9 (4-23) years and 80 (51.6%) were men. While 40.6% of the individuals with disabilities had only physical disability, 9.2% had other disabilities in addition to physical disability. The frequency of the type of disabilities is shown in Table 2.

Table 2. Types of disability of caretakers

Disability type	Number (n)	Proportion (%)
Physical	66	42.6
Mental	31	20
Hearing	9	5.8
Visual	10	6.5
Language and speech	11	7.1
Physical and mental	10	6.5
Physical and hearing	1	0.6
Physical and visual	1	0.6
Physical, language and speech	1	0.6
Mental, language and speech	5	3.2
Physical, language and speech	5	3.2
Physical, mental, language and speech	5	3.2
Total	**155**	**100**

The mean caregiver burden score was 40.45±10.5 and the healthy lifestyle behavior score was 135.7±19.7 (Table 3). There was no relationship between caregiver burden and healthy lifestyle behaviors (r=0.048, p=0.551). There was a weak positive correlation between caregiver burden and nutrition, one of the sub-dimensions of the HPLP II (r=0.038; p=0.016), and a very weak negative correlation between caregiver burden and the health responsibility score (r=-0.170, p=0.034) and the stress management score (r=-0.184, p=0.022) (Table 4).

Discussion

In this study with the aim of evaluating the relationship between perceived burden and healthy lifestyle behavior on family caregivers of young persons with disabilities in Bukhara, there was no correlation between the scores for caregiver burden and healthy lifestyle behavior scales in general. Only a positive correlation was found between caregiver burden and nutrition sub-dimension, whereas negative correlations were found between caregiver burden and health responsibility and stress management sub-dimensions of HPLP II.

On the contrary, Mochari-Greenberger and Mosca (9) correlated caregiver burden and healthy lifestyle behavior among family caregivers of patients with cardiovascular diseases, and found that caregiver burden was a barrier for healthy lifestyle among family members of patients. The findings of our study may be associated with the fact that Uzbekistan is reported to be the country with the happiest people in Central Asia (22). Even during the COVID-19 pandemic, which generally exacerbated the stress of family caregivers (10), scores-even they do not have cut points- for healthy lifestyle behavior were about high (more than half of the total value) while burden scores was low (less than half of the total value).

In the present study, a negative correlation was found between caregiver burden and health responsibility, stress management sub-dimensions of the healthy lifestyle behaviors scale. Dependencies on family members influence on caregiver stress level on behalf of management and care of the people with disabilities (23).

Table 3. Scores of the caregiver burden and healthy lifestyle behavior

	Min-max	Mean ± SD
The Zarit Burden Interview (possible score range 0-88)	14-72	40.45±10.52
HPLP II		
Health responsibility	9-35	23.52±5.03
Physical activity	8-29	16.69±5.25
Nutrition	9-36	22.44±4.95
Spiritual development	9-36	26.82±4.25
Interpersonal relations	9-35	24.98±4.21
Stress management	8-32	21.26±4.08
Total HPLP II (52-208)	52-203	135.71±19.73

Min: Minimum, Max: Maximum, HPLP II: Health Promoting Lifestyle Profile II, SD: Standard deviation

Table 4. The relationship between caregiver burden and healthy lifestyle behavior

		Health responsibility	Physical activity	Nutrition	Spiritual development	Inter-personal relations	Stress management	Total HPLP
Caregiver Burden	r	**-0.170**^{*b}	0.038^{b}	**0.193**^{*a}	-0.056^{b}	-0.139^{a}	**-0.184**^{*a}	**-0.048b**
	p	**0.034**	0.635	0.016	0.486	0.085	0.022	0.551
Health responsibility	r	1	0.366^{**b}	**0.462**^{**b}	**0.295**^{**b}	**0.460**^{**b}	**0.415**^{**b}	0.756^{**b}
	p		0.000	0.000	0.000	0.000	0.000	0.000
Physical activity	r	0.366^{**b}	1	0.426^{**b}	**0.240**^{**b}	**0.245**^{**b}	0.397^{**b}	**0.643**^{**b}
	p	0.000		0.000	0.000	0.000	0.000	0.000
Nutrition	r	0.462^{**b}	0.426^{**b}	1	**0.198**^{*b}	0.148^{a}	**0.423**^{**a}	0.620^{**b}
	p	0.000	0.000		0.013	0.067	0.000	0.000
Spiritual development	r	0.295^{**b}	0.240^{**b}	0.198^{*b}	1	0.328^{**b}	**0.429**^{**b}	**0.593**^{**b}
	p	0.000	0.000	0.013		0.000	0.000	0.000
Interpersonal relations	r	0.460^{**b}	0.245^{**b}	0.148^{a}	0.328^{**b}	1	0.403**	**0.576**^{**b}
	p	0.000	0.000	0.067	0.000		0.000	0.000
Stress management	r	0.415^{**b}	0.397^{**b}	0.423^{**a}	0.429^{**b}	0.403^{**a}	1	**0.708**^{**b}
	p	0.000	0.000	0.000	0.000	0.000		0.000
Total HLP	r	0.756^{**b}	0.643^{**b}	0.620^{**b}	0.593^{**b}	0.576^{**b}	0.708^{**b}	1
	p	0.000	0.000	0.000	0.000	0.000	0.000	

^{a}: Pearson correlation analysis, ^{b}: Spearman correlation analysis, HPLP: Health Promoting Lifestyle Profile, *: p<0.05 statistically significant

Bourke-Taylor et al. (24) reported that mothers of children with disabilities with less challenging behavior were psychologically healthier and this situation was inversely proportional to their stress. A study from Türkiye showed that as the motor and communication skills of the young person who was cared for decreased, the caregiver burden increased and the psychological state of the caregiver was negatively affected (25). Consequently, it was recommended that the necessary psychological support for caregivers should be provided (4, 26). Nadeem et al. (27) also showed that parents with children with disabilities reported higher levels of stress than parents with normal children. Although the degree of disability of the young people of our study was unknown and we could not analyze the data in detail, our results supported earlier findings. That is, stress management and health responsibilities scores were about high in those with low caregiver burden scores in our study. These results show that family caregivers of young people with disabilities might require additional support for stress management. We realize that the presence of a relationship between the sub-dimensions reinforces the importance of detailed examination.

The finding that the caregiver burden was inversely related to health responsibilities suggested the necessity of supportive programs. A previous study supports our view, stating that the educational program for mothers of children with disabilities improved the lifestyle goals of the mothers and had a positive effect on their health status (24).

The mean caregiver burden was 40.45±10.5 out of 88 in our study. Piran et al. (2) reported that there was a moderate caregiver burden in families with children with chronic diseases. In addition, considering the factors that increased the caregiver burden, they recommended that caregivers might be supported with holistic and family-centered programs.

In the study of Miodrag and Hodapp (13), it was reported that mothers with children with disabilities experienced more physical health problems and these problems might hinder the mothers' caregiving and parent-child relationship In our study, perception of healthy lifestyle behavior of caregivers was about high. However, it might be necessary to address the health status and problems, if any, of caregivers.

The fact that there was no relationship between the disease affecting the young person and family caregivers' history of chronic diseases might be attributed to the inadequacy of diagnosis due to the under-developed health system (28) in Bukhara, but evidence-based studies are essential to support this hypothesis. Lee et al. (1) reported that various chronic diseases were significantly higher in family caregivers of children with disabilities, and they were more likely to engage in risky health behavior, such as smoking. In our study, the number of smokers was 5.2%.

Bozkurt Zincir et al. (5) found positive correlations between caregiving time and caregiver burden; negative correlation between educational level of caregivers and perceived burden of caregivers. Caregiver gender, marital status, and burden also influenced the depression in caregivers. Therefore, we recommend assessing the health status of caregivers in detail in future studies.

The health of children with disabilities is related to the physical, psychological, and social health of their caregivers and so both health screening for, and further research into caregivers are

recommended (1, 10). Caregivers may engage in negative health behavior, such as emotional eating, in order to cope with the difficulty of caregiving (29). The finding in our study that a positive correlation between caregiver burden and nutrition sub-dimension of the healthy lifestyle behaviors scale highlights the importance of this situation by examining the relationship between caregiver burden and healthy lifestyle behavior. Nutrition was another sub-dimension of healthy lifestyle behavior which was directly proportional to the caregiver burden in our study. We think Uzbeks attach importance to nutrition in all conditions. Studies are needed on the cultural eating habits of this population. We have seen that studies in the literature are often focused on caretakers' nutrition rather than caregiver's nutrition (30).

Healthy lifestyle behaviors, such as undertaking physical activity, may become complicated in some families of children with disabilities (31). Denham et al. (32) noted that nearly all caregivers reported low levels of physical activity. Our results showed that the rate of physical activity of caregivers was low, but there was no correlation with caregiving burden. Michalsen et al. (33) stated that the encouragement for physical activity in individuals with disabilities should be provided with cooperation and interaction between the individual and the family, while the behavior of the families in this regard was also important. The state of Uzbekistan supports programs developed to strengthen the welfare and health of society, provide a healthy lifestyle, and increase the level of physical activity of the population (34).

In our study, the proportion of women caregivers was higher than for men. Similarly, Toledano-Toledano and Domínguez-Guedea (35) reported that 81.7% of caregivers were women. Bozkurt Zincir et al. (5) found that burden scores of women caregivers were higher than male caregivers. However, the traditional role of women as caregivers, especially in Central Asia, may be a reason for our findings (36). A study underlined the need to promote alternatives and opportunities so that care was shared and did not fall only on women (37). Gender-specific studies should be undertaken.

Falzarano et al. (38) found that greater levels of familism might exert a protective influence against adverse caregiving outcomes. The results of our study which was undertaken exclusively in the population of Bukhara, could not be generalized to the country. It should be remembered that there are no earlier studies in the same field from Uzbekistan. Therefore, it is hoped that this research will inspire further studies based on our study. Comparing the results of our study with the results of different countries might contribute a cultural difference dimension to the lifestyle behavior identified.

Study Limitations

The fact that we did not come across any study related to caregivers across the country was a strength of the study. However, our inability to make comparisons limited us. We recommend that the results be supported by controlled studies or studies comparing the results of individuals in different disability groups. The second limitation was a subjective one as we did not examine that caregivers might not be able to give clear answers for parameters such as burden, nutrition, and stress management, because they felt uncomfortable with strangers asking personal questions. Moreover, the disability status of the young people should be looked into in greater detail and the effect of this on caregiver burden may be evaluated more accurately. Another limitation was the inability to assess the health status of caregivers in detail. There is a need for advanced studies that examine the factors affecting caregiver burden and lifestyle habits of family caregivers of persons with disabilities.

Conclusion

The burden experienced by caregivers of young people with disabilities in Bukhara was positively correlated with nutrition, and negatively correlated with health responsibility and stress management sub-dimensions scores of a validated healthy lifestyle behavior questionnaire. In our study, perception of healthy lifestyle behavior of caregivers was about high. There were less likely to engage in risky health behavior, such as smoking on the other hand, the rate of physical activity of caregivers was low. Thus, there is a need for further evaluation of caregivers in this population which may aim to increase support for this role, as their health status may be adversely affected by the caregiver burden.

Ethics

Ethics Committee Approval: Ethical approval was obtained from University of Health Sciences Türkiye, Hamidiye Faculty of Health Sciences Scientific Research Ethics Committee (approval number: 21/720, date: 26.11.2021).

Informed Consent: After obtaining informed consent from the participants, they were asked to fill in a study-specific personal details form.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: H.Y., N.K., Concept: H.Y., N.K., Design: H.Y., N.K., Data Collection or Processing: H.Y., Analysis or Interpretation: H.Y., N.K., Literature Search: H.Y., N.K., Writing: H.Y., N.K.

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Original Article

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BDNF Levels and Cognitive Function in Patients with Type 2 Diabetes Treated with SGLT2 Inhibitors

SGLT2 İnhibitörleri ile Tedavi Edilen Tip 2 Diyabet Hastalarında BDNF Düzeyleri ve Kognitif İşlevler

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ABSTRACT

Objective: As cognitive impairment becoming more widely recognized as a complication of type 2 diabetes mellitus (T2DM), it is discussed in the literature that antihyperglycemic treatment may also improve cognitive functions. Clinical research on this topic is particularly limited regarding sodium-glucose-cotransporter-2 (SGLT2) inhibitors. Brain-derived neurotrophic factor (BDNF) is a protein essential for cognitive functions and glucose metabolism. The aim of our research was to examine cognitive performance and BDNF levels in users of SGLT2 inhibitors.

Methods: This cross-sectional study was conducted with 86 patients with T2DM, including 41 patients using metformin and 45 patients using SGLT2 inhibitors. Patients' cognitive performance was assessed with the Montreal cognitive assessment (MoCA) test, and their serum BDNF levels were measured using the ELISA method.

Results: There were no significant differences between SGLT2 inhibitor users and metformin users in MoCA total scores, as well as in the Visuospatial/Executive, Naming, Attention, Language, Abstraction, Memory, and Orientation subdomains. Although BDNF levels were relatively higher in the SGLT2 inhibitor group, the difference was not statistically significant. Significant correlations were observed between BDNF levels and the levels of microalbumin, microalbumin/creatinine, estimated glomerular

ÖZ

Amaç: Kognitif bozukluk tip 2 diyabetes mellitusun (T2DM) bir komplikasyonu olarak giderek daha fazla kabul görmekte ve literatürde antihiperglisemik tedavinin bilişsel işlevleri de iyileştirebileceği tartışılmaktadır. Bu konudaki özellikle sodyum-glukoz-kotransporter-2 (SGLT2) inhibitörleri ile ilgili klinik araştırmalar sınırlıdır. Beyin-kaynaklı nörotrofik faktör (BDNF), kognitif işlevler ve glukoz metabolizması için önemli bir proteindir. Araştırmamızın amacı SGLT2 inhibitörü kullananlarda kognitif performans ve BDNF düzeylerini incelemektir.

Yöntemler: Bu kesitsel çalışma, metformin kullanan 41 hasta ve SGLT2 inhibitörü kullanan 45 hasta olmak üzere toplam 86 T2DM hastası ile gerçekleştirilmiştir. Hastaların kognitif performansı Montreal bilişsel değerlendirme (MoCA) testi ile serum BDNF düzeyleri ELISA yöntemi kullanılarak ölçüldü.

Bulgular: SGLT2 inhibitörü kullananlar ile metformin kullananlar arasında MoCA toplam skorlarında ve Vizuospasyal/Yürütücü, İsimlendirme, Dikkat, Dil, Soyutlama, Hafıza ve Oryantasyon alt alanlarında anlamlı bir fark bulunmadı. SGLT2 inhibitörü grubunda BDNF seviyeleri nispeten daha yüksek olmasına rağmen, fark istatistiksel olarak anlamlı değildi. BDNF düzeyleri ile mikroalbümin, mikroalbümin/kreatinin, glomerüler filtrasyon hızı (eGFR), lenfosit düzeyleri arasında anlamlı korelasyonlar gözlenmiştir. Lineer regresyon analizine göre, eGFR düzeylerinin BDNF düzeylerini öngörmedeki etkisi anlamlıdır.

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ABSTRACT

filtration rate (eGFR), lymphocytes, According to linear regression analysis, effect of eGFR levels in predicting BDNF levels was significant.

Conclusion: Our results indicated that cognitive performance and BDNF levels were similar between users of metformin and SGLT2 inhibitors. Clinical research investigating the effect of SGLT2 inhibitors on cognitive functions in T2DM is limited. Future prospective follow-up studies with SGLT2 inhibitors may provide more comprehensive information.

Keywords: Sodium-glucose transporter 2 inhibitors, cognition, type 2 diabetes, brain-derived neurotrophic factor

ÖZ

Sonuç: Sonuçlarımız, bilişsel performans BDNF düzeylerinin metformin ve SGLT2 inhibitörleri kullanıcıları arasında benzer olduğunu göstermiştir. SGLT2 inhibitörlerinin T2DM'de bilişsel işlevler üzerindeki etkisini araştıran klinik araştırmalar sınırlıdır. SGLT2 inhibitörleri ile gelecekte yapılacak prospektif takip çalışmaları daha kapsamlı bilgi sağlayabilir.

Anahtar Kelimeler: Sodyum glukoz ko-trasnporter 2 inhibitörleri, kognisyon, tip 2 diyabet, beyin kaynaklı nörotrofik faktör

Introduction

Cognitive impairment is one of the complications of type 2 diabetes mellitus (T2DM) that has recently received increased attention. Compared to people without diabetes, individuals with T2DM have been reported to be at a greater risk of neurological dysfunctions (1) and have an approximately twofold higher risk of developing dementia (2). Noting a possible link between glucose regulation and cognitive function, higher HbA1c levels have been associated with poorer performance on cognitive tests among patients with T2DM (3). Insulin resistance and high blood glucose play a significant role in the development of cognitive impairment and dementia (1,4).

In the current literature, the relationship between T2DM and cognitive impairment has been emphasized, and it has been stated that antidiabetic treatment may have positive effects on cognitive functions (5). Several trials have assessed the effects of insulin therapy on cognitive function in patients with T2DM, but few have examined the impact of non-insulin antidiabetic agents on cognitive functions. Sodium-glucose cotransporter 2 (SGLT2) inhibitors are the latest class of oral antihyperglycemic agents approved for diabetes treatment. The primary mechanism of action of SGLT2 inhibitors is to limit glucose reabsorption by inhibiting SGLT2 receptors in the proximal tubules of the kidneys, thereby lowering glucose levels independently of insulin. SGLT2 inhibitors are fat-soluble and can cross the blood-brain barrier. SGLT1 and SGLT2 co-receptors are present in the human central nervous system and are crucial for maintaining glucose homeostasis (6). Although there are some preclinical studies reporting that SGLT2 inhibitors have a positive effect on cognition (7,8), clinical studies with SGLT2 inhibitors are limited.

Brain-derived neurotrophic factor (BDNF) is a protein essential for the growth, maintenance, and survival of neurons (9). It plays a key role in cognitive functions like learning and memory and is involved in synaptic plasticity. Also BDNF is important for glucose metabolism and has been associated with T2DM (10). Changes in BDNF levels have been linked to T2DM and neurodegenerative diseases like Alzheimer's disease (AD) (9-11). However, studies on the relationship between serum BDNF levels and glucose in T2DM have yielded varied results. No clinical studies investigating the effect of SGLT2 inhibitors on BDNF levels were found in the literature. The aim of our study was to investigate cognitive performance and BDNF levels in patients using metformin and SGLT2 inhibitors in the treatment of T2DM.

Methods

Patients

This cross-sectional study was conducted between October 2022 and September 2023 with 86 patients with T2DM who were admitted to the internal medicine outpatient clinic of Bezmialem Vakıf University Faculty of Medicine. The research groups consisted of 41 patients using metformin and 45 patients using SGLT2 inhibitors, either alone or in combination. Inclusion criteria were patients over 30 years of age, with at least a primary school education, and who had been taking metformin or SGLT2 inhibitors for at least three months. Exclusion criteria included patients with a history of severe psychiatric disorders (e.g., major depressive disorder) or neurological diseases (such as dementia, cerebrovascular disease, intracranial infection, demyelinating disease, brain tumor, head trauma), those receiving insulin therapy, individuals with visual and hearing impairments that would interfere with neuropsychological tests, patients with vitamin B12 and folic acid deficiencies, those who had experienced hypoglycemia or hyperglycemia attacks, and those with alcohol, substance, or drug addiction. Informed written consent was obtained from the participants, and the study received approval from the Bezmialem Vakıf University Clinical Ethics Committee (approval no: 17/6, date: 21.09.2022).

Cognitive Assessment

Cognition of the patients was evaluated with the Montreal cognitive assessment (MoCA) test. MoCA is a screening test that evaluates different areas such as attention, executive functions, language and orientation. The MoCA test developed by Nasreddine et al. (12) is evaluated over 30 points. According to a validation study in a Turkish population, a score of 21 and above is associated with normal cognition (12,13).

Laboratory

Blood samples were collected in the morning after 8 hours of fasting in gel biochemistry tubes, centrifuged at 3500xg for 10 minutes at room temperature, and stored at -80° C until the study's completion. BDNF levels were determined using the sandwich ELISA method with human BDNF ELISA kits. All steps of the analysis were performed according to the protocol provided by the kit manufacturer (sample protocol: https://www.elabscience.com/protocols-elisa-155.html).

Statistical Analysis

All analyses were performed using IBM SPSS 22.0 program. Normality was evaluated by Shapiro-Wilk test. Categorical variables are shown as number (percentage) and compared with chi-square test. SGLT2 inhibitor and metformin users are compared with t-test in normally distributed variables and Mann-Whitney U test in non-normally distributed variables. Linear regression analysis are used to determine the variables predicting BDNF levels. A p-value ≤0.05 is accepted statistically significant.

Results

A total of 86 patients with T2DM participated in our study, with a mean age of 51.95 years. The cohort consisted of 46 men (53.5%) and 40 women (46.5%). Of the T2DM patients, 41 (48%) were using metformin and 45 (52%) were using SGLT2 inhibitors. There were no significant differences between the groups in terms of age, education, and gender (p>0.05). The clinical and demographic data of patients using metformin and SGLT2 inhibitors are presented in Table 1. No significant differences were found between the clinical data of the two groups (p>0.05).

A family history of diabetes was present in 8 patients (19%) on metformin and 7 patients (15%) on SGLT2 inhibitors, with no significant difference between the groups (p=0.699). When analyzing comorbid conditions, hypertension was found in 21 patients in both groups. Hyperlipidemia was present in 19 metformin users and 29 SGLT2 inhibitor users. Thyroid disorders were observed in 7 (17%) metformin users and 6 (13%) SGLT2 inhibitor users. There were no significant differences between the groups in terms of hypertension, hyperlipidemia, and thyroid disorders (p>0.05).

The MoCA total and subdomain scores of the groups were compared and presented in Table 2. There were no significant differences between SGLT2 inhibitor users and metformin users in MoCA total scores, as well as in the Visuospatial/Executive, Naming, Attention, Language, Abstraction, Memory, and Orientation subdomains.

Serum BDNF levels of the groups were compared and shown in Figure 1. The BDNF level in the metformin group was 2895±1291 pg/mL, while it was 3056±1449 pg/mL in the SGLT2 inhibitor group. Although BDNF levels were relatively higher in the SGLT2 inhibitor group, the difference was not statistically significant (p=0.599).

Table 1. Clinical and demographic data of T2DM patients treated with metformin and SGLT2 inhibitors

Variables	Metformin (n=41)	SGLT2 inh (n=45)	p-value
Age	51.85±8.15	52±10	0.941
Gender (female)	22 (53.7%)	18 (40%)	0.205
Education (years)	7.68±3.64	8.30±3.75	0.445
Fasting glucose (mg/dL)	152.17±55.66	142.09±37.48	0.557
HbA1c (%)	7.23 ±1.66	7.47±1.31	0.161
Iron	74.81±34.25	97.04±44.90	0.07
LDL (mg/dL)	123.85±33.98	127.0155.03	0.674
HDL(mg/dL)	44.15±10.95	45.69±12.24	0.686
TSH (mIU/L)	3.65±7.67	1.93±1.04	0.194
Trigliyseride (mg/dL)	156.28±75.36	182.61±97.33	0.276
B12 (ng/L)	368.01±233.22	354.05±130.74	0.506
Folic acid (µg/L)	8.46±3.56	8.47±1.76	0.469
eGFR	89.82±16.48	93.82±14.15	0.447
Creatine	0.86±0.18	0.82±0.15	0.353
Neutrophile (10^3/µL)	4.59±1.24	4.442.831.15	0.462
Lymphocyte (10^3/µL)	2.65±0.71	2.83±0.87	0.583
NLR	1.91±1.02	1.68±0.46	0.603
Hemoglobin (g/dL)	13.79±1.58	14.64±1.43	0.015
Platelet (10^3/µL)	268.57±76.00	269.84±56.36	0.933
Microalbumine	51.03±103.51	23.11±29.24	0.299
Microalbumine/creatine	59.92±113.01	21.89±25.81	0.401

Categorical variables are expressed as n (%), numerical variables as mean ± Standard deviation, T2DM: type 2 diabetes mellitus, BDNF: Brain-derived neurotrophic factor, eGFR: Estimated glomerular filtration rate, LDL: Low-density lipoprotein cholesterol, HDL: High-density lipoprotein cholesterol, TSH: Thyroid stimulating hormone

Table 2. Comparison of cognitive performance of metformin and SGLT2 inh groups

Variables	Metformin (n=41)	SGLT2 inh (n=45)	p-value
MoCA total score	21.95±3.85	22.14±4.35	0.834
Visuospatial/executive	3.49±1.16	3.35±1.30	0.726
Naming	2.51±0.55	2.58±0.62	0.409
Attention	4.66±1.51	4.65±1.37	0.981
Language	1.49±0.87	1.67±1.12	0.303
Abstraction	1.37±0.69	1.37±0.75	0.863
Memory	2.63±1.37	2.53±1.59	0.790
Orientation	5.80±0.51	5.91±0.29	0.421

SGLT2: Sodium-glucose-cotransporter-2, MoCA: Montreal cognitive assessment

The relationship between BDNF levels and laboratory findings was examined. Significant correlations were found between BDNF and microalbumin (Spearman's r=0.318, p=0.012), BDNF and microalbumin/creatinine (Spearman's r=0.331, p=0.009), BDNF and estimated glomerular filtration rate (eGFR) (spearman r=0.257, p=0.025), BDNF and neutrophile (r=-0.239, p=0.038) as well as BDNF and lymphocyte (r=0.265, p=0.021) and these correlations are presented in Figure 2. The correlation of BDNF levels with lymphocyte and neutrophil levels was negative, while the correlation with microalbumin, microalbumin/creatinine and eGFR values was positive. BDNF-related these variables were added to the linear regression model and the results of the model are presented in Table 3. According to this, the effect of eGFR levels in predicting BDNF levels is significant.

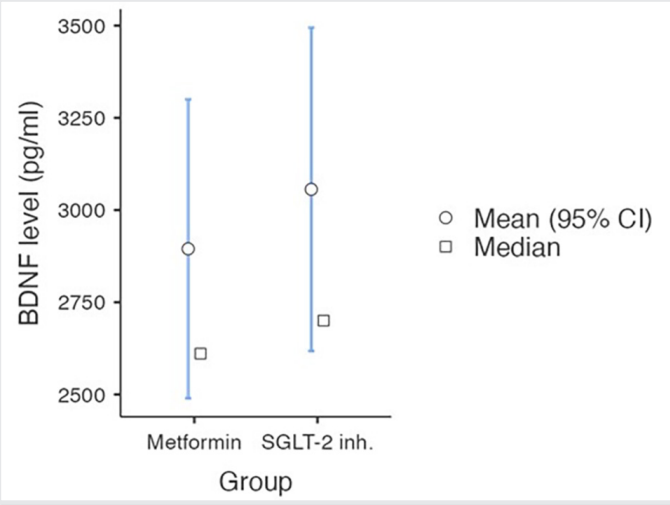

Figure 1. Comparison of BDNF levels between Metformin and SGLT2 inhibitors users

BDNF: Brain-derived neurotrophic factor, SGLT2: Sodium-glucose-cotransporter-2, CI: Confidence interval

Table 3. Linear regression analysis for BDNF level (pg/mL)

Predictor	B	SE	p-value
Lymphocyte	-7.23	72.07	0.920
Neutrophile	-44.81	64.45	0.490
eGFR	33.49	13.20	0.014
Microalbumine	1.04	2.58	0.689
Microalbumine/ Creatine	1.48	4.71	0.754

Adjusted R^2= 0.117, F= 2.56, p=0.038, BDNF: Brain-derived neurotrophic factor, eGFR: Estimated glomerular filtration rate

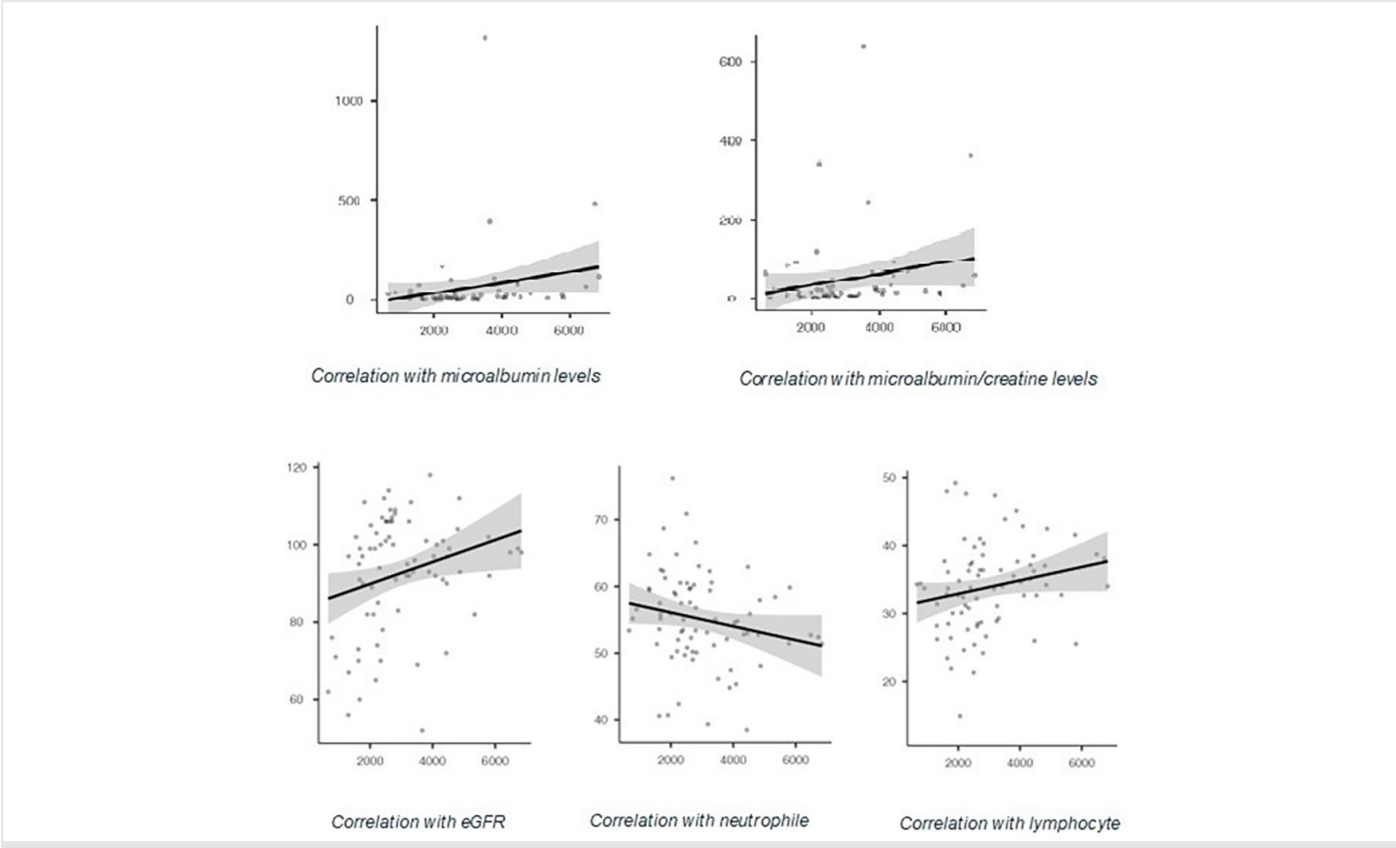

Figure 2. Correlation of microalbumin, microalbumin/creatinine, eGFR neutrophile and lymphocyte levels with BDNF

BDNF: Brain-derived neurotrophic factor, eGFR: Estimated glomerular filtration rate

Discussion

As the cognitive complications of T2DM receive increasing attention, the impact of antidiabetic therapies on cognitive performance has become a significant topic of interest. In this study, we compared the cognitive performance and BDNF levels of individuals using SGLT2 inhibitors with those using metformin. There were no significant differences between SGLT2 inhibitor users and metformin users in cognitive performance and BDNF levels.

A range of cognitive dysfunctions, from mild cognitive impairment (MCI) to dementia, are increasingly acknowledged as significant comorbidities and complications of diabetes. Therefore, recent guidelines recommend screening for cognitive impairment in diabetic patients (14). Recently, it has also been suggested that antidiabetic treatment may have positive effects on cognitive functions. There are a limited number of studies in the literature examining the effect of SGLT2 inhibitors on cognitive functions. When SGLT2 inhibitors were administered to a complex animal model of Alzheimer's and diabetes, a reduction in cortical thinning and neuronal loss was observed in diabetic mice, along with the preservation of insulin levels (15). In several animal studies with empagliflozin, inflammatory mediators and oxidative stress decreased, BDNF levels increased, cognitive functions improved and neuroprotective effects were found (16-18). At the same time, an animal study comparing the effects of dapagliflozin and vildagliptin found that dapagliflozin was more effective in preserving synaptic plasticity, while preventing cognitive functions equally, probably through the same mechanisms (19). These preclinical findings suggest that SGLT2 inhibitors may have neuroprotective effects in diabetic patients.

Clinical research investigating the effect of SGLT2 inhibitors on cognitive functions in T2DM is limited. Two studies in the literature comparing the cognitive effects of SGLT2 inhibitors versus incretins found no statistically significant difference (20,21). A study by Mone et al. (8) published in 2022 investigating the effect of empagliflozin compared its effect against metformin and showed for the first time that SGLT2 inhibitors had beneficial effects on cognitive impairment in patients with diabetes and heart failure. In a study, SGLT2 inhibitors demonstrated significant preventive benefits against newly diagnosed dementia ($p<0.001$) and Parkinson's disease ($p=0.034$) compared to DPP-4 inhibitors (7). In our study, we did not find any difference in cognitive performance between using SGLT2 inhibitors versus metformin. Notably, DPP-4 inhibitors and Glucagon-like peptide-1 receptor agonists GLP1RA drugs have been reported to benefit cognitive functions and improve performance in various cognitive domains (22-25), but clinical studies with SGLT2 inhibitors are limited.

As in many clinical trials evaluating the effects of antihyperglycemic agents, we included metformin users as a control group against SGLT2 inhibitors. But results evaluating the effect of metformin on cognitive performance are conflicting. While some studies reported that metformin improved cognitive performance (8,26-30), others found no significant association between metformin use and cognitive function. Additionally, some studies indicated that metformin use might increase the risk of MCI or result in decreased cognitive test score.

The number of studies exploring biomarkers that may help understand brain changes in T2DM patients is rapidly growing. Researchers are examining biomarkers linked to the primary pathologies of dementia, including AD and vascular disease, as well as various biomarkers related to brain tissue damage, blood flow, and metabolism, to assess their relationship with cognitive status in patients with T2DM (31). Brain regions crucial for memory, like the hippocampus, have high expression of insulin receptors. Consequently, disrupted insulin levels or signaling in the brain could result in neuronal and synaptic losses, leading to cognitive impairments. BDNF is a protein known to be associated with cognition, thought to be responsible for neuronal degeneration and plasticity, and is also important for glucose metabolism. Animal studies have identified BDNF as one of the molecular factors linking T2DM to AD's neuropathology (32). Considering that BDNF expression in the brain is high in hippocampal neurons, it has been suggested that BDNF levels might be an early biomarker of cognitive impairments in diabetes (9). Our hypothesis was that BDNF could be an important biomarker in detecting the potential neuroprotective effects of SGLT2 inhibitors. There are no studies in the literature that evaluate the changes in cognitive functions and BDNF levels in patients with T2DM treated with SGLT2 inhibitors, nor any that investigate their relationship. In our study, we did not find any differences in cognitive performance and BDNF levels between the groups. Consequently, we currently lack data to support or refute our hypothesis. Prospective future studies that monitor changes in cognitive performance and BDNF levels may provide more definitive evidence on this matter.

Study Limitation

The cross-sectional design is a limitation of this study. Future studies with a prospective design may provide clearer data on the changes in congitive performance and BDNF levels in the follow-up of patients. Patients with T2DM can use non-insulin antihyperglycemic agents in many different combinations. The fact that we were not able to standardize the other drugs used by patients using SGLT2 inhibitors in our study was also a limitation of this study.

Conclusion

The impact of antidiabetic medications on cognitive complications in diabetic patients remains an important area of research. Studies evaluating the effects of SGLT2 inhibitors, one of the newer and frequently used classes of oral antidiabetics, on cognitive complications are limited. In this study, we compared the effects of SGLT2 inhibitors on cognitive performance with those of metformin. Our results indicated that cognitive performance was similar between users of metformin and SGLT2 inhibitors. We also compared BDNF levels, hypothesizing that BDNF, an important protein for cognitive functions and glucose

metabolism, might serve as a biomarker for evaluating cognitive functions. BDNF levels were similar between the groups. Future prospective follow-up studies with SGLT2 inhibitors may provide more comprehensive information on this topic.

Ethics

Ethics Committee Approval: The study received approval from the Bezmialem Vakıf University Clinical Ethics Committee (approval no: 17/6, date: 21.09.2022).

Informed Consent: Informed written consent was obtained from the participants.

Footnotes

Authorship Contributions

Concept: B.S.Ş., A.Ş, Design: B.S.Ş., Data Collection or Processing: B.S.Ş., Ş.D., Analysis or Interpretation: B.S.Ş., A.Ş., Literature Search: B.S.Ş., E.Ç., Writing: B.S.Ş., E.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Breastfeeding During Pregnancy: Opinions and Behaviors of Mothers

Gebelikte Emzirme: Annelerin Düşünce ve Davranışları

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ABSTRACT

Objective: This study was performed to determine opinions and behaviors of mothers about maintenance of breastfeeding during pregnancy.

Methods: The research was descriptive and the data were collected between March 2019 and December 2019. Data were collected by using a questionnaire at face-to-face interviews. A total of 358 mothers just giving birth at an obstetrics hospital in İstanbul and fulfilling the inclusion criteria were included in the study. Obtained data were analyzed with descriptive statistics and chi-square test.

Results: Of all the mothers included in the study, 74.6% reported that women becoming pregnant during lactation should not breastfeed their babies. Thirty-two percent of the mothers with a history of multiparity had the experience of becoming pregnant during lactation and 49% of them reported that they immediately stopped breastfeeding and the rest maintained breastfeeding their babies for a short time. Twenty-one-point-four percent of the mothers said that they weaned their previous baby because they were pregnant, wanted to get pregnant again, or thought they were pregnant.

Conclusion: The mothers believed that women becoming pregnant during lactation should not continue to breastfeed their babies. Besides, the mothers having the experience of pregnancy during lactation were found to stop breastfeeding immediately or short time after they learned about their pregnancy.

Keywords: Mothers' health, infants' health, breastfeeding, pregnancy, tandem breastfeeding

ÖZ

Amaç: Bu araştırma, annelerin gebelikte emzirmenin sürdürülmesine ilişkin görüş ve davranışlarını belirlemek amacıyla yapılmıştır.

Yöntemler: Araştırma tanımlayıcı tipte olup, veriler Mart 2019-Aralık 2019 tarihleri arasında toplanmıştır. Veriler, yüz yüze görüşme yöntemiyle anket kullanılarak toplanmıştır. İstanbul'da bir doğum hastanesinde yeni doğum yapan ve dahil edilme kriterlerini karşılayan toplam 358 anne çalışmaya dahil edilmiştir. Elde edilen veriler tanımlayıcı istatistikler ve ki-kare testi ile analiz edilmiştir.

Bulgular: Araştırmaya katılan annelerin %74,6'sı emzirme döneminde gebe kalan kadınların bebeklerini emzirmemeleri gerektiğini bildirmiştir. Multiparite öyküsü olan annelerin %32'si emzirme döneminde gebe kalma deneyimi yaşamış, %49'u emzirmeyi hemen bıraktığını, geri kalanlar bebeklerini kısa bir süre daha emzirmeye devam ettiklerini bildirmiştir. Annelerin %21,4'ü önceki bebeklerini hamile kaldıkları için, tekrar hamile kalmak istedikleri için veya hamile olduklarını düşündükleri için sütten kestiklerini belirtmişlerdir.

Sonuç: Anneler, emzirme döneminde hamile kalan kadınların bebeklerini emzirmeye devam etmemeleri gerektiğine inanıyordu. Ayrıca emzirme döneminde gebelik deneyimi yaşayan annelerin gebeliklerini öğrendikten hemen sonra veya kısa bir süre sonra emzirmeyi bıraktıkları saptanmıştır.

Anahtar Kelimeler: Anne sağlığı, bebek sağlığı, emzirme, gebelik, tandem emzirme

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Introduction

It is known that breastfeeding should be started in the first half an hour after birth and that babies should be offered exclusive breastfeeding in the first six months of their lives and breastfed in addition to appropriate complimentary food until two years of age or longer (1,2). However, according to a report by the World Health Organization and the UNICEF (2018), only 41% of the babies younger than 6 months are exclusively breastfed and only 45% of the babies are breastfed till the age of two years in the world (3). In Türkiye, only 41% of the babies are exclusively breastfed for the first five months of their life and only 34% of the babies are breastfed for two years (4). The above mentioned evidence shows that babies both in Türkiye and the rest of the world are weaned and fed with additional food earlier than expected (3,4).

Many factors affect initiation of feeding with additional food and/or weaning babies (5-11). One of the factors causing early weaning is that 1.3-55.7% of the mothers become pregnant during their lactation period (7,8,12). This causes mothers to wean their babies earlier and breastfeed them for shorter than two years. There have not been any studies on the opinions of mothers about how to behave regarding maintenance of breastfeeding if they become pregnant during the lactation period. Knowing opinions and tendencies of mothers about this issue can guide prenatal education for pregnant women. Mothers who become pregnant while breastfeeding want to have information about whether they will continue to breastfeed during pregnancy when they expect a new baby. The results of this study contain important information for educating the mother and other family members in such a situation. The present study was performed to determine opinions and behaviors of the mothers about maintenance of breastfeeding if they became pregnant during lactation. To achieve this aim, answers to the following questions were sought:

1.What do the mothers think about maintenance of breastfeeding if they become pregnant during lactation?

2.How did the mothers who became pregnant during lactation in the past behave regarding maintenance of breastfeeding?

Methods

Study Population and Sample

This descriptive study was carried out in a state obstetrics and gynecology hospital in Istanbul. The study population comprised 4441 women giving birth in this hospital in 2017. The sample calculation method, whose universe is known, was used to determine the number of samples (n= N. t^2. pq/ y^2. (N-1) + t^2. Pq). The sample size was calculated at minimum 354. Inclusion criteria were the age of 18 years or older, understanding and speaking Turkish, experiencing pregnancy ending in the 37th-41st gestational weeks, giving birth to a healthy, living singleton, staying with the baby in the same room since giving birth, not having a mental disease or a medical condition likely to prevent breastfeeding and voluntarily accepting to participate in the study. The sample included 358 mothers fulfilling the inclusion criteria.

Data Collection

Data were collected at face to face interviews with a questionnaire created by the researchers. Until the calculated sample size was obtained, the researchers visited the hospital and met the women giving birth every day between 1 March 2019 and 27 December 2019. Informed consent was obtained from the mothers meeting the inclusion criteria and accepting to participate in the study.

Statistical Analysis

Data analysis was performed by using Statistical Package for Social Sciences 20.0 for Windows. Descriptive statistics like frequencies and percentages and chi-square test were utilized for the analysis. Obtained results were evaluated at the confidence interval of 95% and the significance level of $p<0.05$.

Ethical Approval

The ethical review of study was approved by the University of Health Sciences Türkiye, Istanbul Training and Research Hospital Ethic Committees (number: E.4117, date: 25.12.2018) and from the relevant health institution (number: 16867222-604.01.01, date: 12.03.2019).

Results

The mean age of the mothers was 29.39±5.79 years [minumum (min):18 years; maximum (max):45 years]. Of all the mothers, 30.2% were primary school graduates, 81.8% were housewives and 69.8% had a nuclear family. Concerning obstetric features, the number of pregnancies ranged from one to eight, 25.4% of the mothers were primiparous (min:1 birth and max:6 births) and 34.6% had two live children. Seventy-eight-point-eight percent of the mothers reported that babies should be exclusively breastfed for the first six months of their lives ($\bar{x}$=177.68±54.37 days) and 69.6% of the mothers reported that babies should be breastfed until they were two years old (Table 1).

When 266 mothers with more than one living baby were asked about the age of their prior baby, one of every five mothers (17.7%) reported that their prior baby was younger than 24 months (min=11 months; max=24 months). Forty-one-point-four percent of the mothers exclusively breastfed their prior baby for six months without giving water or additional food and 32.3% of the mothers breastfed their baby for over 24 months (max=60 months). When the mothers were asked about the reasons for weaning their prior baby, the mothers reported that their babies were old enough for weaning (28.2%) and gave up mother's milk (16.9%). They also said that they became pregnant/suspected of becoming pregnant or wanted to become pregnant (21.4%) and mother's milk was insufficient (15%) (Table 2).

Regarding whether to maintain or cease breastfeeding during pregnancy, 74.6% of the mothers said that women becoming pregnant during lactation had to wean their baby (Table 3). Ninety-one mothers (25.4%) reported that breastfeeding could be maintained for 1-8 months during pregnancy, until health

Table 1. Sociodemographic, obstetric and breastfeeding features of the mothers (n=358)

Features of the mothers	n	%
Age (years)[a]		
18-25	102	28.5
26-35	193	53.9
36-45	63	17.6
Education		
Literate/illiterate	65	18.2
Primary school	108	30.2
Secondary school	77	21.5
High school	66	18.4
University	42	11.7
The place the longest time spent in till the age of 15 years		
Village	82	22.9
Town	39	10.9
City	212	59.2
Foreign country	25	7.0
Paid employment		
Yes	65	18.2
No	293	81.8
Type of family		
Nuclear family	250	69.8
Extended family	108	30.2
Number of pregnancies		
1	77	21.5
2	95	26.5
3	89	24.9
4-8	97	27.1
Number of childbirths		
1	91	25.4
2	126	35.2
3	84	23.5
4-6	57	15.9
Number of live children		
1	92	25.7
2	124	34.6
3	84	23.5
4-6	58	16.2
How long a baby must be exclusively breastfed (months)[b]		
0-5	53	14.8
6	282	78.8
7-24	23	6.4
How long a baby must be breastfed (months)[c]		
6	3	0.8
7-23	72	20.1
24	249	69.6
25-48	34	9.5

[a]The mean age of the mothers was 29.39±5.79 years. [b]The mothers reported that babies should be exclusively breastfed for a mean of 177.68±54.37 days. [c]The mothers reported that babies should be breastfed for a mean of 23.01±5.89 months

Table 2. Age and breastfeeding features of prior children (n=266)

Age and breastfeeding of prior children	n	%
Age (months)[a]		
≤24	47	17.7
25-36	56	21.1
37-48	36	13.5
49-60	39	14.7
61-216	88	33.0
Duration of exclusive breastfeeding (months)[b]		
0	37	13.9
1	17	6.4
2	16	6.0
3	20	7.5
4	23	8.6
5	26	9.8
6	110	41.4
7-8	12	4.5
9-11	5	1.9
Duration of breastfeeding (months)[c]		
0-6	50	18.8
7-12	35	13.2
13-18	74	27.8
19-23	21	7.9
24-60	86	32.3
Reasons for weaning[d]		
The baby was old enough to be weaned	75	28.2
I became pregnant, wanted to become pregnant and thought I was pregnant[e]	57	21.4
The baby stopped breastfeeding	45	16.9
The mother's milk was insufficient	40	15.0
The mother had a health problem (illness, receiving medications, weakness and fatigue)	19	7.1
A health professional recommended weaning	14	5.2
The baby had a health problem (illness and surgery etc.)	14	5.2
The baby did not eat and gain weight	11	4.1
The mother returned to work	9	3.3
The mother had a problem with her breasts/nipples	9	3.3
The mother wanted to stop breastfeeding	8	3.0
The baby was given formula at hospital	6	2.2
The baby wanted to be breastfed frequently	6	2.2
Social/religious factors	5	1.8
The mother thought her milk changed and became useless	3	1.1
The mother gave birth	1	0.3

Findings presented in this table reflect responses of the mothers with more than one live child.
[a]The mean age of the prior baby was $\bar{x}$=57.72±38.39 months. [b]The mean duration of exclusive breastfeeding for the prior baby was 127.43±73.47 days. [c]The mean duration of breastfeeding for the prior baby was 17.14±9.23 months. [d]Some of the mothers mentioned more than one reason and the percentages are based on the total number of the responses. [e]The answers "I became pregnant" (n=53), "I wanted to become pregnant" (n=2) and "I thought I was pregnant" (n=2) were included

Table 3. Opinions of the mothers about maintenance of breastfeeding during pregnancy (n=358)

Opinions of the mothers	n	%
Do you think a woman becoming pregnant during lactation can continue to breastfeed their babies?		
No	267	74.6
Yes	91	25.4
How long do you think breastfeeding can be maintained during pregnancy? (n=91)		
For some time after learning about pregnancy (minimum: 1 month; maximum: 8 months)	33	36.3
Until the baby is six months old	16	17.5
Until delivery of the baby	15	16.5
Until a health professional recommends stopping breastfeeding	9	9.9
Until the baby stops breastfeeding	7	7.7
Other[a]	3	3.3
Until the baby is 4-5, 12 or 24 months[b]	8	8.8
Do you think maintenance of breastfeeding harms the mother, fetus and the baby breastfed?		
No	62	17.3
Yes	296	82.6
Does breastfeeding during pregnancy harm the mother? (n=296)[‡]		
No	216	73.0
Yes	80	27.0
What are the harms of breastfeeding during pregnancy in the mother? (n=80)		
The mother's health is affected	63	78.8
Abortion/preterm delivery occurs	11	13.8
Religious reasons	1	1.3
Other[§]	5	6.3
Does breastfeeding during pregnancy harm the fetus? (n=296)[‡]		
No	131	44.3
Yes	165	55.7
What are the harms of breastfeeding during pregnancy in the fetus? (n=165)		
The fetus' health is affected	137	83.0
Abortion/preterm delivery	13	7.9
Religious reasons	7	4.2
Other	8	4.8
Does breastfeeding during pregnancy harm the baby breastfed? (n=296)[‡]		
No	49	16.6
Yes	247	83.4
What are the harms of breastfeeding during pregnancy in the baby breastfed? (n=247)		
The baby's health s affected	134	54.3
The content of the mother's milk is affected. The mother's milk is not beneficial	91	36.8
Religious reasons	15	6.1
Other[c]	7	2.8
Do you think a mother can breastfeed both their newborn and the prior baby?		
No	282	78.8
Yes	76	21.2
If you become pregnant during lactation, do you want to continue breastfeeding your baby?		
Yes	123	34.4
No	235	65.6
Do you continue to breastfeed your baby you have breastfed during your pregnancy together with your newborn? (n=90)[d]		
Yes	5	5.6
No	85	94.4

[a]The answers "Until the mother feel uncomfortable" (n=1), "as long as the mother can hold her baby" (n=1) and "until the content of mother's milk changes" (n=1) were included. [b]The mothers reporting that breastfeeding can be breastfed until the baby is 4-5 months old (n=3), 12 months old (n=3) and 24 months old (n=2) were included in this group. [c]The answers "I don't know why but I think it will give harm" (n=1), "I've heard from the gynecologist that the baby can have diarrhea" (n=1), "If the baby is not weaned earlier, he/she mu become jealous of the newborn" (n=1), "The medications used by the mother can be transmitted to the baby through the mother's milk and can harm it" (n=1), "Hormonal changes give harm" (n=1), "The doctor said the baby will become ill" (n=1) and "There won't be sufficient milk left for the newborn" (n=1) were included in this group. [d]The answers given to this question by 90 mothers recently becoming pregnant during lactation were included

professionals told the woman to stop it, until the baby quited it or reaches a certain age, until the woman gives birth or after giving birth. Concerning the reasons for maintenance of breastfeeding, the mothers noted that the baby was too young for weaning, had the right to receive mother's milk and needed to grow up and develop.

Two-hundred and six mothers (82.6%), reported that maintenance of breastfeeding during pregnancy would definitely harm the mother, the fetus or the baby breastfed. Ninety-one mothers said (36.8%) that the amount, composition, content and taste of mother's milk would change, and that mother's milk would be less beneficial for the baby, would even be harmful or poison the baby. Seventy-eight-point-eight percent of the mothers reported that tandem breastfeeding was not right (breastfeeding the newborn and the prior baby together), which would cause mother's milk to be insufficient, would be difficult for both the mother and her babies, and that the newborn would need mother's milk more. They also said that the baby already weaned during pregnancy would not want mother's milk and that the difference between the ages of the babies would create problems. Seventy-six mothers (21.2%) in favor of tandem breastfeeding reported that if mother's milk was sufficient, the babies could be breastfed together since they were siblings and so that they should not become jealous of each other.

More than half of the mothers (65.6%) reported that they would not continue to breastfeed their prior baby if they became pregnant since their milk would poison their baby, become harmful to it, change and become useless. They also said that maintenance of breastfeeding would affect their pregnancy and the fetal growth and was not right in terms of their religious beliefs (Table 3). Only education had a significant effect on the opinions of the mothers about whether a woman becoming pregnant during lactation should continue breastfeeding ($p<0.00$ $\chi^2=20,719$). A significantly higher rate of the university graduates noted that women becoming pregnant during lactation (64.1%) should maintain breastfeeding compared to the literate/illiterate mothers (35.9%) ($p<0.00$ $\chi^2=15.893$), primary school graduates (56.1%) ($p<0.01$ $\chi^2=11,470$) and secondary school graduates (47.9%) ($p<0.02$ $\chi^2=9,930$). Similarly, education significantly affected the opinions of the mothers about whether they themselves would maintain breastfeeding if they became pregnant ($p<0.00$ $\chi^2=20,975$). A significantly higher rate of the university graduates (61.8%) were willing to breastfeed their prior baby if they became pregnant compared to the literate/illiterate mothers (38.2%) ($p<0.01$ $\chi^2=10,592$), primary school graduates (48.8%) ($p<0.00$ $\chi^2=15,089$) and secondary school graduates (41.7%) ($p<0.01$ $\chi^2=11,997$).

Thirty-two percent of the mothers having a history of multiparity (n=90) reported that they became pregnant while breastfeeding their current or prior babies. They noted that their babies were aged 2-24 months when they learned about their pregnancy. The highest rate of them (45.6%) became pregnant when their babies were aged 13-24 months (min= 2 max= 24). More than half of the mothers becoming pregnant during lactation (53.3%) said that they learned about their pregnancy in the sixth gestational week or later (min=5; max=35) (Table 4).

Sixty mothers (66.7%) did not observe any changes in their babies until they learned about their pregnancy. However, 30 mothers (33.3%) said that their babies did not want mother's milk, lost weight and became ill (vomiting, diarrhea and high temperature). After learning about their pregnancy, 64 mothers (71.1%) did not recognize any changes in their milk or babies, but 26 mothers said (28.9%) that their milk changed in terms of its color, quantity and viscosity and their baby became ill (vomiting and diarrhea) (Table 4).

Table 4. The mothers' behaviors concerning maintenance of breastfeeding during pregnancy (n=281)

The mothers' behaviors	n	%
Have you ever become pregnant during lactation?		
Yes	90	32.0
No	191	**68.0**
How old was the baby you breastfed when you learned about your pregnancy? (n=90)		
≤6 months	17	18.9
7-12 months	32	35.5
13-24 months	41	**45.6**
Median: 12		
What was the gestational week when you learned about your pregnancy? (n=90)		
≤6 weeks	48	**53.3**
≥7 weeks[a]	42	46.7
Median:6		
Did you notice any changes in the baby you breastfed before you learned about your pregnancy? (n=90)		
Yes	30	33.3
No	60	**66.7**

Table 4. Continued

The mothers' behaviors	n	%
Did you notice any changes in your milk before you learned about your pregnancy? (n=90)		
Yes[b]	16	17.8
No	74	82.2
Did you notice any changes in your milk or baby after you learned about your pregnancy? (n=90)		
Yes[c]	26	28.9
No	64	**71.1**
Did you ask someone about maintenance of breastfeeding during your pregnancy? (n=90)		
Yes	62	**68.9**
No	28	31.1
Who did you ask about maintenance of breastfeeding during your pregnancy? (n=62)		
Nurse	5	8.1
Midwife	2	3.2
Doctor	26	41.9
Relative[d]	29	**46.8**
What did the health professional/relative recommend about maintenance of breastfeeding during your pregnancy? (n=62)		
He/she recommended stopping breastfeeding.	48	77.4
He/she recommended continuing to breastfeed.	14	22.6
Who recommended stopping breastfeeding? (n=48)		
Health professional	24	50.0
Relative	24	50.0
Did you continue to breastfeed your baby after you learned about your pregnancy? (n=90)		
Yes	46	**51.1**
No	44	48.9
At what gestational week did you stopped breastfeeding your baby? (n=46)		
7-14	25	**54.3**
15-22	16	34.8
23-36	5	10.9
How old was your baby when you weaned it? (n=90)		
<6 months	9	10.0
6 months	6	6.7
7-12 months	25	27.8
13-23 months	47	**52.2**
24≥ months	3	3.3
How did people react when you continued to breastfeed your baby during your pregnancy? (n=90)		
Positive	16	17.8
Indecisive/unresponsive	20	22.2
Negative	54	**60.0**
How did the health professionals react when you continued to breastfeed your baby during your pregnancy? (n=90)		
Positive	19	21.1
Indecisive/unresponsive[e]	47	**52.2**
Negative	24	26.7

Findings presented in this table reflect responses of the mothers giving birth at least once. [a]Thirty-nine mothers learned their pregnancy in the 7th-12th gestational week, one mother in the 16th gestational week, one mother in the 22nd week and one mother in the 35th gestational week. [b]The mothers reported that the mother's milk was yellowish, dark in color or water and changed in its taste (n=16). [c]The mothers reported that the mother's milk changed in color, amount and viscosity and that the bay become ill (vomiting, diarrhea) (n=26). [d]The first-degree relatives like mother, mother-in-law, sister-in-law (n=29) were included in this group. [e]The answers "I didn't ask about maintenance of breastfeeding" (n=7), "indecisive/unresponsive" (n=38), "The gynecologist told me to breastfeed my baby, but the family physician told me not to breastfeed" (n=1) and "The nurse said that it is not harmful but that I mustn't breastfeed" (n=1) were included

Out of 90 mothers (32%) becoming pregnant during lactation, 62 asked (68.9%) someone for advice about maintenance of breastfeeding and 77.4% of them said that the people they requested advice from (health professionals and relatives) told them not to breastfeed or stop breastfeeding after the third month of their pregnancy. Some mothers said that they asked health professionals for advice and that while half of the health professional advised them to quit breastfeeding, the others recommended maintaining breastfeeding (Table 4).

Of 90 mothers (32%) becoming pregnant during lactation, 46 reported (51.1%) continuing to breastfeed after learning about their pregnancy and more than half of them (54.3%) noted that they maintained breastfeeding until the 7th-14th weeks of their pregnancy (min= 7; max= 36). A Syrian, primary school graduate mother breastfeeding until the 36th week of her pregnancy noted that she asked her gynecologist about breastfeeding during pregnancy since she thought it would not be harmful and her baby would need it. She added that she maintained breastfeeding her baby during her pregnancy since her doctor recommended continuing to breastfeed as long as she took vitamins. Sixty percent of the mothers reported people around them did not approve of their breastfeeding throughout their pregnancy. Fifty-two-point-two percent of the mothers said that health professionals did not display a negative or a positive reaction to their breastfeeding (Table 4). Only family type significantly affected the behavior of maintenance of breastfeeding in the mothers becoming pregnant during lactation ($p<0.033$ $\chi^2=4,535$). A higher rate of the mothers having a nuclear family maintained breastfeeding after becoming pregnant than those having an extended family.

Discussion

The present study was performed to seek an answer to the question "What are the opinions and behaviors of the mothers about maintenance of breastfeeding during lactation". The study included 358 early postpartum mothers. It was shown that three of every four survey takers believed that mothers should stop breastfeeding after becoming pregnant. Besides, most of the mothers becoming pregnant in the past were found to cease breastfeeding short time after they learned about their pregnancy. However, most of the mothers knew how long a baby should receive exclusive breastfeeding or should be breastfed. It can be suggested that cultural and environmental factors rather than knowledge of the mothers are effective in their opinions and behaviors regarding continuation of breastfeeding during lactation.

About one third of the mothers were either high school graduates or had a higher level of education. More than half of the mothers spent their childhood in a big city like Istanbul, where they could easily access education, healthcare and socia-cultural activities. One of every four mothers became a mother for the first time. Eight of every ten mothers were aware that babies should be exclusively breastfed for six months and seven of every ten mothers knew that they should breastfeed their babies for at least 24 months. Most of the mothers having above mentioned features said that women becoming pregnant during lactation should discontinue breastfeeding and that they would stop breastfeeding their prior baby if became pregnant. This finding was important since it showed that knowledge of the mothers did not have an influence on their opinions. The main factors affecting the mothers' opinions was social and cultural beliefs. There have not been any studies on opinions of women about maintenance of breastfeeding during pregnancy. Consistent with the finding of the present study, several studies on the reasons for weaning the baby showed that pregnancy was a cause of weaning (7,8,12,13).

Opinions of Mothers about Maintenance of Breastfeeding during Pregnancy

The mothers in favor of maintenance of breastfeeding during pregnancy differed in their opinions about the duration of breastfeeding. A group of the mothers maintained that breastfeeding could be continued for 1-8 months depending on the fetus, mother/baby health status and pregnancy. Although they had accurate knowledge of infant feeding, they held the view that breastfeeding maintenance did not rely on the need for exclusive breastfeeding and duration of breastfeeding but relied on the fetus, infant, mother or the course of pregnancy. This view might have resulted from their cultural background and social environment. Health status of the pregnant mothers, their fetuses and babies, tiredness and workload of the pregnant mothers, social pressure, any harm to the mothers, their babies and their fetuses according to the mothers' opinions and other personal reasons seem to be the frequent factors affecting their views. However, it was shown in the literature that breastfeeding maintenance did not have a negative effect on mothers continuing to breastfeed, their babies, fetal growth/development or pregnancy (14-16). Besides, the finding that the mothers learning about their pregnancy in the 35th gestational week and continuing to breastfeed until then and their babies were healthy shows that breastfeeding can be maintained during pregnancy. The mothers' worries can be due to their insufficient information about the issue.

The other group of the mothers noted that the age of the baby was important for breastfeeding maintenance by taking account of young age of the baby for weaning, its right to receive mother's milk and its growth and development. Most of the mothers were aware of the accurate information about duration of breastfeeding. In addition, the university graduates mainly supported the idea that breastfeeding should be maintained if they or other women became pregnant during lactation. This finding showed that knowledge of the mothers had an influence on their opinions and behaviors as their education level increased. Consistent with this finding, several studies have revealed that mothers' education level affects their opinions about their health, breastfeeding behavior and attitudes and their children's health (17-19).

In the present study, eight of every ten mothers had the opinion that maintenance of breastfeeding during pregnancy would harm the mother, the fetus and the baby breastfed. It has been shown in the literature that continuation of breastfeeding during pregnancy does not create a risk regarding maternal health, pregnancy, abortion, pregnancy complications (intrauterine

death, intrauterine growth retardation and missed abortion) and preterm labor (14-16, 20-23). In a case report about two mothers becoming pregnant during lactation and breastfeeding their babies during their pregnancy, Devecioğlu et al. (24) stated that the mothers took vitamin and iron supplements and continued to breastfeed their babies throughout their pregnancy without any complications. However, Ayrim et al. (25) showed in their study that maintanence of breastfeeding during pregnancy significantly decreased weight gain and increased the risk of anemia in the mothers during pregnancy. They recommended that the mothers should be followed in terms of nutrition and anemia and provided with education about them. There have not been any other studies justifying worries of the mothers regarding brestfeeding during pregnancy except for the study by Ayrim et al. (25).

The mothers included in the present study had the opinion that the taste and content of the mother's milk changed during pregnancy and made the breastfed baby ill or poison it. They also thought that the fetus had the right for breastfeeding and that breastfeeding during pregnancy was against the principles of Islam, which was consistent with several studies in the literature (13,24,26,27). It is stated in a review that the mother's milk can decrease in quantity, change in terms of color and content during pregnancy and turn into colostrum towards the end of pregnancy (28). However, it is reported in the literature that these changes in the mother's milk do not have a negative impact on the health of the baby breastfed during pregnancy (23,29). Several studies about mothers' worries that maintenance of breastfeeding during pregnancy may have harmful effects have shown that birth weight and Apgar score of the newborn are not affected (14,25,30). It is known that some women becoming pregnant during lactation continue breastfeeding their baby during pregnancy and even both their baby and the newborn after giving birth (23,26,31). This is called tandem breastfeeding in the literature (31,32). Three fourth of the mothers in the present study had worries about tandem breastfeeding and noted that it should not be performed. In two case reports, it was stated that the mothers became pregnant during lactation, continued breastfeeding during their pregnancy and performed tandem brestfeeding after giving birth. The mothers were reported to believe that tandem brestfeeding was indicator of respect for the babies (31) and prevented siblings' becoming jealous of each other (24,31). Congruent with the literature, few mothers in the current study were in favor of tandem breastfeeding.

Behaviors of Mothers about Maintenance of Breastfeeding during Pregnancy

In this study, 21.4% of the mothers were found to wean their babies since they became pregnant, wanted to become pregnant or thought they became pregnant. This showed that the mothers were not offered sufficient support for contraception in the postpartum period. It is stated in the literature that becoming pregnant during lactation has a considerable effect on early weaning of babies (1.3-55.7%) (7,8,12). Compatible with the literature, the present study also revealed that becoming pregnant while lactating played an important role in early weaning of the babies.

In the current study, of all the multiparous mothers, 32% became pregnant during lactation currently or previously and about half of them stopped breastfeeding soon after they learned about their pregnancy and the rest did so in a few weeks after learning about their pregnancy. However, few mothers maintained breastfeeding until the third trimester. In Ayrim et al.'s (25) study, 27.2% of the mothers were found to become pregnant during their lactation period and in Şengül et al.'s (16) study, 63.9% of the mothers were found to become pregnant while lactating. These findings indicated that a considerable rate of the mothers became pregnant during lactation.

Most of the mothers included in the present study and becoming pregnant during lactation said that they learned about their pregnancy in the sixth gestational week on average. In Devecioğlu et al.'s (24) report of two cases, one mother learned about her pregnancy in the 12th gestational week and the other mother learned about it in the fifth gestational week. It could be suggested that mothers usually learned about their pregnancy in the first trimester. In the present study, one third of the mothers becoming pregnant during lactation reported that their breastfed babies became ill and had some symptoms like nausea, vomiting, diarrhea and high temperature until they learned about their pregnancy. They also said that they observed changes in color, quantity and viscosity in the mother's milk and that even their babies rejected mother's milk since its taste changed. Besides, they mentioned that similar changes appeared in the mother's milk after they learned about their pregnancy. These findings from the present study are consistent with the evidence from the literature.

Moscone and Moore (23) also reported that 49% of the mothers becoming pregnant during lactation experienced changes in the amount of the mother's milk and the frequency of breastfeeding. In a study by Shaaban and Glasier (29), 66.3% of the mothers had changes in the quantity of the mother's milk and the frequency of breastfeeding and only 29.6% of the mothers observed diarrhea, pulmonary infections and slow growth in the breastfed babies. Although it has been reported in the literature that the production and color of the mother's milk can change during pregnancy (33), there is not reliable evidence that the mother's milk produced during pregnancy can be harmful (23,29).

In the present study, more than two thirds of the mothers becoming pregnant during lactation asked health professionals (midwives, nurses or doctors) and/or their first degree relatives about continuation of breastfeeding. They were frequently recommended to stop breastfeeding. Some of the mothers noted that they asked two different health professionals about maintenance of breastfeeding (nurse, midwife, family physician, gynecologist and pediatrician) and that one recommended continuation of breastfeeding while the other recommended quitting it. In Moscone and Moore's (23) study, the women becoming pregnant during lactation also consulted their spouses, relatives and health professionals about breastfeeding during their pregnancy. It was reported by Moscone and Moore (23) that while midwives and the women's spouses were supportive, other health professionals and the women's relatives suggested

weaning. Besides, some of the women (39%) were reported to conceal their pregnancy from health professionals in order not to receive a negative response to their maintenance of breastfeeding (23). In a report of two cases, Devecioğlu et al. (24) revealed that the mothers becoming pregnant during lactation requested information from gynecologists and that one of them was recommended to maintain it whereas the other was recommended to wean her baby. Pareja et al. (30) showed that 20% of the mothers becoming pregnant during lactation received advice from their friends, relatives or health professionals and that those receiving recommendation from their relatives continued to breastfeed till the end of their pregnancy. It can be suggested that the present study is consistent with the literature. Although health professionals, relatives and mothers seem to disagree about continuation of breastfeeding, they mostly recommend weaning the baby. In the current study, 51.1% of the mothers becoming pregnant during lactation continued to breastfeed their babies for some time after learning about their pregnancy. It is striking that some of these mothers decided to breastfeed depending on the age of the baby or the gestational week. Only one mother was found to continue to breastfeeding her baby until it was two years old (until the 36th gestational week) since she thought her baby needed it and since her gynecologist told her to continue breastfeeding with taking a vitamin supplement. Several other studies also showed that mothers becoming pregnant during lactation maintained breastfeeding for some time during pregnancy (15,21,26). Devecioğlu et al. (24) stated in their case report that the mothers becoming pregnant during lactation breastfed their babies during their pregnancies with taking vitamin and iron supplements and did not experience any health problems. Duration of breastfeeding during pregnancy determined in the present study was similar to that reported in the literature. The breastfeeding behavior of most of the mothers during their pregnancy was not approved by their friends and relatives and many people around them. Half of the mothers asking for advice from health professionals reported receiving a negative response and some of the mothers noted that health professionals were indecisive about or unresponsive to their request for advice. Some women did not ask health professionals about maintenance of breastfeeding in case they told them not to breastfeed their babies. Consistent with the present study, several studies showed that some health professionals and people around women becoming pregnant during lactation advised the women to wean their babies (8,24). However, the health professionals play an active role in the decision of continuing breastfeeding while breastfeeding during pregnancy. For this reason, the pregnant woman should be listened to with interest and her questions should be answered carefully. A supportive and reassuring relationship should be established with the mother, and she should be explained that there is no need to wean her baby if she becomes pregnant while breastfeeding, and that she can continue breastfeeding and that she can breastfeed in tandem after delivery (34,35). Maintenance and duration of breastfeeding during pregnancy can be affected by the type of family bringing up women, society, education, religious values and beliefs of the women about breastfeeding.

Study Limitations

In this survey, mothers were asked about their opinions, past experiences and behaviors regarding continuing breastfeeding during pregnancy.These opinions and behaviors may not be representative of all other mothers, and they may be affected by the time the survey was taken. Therefore, the results cannot be generalized.

Conclusion

In this study, most of the mothers believed that they had to wean their baby if they become pregnant during lactation. However, a small number of the women believed that they could continue to breastfeed their baby if the course of pregnancy and/or the health status of the mother, fetus and the baby breastfed was good. University education was the most important factor affecting the mothers' opinion about maintenance of breastfeeding during pregnancy. Most of the mother also believed that the content of the mother's milk changed during pregnancy and that breastfeeding harmed the mother, the fetus and/or the baby. A considerable rate of the mothers had an experience of becoming pregnant during lactation and weaned their baby in the past. The mothers continuing to breastfeed were disapproved of their breastfeeding behavior. Having a nuclear family was another factor that supported maintenance of breastfeeding.

In conclusion, the main factor determining the mothers' opinions and behaviors regarding maintenance of breastfeeding during pregnancy is not their knowledge about breastfeeding but their worries about their own health or their babies' health, societal, cultural and religious beliefs, recommendations and reactions of health professionals, family and friends. Considering benefits of breastfeeding for the recommended period of time, women becoming pregnant during lactation should be encouraged to continue breastfeeding.

Ethics

Ethics Committee Approval: The ethical review of study was approved by the University of Health Sciences Türkiye, Istanbul Training and Research Hospital Ethic Committees (number: E.4117, date: 25.12.2018).

Informed Consent: Informed consent was obtained from the mothers meeting the inclusion criteria and accepting to participate in the study.

Footnotes

Authorship Contributions

Concept: P.D.K., S.Ö., Design: P.D.K., S.Ö., Data Collection or Processing: P.D.K., Analysis or Interpretation: P.D.K., S.Ö., Literature Search: P.D.K., Writing: P.D.K., S.Ö.

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Original Article

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The Effects of MicroRNAs on Cardiomyopathy in a Rat Model of Streptozotocin-induced Diabetes Mellitus

Streptozotosin ile İndüklenen Diabetes Mellitus Sıçan Modelinde MikroRNA'ların Kardiyomiyopati Üzerine Etkileri

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ABSTRACT

Objective: Diabetic cardiomyopathy (DCM) is characterized by complex pathophysiological events. miRNAs play a role in the DCM. In our study, the potential of miRNAs as a biomarker in the diagnosis and treatment of DCM was evaluated in the diabetes model induced by streptozotocin in rats.

Methods: Therefore, miRNAs obtained from rat heart tissue were analyzed by microarray, and twelve miRNAs (rno-miR-200c-3p, rno-miR-129-5p, rno-miR-150-3p, rno-miR-3584-5p, rno-miR-34c-3p, rno-miR-342-3p, rno-miR-466b-3p, rno-miR-466c-3p, rno-miR-31a-3p, rno-miR-15b-5p, rno-miR-196b-3p, and rno-miR-208a-5p) with changed expression levels were validated by real-time polymerase chain reaction analysis.

Results: As a result of the validation, it was determined that three miRNAs (rno-miR-15b-5p, rno-miR-196b-3p, and rno-miR-208a-5p) were downregulated, one miRNA (rno-miR-200c-3p) was upregulated ($p<0.05$). MiR-15b-5p, miR-196b-3p, miR-200c-3p, and miR-15b-5p are involved in the regulation of DCM, and the *GRAP2* gene is one of the possible targets of these miRNAs.

Conclusion: miR-200c-3p has diagnostic value and may be a biomarker candidate.

Keywords: Diabetic cardiomyopathy, miRNA expression, biomarker, streptozotocin

ÖZ

Amaç: Diyabetik kardiyomiyopati (DKM), karmaşık patofizyolojik olaylarla karakterizedir. miRNA'lar DKM'de rol oynamaktadır. Çalışmamızda sıçanlarda streptozotosin ile oluşturulan diyabet modelinde miRNA'ların DKM'nin tanı ve tedavisinde biyobelirteç olarak potansiyeli değerlendirildi.

Yöntemler: Bu nedenle, sıçan kalp dokusundan elde edilen miRNA'lar mikroarray ile analiz edildi ve on iki miRNA'nın (rno-miR-200c-3p, rno-miR-129-5p, rno-miR-150-3p, rno-miR-3584-5p, rno-miR-34c-3p, rno-miR-342-3p, rno-miR-466b-3p, rno-miR-466c-3p, rno-miR-31a-3p, rno-miR-15b-5p, rno- miR-196b-3p ve rno-miR-208a-5p) değişen ekspresyon seviyeleri gerçek zamanlı polimeraz zincir reaksiyonu analizi ile doğrulandı.

Bulgular: Doğrulama sonucunda, üç miRNA'nın (rno-miR-15b-5p, rno-miR-196b-3p ve rno-miR-208a-5p) downregüle olduğu ve bir miRNA'nın (rno-miR-200c-3p) upregüle olduğu belirlendi ($p<0.05$). miR-15b-5p, miR-196b-3p, miR-200c-3p ve miR-208a-5p, DKM'nin düzenlenmesinde yer almakta ve *GRAP2* geni, bu miRNA'ların olası hedeflerinden biridir.

Sonuç: miR-200c-3p tanısal değere sahiptir ve biyobelirteç adayı olabilir.

Anahtar Kelimeler: Diyabetik kardiyomiyopati, miRNA ekspresyonu, biyobelirteç, streptozotosin

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Introduction

Diabetic cardiomyopathy (DCM) is characterized by diastolic relaxation abnormalities in its early stages and later by dyslipidemia, hypertension, and heart failure (1). Insulin resistance, hyperinsulinemia, and hyperglycemia are independent risk factors for the development of DCM (2). Systemic metabolic disorders, inappropriate activation of the renin-angiotensin-aldosterone system, subcellular component abnormalities, oxidative stress, inflammation, and dysfunctional immune modulation can lead to the development of DCM (3). These abnormalities promote cardiac tissue interstitial fibrosis, heart stiffness/diastolic dysfunction, and then systolic dysfunction, precipitating heart failure (4). The dysregulation of coronary endothelial cells and exosomes contribute to the pathology of DCM (5). Interstitial fibrosis, myocyte hypertrophy, and increased contractile protein glycosylation in heart tissue are seen in DCM (6). Systolic and diastolic dysfunction is observed in patients with DCM (7).

Mitogen-activating protein kinases (MAPKs) signaling pathway is dysregulated in DCM (8). Increased activity of MAPKs has been demonstrated in the streptozotocin (STZ)-induced diabetes model (9).

miRNAs are highly expressed in the cardiovascular system and may play a role in cardiovascular development and diseases (10). miRNAs regulate gene expression by inhibiting translation or allowing mRNA to be degraded (11).

The GRB2-related adapter protein 2 (GRAP2) is a member of the GRB2/Sem5/Drk family. This protein is involved in leukocyte-specific protein tyrosine kinase signaling (https://www.genecards.org/cgi-bin/carddisp.pl?gene=GRAP2). In cardiac hypertrophy, it manifests itself with an increase in response to pressure overload against mechanical stress. GRAP2 performs this function by activating MAPK through RAS, ROS, and other signaling molecules. GRAP2 regulates fibrosis, fetal gene induction, and cardiomyocyte growth (12). Adapter proteins regulate insulin signaling in diabetic patients work in coordination with substrates such as GRAP2 and increase IRS2 phosphorylation. The uptake of GRAP2, which is an obstacle to maintaining the balance between cell death and survival, is inhibited, insulin signaling is impaired, and the diabetic neuropathy process is contributed (13). The ability of GRAP2 to be regulated by miRNAs is an important target both in terms of understanding its potential in diseases and terms of its diagnostic value.

In this study, the expression levels of DCM-related miRNAs were determined in the STZ-induced diabetes model. Additionally, target genes and signaling pathways of miRNA were identified by bioinformatic analysis.

Methods

Animals and Experimental Modeling of Diabetes

Fifteen male Sprague Dawley rats (300-400 g) were used. The rats were obtained from Marmara University Experimental Animal Research Center. Rats were kept under controlled temperature (22 °C±1 °C) and humidity (60%) conditions on a 12-hour light/dark cycle with access to food and water. Rats were divided into two groups; diabetes (n=9) and control (n=6). The rats were given a single i.p. injection of 60 mg/kg streptozotocin (STZ; Sigma, St. Louis, MO) in 10 mM sodium citrate (Sigma, St, Louis, MO) buffer (pH 4.6) (14). Two days after the STZ injection, blood glucose levels were measured with an Accu-Chek Go (Roche) glucometer. Rats with blood glucose levels above 200 mg/dL were considered diabetic. After 6 weeks, the weights of the rats were measured. Intracardiac blood was drawn from the anesthetized rats, and the heart tissues were removed. The left ventricle of the heart was dissected and stored at -80 °C for gene expression analysis. A portion of the left ventricle was taken in 10% formaldehyde for histological analysis.

RNA Extraction Procedure

Homogenization was performed by adding 50 mg of heart tissue and 450 µL of lysis buffer solution to tubes with ceramic beads. Total RNA isolation was performed according to the kit (GenUP TM Total RNAKit-biotechrabbit) protocol. RNA purity and quantification were performed with a spectrophotometer (Thermo Scientific™ NanoDrop™ 2000, Massachusetts, USA). The RNA pool was created by mixing an equal amount of RNA from the heart tissues of rats in diabetes and control groups in an equal amount of 100 ng/µL in a tube for each group.

Microarray Profiling of miRNAs

The Affymetrix GeneChip microRNA 4.0 Array was used to analyze miRNA expressions (Ay-ka Limited Company). In this array, 1.250 miRNAs belonging to Rattus norvegicus species were screened and 2-fold or more changes were detected in 81 miRNAs. The 12 most upregulated and most downregulated miRNAs were selected. The criteria for this selection were folded change ≥2 and $p<0.05$.

Complementary DNA (cDNA) Analysis

cDNA synthesis was performed from 10 ng total RNA by reverse transcription PCR using the kit (qScript™ microRNA Synthesis Kit- VWR).

miRNA Expression Analysis

miRNA expression analyses were performed by real-time polymerase chain reaction (RT-PCR). RT-PCR amplifications were performed with Syber Green PCR Master mix (Perfecta® SYBR Green FastMix® Reaction Mixes-VWR, Beverly, California) using the BIORAD-CFX Connect RT-PCR system. Each sample was analyzed in duplicate. For the relative analysis of miRNA expressions in heart tissue, an endogenous control *U6* gene (15) was used. The reaction mixture was prepared as 20 µL total volume, 10 µL 2X SYBR Green SuperMix, 0.4 µL advanced primer (Rat U6 for tissue endogenous control), 0.4 µL reverse primer (Universal PCR primer), 8.2 µL nuclease-free water, and 1 µL cDNA. Thermal cycling conditions were programmed for 10 min at 95 °C, followed by 5 seconds at 95 °C, 15 seconds at 60 °C, and 15 seconds at 70 °C 40 cycles.

By performing a melting curve analysis in the extension step, RT was programmed to make a reading from 70 °C to 90 °C in 0.2 seconds in 0.5 °C increments. Up and down-regulation of miRNAs using the obtained cDNAs was determined by RT-PCR. Each sample was analyzed in duplicate. Samples were standardized by U6 endogenous control (ΔCT). The relative quantification of the standardized samples was calculated by the $2^{-\Delta\Delta CT}$ method (16).

Bioinformatic Analysis

The miRDB database was used to find potential target genes for 4 miRNAs (http://mirdb.org/cgi-bin/search.cgi). In selecting target genes, those with miRNA: target gene matching scores between 70% and 100% were preferred. Obtained results were verified with TargetScan 7.2 (http://www.targetscan.org/vert_72/). The common target of these 4 miRNAs was determined to be the *GRAP2* gene. These miRNAs and target genes were searched in CDM by searching the HMDD database (https://www.cuilab.cn/hmdd). MiRPathDB (https://mpd.bioinf.uni-sb.de/mirnas.html?organism=hsa), Reactome (https://reactome.org/), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases (https://www.genome.jp) /kegg/pathway.html) was used to detect metabolic pathways involving the *GRAP2* gene. These genes were evaluated in both species using the Human-Mouse: Disease Connection database (http://www.informatics.jax.org/mgihome/projects/aboutHMDC.shtml). Finally, because multiple miRNAs target the same gene, the mirDIP database (microRNA Data Integration Portal) was used to identify the target of this miRNA (http://ophid.utoronto.ca/mirDIP/index.jsp#r).

Histological Analysis

Heart tissues were fixed in 10% formaldehyde and then passed through an increasing alcohol series. Heart tissue was embedded in paraffin and 4 µm sections were cut. The slides were treated with hematoxylin eosin, and Masson trichrome stain (Atom Scientific, UK). The samples were examined under a light microscope according to the Kiernan protocol (17) (Nikon Model Eclipse E200MV R and Nikon Digital Imagine Camera). To evaluate histopathological changes in the heart muscle and fibers, slides were graded histopathologically, and "0" was considered negative, "1" mild, "2" moderate, and "3" severe (17).

Statistical Analysis

Statistical analysis was performed using Graphpad Prism 5.0 (Graphpad Software, San Diego, Ca, USA). All data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's test were used in the evaluation between the groups. Mann-Whitney U test was used for comparisons between the means of myocyte diameters of groups. p-value was accepted as 0.05. Receiver operating characteristic (ROC) curve was performed, and area under the curve was assessed for the specificity and sensitivity of miRNAs. The data were analyzed using the statistical package SPSS (Release 11.5, SPSS Inc, Chicago, IL, USA) for Windows.

Results

Bodyweight measurements and blood glucose values at the beginning of the study and 6 weeks after STZ administration are given in Table 1. At the end of 6 weeks, a significant decrease in body weight and hyperglycemia were observed in the diabetes group. The blood glucose levels of the diabetic rats showed a significant increase compared to the control group.

Gene Chip microRNA 4.0 Array Analysis

Twelve miRNA genes, 4 of which were the most upregulated and 8 were the most downregulated genes, were selected. Fold change ≥2 and $p<0.05$ values were used in this selection (Table 2).

miRNA Expression Analysis

It was found that miR-200c-3p, miR-15b-5p, miR-196b-3p, and miR-208a-5p showed a significant difference in diabetic rats compared with the control group ($p<0.05$) (Figure 1). The regulation status of these miRNAs was demonstrated Table 3, Figure 2.

miRNA Bioinformatics Analysis

In the target gene analyses for 4 miRNA and DCM, 220 genes for DCM, 36 genes for miR-200c-3p, 3.573 genes for miR-15b-5p, 649 genes for miR-196b-3p, 4786 genes for miR-208a-5p were compared. The *GRAP2* gene was detected in both miRNAs and DCM (Supplement table 1). In the pathway analysis for GRAP2, 4 signal pathways and 11 sub-pathways that could be related to DCM were detected. According to the Reactome database, it was determined that the intercellular signal transduction pathway was receptor tyrosine kinase. Then, 11 effective sub-pathways and signal diagrams were obtained for these signal pathways. Signal diagrams were detected as TCR signaling, Fc epsilon receptor (FCER1) signaling, DAP12 interaction, and FLT3 signaling. The subpathways were identified as FGFR, NTRK2, EGFR, ERBB2, MET, PDGF, IGF12, Insulin receptor, VEGF, EBB4, and SCF-KIT. These results were the same for homo sapiens and

Table 1. Changes in body weight and blood glucose values of rats before and after STZ administration

Groups	Bodyweight (g)		Blood glucose (mg/dL)	
	First	After 6 weeks	First	After 6 weeks
Control	346.3±11.6	358.8±7.7	93.80±2.01	96.88±1.64
Diabetes	371.9±5.08	271.5±6.32*	98.99±1.81	471.48±30.26+

Data are presented as mean ± standard deviation,
*p<0.001, different according to the first application in the diabetes group,
+p<0.001, different according to the control group.
STZ: Streptozotocin

Table 2. miRNA fold changes according to microarray analysis

miRNAs	Fold change	Accession numbers	Primer sequences
rno-miR-200c-3p	4.15	MIMAT0000873	TAATACTGCCGGGTAATGATG
rno-miR-129-5p	3.89	MIMAT0000600	CTTTTTGCGGTCTGGGCTTGC
rno-miR-150-3p	3.82	MIMAT0017133	CTGGTACAGGCCTGGGGA
rno-miR-3584-5p	3.55	MIMAT0017875	GGGAGGAGTCCAGGAGGC
rno-miR-34c-3p	-7.02	MIMAT0004723	AATCACTAACCACACAGCCAGG
rno-miR-342-3p	-8.07	MIMAT0000589	TCTCACACAGAAATCCACCCGT
rno-miR-466b-3p	-36.47	MIMAT0017285	ATACATACACACACACATACAC
rno-miR-466c-3p	-9.58	MIMAT0017287	TATACATGCACACATACACAC
rno-miR-31a-3p	-9.72	MIMAT0000810	AGCAAGATGCTGGCATAGCTG
rno-miR-15b-5p	-9.9	MIMAT0000784	TAGCAGCACATCATGGTTTACA
rno-miR-196b-3p	-19.37	MIMAT0017171	TCGACAGCACGACACTCCTTCA
rno-miR-208a-5p	-60.8	MIMAT0017155	GAGCTTTTGGCCCGGGTTATAAC

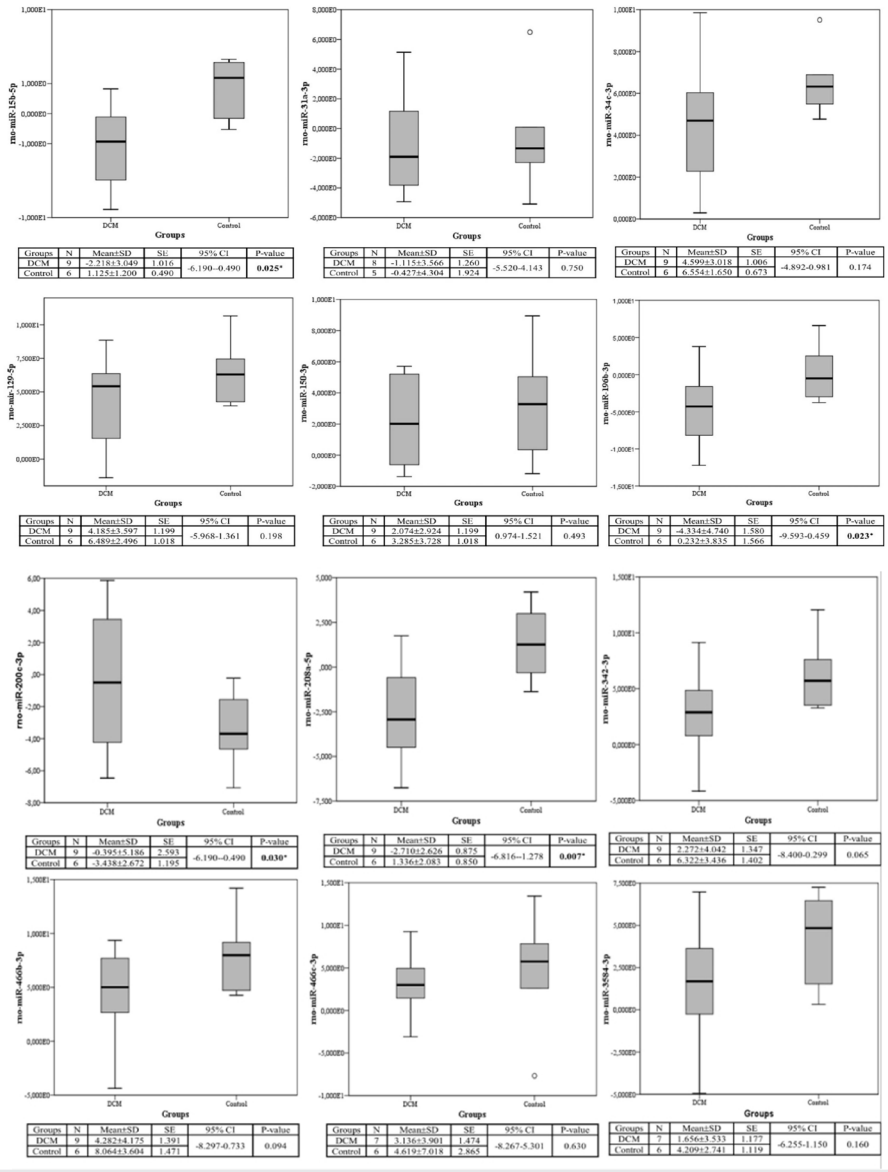

Figure 1. miRNA expression differences diabetes and control groups

Mus musculus (miRNAs have a high degree of seed sequence homology for both species). KEGG pathway analysis showed that the *GRAP2* gene (K07366) was primarily effective in the T-cell receptor signaling pathway. Two signaling pathways were featured in this study: Calcium signaling (nt06120) and TGR-NFAT signaling pathway (N01106). The results were checked in the Reactome database and it was determined that the *GRAP2* gene was effective in the secondary messenger system. The cellular localization of GRAP2 was shown to be cytoplasm, cytosol, endosome, nucleoplasm, nucleus, and plasma membrane. It was determined that GRAP2 was highly expressed in the heart and pericardium and its molecular function is protein binding (Figure 3) (Reactome) (18). In our analysis to determine which of the four miRNAs targeting the same gene could bind primarily, miR-200c-3p had a high degree of target activity, while the other 3 miRNAs (miR-15b-5p, miR-196b-3p, and miR-208a-5p) were found to have moderate target efficiency.

Table 3. miRNA fold change and regulation status according to expression analysis

miRNAs	Diabetes (fold change)	Regulation
rno-miR-200c-3p	16.56	Upregulated
rno-miR-129-5p	4.93	Upregulated
rno-miR-150-3p	0.23	Upregulated
rno-miR-3584-5p	5.86	Upregulated
rno-miR-34c-3p	3.87	Downregulated
rno-miR-342-3p	0.22	Downregulated
rno-miR-466c-3p	3.81	Downregulated
rno-miR-31a-3p	1.61	Downregulated
rno-miR-15b-5p	10.14	Downregulated
rno-miR-196b-3p	23.70	Downregulated
rno-miR-466b-3p	3.75	Downregulated
rno-miR-208a-5p	16.53	Downregulated

ROC Curve Analysis

In the ROC analysis for four miRNAs, miR-200c-3p had the potential to be biomarkers ($p<0.05$) (Table 4, Figure 4), and heart tissue miRNAs were expressed differently in DCM. In addition, this miRNA was thought to be associated with the DCM.

Histopathological Analysis

Myocyte diameters were measured in each of the sections taken from six different heart tissues obtained from the control group (16.35±0.66) and nine different heart tissues obtained from the

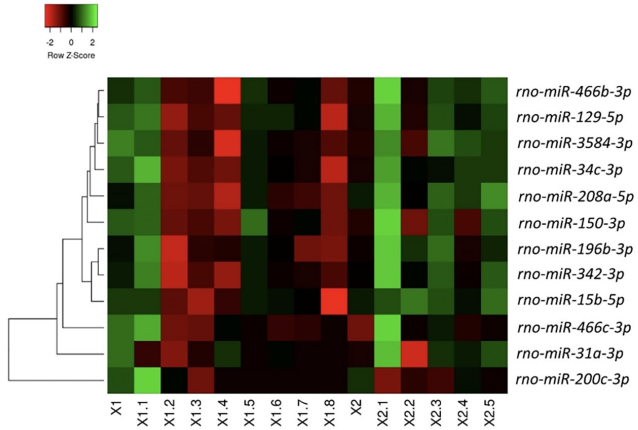

Figure 2. The Heatmap differentially expression of miRNAs. Average linkage and spearman rank correlation were used in the analysis of the data. X1-X1.8; diabetes group, X2-X2.5; control group

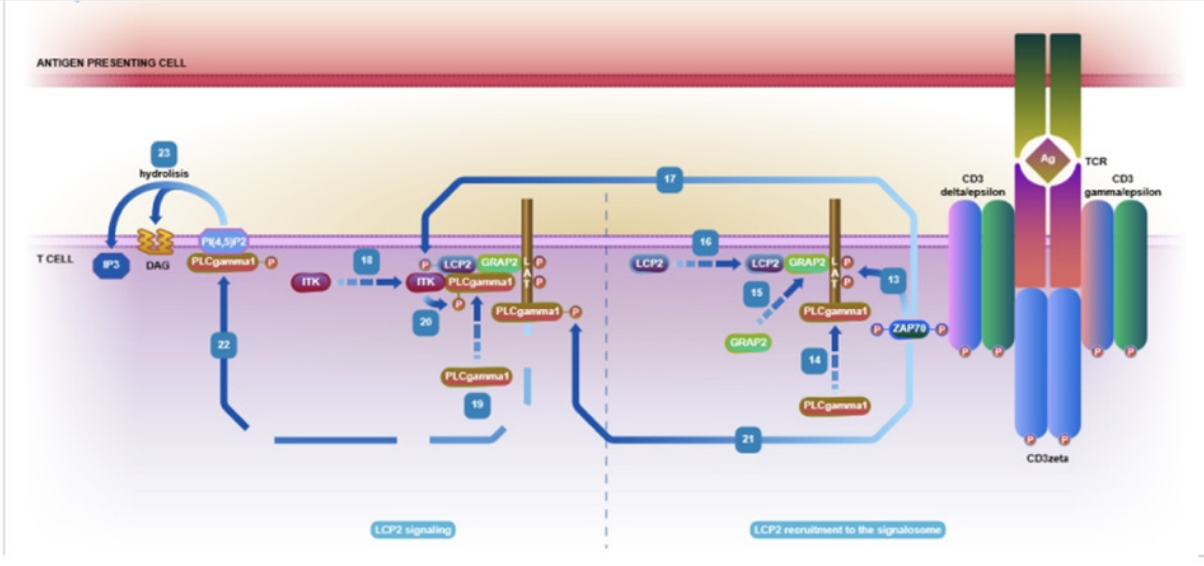

Figure 3. GRAP2 function in T-cell
GRAP2: GRB2-related adapter protein 2

diabetes group (31.45±0.74) group. The mean myocyte diameter in diabetes was found to be high ($p<0.05$). Additionally, hypertrophic cardiomyocytes were found in the ventricular region of rats in the diabetes group (Figure 5C, D, E, F) and cardiomyocytes showing pale acidophilic staining in these regions were also remarkable (Figure 5D). Occasional vacuolization was observed in some cardiomyocytes (Figure 6D). In the diabetes group, more intense connective tissue staining and increased interstitial cardiac fibrosis were observed (Figure 6E, F).

Discussion

Diabetes mellitus is a progressive, chronic, and metabolic disease characterized by hyperglycemia and glycosuria, which develops due to insufficient insulin secretion in the pancreas and/or insufficient response of tissues to insulin (19). Controlling hyperglycemia significantly decreases diabetes-related mortality rates and acute complications (20).

The incidence of morbidity and mortality in diabetic patients increases with complications (21). Increased levels of free radicals contribute to the development of diabetes complications by causing oxidation-related cell damage in many tissues, especially the endothelium (22). Type 1 and type 2 diabetes are primary risk factors for cardiovascular diseases (23). Studies have reported that miRNAs regulate cardiomyocyte hypertrophy, myocardial fibrosis, cardiomyocyte apoptosis, mitochondrial dysfunction, myocardial electrical remodeling, epigenetic modification, and

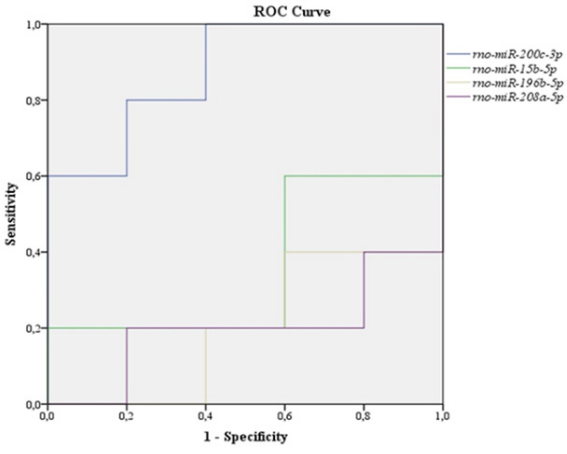

Figure 4. Comparison of sensitivity and specificity of the four miRNAs diabetes and control groups

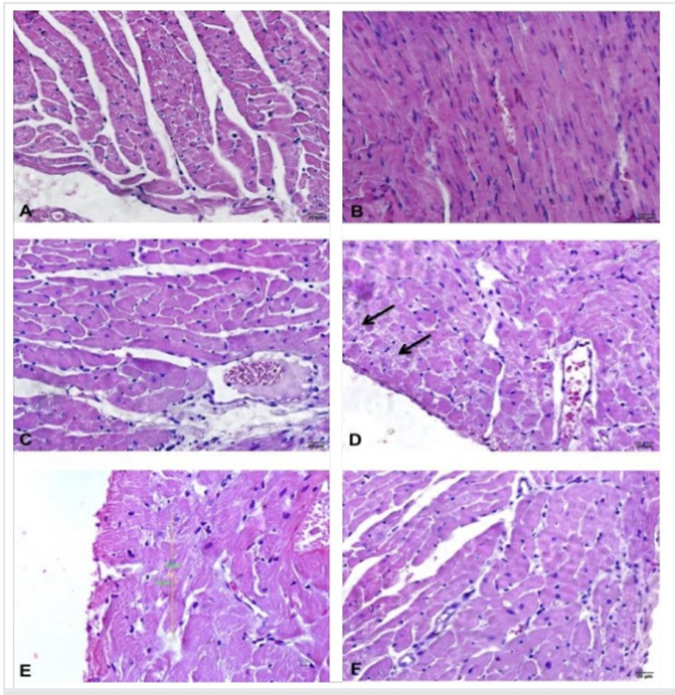

Figure 5. Hematoxylene and eosin staining in control and diabetes samples of myofibril

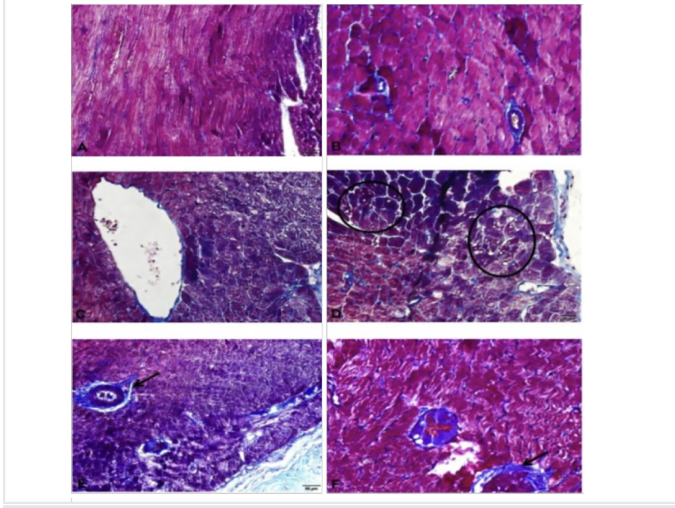

Figure 6. Hematoxylene and eosin staining in control and diabetes samples for cardiomyocyte hypertrophy

Table 4. ROC curve analysis of four miRNAs for control and diabetes groups

Compared groups	Tissue	Test variables	AUC	95% CI	p-value	Specificity	Sensitivity	Criterion
Diabet versus control	Heart	rno-miR-200c-3p	0.815	[0.535-0.963]	0.018	77.78	83.33	>27.70
		rno-miR-15b-5p	0.537	[0.269-0.790]	0.823	22.22	100	>23.28
		rno-miR-196b-3p	0.630	[0.349-0.857]	0.394	66.70	66.70	≤29.00
		rno-miR-208a-5p	0.611	[0.333-0.844]	0.492	77.78	50	≤27.13

AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic

various other pathophysiological processes (24). Additionally, the expression levels of DCM-related miRNAs affect the pathophysiology of the diabetes (25).

miRNA expression levels change similarly in cardiac pathologies such as cardiac hypertrophy, heart failure, and myocardial infarction (26). The cardiac expression of miR-200c is significantly upregulated in diabetic rats (27). miR-200c has specific activity on the functions of dual-specificity phosphatase-1 (DUSP-1), a MAPK phosphatase that regulates the phosphorylation of p-ERK, p-JNK, and p-p38. The expression of miR-200c is high in this model. miR-200c inhibition reduces cardiac hypertrophy by activating DUSP-1 in cardiomyocytes in the diabetes model. In another study, the downregulation of miR-200c protects cardiomyocytes from apoptosis due to hypoxia (28). In our study, miR-200c-3p was upregulated. It can be thought that the upregulation of miR-200c is associated with DCM pathology.

It is reported that Sp1-mediated matrix metalloproteinase-9 (MMP-9) expression plays an important role in diabetic wound healing. miR-129 and miR-335 are known to inhibit MMP-9 expression by targeting Sp1 (29). These two miRNAs upregulated lead to MMP-9 downregulation, which in turn promotes wound healing. There are no studies on the expression of this miRNA in DCM. However, computational studies have shown that miR-129 is upregulated during heart failure (30). Our results showed that the upregulation of miR-129a-5p might be involved in the DCM process, although it was not statistically significant.

In a hypertension model in rats, miR-3584 was reported to be upregulated (31). In another study, miR-3584 was upregulated in plasma in patients with pulmonary arterial hypertension (32).

A study indicated that 19 miRNAs in C2C12 myotubes regulated mitochondrial metabolism in skeletal muscle.

Four of these miRNAs (miR-34c-3p, miR-150-5p, miR-196b-3p, and miR-320a) were reported to be associated with skeletal muscle mitochondrial function in humans (33). Another study found that miR-34c was overexpressed and suppressed in hypertrophic cardiomyopathy, resulting in improved cardiac function (34). In our study, miRNA-34c and miRNA-196b expressions were downregulated, and miR-150 expression was upregulated. Therefore, it can be thought that miR-34c and miR-196b have a triggering role in the pathogenesis of DCM.

miR-15 is dysregulated in cardiovascular and neurological diseases (35). Dysregulation of miR-15b has been shown to cause ROS generation, which controls ATP production, leading to heart disease (35). In our study, miR-15b expression was shown to be downregulated. Contrary to these studies, miR-15b-5p targets the 5' untranslated (UTR) regions of genes. The 3' and 5' regions in the gene have different functions. 5' UTRs are important in gene regulation by miRNAs as they are translational control regions of gene expression. Thus, the stability of the gene can be checked (36). Since a miRNA can target more than one gene, tissue-specific miRNAs have a fundamental role in demonstrating their activity in diseases (37). The expression of this miRNA in the cardiac tissue is high and our results show that its expression in DCM is quite high compared to the controls (Fold change =10.14). miR-15b-5p can be an epigenetic regulator in the pathology of DCM.

The target genes of miR-466-3p are transcription factors and kinases that are actively involved in cell cycle, apoptosis and other cellular events (38). miR-466-3p is thought to be responsible for lung and breast cancer and cardiovascular system diseases. miR-466 may play an important role in DCM as it affects proteins known to be closely associated with inflammation. In our study, miR-466 was downregulated and this miRNA might play a role in the pathogenesis of DCM.

miRNA analyses were performed in rat cardiomyocytes on postnatal day 0 and day 10 (39). miR-31a-5p expression increased on the 10th day in isolated cardiomyocytes and this increase terminated the cell cycle. However, it is unclear how miR-31a-5p affects mature cardiomyocyte proliferation. miR-31a-5p is a negative modulator in the cardiac fibrogenic epithelial-mesenchymal transition of epicardial mesothelial cells. miR-31a-5p was found to be specifically increased in human atrial fibrillation; this was thought to cause loss of atrial dystrophin and neuronal nitric oxide synthase (40).

In our study, the downregulation of miR-31a-5p was also detected. The opposite of the results reported in previous studies could be explained by the presence of a possible compensatory mechanism. In this way, it may be aimed to increase dystrophin and neuronal nitric oxide synthase levels in response to the development of cardiomyopathy. Another study on atheromatous plaques suggested that miR-31 had an anti-inflammatory effect. Therefore, the downregulation of miR-31a-5p in our study can be explained by the fact that inflammation is one of the main factors in cardiomyopathy. miR-31a-5p plasma level was significantly decreased in heart failure patients compared to healthy volunteers (41). Our results are similar to this study.

In miR-208a silenced mice, the loss of miR-208a caused significant expression of multiple skeletal muscle contractile protein genes that were not normally expressed in the heart (42). In our study, it was thought that downregulation of miR-208a was a compensatory mechanism that develops against the pathogenesis of cardiomyopathy. Also, miR-208a is released from damaged cardiomyocytes in the bloodstream. The increased levels of miR-208a may be an important marker in the diagnosis of DCM. Similarly, in a study with mice, it was found that the plasma concentration of miR-208-a increased in myocardial damage and showed a significant correlation with cardiac troponin plasma concentration, and this might be an important indicator of myocardial damage (43,44).

Considering the histopathological results; in the control group, the nuclei of the heart muscle cells were centrally located, their cytoplasm was pale and the intercalated disc was normal. Hydropic degeneration, increase in nuclear size, and occasional vesiculation were observed in myocytes in the diabetes group. Additionally, trichrome staining revealed mild to moderate fibrosis in the interstitial area. Different studies have shown that different miRNAs

are involved in the pathogenesis of cardiomyocyte hypertrophy in DCM (45-49). One of the main features of DCM is cardiac fibrosis and its cause is excessive accumulation of extracellular matrix proteins. In diabetic heart tissue, miRNA regulation may occur under the influence of diabetes. It is inevitable to regulate fibrosis through signaling pathways in cardiac endothelial cells, fibroblasts and myocytes. miRNA dysregulation is associated with structural changes in cardiac tissue.

miR-150 expression level was significantly decreased in cardiomyocyte hypertrophy (45). In our study, cardiomyocyte hypertrophy was observed in the diabetes group, consistent with the results of this study. In another study, degenerative changes were reported in the STZ-induced diabetes model (50). In other studies, the intracellular pH level decreases with acidosis of the cell, and multiple enzyme activities occur (51). Basic groups are given to the environment by the denaturing of the structural and enzymatic cytoplasm proteins by activated enzymes showing affinity to acid dyes (eosin), causing acidophilic staining of the cytoplasm (52). In our study, pale acidophilic staining was observed in diabetic rats. In the STZ-induced diabetes model, there was minimal change in ventricular muscle cells in the 8-10-week period in rats (53).

Where more than one miRNA targets a single gene, free energy and sequence specificity may indicate that the miRNA is efficiently bound (54). In our study, we determined the conservation of miRNAs with the target gene, GRAP2, using TargetScan. miR-200c-3p has a higher probability of matching than other miRNAs. It is predicted that the pathological effects of GRAP2, which is regulated by these 4 miRNAs, on DCM are realized in T-cell signaling. In DCM, cytokines and chemokines are secreted by inflammatory cells in the heart tissue. These substances cause the development of cardiomyocyte hypertrophy and remodeling of the extracellular matrix. This activation triggers the release of several cytokines by affecting various inflammatory cells and cardiac damage is induced. In STZ-induced mice, the myocardium has a higher T-cell infiltration (55).

Study Limitations

The expression level of the *GRAP2* gene, which is the target gene of miR-200c-3p, can be examined.

Conclusion

The *GRAP2* gene may play a role in the T-cell receptor function of miRNAs and that their downregulation may contribute to the pathology of DCM. GRAP2 expression can be examined. It is also possible that miR-200c-3p can be used as epigenetic regulators or biomarkers in DCM.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Ş.D., M.P., A.B., H.K.E., Concept: M.P., H.K.E., Design: M.P., Ş.G.Y., H.K.E., Data Collection or Processing: M.P., Analysis or Interpretation: M.P., Ş.G.Y., A.K., Y.P., H.K.E., Literature Search: A.Ş.D., M.P., Writing: M.P., Ş.G.Y., H.K.E.

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Original Article

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In vitro Evaluation of Repair Bond Strength to Bulk-fill Composites Using Two Silane-Free Universal Adhesives, with and without Silane Application

Silan Uygulaması ile Birlikte ve Silan Uygulaması Olmadan Universal Adeziv Kullanarak Bulk-fill Kompozitlere Tamir Bağlantı Dayanımının *In vitro* Değerlendirilmesi

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ABSTRACT

Objective: To evaluate the effect of silane-free universal adhesives systems on the repair shear bond strength (SBS) of sonic-activated bulk-fill [SonicFill (SF) Kerr, USA] composite applying additional silane in the repair of bulk-fill composite.

Methods: Sonic-activated bulk-fill composite samples (n=40) were prepared using a teflon mold (6 mm x 4 mm) and polymerized. Then all samples were kept in an incubator to simulate the aging process at 37 °C for one month. Aged composite samples were embedded in acrylic resin. For the repair, the specimen surfaces were roughened with a diamond bur. They were divided into 2 groups (n=20) according to the adhesive system used and then into two subgroups (n=10) as additional silane was applied or unapplied.

Adhesive systems Ambar Universal Bond (AUB), (FGM, Brazil), G-Premio Bond (GPB) (GC, USA) and silane (G-Multi primer GC, Tokyo, Japan) were applied according to the manufacturer's instructions. Samples were subjected to a shear bond test in a universal testing device. For statistical analysis, one-way ANOVA test was performed ($p<0.05$).

Results: The highest SBS value was obtained in the silane-treated AUB group (21.88±6.4), while the lowest SBS value was obtained in the silane-treated GPB group (16.07±6.2). No statistically

ÖZ

Amaç: Silan içermeyen universal adeziv sistemlerinin, sonic enerji ile aktive edilmiş bulk-fill (SonicFill (SF), Kerr, ABD) kompozitinin tamirinde ilave silan uygulamasının tamir bağlanma dayanımı (TBD) üzerindeki etkisini değerlendirmektir.

Yöntemler: Sonic enerji ile aktive edilmiş bulk-fill kompozit örnekler (n=40) bir teflon kalıp (6 mm x 4 mm) kullanılarak hazırlandı ve polimerize edildi. Daha sonra tüm numuneler yaşlanma sürecini simüle etmek için bir inkübatörde 37 °C'de bir ay süreyle tutuldu. Yaşlandırılmış kompozit numuneler akrilik içerisine gömüldü. Tamir için numune yüzeyleri elmas frez ile pürüzlendirildi. Numuneler, kullanılan adeziv sistemine göre iki gruba (n=20) ayrıldı ve ardından ilave silan uygulanan ve uygulanmayan olarak iki alt gruba (n=10) ayrıldı. Ambar Universal Bond (AUB) (FGM, Brezilya), G-Premio Bond (GPB) (GC, ABD) ve silan (G-Multi Primer, GC, Tokyo, Japonya) üretici talimatlarına uygun olarak uygulandı. Örnekler universal bir test cihazında kesme bağlanma testine tabi tutuldu. İstatistiksel analiz için tek yönlü ANOVA testi kullanıldı ($p<0,05$).

Bulgular: En yüksek TBD değeri silan uygulanan AUB grubunda (21,88±6,4), en düşük TBD değeri silan uygulanan GBO grubunda

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ABSTRACT

significant difference was observed between all groups ($p<0.05$).

Conclusion: Additional silane application does not affect the SBS of universal adhesives on an SF composite material.

Keywords: Bulk-fill composite repair, sonic-activated bulk-fill composite, surface treatments, silane agent, universal adhesive

ÖZ

(16,07±6,2) elde edildi. Tüm gruplar arasında istatistiksel olarak anlamlı fark gözlenmedi ($p<0,05$).

Sonuç: İlave silan uygulaması, SF kompozit malzeme üzerindeki universal adezivlerin TBD'sini etkilememektedir.

Anahtar Sözcükler: Bulk-fill kompozit tamiri, sonic ile aktive bulk-fill kompozit, yüzey işlemleri, silan ajanı, universal adeziv

Introduction

In recent times, with the rise in both patients' and practitioners' aesthetic expectations, along with advancements in adhesive systems, composite resin has become increasingly common in restoring dental tissue losses (1-3). Especially for endodontically treated teeth and moderate substance loss, using the layering method for composite resin restoration can lead to gap formation between the resin layers due to the prolonged procedure.

Bulk-fill composites can be applied in a single layer up to a thickness of 4 mm, which reduces clinical procedure time, enhances comfort for both the patient and the clinician, and eliminates the risk of moisture contamination and gap formation associated with the layering technique, thereby providing more uniform restorations (4-6). Bulk-fill composites with high and low-viscosity formulations are currently available on the market. They employ various methods to increase the polymerization depth of each bulk-fill composite. These include enhancing the composite's translucency, utilizing specific polymerization modulators, or employing robust initiator systems (7). Generally, low-viscosity bulk-fillers have a low filler ratio to provide flowability; however, there is also a bulk-fill composite, which has a high filler ratio but increases its flowability with the effect of the sonic stimulator (SonicFill, Kerr, USA). This sonic-activated composite is one of the bulk-fill composites that can be used with an air-driven handpiece, which allows it to be applied to the cavity by reducing the viscosity of the composite with the effect of sonic vibration.

In clinical practice, the failure of composite restorations is a significant concern. Dentists may consider repairing these restorations since replacing the restoration can result in pulp exposure, weakened tooth structure, and loss of healthy tooth tissue (8). Repair is a minimally invasive procedure that preserves the tooth structure and extends the life of the restoration. It is also more cost-effective and significantly prolongs the lifespan of both the tooth and the restoration, making it a common choice over full restoration removal (9,10). Many methods are used for surface pretreatment of composite resin restorations before the repair process: mechanical roughening with diamond bur or air abrasion, mechanochemical roughening with sandblasting, chemical roughening with phosphoric acid, hydrofluoric acid or acidified phosphate fluoride (11,12).

Loomans and Özcan (13) stated that the effects of different repair techniques depended on the material, and none of the surface preparation methods were universally accepted.

"Universal" or "Multimode" adhesive systems have been introduced to the market to eliminate the disadvantages of one-step self-etch adhesive systems that provide clinical ease of use. Most acidic functional monomers contain 10-methacryloxydecyl dihydrogen phosphate (10-MDP) and silane. In addition to mechanical and chemical bonding in enamel and dentin, the most important advantage of universal adhesives is the variety of restorative procedures and adhesion strategies (14). The adhesive intermediate layer provides micromechanical retention by chemical bonding to the resin matrix and, if silane-containing, exposed filler particles and monomer penetration into the microporosities on the composite resin (15).

There is limited literature on studies evaluating the shear bond strength (SBS) in the repair of sonic energy-activated bulk-fill materials using both silane-containing and silane-free universal adhesives. The purpose of this study is to evaluate the repair bond strength (RBS) of two silane-free universal adhesive to a sonic-activated bulk-fill (SABF) composite resin after additional silane application. The null hypothesis of the study is that the additional application of silane in the repair of SABF composites would not affect the RBS of silane-free universal adhesives.

Methods

Forty SABF (SonicFill, Kerr USA) composite resin specimens (6 mm x 4 mm) were prepared using a teflon mold and polymerized for 40 seconds with a light emitting diode light device (Demi Ultra Capacitor, Kerr, USA, 450-470 nm wavelength). The prepared composite specimens were subjected to 10,000 cycles of thermocycling over the course of one month to simulate the aging process (16). 2 mm of the aged composite specimens were embedded in acrylic resin (İntegra, İstanbul, Türkiye). They were divided into 2 groups (n=20) according to the adhesive system used and then into two subgroups (n=10) as additional silane was applied or unapplied. The specimen surfaces were roughened with a green band diamond fissure bur (G&Z instruments Gmbh 6890 Lustenau/Austria) (17). Adhesive systems Ambar Universal Bond (AUB), (FGM, Brazil), G-Premio Bond (GPB) (GC, USA) and silane (G-Multi primer GC, Tokyo, Japan) were applied according to the manufacturer's instructions (18). The specimens were divided into four groups based on the type of bonding agent applied and the presence of a silane agent.

- Grup AUB

- Grup AUB + silane

- Grup GPB

- Grup GPB + silane (GPBS)

The main components of the materials used in this study are shown in the table below (Table 1). Specimens were subjected to a SBS test using a universal testing machine (Shimadzu AGS-X Universal, Tokyo, Japan) (crosshead speed: 1 mm/min) with a wedge-shaped tip aligned parallel to the longitudinal axis of the interproximal surfaces of the restorations until failure occurred. Failure modes were evaluated under a stereomicroscope. The obtained values were recorded as MPa.

Repair Procedures

A 2 mm x 2 mm silicone mold was used. Afterward applying the relevant surface treatments and adhesive procedures to the surface of the acrylic-embedded and aged bulk-fill composite specimen, a 2 mm restoration was performed with a bulk-fill (SonicFill, Kerr USA) composite using each specimen mold.

Statistical Analysis

In calculating the specimen size, the probability of type 1 error (α=0.05) and the power of the test (1-β) were considered 0.95. Using the G Power 3.1.9.2 program, it was calculated that the total specimen size should be at least 10. Therefore, the specimen size was determined as 10.

The data were analyzed using IBM statistical package for social sciences (SPSS) 26 Software for Windows. By performing the Shapiro-Wilks test, it was determined that the obtained data showed a normal distribution. Parametric tests were conducted among the groups for pairwise comparisons using ANOVA. The results were evaluated with a significance of $p<0.05$.

Failure Modes

After bond strength testing, the failure mode of all specimens was assessed under a light microscope at a magnification of x15 (Stemi 305, ZEISS, Germany) and classified into the following categories: Failures were classified as "adhesive" if the fracture occurred at the interface between the restorative material and the repair material. A "cohesive" failure was identified if the fracture occurred within either the restorative material or the repair material. If the fracture occurred both within the materials and at their interface, it was classified as a "mixed" failure.

Table 1. The main components of the materials used in this study

Materials	Manufacture	Batch no	Main components	Instructions for use
SonicFill 2	Kerr, Orange, CA, USA	8032275	Bis-GMA, TEGDMA, Bis-EMA, SiO2, glass, oxide	1. Activate SonicFill Handpiece by entirely depressing the foot pedal and filling the entire cavity (up to 5 mm). 2. After placement, press and sculpt, using a hand instrument to define the anatomy. 3. Light cure 40s 4. Finish and polish in the usual manner
Ambar Universal Bond	FGM,Joinville,SC, Brazil	230821	10-MDP, HEMA, UDMA, methacrylic monomers, photoinitiators, co-initiators, stabilizers, silica nanoparticles, and ethanol. pH= 2.6 - 3.0	1. Apply and rub for 20 s (repeat) 2. Air dry for 10 s 3. Light cure 10 s
G-Premio Bond	GC, Tokyo, Japonya	2208101	MDTP, 4-MET, MDP, acetone, photoinitiators, water, dimethacrylate monomers, silicon dioxide	1. Apply adhesive and leave undisturbed for 10 s 2. Dry thoroughly with maximum air pressure 3. Light cure for 10 s
G-Multi Primer (Silane)	GC, Tokyo, Japonya	2011111	Phosphoric ester monomer, Ethanol, Methacrylate monomer, γ-Methacryloxypropyl trimethoxysilane	1. Dispense one drop of G-Multi PRIMER into a dispensing dish 2. Apply a thin layer to the fractured surface of the restoration using a micro-tip applicator and dry with an oil-free air syringe 3. Continue the repair using a light-cured adhesive and a light-cured composite, referring to their respective manufacturer's instructions

Bis-GMA: Bisphenol A-glycidyl methacrylate, TEGDMA: Triethylene glycol dimethacrylate, Bis-EMA: Bisphenol A-diglycidyl methacrylate ethoxylated, 10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate, HEMA: 2-hydroxyethyl methacrylate, UDMA: Urethane dimethacrylate, MDTP: Methacryloyloxydecyl dihydrogen thiophosphate, 4-MET: 4-methacryloxyethyl trimellitic acid

Results

The descriptive statistics values of the SBS of the SABF composite material are shown in Table 2, 3. The highest SBS value was obtained in the AUB group (21.88±6.4 MPa) without silane application, while the lowest SBS value was obtained in the silane-treated GPBS group (16.07±6.2 MPa). No statistically significant difference was observed between all groups ($p<0.05$).

In the GPB group without silane application, only adhesive failures were observed. In other groups, adhesive failures were generally observed.

Discussion

It was determined that the application of additional silane to the repair process performed with two silane-free adhesives did not alter the RBS of the SABF composite. Thus, the null hypothesis was rejected. Composite resin restorations may deteriorate due to mechanical, thermal, and chemical stresses in the oral environment. In this respect, thermal cycling of bonded specimens is the one of the best method to mimic the aging of interfacial bonds. Additionally, the success of composite repair procedures depends on several factors, including surface properties, wettability of chemical bonding agents, and the chemical composition of composites (19-21). Surface roughness is very important for composite repair and the roughening process can be achieved mechanically with the diamond burs used for this purpose (19,22-24). Chemical bonding is also an ideal method for enhancing the bond strength of restored composite materials, apart from micromechanical interlocking (25). Silane agents chemically bond the fillers of the old composite resin to the organic resin matrix of the new composite resin (26,27). For this reason, the use of a silane coupling agent is recommended for composite resin repair (19).

An essential factor for the successful restoration of dental composite is to retain a strong bond with restorations (28). Regarding the repair of composite resins, consensus has not been reached on the optimal protocol or materials for surface preparation of existing and aged composite resin surfaces in clinical practice. In this study, as stated in the literature, roughening of the surface with a bur was used in the surface preparation for the repair process (17). The main factor influencing the RBS has been proposed to be the type of composite resin (29) and some research has indicated that the repair should be done with composite resin of the exact origin as the composite used to perform the original restoration (30).

In this study, the same composite material was used for repair according to the literature. The application of the same bulk-fill composite, which may be explained by the presence of similar monomers in its composition, is expected to increase the effectiveness of the repair procedure that allows adequate copolymerization of methacrylate groups from new and aged composite materials (31). There was no statistically significant difference between the two silane-free universal adhesives used in this study and the repair SBS obtained by applying additional silane.

Furthermore, the absence of any favorable effect of silane in universal adhesives can be clarified by its low stability in aqueous acidic adhesive solution, where the silanol groups formed by hydrolysis can undergo dihydroxylation and condensation to form an oligomer that cannot adhere to glass (32). While some studies (33,34) found no difference in RBS using silane-containing and silane-free universal adhesive, another study showed that previous silane application improved immediate RBS. Nevertheless, once the bottle is opened, the hydrolyzed silane solutions become less and less reactive and prevent optimal adhesion in the long run (26). Cuevas-Suárez et al. (35) claimed that pre-treatment with a silane coupling agent and application of a hydrophobic resin could increase the bond strength of bulk-fill restorations. Contrary to the findings of Cuevas-Suárez et al., (35) it was found that additional silane application did not affect the bond strength in this study. A major factor influencing the bond strength of repair is considered to be the type of resin (36) and some research has suggested that the repair be done with the same composite resin used to perform it (30). It has also been observed that using the same bulk-fill composites in the repair procedure can increase the system's effectiveness. We can explain this with the existence of similar monomers, which allows for the

Table 2. Descriptive statistics values of shear bond strength of sonic-activated bulk-fill composite material

Groups	Ambar Universal (mean ± SD)	G-Premio Bond (mean ± SD)	p-value
Silan +	19.45±10.9	16.07±6.2	0.433
Silan -	21.88±6.4	18.8±6.6	

SD: Standard deviation

Table 3. Failure mode analysis of fractured surfaces after SBS test for all tested groups (%)

	Ambar Universal		G-Premio	
	Silane (+)	Silane (-)	Silane (+)	Silane (-)
Adhesive failure	70	50	70	100
Cohesive failure	20	40	20	0
Mixed failure	10	10	10	0

same copolymerization between methacrylate groups in new and existing composite materials (31).

In this study, stereomicroscope observations indicated that the failure modes were predominantly adhesive. In addition to adhesive failures, cohesive failures in the restorative material were also observed, and the application of additional silane did not reduce the adhesive failure rate. This is consistent with our SBS test results.

In this regard, it is worth noting that when performing a composite restoration, it is essential for the operator to accurately document the type of material used so that the repair can provide adequate bond strength if needed. In this study, it was found that there was no statistically significant difference between the groups with additional silane application and the groups without silane application. Therefore, it can be concluded that applying silane may not be necessary for the repair process of the bulk-fill composite resin. The results of this study show that the bulk-fill composite can be repaired with the same bulk-fill composite without the need for additional silane application. Including silane in universal adhesives may have questionable importance in clinical practice. Silanes in acidic conditions can become unstable due to self-condensation. The reaction of silanol groups (37) causes bond degradation over time (31). Therefore, further studies are needed to evaluate other roughening methods and materials.

Study Limitations

This study was limited to the use of a single composite resin. Only two silane-free adhesive systems were evaluated in this study. The surface treatment was restricted to bur-roughening. The SBS test was limited by the negative characteristics and inhomogeneity of stress distribution at the bond interface.

Conclusion

When repairing a SABF composite material with the same bulk-fil material, it can be repaired with silane-free universal adhesives without the need for an additional silane application.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Footnotes

Authorship Contributions

Concept: N.D., Design: N.D., Data Collection or Processing: A.A., N.D., Analysis or Interpretation: A.A., N.D., Literature Search: A.A., N.D., Writing: A.A., N.D.

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Original Article

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Surgical and Non-surgical Treatments in Pleural Empyema

Plevral Ampiyemde Cerrahi ve Cerrahi Olmayan Tedaviler

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ABSTRACT

Objective: Increasing number of patients are developing complicated pleural infection. We aimed at revealing the differences of treatment modalities performed in patients with empyema.

Methods: Patients those had been diagnosed and treated for empyema were assessed in a retrospective design. For the definitive diagnosis of empyema, thoracentesis was made. We categorized the patients in two groups as therapeutic drainage (group 1) and open surgery (group 2).

Results: A total of 360 patients, 57 of whom were women, were included. Tube drainage was applied to the patients in group 1, and therapeutic thoracentesis was applied to those not suitable for drainage. Patients who did not provide adequate drainage due to loculation were deloculated with fibrinolytic agent or video-assisted thoracoscopic surgery. In patients with advanced empyema with multiloculation and remarkable pleural thickening, which constituted group 2, decortication was performed by thoracotomy. Thoracoplasty was added in those who had insufficient lung volume. There were 292 (81.2%) patients in group-1 and 68 (18.8%) patients in group 2. In comparison of two groups, a significant difference was detected for lactate dehydrogenase ($p<0.001$) in pleural fluid and leukocyte count ($p=0.05$), hemoglobin ($p=0.01$), albumin ($p=0.002$), urea ($p=0.3$), and creatinine ($p=0.21$) levels in blood. The treatment results revealed no significant difference between three groups (recovered, sequelae changes, death), except for blood neutrophil count and antibiotic duration.

ÖZ

Amaç: Artan sayıda hastada komplike plevral enfeksiyon gelişmektedir. Biz ampiyemli hastalarda tedavi modaliteleri arasındaki farkları belirlemeyi hedefledik.

Yöntemler: Ampiyem teşhisi konulan ve tedavi edilen hastalar retrospektif bir tasarım ile değerlendirildi. Ampiyemin kesin teşhisi için torasentez yapıldı. Hastalar iki gruba ayrıldı; terapötik drenaj (grup 1) ve açık cerrahi (grup 2).

Bulgular: Çalışmada 57 kadın, toplam 360 hasta yer aldı. Grup 1'deki hastalara tüp drenaj ve tüp drenaj uygun olmayanlara terapötik torasentez uygulandı. Lokülasyon nedeniyle uygun drene edilemeyenlerde fibrinolitik ajanlar ya da video yardımlı torakoskopik cerrahi kullanıldı. Multilokülasyon ve belirgin plevral kalınlaşmaya sahip ileri ampiyemde (grup 2) torakotomi yoluyla dekortikasyon yapıldı. Yetersiz akciğer volümlü hastalarda torakoplasti uygulandı. Grup 1'de 292 hasta (%81,2) ve grup 2'de 68 hasta (%18,8) yer aldı. İki grup değişkenleri karşılaştırıldığında; plevral sıvıda laktat dehidrojenaz ($p<0,001$) ve kanda lökosit sayısı ($p=0,05$), hemoglobin ($p=0,01$), albumin ($p=0,002$), üre ($p=0,3$) ve kreatinin ($p=0,21$) düzeyleri açısından anlamlı farklılıklar izlendi. Tedavi sonuçları üç grup arasında (iyileşen, sekel gelişen, ölen) - kan nötrofil düzeyi ve antibiyotik süresi hariç - anlamlı farklılık göstermedi.

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ABSTRACT

Conclusion: Medical treatment plus therapeutic drainage therapy may be chosen as first treatment instead of open surgery in empyema.

Keywords: Thoracic empyema, surgery, video-assisted thoracic surgery, pleural fluid

ÖZ

Sonuç: Medikal tedavi ve terapötik drenaj tedavisi ampiyemde açık cerrahi yerine ilk tedavi yöntemi olarak tercih edilebilir.

Anahtar Sözcükler: Torasik ampiyem, cerrahi, video yardımlı torasik cerrahi, plevral sıvı

Introduction

Nearly 100 years ago, the management recommendations for empyema were early closed pleural drainage, avoidance of early open drainage, and sterilization of the empyema area. Though significant developments on antibiotherapy, radiology, intrapleural fibrinolytics, and minimally invasive surgery, these are still core principles. On the other hand, the morbidity, mortality, and burden of pleural infection remain high (1-3).

Patients with complicated pleural infection/empyema have been increasing, especially in children and older patients, requiring medical and surgical management. The optimal duration of therapy is not clearly defined. It is generally individualized based on the type of effusion, the adequacy of drainage, clinical and radiographic improvement and the immunity (4,5). Medical and surgical treatment both have complications. Also there are long-term complications of infection affecting pleural space including residual pleural thickening, and rarely, fibrothorax, bronchopleural fistula formation, empyema necessitatis and pleural calcification. The pleural involvement usually resolves over three to six months. Patients should be considered for decortication when they fail to improve and are symptomatic after six months. If an adequate and prompt therapy is administered, the long-term survival is good. Mortality is highest in those requiring open surgery or decortication. Comorbid conditions or surgical complications are usually the primary cause of death rather than empyema itself and is highest during the first 400 days (6,7).

Clinicians select type of antibiotic based on the site of acquisition, epidemiology, severity, individual risk factors for drug-resistant pathogens or infection with other specific organisms. Immunocompromised patients have higher hospitalization rates and mortality, and longer hospital stays. Pleural fluid biochemistry in the fibrinopurulent and organizing phases differ from those of the exudative phase requiring placement of a chest tube (4,5).

As empyema has poor prognosis, treatment should be started promptly at the time of diagnosis. Besides most patients recover, clinical outcomes remain poor with one in five patients requiring surgical intervention and 20% die within the first year of diagnosis (8).

The macroscopic appearance and smear/culture of pleural fluid may help to diagnose, while other biochemical and radiological characteristics are not specific. We aimed to reveal the role of blood/pleural fluid tests and patient-based properties as a prognostic marker in empyema. We aimed to reveal the consequences/differences of treatment modalities in empyema.

Methods

Design

The study was conducted in a retrospective design, which was approved by the institutional review board. Patients diagnosed as having empyema in clinic were included. Demographic characteristics of the patient population as well as routine blood test results, radiological imaging properties (thoracic ultrasonography and computed tomography) at the time of the first admission were retrieved from the hospital records. For the definitive diagnosis of empyema, the criteria for aspirating purulent fluid in thoracentesis with bacterial growth in the liquid sample taken and/or pH <7.20 and/or leukocytes >1,000/mm^3 was used.

Pulmonary function tests (PFTs) were performed using spirometry (Zan 500, Germany). Approval was obtained from the University of Health Sciences Türkiye, Dr. Suat Seren Chest Diseases and Thoracic Surgery Training and Research Hospital Ethics Committee (decision number: E.9801, date: 06.10.2017).

Definition of mortality: If a patient died any time from diagnosis to end of treatment, without any other reason, the result was evaluated as death.

The patients were evaluated as healed or sequelae according to the chest X-ray taken on 2nd week, 4th week and 3rd month control visits.

Thoracentesis: It was performed with a 16G branule from the region marked by thoracic ultrasonography under local anesthesia. Biochemical examinations, cytological examination and microbiological results of the aspirated fluid were evaluated. The number and duration of antibiotics given, and the surgical method applied were recorded.

Patients and Categorization

The patients were divided into two groups according to the treatment modalities, as therapeutic drainage (group 1) and open surgery (group 2). Tube drainage was applied to the patients in group 1, and therapeutic thoracentesis was applied to those not suitable for drainage. Patients who did not provide adequate drainage due to loculation were deloculated with fibrinolytic agent or video-assisted thoracoscopic surgery (VATS). In patients with advanced empyema with multiloculation and remarkable pleural thickening, which constituted group 2, decortication

was performed by thoracotomy. Addionally, thoracoplasty was added to patients who had insufficient lung volume. The RAPID score for pleural infection was used to predict mortality (9).

Statistical Analysis

The data were imported to a database formed by the Statistical Package for Social Sciences (SPSS) program V22 (IBM Corp, Armonk, New York, USA). Nominal variables were evaluated by their frequencies and percentages and compared by using cross tables. Continuous variables were used, and the normal distribution was examined by a normality test, graphical analysis, and by considering the sample size. Comparison of the variables was performed with parametric tests. One-Way ANOVA test was used for comparison of 3 groups. For all the statistical comparison tests, the probability of a type 1 error was α=0.05 and two sided. A p-value of <0.05 was considered statistically significant.

Results

A total of 360 patients, 57 of whom were women, were included. The mean age was 55.5±15.4 years; and 84.2% (n=303) was male. One hundred ninety-four (53.6%) patients had at least one comorbidity and 155 (61.5%) had smoking history. Most of the effusions were right sided (57.8%) while 43.3% of the effusions were free flowing. Most of the patients were treated with two or more antibiotics (n=248, 68.8%). Two hundred twenty-two (61.7%) patients were in the low-risk category of RAPID score, 17 patients (4.7%) were in high-risk group. There were 25 deaths (6.9%), and 143 patients (39.7%) were healed with sequelae.

Therapeutic drainage (therapeutic thoracentesis, VATS drainage, tube thoracostomy) was performed in 292 (81.2%, group 1) patients and open surgery (decortication with thoracotomy, thoracoplasty) was performed in 68 (18.8%, group 2) patients.

Group 1 and group 2 revealed no difference of outcome, as recovering with or without sequelae and death (p=0.36). But the patients in group 2 were mostly composed of low-risk patients according to the RAPID score (p=0.01). Pleural fluid lactate dehydrogenase (LDH) was significantly lower in patients of group 2 (median 2841 vs. 1670; p<0.001). Also, significant differences were present in blood leukocyteand neutrophil counts, hemoglobin, and albumin levels, all were higher in group 2 (respectively, p=0.05, p<0.001, p=0.01 and p=0.002). There was no difference in radiographic findings, PFTs, comorbidities, smoking history or antibiotherapy duration between the groups. Demographic characteristics of the study population and comparison of groups are presented in Table 1.

Study population was also categorized according to the treatment outcomes, as patients fully recovered without sequelae (n=192), patients with sequelae on radiography (n=143) and patients who died (n=25). Comparison of these groups revealed no difference in terms of age, gender, comorbidities, PFTs, pleural fluid analysis or RAPID score. Pleural fluid pH and albumin levels tended to be lower in patients with sequelae at the edge of significance

Table 1. The demography and comparison of group 1 (patients treated with therapeutic drainage) and group 2 (patients treated with open surgery)

Variables		Study population (n=360)	Group 1 (n=293)	Group 2 (n=67)	p-value
Age, years ± SD		55.5±15.4	57.1±15.1	48.6±1.8	<0.001
Gender, male		303 (84.2%)	243 (82.9%)	60 (89.6%)	0.2
Smoking history	Active	82 (32.5%)	64 (32.7%)	18 (32.1%)	0.69
	Ex-smoker	73 (29%)	59 (30.1%)	14 (25%)	
	Never smoker	97 (38.5%)	73 (37.2%)	24 (42.9%)	
Presence of comorbidities	None	167 (46.4%)	129 (44%)	38 (56.7%)	0.44
	1	155 (43.1%)	132 (45.1%)	23 (34.3%)	
	2 or more	38 (10.6%)	32 (10.9%)	6 (9%)	
Localization	Right	207 (57.8%)	169 (58.1%)	38 (56.7%)	0.25
	Left	137 (38.3%)	113 (38.8%)	24 (35.8%)	
	Bilateral	14 (3.9%)	9 (3.1%)	5 (7.5%)	
Radiographic appearance	Free	154 (43.3%)	125 (43.1%)	29 (43.9%)	0.99
	Loculated	137 (38.3%)	112 (38.6%)	25 (37.9%)	
	Multiloculated	65 (18.3%)	53 (18.3%)	12 (18.2%)	
Pulmonary function tests					
FEV1 (% ± SD)		57.0±17.6	56.4±17.7	58.5±17.3	0.49
FVC (% ± SD)		58.3±18.3	57.8±17.4	60.0±20.7	0.52
FEV1/FVC (n ± SD)		81.3±14.0	80.2±14.0	84.5±13.4	0.07

Table 1. Continued

Variables		Study population (n=360)	Group 1 (n=293)	Group 2 (n=67)	p-value
Laboratory parameters					
Pleural fluid					
pH		6.8±0.52	6.7±0.55	6.8±0.39	0.355
Glucose, mg/dL		5	5	2	0.751
Albumin, g/dL		1.7	1.7	1.9	0.559
LDH, u/L		2425	2841	1670	<0.001
Blood sample					
Leucocytes, /mm^3		12.400	12.000	14.000	0.05
Hemoglobin, gr/dL		11.4	11.4	11.8	0.01
Neutrophils, /mm^3		4.200	3.000	6.900	<0.001
CRP, mg/dL		14.7	15	12.8	0.4
LDH, u/L		185.5	182	187	0.61
Albumin, gr/dL		3.0	2.8	3.2	0.002
Protein, gr/dL		6.7	6.6	6.8	0.3
Urea, mg/dL		32	34	31	0.3
Creatinine, mg/dL		0.9	0.89	0.84	0.21
Polymicrobial growth		11	10	1	0.69
Number of antibiotics used	1	102	81	21	0.38
	2	183	154	29	
	3	52	40	12	
	4	13	9	4	
Treatment duration, months		1	1	1	0.35
RAPID score	0	48 (13.3%)	32 (10.9%)	16 (23.9%)	0.007
	1	81 (22.5%)	64 (21.8)	17 (25.4%)	
	2	93	75 (25.6%)	18 (26.9%)	
	3	74	61 (20.8%)	13 (19.4%)	
	4	47 (13.1%)	44 (15%)	3 (4.5%)	
	5	17 (4.7%)	17 (5.8%)	0	
RAPID risk category	Low-risk	222 (61.7%)	171 (58.4%)	51 (76.1%)	0.01
	Medium-risk	121 (33.6%)	105 (35.8%)	16 (23.9%)	
	High-risk	17 (4.7%)	17 (5.8%)	0	
Outcome	Recovered	192 (53.3%)	154 (52.6%)	38 (56.7%)	0.36
	Sequelae	143 (39.7%)	116 (39.6%)	27 (40.3%)	
	Death	25 (6.9%)	23 (7.8%)	2 (3%)	

FEV_1: Forced expiratory volume, FVC: Forced vital capacity, SD: Standard deviation

(respectively, p=0.09 and p=0.07). Among laboratory results, blood neutrophil count tended to be higher in patients who did not survive (p=0.03). Also, antibiotic duration was also shorter in this group of patients (p=0.03). Comparison of outcomes is presented in Table 2.

Discussion

When we compared the characteristics of patients treated with therapeutic drainage and patients who needed open surgery during the management of empyema, patients managed with open surgery were younger, with lower plural fluid LDH

Table 2. The outcomes of three groups: Recovered, sequelae changes, death

		Mean ± SD			
Variables		**Recovered (n=192)**	**Death (n=25)**	**Sequelae (n=143)**	**p-value**
Age (n)		55.9±15.3	55.7±15	55.2±15.5	0.951
Smoking, pack year (n)		38.4±28.8	39.5±31.4	39±23.8	0.991
FEV_1 (%)		60±15.3	56±14	56±19	0.576
FVC (%)		60±15	58±14	57±20	0.677
FEV_1/FVC (%)		82±14	80±13	81±14	0.885
Pleural fluid					
pH		6.8	6.9	6.8	0.09
Glucose (mg/dL)		7	6	2	0.42
Albumin (g/dL)		2.1	2.3	1.6	0.07
Lactate dehydrogenase (u/L)		1.848	2.876	3.167	0.37
Blood sample					
Leukocytes (x10^3/uL)		12.600	11.900	12.050	0.57
Hemoglobin (g/dL)		11.5	11.4	11.4	0.85
Neutrophile (x10^3/uL)		3.460	7.200	3.550	0.03
C-reactive protein (mg/dL)		13.7	12.4	16.2	0.79
Lactate dehydrogenase (u/L)		182	205	180	0.26
Albumin (g/dL)		2.9	2.6	2.9	0.25
Urea (mg/dL)		33	42.5	34.5	0.58
Creatinine (mg/dL)		0.87	1,04	0.89	0.36
Antibiotic duration (n)		1	0.85	1	0.03
RAPID score	Low risk	121 (63%)	12 (48%)	89 (62.2%)	0.26
	Medium risk	65 (33.9%)	10 (40%)	46 (32.2%)	
	High risk	6 (3.1%)	3 (12%)	8 (5.6%)	

FEV_1: Forced expiratory volume, FVC: Forced vital capacity, SD: Standard deviation

levels, and higher blood leukocyte and neutrophil counts, and hemoglobin and albumin levels. Also, their RAPID score was lower than patients treated with therapeutic drainage. There was not any difference of mortality among groups. No difference was found between groups of patients who died and who survived with or without sequelae, except blood neutrophil count and antibiotic treatment duration. The death rate was 6.9% in total, which is comparable to death rates in literature (10,11).

The decision to drain is based on the characteristics of the pleural fluid. The fluid should be drained immediately upon the appearance of pus. Biochemical properties of pleural fluid change in the presence of bacteria and inflammation. Pleural fluid pH, glucose and LDH levels are considered a priority in terms of drainage. In all patients with suspected (or diagnosed) empyema, initiation of antibiotics should be promptly and not be delayed for sampling or drainage procedures, etc. Prompt drainage of pleural fluid in addition to antibiotic therapy is also suggested. The initial procedure of choice is usually a single tube or catheter thoracostomy. Image-guided placement of a catheter(s) may be needed when pleural loculations prevent adequate drainage by a single tube (3,6,7,12,13). Simple surgical approach was performed in 81.4% and invasive surgical methods were performed in 18.6%. The average antibiotic duration was 1.77 months. In our patient population, patients who were treated with open surgery had comparatively better clinical condition with lower ages who comprised the patients suitable for major surgery. In addition, although higher mortality rates were reported in patients with empyema who were treated with pleural drainage with thoracoscopy alone (12-15), we observed no difference in mortality between treatment groups. The hospital mortality rate was lower in patients who underwent surgery than in those who underwent non-operative drainage (16). There are studies claiming otherwise. Although surgery was not possible because of poor clinical conditions in the non-survivor group, the mortality rate might have differed if surgery had been performed in anyone (17). It was also reported that the readmission and reintervention rates were higher in patients managed with chest tubes, suggesting a possible benefit from earlier surgical intervention (14,18).

In a serial of 71 patients, 11% died during treatment in hospital, where 89% underwent thoracic drainage; 70% were administered urokinase in the pleural cavity; and 11% underwent surgeries (17). The duration of antibiotic therapy and the length of hospital stay were 30 days and 23 days, respectively. Patients with poor performance score and patients with aspiration were significantly more common in the non-survivor group. Clinical condition, surgical intervention, and persistent pleural spaces were independent prognostic factors for empyema recurrence. In persistent pleural space, decortication and early thoracoplasty were advised (19).

Older age, high blood urea nitrogen (BUN) level, low serum albumin level, hospital-acquired infection, and the absence of purulence in RAPID scoring predicted poor prognosis at three months. High BUN level, an indicative of dehydration, is supposed to negatively affect prognosis (9,17). Although we found no difference in RAPID scores according to the patient outcomes, patients treated with open surgery tended to have lower RAPID scores which also reflected better clinical status of these patients.

There is a relation between albumin level, a plasma protein/marker of the nutritional status and infection. Importantly, polymicrobial etiology leads a poor prognosis for empyema (14,17,20). In categoric variables, gender, number of microorganisms isolated in culture, number of comorbidities, radiological location/appearance and number of antibiotics used were similar between two patient groups. Our culture results with polymicrobial growth were rare, so it was unavailable to retrieve robust data for these data.

Study Limitations

Being a monocentric retrospective study was a limitation. Although we had considerably high number of patients with real life data, it was impossible to eliminate selection bias.

On the other hand, we would like to point out that due to the retrospective design, surgery was performed as a necessity rather than an option for the patients included in the analysis. Indeed, various complications, sequelae and death were inevitable if good drainage was not provided. The similarity of these negative outcomes in treatment results was not an evidence of no need for surgery and drainage would be sufficient.

Conclusion

As a conclusion, it can be stated that medical treatment plus simple surgical intervention such as thoracentesis, tube thoracostomy, VATS, fibrinolytic therapy could be chosen as first treatment instead of more invasive intervention such as thoracoplasty, open surgery, decortication of the lung. More invasive techniques should be considered on individual cases, till more convincing evidence for better outcome are reported.

Ethics

Ethics Committee Approval: Approval was obtained from the University of Health Sciences Türkiye, Dr. Suat Seren Chest Diseases and Thoracic Surgery Training and Research Hospital Ethics Committee (decision number: E.9801, date: 06.10.2017).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.Ç.Ç., A.E.E., A.Ü., Concept: S.D., N.A., Design: S.D., N.A., Data Collection or Processing: S.D., N.A., Ö.Ö., G.V.Ş., E.Ç.Ç., M.O.G., F.G., Analysis or Interpretation: S.D., N.A., Ö.Ö., G.V.Ş., M.O.G., F.G., A.E.E., Writing: S.D., A.E.E.

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Original Article

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The Effects of Microseconds Electroporation on Pore Size, Viability and Mitochondrial Membrane Potential of Cervical Cancer Cells

Mikrosaniye Elektroporasyonun Rahim Ağzı Kanseri Hücrelerinin Gözenek Boyutu, Canlılığı ve Mitokondri Membran Potansiyeli Üzerine Etkileri

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ABSTRACT

Objective: Electroporation (EP) is a method in which the membrane permeability is increased by applying electrical pulses. The determination of modifications that occur in cells subsequent to EP with varying pulse parameters holds significant importance in establishing the foundations of EP theory. Therefore, we sought clarification regarding the phenomenon of pore formation on the membrane of the electroporated human cervical cancer cell line (HeLa) cells.

Methods: The pores created on the cell membrane due to EP was observed using a scanning electron microscope. The change in the viability and mitochondrial membrane potential (ΔΨm) of cells was determined by WST-8 and JC-1 assays.

Results: The surface of the electroporated cell membrane exhibited a relatively uniform pore population. The viability of HeLa cells was significantly reduced with increasing electric field intensities. A slight decrease in ΔΨm was observed between the control and the 0.8 and 1.6 kV/cm EP groups, but ΔΨm was higher in the 2.4 and 3.2 kV/cm EP groups compared to the control group.

Conclusion: In conclusion, our study showed that the application of EP to the cervical cancer cell line resulted in the formation of pores of varying sizes on the membrane. While cell viability decreased with increasing electric field amplitude, no significant

ÖZ

Amaç: Elektroporasyon (EP), elektrik darbeleri uygulanarak membran geçirgenliğinin artırıldığı bir yöntemdir. EP sonrası hücrelerde değişen nabız parametreleriyle meydana gelen değişikliklerin belirlenmesi, EP teorisinin temellerinin oluşturulmasında büyük önem taşımaktadır. Bu nedenle, EP'ye tabi tutulan insan rahim ağzı kanseri hücre dizisi (HeLa) hücrelerinin zarı üzerinde gözenek oluşumu olgusuna ilişkin açıklama aradık.

Yöntemler: EP'ye bağlı olarak hücre zarında oluşan gözenekler taramalı elektron mikroskobu kullanılarak gözlemlendi. Hücrelerin canlılığı ve mitokondriyal membran potansiyelindeki (ΔΨm) değişiklik, WST-8 ve JC-1 analizleri ile belirlendi.

Bulgular: EP'ye tabi tutulmuş hücre zarının yüzeyi nispeten tekdüze bir gözenek popülasyonu sergiledi. HeLa hücrelerinin canlılığı, artan elektrik alan yoğunluklarıyla önemli ölçüde azaldı. Kontrol grubu ile 0,8 ve 1,6 kV/cm EP grupları arasında ΔΨm'de hafif bir azalma gözlendi ancak 2,4 ve 3,2 kV/cm EP gruplarında ΔΨm kontrol grubuna göre daha yüksekti.

Sonuç: Sonuç olarak çalışmamız, EP'nin rahim ağzı kanseri hücre hattına uygulanmasının, membran üzerinde değişen boyutlarda gözeneklerin oluşmasına neden olduğunu gösterdi. Elektrik alan genliğinin artmasıyla hücre canlılığı azalırken, EP tedavisi ve kontrol grupları arasında ΔΨm'de anlamlı bir değişiklik gözlenmedi. EP'ye

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ABSTRACT

change was observed in ΔΨm between EP treatment and control groups. It should be noted that further research is needed to determine the pore distributions in electroporated cells and the resulting changes at different electric field amplitudes.

Keywords: Electroporation, cervical cancer, pore size, cell viability, mitochondrial membrane potential

ÖZ

tabi tutulmuş hücrelerdeki gözenek dağılımlarını ve farklı elektrik alanı genliklerinde ortaya çıkan değişiklikleri belirlemek için daha fazla araştırmaya ihtiyaç olduğu unutulmamalıdır.

Anahtar Sözcükler: Elektroporasyon, rahim ağzı kanseri, gözenek boyutu, hücre canlılığı, mitokondri membran potansiyeli

Introduction

The membrane permeability is risen by the application of electrical field pulses of appropriate amplitude and parameters. This method is referred to as "electropermeabilization" or "electroporation" (EP). With EP application, particles that cannot cross the membrane under normal cell conditions are allowed to pass through the membrane. Intense and short-term electrical pulses result in an rise in the transmembrane potential (TMP) on the cell membrane (1-5). When the TMP reaches a critical value, the formation of aqueous pores will allow molecular transitions through the membrane. Despite the fact that a precise mechanism at the molecular level cannot be fully expressed, molecular flow has been demonstrated in the membrane regions where the highest TMP is observed (6-8). The effectiveness of EP depends on the applied electrical pulse parameters (duration, intensity pulse shape and pulse number). Based on the impact of these parameters, EP can be reversible or irreversible (9-11). Reversible EP has many applications in the fields of medicine and biotechnology including electrogenotherapy and electrochemotherapy (ECT) (5,12). Irreversible EP is utilized for tumor ablation (due to its non-thermal effect) and sterilization purposes (11-13).

The amount of molecules penetrating into the cell by EP is related to the size of the pores formed on the membrane. Hence, it is very important to determine the size of pores created on cell membrane owing to application of short-term and high-intensity electrical pulses to enhance the efficacy of EP. There are several theoretical and experimental studies on the pore formation kinetics and reclosing in the literature. Different pore sizes and resealing times have been reported depending on different cell lines and EP parameters in these studies. Furthermore, there are a limited number of studies that demonstrate the presence of electropores (14-19).

The cells frequently adapt to physiological requirements in order to maintain their vitality and internal balance. Cell injury is defined as the adverse effects of internal or external stimuli that are severe enough to disrupt cell homeostasis or where the cell is unable to adjust without being damaged. As a consequence of cell injury, the cell may or may not be repaired and the cell death may occur. The primary causes of cell damage include damage to membranes, DNA, proteins, and mitochondria, as well as an upraised amount of intracellular calcium and reactive oxygen species (ROS) (20-22).

The application of EP may result in lipid peroxidation and damages to proteins embedded within the cell membrane. Therefore, the formation of pores due to EP is a cell injury (23-25). The main cause of cell death after EP remains unclear, as it overlaps with many types of cell damage and cell death. The excessive entry of Ca^{2+} into the cell may result in cell death due to EP. Ca^{2+} regulates many cellular mechanisms including cell proliferation, cell death and cell cycle. The pulsed electric fields (PEFs) may also trigger oxidative stress and initiate the production of ROS. Furthermore, EP administration may cause damage to mitochondria, which are known to play an important role in apoptosis. The causes of cell death after EP are closely related to each other. For this reason, it is quite complex to determine which death stimulus is the effect or result of the pathological condition that occurs after EP (26-30). Mitochondria have an important role in the intrinsic death pathways. It may be beneficial to understand the cellular mechanisms of action of EP by determining the change in mitochondrial membrane potential (ΔΨm) due to the application of PEF, EP may cause a loss of ΔΨm.

Chemotherapy is a prevalent treatment option for cervical cancer. The administration of substantial doses of chemotherapeutic agents utilized in treatment can nevertheless result in significant side effects. Research has shown that ECT is an influential treatment method for certain types of cancer (12,31) providing low doses and a significant decrease in side effects. Nevertheless, the lack of a study examining the formation of pores on electroporated human cervical cancer cell line (HeLa) cell membrane, suggests a deficiency in this field. We aimed to investigate the effects of EP application on the cell viability and ΔΨm in HeLa cells and to determine the size of electropores created on the surface of the HeLa cell membrane after EP application.

Methods

Cell Culture and Electroporation Protocol

The human cervical cancer cell line HeLa was used in our study. The cells were incubated with culture medium at 5% CO_2 and 37 °C [cell culture medium; (Dulbecco's Modified Eagle's medium (DMEM), Capricorn DMEM-HA) containing 10% fetal bovine serum (Capricorn FBS-11A) and 1% penicillin/streptomycin antibiotic (PSA, HyClone)]. 10^5 cells at 100 µL were incorporated into EP cuvettes with a gap of 4 mm and subsequently placed in the BTX pulse generator chamber. Then, increasing electric field amplitudes of 0.8 and 3.2 kV/cm at 100 µs pulse width, eight square waves and 1 Hz repetition frequency were applied to each EP cuvette. After each application, the cells were kept in the room environment for approximately 10

minutes. Then, ten thousand cells per 100 µL were seeded into 96-well plates and incubated for 24 hours. Control cells were placed in the EP chamber in the bathtub but no EP treatment was made.

Scanning Electron Microscope and the Distribution of Pore Size

The pores created on the plasma membrane of HeLa cells at an electric field strength of 1.6 kV/cm were observed by scanning electron microscope (SEM). The electric field strength of 1.6 kV/cm was found to be the optimal electric field strength with minimal cell death and maximum membrane permeability for HeLa ECT (32). SEM is a highly effective technique for obtaining both quantitative and qualitative data regarding porous structures. It is commonly used for the determination of the average size of pores and their distributions. In order to prevent the closure of pores, cells were placed on ice for a duration of 10 minutes subsequent to the application of EP prior to undergoing SEM observations (14,15). Cells were washed with PBS solution three times and fixed with 2.5% glutaraldehyde (Capricorn PBS-1A) After that, the samples were rinsed with PBS and the glutaraldehyde was removed. This was followed by the dehydration of samples with increasing concentrations of ethanol from 30% to 100%. After dehydration, the samples were dried and subsequently photographed using a high resolution SEM at various positions with a suitable magnification (x100.000-x200.000). The measurements of the size of pores created by electrical pulses were determined from SEM images using ImageJ, which is unable to provide an accurate analysis of scientific images. We analyzed 10 SEM micrographs and measured pore size at randomly selected locations in each SEM micrograph. The size distribution of pores created by electrical pulses was determined by measuring the radius of 250 pores on the entire cell membrane.

The Measurement of Cell Viability and ΔΨm

Cell viability was measured 24 hours after EP treatment using the WST-8 assay. 10 µL of cell proliferation reagent WST-8 (ABP Biosciences) was added to each well, followed by a three-hour incubation. After a period of waiting, the absorbance values at the appropriate wavelength (450 nm) were determined using an ELISA reader. The cell viability results were presented as a percentage relative to the control cells. ΔΨm of the cells was measured JC-1 (ABP Biosciences) assay 24 hours after EP. After incubation, the cell culture medium was aspirated, 200 µL of PBS and 2 µL of JC-1 stock solution was added to each well (Capricorn PBS-1A). After half an hour of incubation in the incubator, the well was washed twice with PBS and the same amount of PBS was added each well. The ratio of red to green was determined by measuring the green and red fluorescence values with a spectrofluorometric plate reader.

Statistical Analysis

All experiments were performed in triplicate and data are presented as mean standard deviation. Control comparisons were made using One-Way ANOVA test and Tukey test was used as post-hoc. $P<0.01$ was considered statistically significant. GraphPad Prism 9 program was used for statistical analysis.

Results

The surface of the electroporated membrane was observed by means of SEM. High intensity µs EP induced nanopores on HeLa cell membrane (Figure 1). The surface of the plasma membrane of the electroporated HeLa cells showed relatively homogeneous pore populations. On the non-electroporated control HeLa cells, no pore-like structures were observed. The surface of the non-electroporated cell membrane exhibited a rougher surface than

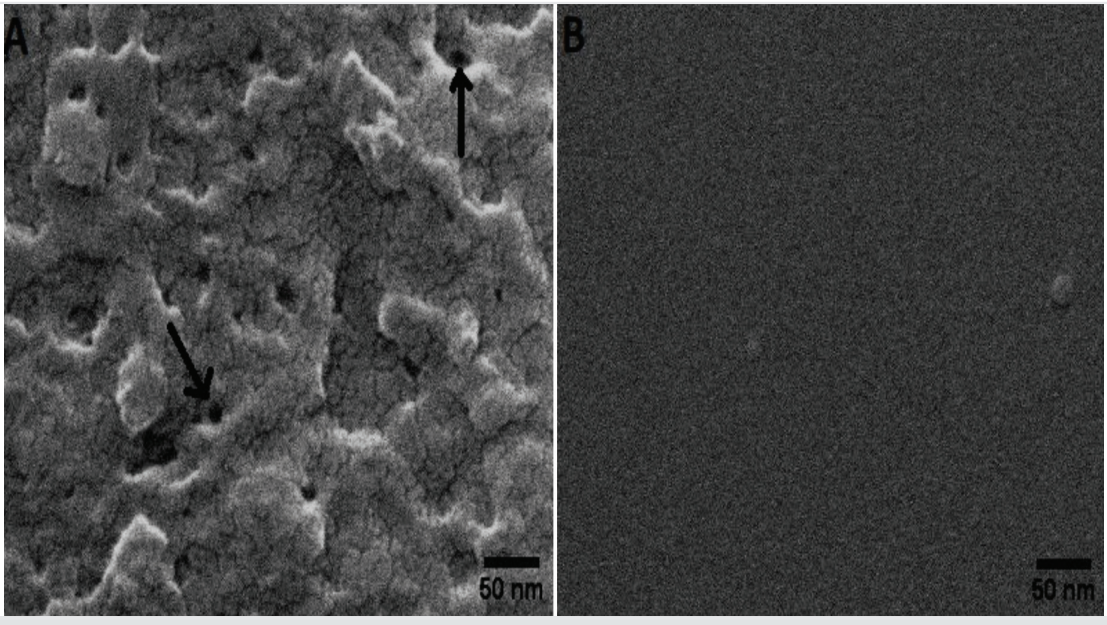

Figure 1. SEM images of A) electroporated and B) non-electroporated HeLa cell membrane, the black arrows indicate pores
SEM: Scanning electron microscope, HeLa: Human cervical cancer cell line

that of the electroporated cell membrane. The mean radius of electropores created on the electroporated HeLa cell membrane was 9.23 nm (±2.8 nm) (Figure 2). The mean pore size observed in this study was large enough to enable the passage of many chemotherapeutic agents through the cell membrane.

The viability of HeLa cells for four different electric field strengths having repetition frequency of 1 Hz, an eight square waves and pulse duration of 100 μs parameters which are used in classical ECT treatment was given in Figure 3. As shown in figure, a slight reduce in cell viability was observed at the electric field intensity of 0.8 kV/cm, even so, it was not statistically significant ($p>0.05$). The change in cell viability at 1.6 kV/cm of electric field strength was statistically significant when compared to the control group. We found that the cell viability was 27.09% at 3.2 kV/cm, which we selected as the highest field strength ($p<0.0001$). Figure 4 depicts the morphological modifications of the cells in response to four distinct field strengths applied under an inverted microscope. The live cells had transparent, light-colored, and clearly defined membranes. At high electric field strength levels (2.4 and 3.2 kV/cm), the cell shape was distorted. The electric field amplitude of 1.6 kV/cm was the critical amplitude for cell viability. The viability of cells was significantly reduced above this electric field amplitude due to EP application.

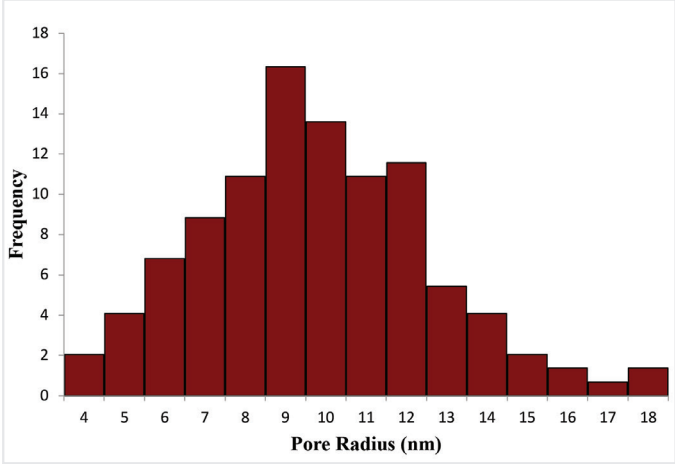

Figure 2. Pore size distribution histogram of electropores created on HeLa cell membrane

HeLa: Human cervical cancer cell line

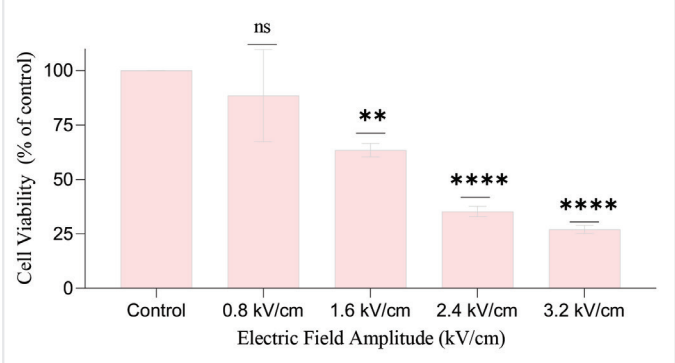

Figure 3. Cell viability in cells at increasing electric field amplitudes (0-3.2 kV/cm)

The change in ΔΨm, which occurs at these intensities by changing only the electric field intensities from the pulse parameters, was depicted in Figure 5. The slight depolarization tendency was determined in ΔΨm in EP applications with an electric field amplitude between 0.8 and 1.6 kV/cm ($p>0.05$). When we further increased the electric field amplitude, the change in ΔΨm tended to increase, but this increase was not statistically significant compared to the control group ($p>0.05$). At low electric field intensities, a slight depolarization tendency was observed in the ΔΨm. However, fluctuating changes occurred at high electric field amplitudes.

Discussion

EP treatment has the ability to induce various types of cell death, inclusive of apoptosis and necrosis. This circumstance is closely associated with the cell type application conditions and EP parameters employed in the study (23,33,34). In this study, we examined the impact of electrical pulses having amplitudes of 0.8, 1.6, 2.4, and 3.2 kV/cm, a pulse width 100 μs, an eight square waves and a repetition frequency of 1 Hz on the viability and ΔΨm of HeLa cells. The EP pulse parameters; 100 μs pulse width, eight square waves and 1 Hz repetition frequency are commonly employed in ECT applications. The viability of HeLa cells decreased with the increase in the applied electric field amplitude. The cell viability was found to be 88.5% and 63.49%, respectively, at electric field intensities of 0.8 and 1.6 kV/cm. These field intensities were among the most efficient values for ECT applications. The application of EP had a greater damaging effect on HeLa cells at higher electric field amplitudes. The cell viability was significantly decreased to 35.35% (compared to control cells) at a field strength of 2.4 kV/cm.

Zhou et al. (35) studied the effects of EP pulse amplitude (0-1000 V) with a pulse duration of 100 s and a repetition frequency of 1 Hz on HeLa cells. They found that 400 V was the threshold pulse amplitude level for reversible EP, and cell viability was decreased with an increase in pulse amplitude. Miller et al. (36) demonstrated that besides the thermal effects of an IRE application, whole-cell ablation could occur in different electrical parameters. Another study investigated the reversible and irreversible EP parameters of HepG2 cells. This study revealed that the IRE electric field amplitude was approximately 4 kV/cm for almost all types of cell death. Furthermore, it was found that the maximum electric field amplitude for reversible EP was 1 kV/cm (37). Saczko and colleagues conducted an ECT study on OvBH-1 and SKOV-3 cells, and reported that EP was safe up to 2 kV/cm (38).

In this study, we examined the change in ΔΨm resulting from EP application for four distinct electric field amplitudes. A slight decrease in ΔΨm was observed between the control and the 0.8 and 1.6 kV/cm EP groups, but ΔΨm was higher in the 2.4 and 3.2 kV/cm EP groups compared to the control group. Beebe et al. (26) reported an enhancement in cell

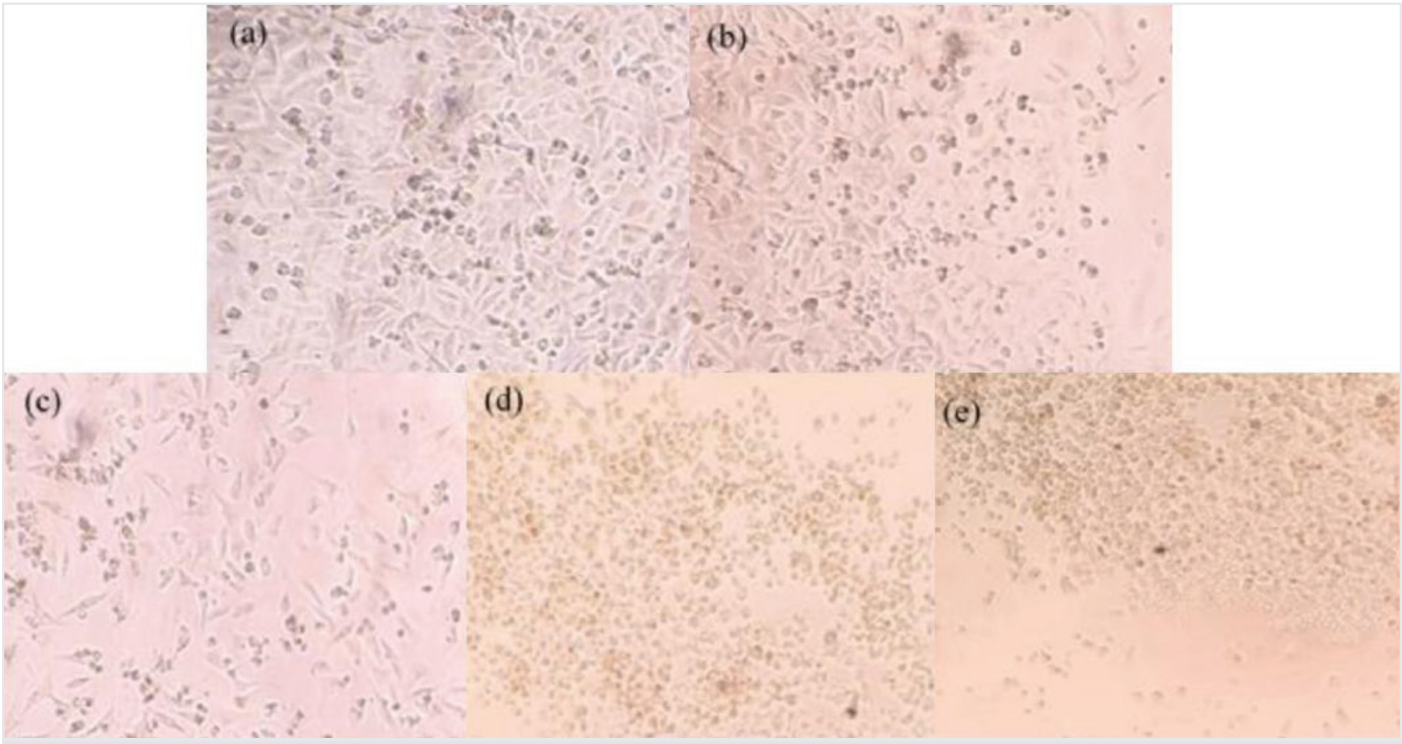

Figure 4. Invert microscope images of EP applications at x100 magnification in HeLa cell line, (a) control, (b) 0.8 kV/cm, (c) 1.6 kV/cm, (d) 2.4 kV/cm, (e) 3.2 kV/cm. It has seen that the number of HeLa cells decreases and cell morphologies are distrupted as the electric field value increases

EP: Electroporation, HeLa: Human cervical cancer cell line

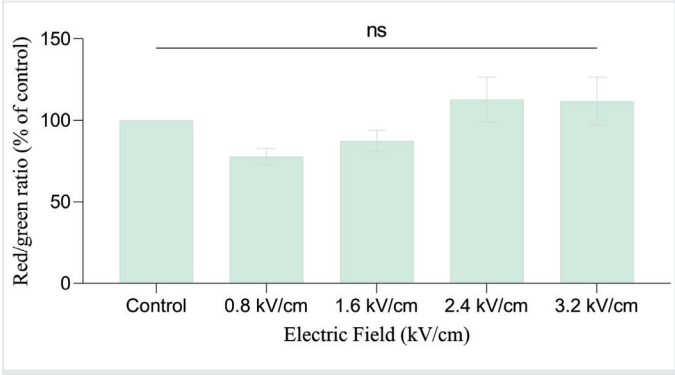

Figure 5. Mitochondrial membrane potential change at increasing electric field amplitudes in HeLa cells (0-3.2 kV/cm)

HeLa: Human cervical cancer cell line

viability and distruption of ΔΨm following the application of a nanosecond PEF (nsPEF) for varying exposure periods in N1-S1 hepatocellular carcinoma cell line. They observed that the primary trigger for cell death was the loss of ΔΨm resulting from the application of EP. Another study performed in human T lymphocyte cells reported decreased ΔΨm and increased mitochondrial membrane permeability following to application of nsPEF (39). In their study on the SMMC-7721 cell line, Mi et al. (30) found a gradual decrease in ΔΨm depending on the voltage amplitude and pulse duration. They found that higher field strengths and longer exposure periods were more effective. The study performed on HepG2 cell line demonstrated that the application of prolonged electrical pulses with an electric field amplitude of 10 kV/cm, a pulse duration of 500 ns, and a repetition frequency of 1 Hz induced apoptosis (40). The study also reported an increase in intracellular Ca^{2+} level and loss of ΔΨm. Gibot et al. (41) applied EP in combination with Ca^{2+} to human dermal fibroblasts and HCT-116 line and showed that the combined application of EP in combination with Ca^{2+} induced cell death without induction of genotoxicity. They concluded that the cytotoxicity resulted from the application of EP in combination with Ca^{2+} was associated with a dramatic decrease in ΔΨm and ATP depletion (41). Until now, it has been demonstrated that a reduction in ΔΨm leads to apoptotic cell death. However, recent scientific studies have demonstrated that an increase in m may be responsible for apoptosis (42-44). Furthermore, excess levels of ROS, ATP and Ca^{2+} may affect mitochondrial activity. All of these factors make up a complex network capable of influencing each other. This intricate sequence renders the explanation of mitochondrial dysfunction arduous (29).

We observed a pore size distribution ranging from 4 to 18 nm, and the most common pore size was around 9 nm in this study. There are a limited number of studies that have examined the impact of EP on pore size. This is the reason we conducted this study. An electrical pulse EP of 40 kV/m for a duration of one microsecond caused the formation of electropores with an

average radius of 22 nm (15). The study conducted by Chang and Reese (14) revealed the existence of a non-homogeneous pore distribution on the membrane of human red blood cells. Some other authors also reported different average pore sizes depending on pulse parameters such as amplitude and duration of electrical pulses used in the studies (15,45-50).

Study Limitations

In this study, the distribution of pore sizes formed in the membrane by EP application in HeLa cells was examined. In addition, in our study, changes in cell viability and mitochondria membrane potential were demonstrated with EP application. Examining pore formation is a very difficult and complex process. Therefore, the limitation of the study was that the pore formation was examined at a single electric field value. This study lays an important foundation for examining membrane pore formation in cervical cancer cells by EP. We think that examining cellular changes in different EP parameters will allow more comprehensive findings to be obtained.

Conclusion

In summary, it was demonstrated that the application of EP to the cervical cancer cell line resulted in the formation of pores of varying sizes on the membrane. It was observed that increasing the electric field amplitude led to a significant decrease in cell viability after a critical value, but no significant change in $\Delta\Psi m$ was observed between EP treatment and control groups.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

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Footnotes

Authorship Contributions

Design: G.G., M.A.E., Data Collection or Processing: G.G., M.A.E., Z.Ç., Analysis or Interpretation: G.G., M.A.E., Z.Ç., Literature Search: G.G., M.A.E., Z.Ç., Writing: G.G., M.A.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Is There a Relationship between the Staining Pattern of Classical Neuroendocrine Markers and Clinicopathological Findings in Neuroendocrine Tumors of the Appendix?

Apendiksin Nöroendokrin Tümörlerinde Klasik Nöroendokrin Belirteçlerin Boyanma Paterni ile Klinikopatolojik Bulgular Arasında Bir İlişki Var Mıdır?

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ABSTRACT

Objective: This study investigates the association between staining patterns of classical neuroendocrine markers, synaptophysin (Snp) and chromogranin-A (Chr), and clinicopathological findings in appendiceal neuroendocrine tumors (NETs). These tumors, often diagnosed incidentally post-appendectomy, pose diagnostic challenges due to their diverse histomorphologic patterns. The study aims to enhance understanding of the relationship between staining patterns and key pathological parameters.

Methods: A retrospective analysis included 28 cases of appendiceal NETs diagnosed over an 8-year period. Histopathological features, including grade, lymphovascular invasion, stage, localization, size, Ki67 proliferation index, and morphological pattern, were reassessed. Immunohistochemical staining of Snp and Chr was examined for extensity and intensity in NET areas.

Results: The study comprised 17 female and 11 male patients, with a mean age of 34 years. Histomorphological patterns included small nests, large nests, and trabecular-palisading patterns. Statistically significant correlations were observed between Snp staining intensity and tumor size, and Chr staining extensity and histomorphologic patterns. Chr staining extensity exceeding 95% was identified in all cases with large nest patterns. Additionally,

ÖZ

Amaç: Bu çalışmada, apendiks nöroendokrin tümörlerinde (NET) klasik nöroendokrin belirteçler olan sinaptofizin (Snp) ve kromogranin-A (Chr) boyanma paternleri ile klinikopatolojik bulgular arasındaki ilişki araştırılmıştır. Genellikle apendektomi sonrası tesadüfen teşhis edilen bu tümörler, çeşitli histomorfolojik paternleri nedeniyle tanısal zorluklar oluşturmaktadır. Bu çalışma, boyanma paternleri ile anahtar patolojik parametreler arasındaki ilişkinin daha iyi anlaşılmasını amaçlamaktadır.

Yöntemler: Retrospektif bir analiz, 8 yıllık bir süre içinde tanı konulan 28 apendiks NET olgusunu içermektedir. Derece, lenfovasküler invazyon, evre, lokalizasyon, boyut, Ki67 proliferasyon indeksi ve morfolojik patern dahil olmak üzere histopatolojik özellikler yeniden değerlendirildi. Snp ve Chr'nin immünohistokimyasal boyanması NET alanlarındaki yaygınlık ve yoğunluk açısından incelendi.

Bulgular: Çalışmaya yaş ortalaması 34 olan 17 kadın ve 11 erkek hasta dahil edildi. Histomorfolojik paternler arasında küçük yuvalar, büyük yuvalar ve trabeküler-palizadlanma vardı. Snp boyanma yoğunluğu ile tümör boyutu ve Chr boyanma yoğunluğu ile histomorfolojik paternler arasında istatistiksel olarak anlamlı korelasyonlar gözlendi. Büyük yuva paterni olan tüm olgularda Chr

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ABSTRACT

Chr staining extensity increased with advancing pathological stage, notably between pT1-pT4 groups.

Conclusion: This study emphasizes the importance of immunohistochemical evaluation, particularly in distinguishing between L and EC cells in appendiceal NETs. The unique trabecular-palisading pattern, associated with L cell histomorphology, demonstrated significant correlations with Chr staining patterns. Snp staining intensity correlated with tumor size, while Chr staining percentage increased with advanced pathological stages. The findings suggest that it will create awareness in pathology practice in terms of both diagnosis and pathological prognostic parameters (tumor size, stage).

Keywords: Appendiceal neuroendocrine tumors, synaptophysin, chromogranin-A, histopathology, immunohistochemistry

ÖZ

boyanma yaygınlığının %95'i aştığı tespit edilmiştir. Ek olarak, Chr boyanma yaygınlığı, özellikle pT1-pT4 grupları arasında olmak üzere, ilerleyen patolojik evre ile artmıştır.

Sonuç: Bu çalışma, apendiks NET'lerinde özellikle L ve EC hücrelerinin ayırt edilmesinde immünohistokimyasal değerlendirmenin önemini vurgulamaktadır. L hücre histomorfolojisi ile ilişkili benzersiz trabeküler-palizatlanma paterni, Chr boyanma paterni ile anlamlı korelasyonlar göstermiştir. Snp boyanma yoğunluğu tümör boyutu ile korelasyon gösterirken, Chr boyanma yüzdesi ileri patolojik evrelerde artmıştır. Bulgular patoloji pratiğinde hem tanı hem de patolojik prognostik parametreler (tümör boyutu, evre) açısından farkındalık yaratacağını düşündürmektedir.

Anahtar Sözcükler: Apendiks nöroendokrin tümörleri, sinaptofizin, kromogranin-A, histopatoloji, immünohistokimya

Introduction

Appendiceal neuroendocrine tumors (NETs) are usually diagnosed incidentally after appendectomies for acute appendicitis (1). They are found in approximately 2% of appendectomies (2). Most tumors are too small to be detected clinically and since the tumor is usually located at the apex, it is unlikely to cause obstruction due to mass effect (2,3). Therefore, the follow-up of the patient after appendectomy depends on the parameters in the pathology report. Appendiceal NETs usually develop from enterochromaffin-like serotonin-secreting EC cells that form insular pattern, solid nests and nodules, and more rarely from glucagon like protein-1 and other proglucagon-related peptide secreting L cells, which usually show trabecular growth pattern (1). It has also been observed that both cell types can cause tumors in a glandular-tubular pattern. Synaptophysin (Snp) and chromogranin-A (Chr), known as classical neuroendocrine markers, are positive in most appendiceal NETs (1). Since Snp can also be positive in tumors other than NETs, its specificity is controversial; therefore, especially Chr positivity is diagnostically important (4). Immunohistochemical and morphologic findings to concretely determine the L cell phenotype are not clear. Glucagon 1, glucagon 2 and peptide YY antibodies have been used to detect L cells and it was found that glucagon 2 was more sensitive (5,6). However, application of these stains in daily use is not suitable in terms of cost and practicality. Appendiceal NETs usually react positively with Snp and Chr, but it is known that L cell types may not stain with Chr as in rectal NETs. This may cause diagnostic difficulties. The aim of this study was to examine the relationship between Snp and Chr staining pattern and clinical findings and to investigate the relationship between staining pattern and histopathologic findings which might cause diagnostic difficulties.

Methods

Material and Case Selection

There were 33 cases of appendiceal NET diagnosed in our pathology clinic within an 8-year period (2015-2023). Five cases were excluded from the study because their slides could not be reached. In our retrospective study, 28 cases were included. Histopathological findings (histological grade, lymphovascular/perineural invasion, stage, tumor localization, tumor size, Ki67 proliferation index, morphological pattern "small-large nest, trabecular-palisading") were re-evaluated on Hematoxylin-Eosin stained slides. Immunohistochemical Chr and Snp stained slides were examined for staining extensity (percentage) and staining intensity (absent, weak, moderate, severe) in NET areas. Clinical findings were obtained from the hospital information system.

Ethical statement: The ethics committee approval of our study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Istanbul Training and Research Hospital with the decision number 357, date: 22.12.2023.

Informed Consent: The archival material of the patients was used and no additional procedures were performed. The study was completely retrospective.

Statistical Analysis

SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) program version 26.0 was used for statistical analysis of the data in our study. In descriptive statistics, mean value, standard deviation, median, minimum and maximum values for continuous variables and number and percentage values for discrete variables were calculated. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normal distribution as initial analysis. Mann-Whitney U and chi-square tests were used to compare the data between two groups. Pearson correlation test was used for correlation analysis of binary data. The results were evaluated at 95% confidence interval and $p<0.05$ was defined as statistical significance.

Results

Seventeen of the patients were female and 11 were male. The mean age was 34 years (range 14 to 70 years). The mean tumor

size was 1.07 cm and ranged between 0.2-5 cm. Seventeen cases were located in the distal (68%), 4 cases in the proximal (16%), 2 cases in the middle part (8%) of the appendix, and 2 cases involved the appendix diffusely (8%). Since three cases were fragmented, no comment could be made about the localization. The number of cases with histologic grade 1 was 21 and grade 2 was 7. In terms of the predominant histomorphologic pattern, 19 cases showed small nest pattern, 7 cases showed large nest pattern and 2 cases showed trabecular - palisading pattern. There were 2 cases with tubular pattern. One of these cases had a predominant pattern of small nest while the other had a predominant pattern of large nest and was included in these groups. Lymphovascular invasion was detected in six cases and 4 of them showed small nest pattern. There were 10 cases in T1 stage, 13 cases in T3 stage, 5 cases in T4 stage and no cases in T2 stage. Both of the cases with trabecular-palisading pattern were in T1 stage and the tumor diameter was 0.5 cm (Table 1). In all cases, Snp showed moderate to strong staining in more than 90% of the tumor, with no weak staining. Chr staining with an extensity of more than 95% was detected in all cases with large nest pattern (Figure 1). There were 6 cases with Chr staining in less than 90% of the tumor area. In 4 of them, less than 50% and weak staining was seen. Two of these 4 cases showed trabecular-palisading pattern and two of them showed small nest pattern (Figure 2).

A statistically significant relationship was found between Snp staining intensity and tumor size (p=0.042). The staining intensity increased as the tumor size increased. When histomorphologic patterns were evaluated in terms of Chr staining extensity (SE), a statistically significant correlation was found (p=0.029). In addition, Chr SE increased with increasing pathologic stage, which was statistically significant especially between pT1-pT4 groups (p=0.038). No significant correlation was found between other parameters and staining patterns.

Discussion

Appendiceal NETs are frequently encountered incidentally as small-sized tumors (1). For pathologic diagnosis, immunohistochemical findings are indispensable for both diagnosis and differential diagnosis (4). EC and L cells are important components reflecting the histopathologic and phenotypic diversity of NETs of the appendix. Although the prognostic significance of hormonal differences in NETs originating from L or EC cells has not been demonstrated, the differences of these cell types play an important role in determining the histopathologic diagnosis (7). They may present diagnostic difficulties due to factors such as patchy-poor staining with Chr. L cells may generally be less reactive to neuroendocrine markers than EC cells (1). In a study conducted by Sohn et al. (6) in rectal NET cases, L cells were detected mostly in G1, G2 tumors located in the mucosa and submucosa below 1 cm. In our study, similarly, tumors showing trabecular-palisading feature (L cell histomorphology) were found to be at early stage (T1), and have small tumor size (0.5 cm) and G1-G2 grades. Although the number of cases showing trabecular - palisading pattern was small in our study, the findings were statistically significant in terms of Chr SE in these cases. In this pattern, Chr expression is highly reduced (especially staining prevalence is between 5-10%) while Snp is positive in these areas (moderate or strong

Table 1. Synaptophysin and chromogranin-A staining patterns according to histopathologic findings

	Synaptophysin staining extensity		Chromogranin-A staining extensity		Synaptophysin staining intensity			Chromogranin-A staining intensity		
	≤90%	>90%	≤90%	>90%	Weak	Moderate	Strong	Weak	Moderate	Strong
Pathologic stage										
T1 (n=10) n (%)	4 (40)	6 (60)	6 (60)	4 (40)	0	8 (80)	2 (20)	4 (40)	2 (20)	4 (40)
T3 (n=13) n (%)	2 (15)	11 (85)	1 (7)	12 (93)	0	6 (46)	7 (54)	0	3 (23)	10 (77)
T4 (n=5) n (%)	0	5 (100)	0	5 (100)	0	2 (40)	3 (60)	0	1 (20)	4 (80)
p-value	0.16		**0.016**		0.19			0.06		
Histologic grade										
Grade 1 (n=21) n (%)	6 (28)	15 (72)	6 (28)	15 (72)	0	12 (58)	9 (42)	4 (20)	3 (14)	14 (66)
Grade 2 (n=7) n (%)	0	7 (100)	1 (15)	6 (85)	0	4 (57)	3 (43)	0	3 (43)	4 (57)
p-value	0.27		0.87		1			0.95		
Histologic patterns										
Small nest (n=19) n (%)	6 (31)	13 (69)	5 (27)	14 (73)	0	12 (63)	7 (37)	3 (16)	4 (21)	12 (63)
Large nest (n=7) n (%)	0	7 (100)	0	7 (100)	0	2 (28)	5 (72)	0	1 (15)	6 (85)
Trabecular-palisading (n=2) n (%)	0	2 (100)	2 (100)	0	0	2 (100)	0	1 (50)	1 (50)	0
p-value	0.17		**0.029**		0.13			0.072		

positivity of 90% or more). This reveals a unique relationship between immunohistochemical and histomorphologic findings. Considering the staining pattern of Chr, we think that it may be useful to enlist the help of other neuroendocrine cell markers such as CD56, INSM1 in addition to Snp in NET suspicious cases with small tumor size.

Although NETs originating mostly from L cells are known to show a tubular-pseudoglandular growth pattern, tumors originating from EC cells may also show this pattern (1,8). Although neuroendocrine marker positivity of EC cells provides an easy solution, L cells may cause diagnostic confusion with conventional adenocarcinomas and especially goblet cell adenocarcinoma showing tubular-glandular growth with Chr negativity. As known in goblet cell adenocarcinomas, varying amounts of neuroendocrine cells are among the tumor components. In addition, L cells may lose CDX2 expression as well as Chr (9). It has also been reported that L cells express markers such as prostatic acid phosphatase and PAX8 (9,10). With these striking findings, NETs arising from L cells can easily be confused with metastatic disease. Recent studies have reported that SATB2 is positive in both rectal and appendiceal NETs and is useful in differentiation from pancreatic and duodenal NETs (10).

In our study, Snp staining intensity increased with increasing tumor size and Chr SE increased with increasing pathological stage. Specific prognostic data for NET are very limited and stage seems to be the only relevant parameter to determine aggressive disease (1). In one study, tumor size greater than 15 mm, presence of lymphovascular invasion and G2 grade were identified as independent indicators to determine the presence of lymph node metastasis (11). Other studies have not associated some of these parameters with disease-related survival (12-14). All these inconsistencies reflect the high heterogeneity of study planning and case selection, which are fundamental biases of retrospective studies.

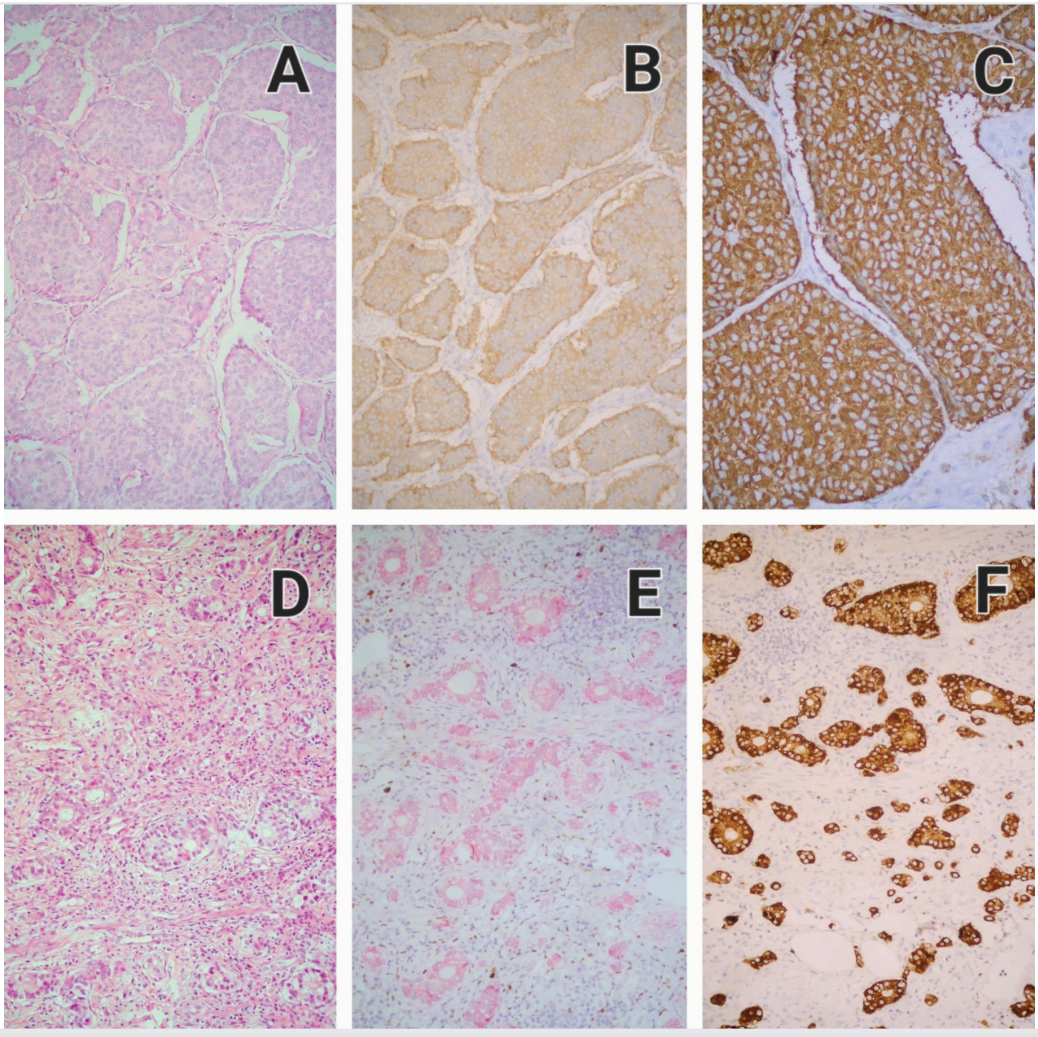

Figure 1. Appendiceal neuroendocrine tumor showing large nest pattern (A: Hematoxylin-Eosin x200. B: Synaptophysin x200, extensity: 100%, intensity: moderate C: Chromogranin-A x200 extensity: 100%, intensity: severe). Appendiceal neuroendocrine tumor showing tubular and predominantly (not in figure) small nest pattern (D: Hematoxylin-Eosin x100. E: Synaptophysin+Ki67 (double staining) x100, extensity: 100%, intensity: moderate F: Chromogranin-A x100, extensity: 100%, intensity: severe)

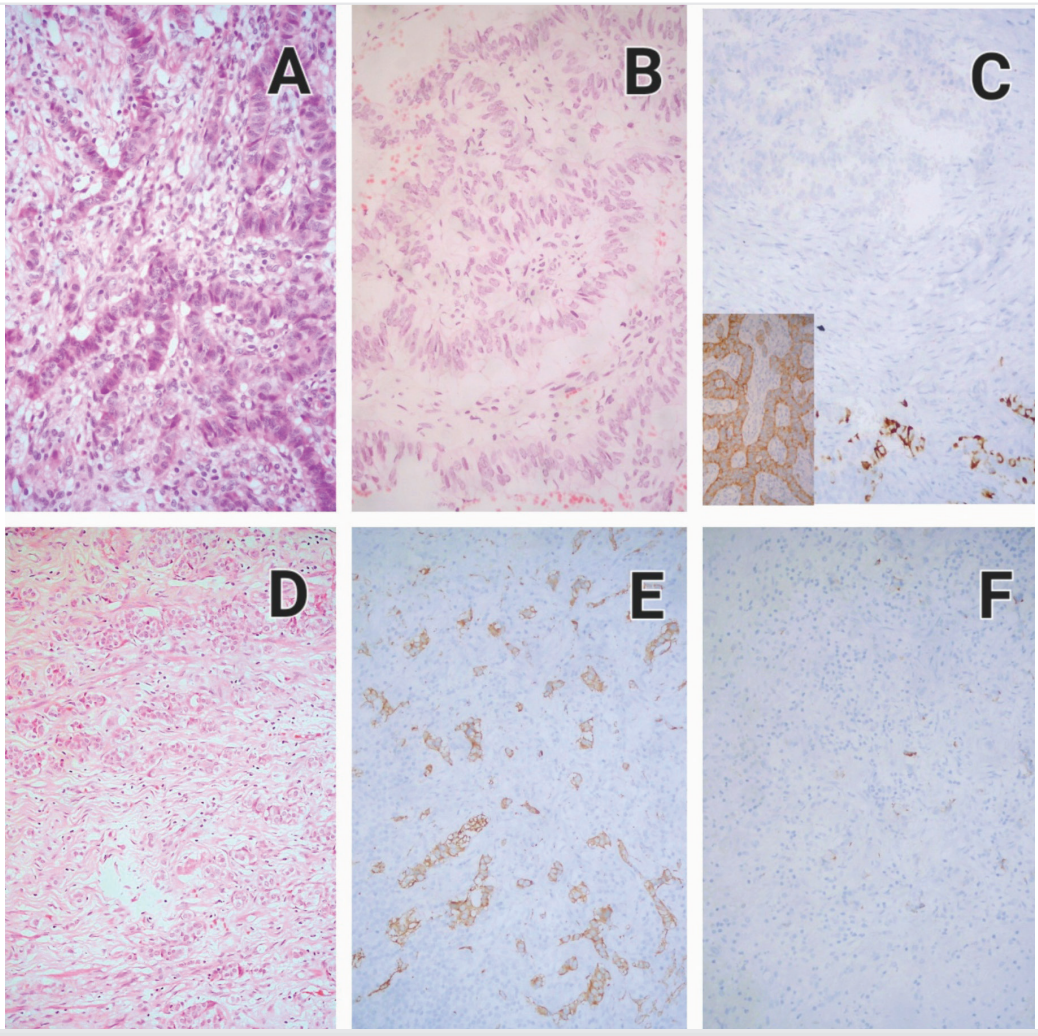

Figure 2. Appendiceal neuroendocrine tumor showing trabecular-palisading pattern [A, B: Hematoxylin-Eosin x200, x400. C: Chromogranin-A "extensity: 10%, intensity: moderate" and Synaptophysin "extensity: 100%, intensity: severe" (inset)]. Appendiceal neuroendocrine tumor showing small nest pattern (D: Hematoxylin-Eosin x100. E: Synaptophysin x100, extensity: 90%, intensity: severe, F: Chromogranin-A x100, extensity: 5%, intensity: weak)

Although right hemicolectomy is strongly recommended especially in tumors larger than 2 cm, some parameters, especially location at the base of the appendix, R1 resection status, lymphovascular invasion, mesoappendiceal invasion (extension over 3 mm) and G2 tumor grade in tumors smaller than 2 cm emphasize that right hemicolectomy should be discussed in an appropriate multidisciplinary setting after appendectomy (1). Since the treatment and follow-up protocol for each patient may differ, all histopathologic parameters should be specified in the pathology report to determine the clinical decision.

Study Limitations

The most important limitation of our study is the small number of cases showing trabecular-palisading pattern. In addition, not including prognostic data (such as recurrence, metastasis, life expectancy, disease-related death) may be another limitation. However, since the aim of our study was to compare immunohistochemical findings with other pathologic parameters, we think that these findings can be ignored.

Conclusion

The NETs of the appendix pose diagnostic challenges and highlight the importance of immunohistochemical evaluations to distinguish between L and EC cells. This study highlights the unique histomorphologic and immunohistochemical features of NETs, such as the trabecular-palisading pattern. Our study also demonstrates that prognostically, Snp staining intensity correlates with tumor size, while Chr staining percentage increases in advanced pathological stages.

Ethics

Ethics Committee Approval: The ethics committee approval of our study was obtained from the Clinical Research Ethics Committee of University of Health Sciences Türkiye, Istanbul Training and Research Hospital with the decision number 357, date: 22.12.2023.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.C., Concept: M.C., Design: M.C., Ö.G., Data Collection or Processing: M.C., E.Y., S.C., Ö.G., Analysis or Interpretation: M.C., E.Y., S.C., Ö.G., Literature Search: M.C., S.C., Ö.G., Writing: M.C., Ö.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Determination of Medical Students' Perception on Disaster Awareness

Tıp Fakültesi Öğrencilerinin Afet Farkındalığı Algısının Belirlenmesi

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ABSTRACT

Objective: Medical students and general practitioners have a vital role in disasters both in the "preparedness and mitigation" and "response and recovery" phases. To prepare students management of disasters as health professionals, the level of disaster preparedness perceptions of the students should be evaluated. So, the undergraduate medical curriculum can be structured to ensure the readiness of the students for disaster. The aim of the study was to determine the level of disaster preparedness perception of medical students.

Methods: This descriptive study was conducted in the Faculty of Medicine of a state university. The population of the study consisted of students studying in the fourth, fifth and sixth grades of the medical faculty which had a training on disaster module in the fifth year. The study was completed with the participation of 288 students. In the data collection process, "personal information form" and the "disaster preparedness perception scale of personnel working in prehospital emergency health services" were used.

Results: It was found that exposure to disasters, presence of individuals exposed to disasters in the family and environment, participation in disaster exercises, receiving disaster training, willingness to volunteer in disasters, the level of participants' perception of themselves as prepared for disasters, the level of perception of the disaster risk of the region of residence, were effective on the mean total score of the scale.

ÖZ

Amaç: Tıp öğrencileri ve pratisyen hekimler afetlerde hem "hazırlık ve zararı azaltma" hem de "müdahale ve iyileştirme" aşamalarında kritik rol oynamaktadır. Öğrencileri sağlık profesyonelleri olarak afet yönetimine hazırlamak için, afet hazırlık algılarının değerlendirilmesi gerekmektedir. Böylece, mezuniyet öncesi tıp eğitimi programı öğrencilerin afete hazırlıklarını sağlayacak şekilde yapılandırılabilir. Bu çalışmanın amacı tıp fakültesi öğrencilerinin afet hazırlık algısı düzeyini belirlemektir.

Yöntemler: Tanımlayıcı türdeki çalışma bir üniversitenin tıp fakültesinde yapıldı. Çalışmanın evrenini beşinci sınıfta afet eğitimi yer alan tıp fakültesinin dördüncü, beşinci ve altıncı sınıfında öğrenim gören öğrenciler oluşturdu. Çalışma 288 öğrencinin katılımıyla tamamlandı. Veri toplama sürecinde "kişisel bilgi formu" ve "hastane öncesi acil sağlık hizmetlerinde çalışan personelin afetlere hazırlık algısı ölçeği" kullanıldı.

Bulgular: Afet geçmişinin olması, aile ve çevrede afet geçmişi olan bireylerin varlığı, afet tatbikatlarına katılım, afet eğitimi alma, afetlerde gönüllü olma isteği, katılımcıların kendilerini afetlere hazırlıklı görme düzeyi ve yaşanılan bölgenin algılanan afet riskinin ölçek toplam puan ortalaması üzerinde etkili olduğu görüldü.

Sonuç: Afet hazırlık algısı düzeyi, afet yönetiminde yetkinlik kazandırılmasında ilk adım olarak görülebilir. Çalışmamızda dönem 5'te yer alan teorik temelli afet eğitiminin öğrencilerinin

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ABSTRACT

Conclusion: Disaster preparedness perception can be seen as a first step toward competency in disaster management in medical students. Conducting simulation-based well-structured training modules is recommended to improve the disaster preparedness perceptions of the students. Further studies are needed to evaluate the effect of simulation-based training in disaster management.

Keywords: Disaster, disaster preparedness, medical education, disaster preparedness perception, medical student

ÖZ

afet hazırlık algı düzeyinde artış sağladığı öne çıkan bulgular arasındadır. Alanyazında simulasyona dayalı eğitim modullerinin yapılandırılması öğrencilerin afet hazırlık algı düzeylerinin geliştirilmesi için önerilmektedir. Simulasyona dayalı eğiitmlerin etkisinin değerlendirilmesi için ileri çalışmalar önerilir.

Anahtar Sözcükler: Afet, afet hazırlığı, tıp eğitimi, afet hazırlık algısı, tıp öğrencisi

Introduction

Disaster is defined as a situation that occurs suddenly, slows down or stops daily life, requires urgent intervention and the struggle is not sufficient to be provided by local resources (1). Millions of people in the world are faced with disasters. As a result of disasters, physical, social, economic and psychological problems arise, and loss of life and property occur. To prevent or reduce the impact of the disasters, an effective disaster management is required (2,3).

Disaster management includes several initiatives to be implemented before, during and after the disaster. In the preparedness and mitigation phase, risk and damage reduction and preparation stages take place. Intervention initiatives during the disaster, and improvement and reconstruction initiatives after the disaster are carried out (4,5). In order to be carried out effectively, the disaster preparedness levels of individuals and societies should be high. Disaster preparedness is of vital importance to increase the resilience of societies and individuals, minimise the loss of life and property, and respond effectively in emergencies. This preparedness requires collaboration between healthcare providers, emergency responders and community members (6).

In the literature, there are studies examining the disaster preparedness levels of individuals from different segments of the society. In a study involving hospital employees, it was stated that more than half of the participants (57.9%) did not have sufficient information about hospital disaster plans. Not knowing the hospital disaster plan completely will cause disruptions in the management of the disaster by the responsible persons in case of a disaster. In this case, deficiencies will be seen in the provision of health services, which are among the services at risk in disasters (6). It is shown that the level of disaster preparedness of psychiatric nurses is high in Türkiye (7). In a different study, it was observed that gender, age, having a history of disaster, working experience in disasters, and receiving disaster training outside the National Medical Rescue Team (NMRT) had effects on the disaster preparedness levels of the NMRT employees (8).

Krishnan et al. (9) showed that interventions for improving disaster preparedness were effective in improving medical students' confidence level in disaster preparedness. Stating there were not any structured training programs in undergraduate medical education for disaster management, the importance of well-structured training programs was emphasised in several studies all over the world (9-11). In Türkiye, the National Core Curriculum for undergraduate medical education recommends a structured program for disasters under the disasters subheading (12). There is structured, lecture-based training with case discussions in the fifth-year curriculum in our study context.

The aim of this study was to determine the levels of disaster preparedness perceptions of future medical doctors, medical students, who had an important role in providing health services in disasters. The research questions were:

1. What are the levels of disaster preparedness perceptions of medical students?

2. What is the effect of disaster management training on disaster preparedness perceptions of medical students?

Methods

Type of Study: This descriptive study was conducted to determine the level of disaster preparedness perception of medical students.

Location of the Study: The study was conducted at the Karadeniz Technical University in Trabzon, located in the Eastern Black Sea Region of Türkiye.

Population and Sample of the Study: Training on disasters module placed in the fifth-year curriculum as a part of emergency medicine rotation. To compare the students who had trained on disasters and who had not; fourth, fifth, and sixth-grade medical students were invited to the study (n=656). OpenEpi programme was used for the sample calculation of the study. As a result of the analysis, it was determined that at least 243 students should be reached to reach 80% power at 95% confidence interval. The research was completed with the participation of 288 students.

Data Collection Tools: In the data collection process of the study, the "personal information form" developed by the researchers as a result of the literature review and the "Disaster Preparedness Perception Scale of Personnel Working in Prehospital Emergency Health Services (DPPSPWPEHS)" developed by Tercan and Şahinöz (13) were used.

Personal Information Form: The form developed by the researchers as a result of the literature review includes 16

questions related to sociodemographic characteristics such as age, gender, and class of study, and situations such as previous exposure to disaster, participation in disaster drills, and receiving disaster training outside of medical education (8,14,15).

Disaster Preparedness Perception Scale of Personnel Working in Prehospital Emergency Health Services: The scale developed by Tercan and Şahinöz (13) consists of five sub-dimensions and a total of 28 items. The sub-dimensions are self-efficacy, willingness, intervention skill, benefit, and importance. The items in the scale are answered as "strongly disagree" (1 point), "disagree" (2 points), "undecide" (3 points), "agree" (4 points) and "strongly agree" (5 points). A minimum of 28 and a maximum of 140 points can be obtained from the scale. As the score obtained from the scale increases, the perception of preparedness for disasters increases. Cronbach alpha coefficient of the scale was found to be 0.925 in the reliability analysis conducted to determine the work consistency of the scale. The scale has a high level of reliability (13).

Statistical Analysis

The data collection form and scale were applied to the students in the classroom environment by face-to-face interview method. It took an average of 15 minutes to complete the form and scale. After the data collection process was completed, the data were entered into the Statistical Package for Social Sciences (SPSS) 18.0 programme. Frequency, percentage, mean, standard deviation and mean rank values were used to analyse the data. In addition, independent samples t-test was used for two-group comparisons and one-way ANOVA test was used for comparisons with three or more groups in which the data showed normal distribution. Mann-Whitney U test was used for two-group comparisons and Kruskal-Wallis H test was used for comparisons with three or more groups in which the data were not normally distributed,

Ethical aspects of the study: Institutional permission (number: E-46362034-299-40418, date: 15.02.2023) was obtained from Dean's Office of the Faculty of Medicine at Karadeniz Technical University for the conduct of the study. Ethical permission was obtained from Karadeniz Technical University Faculty of Medicine Scientific Research Ethics Committee for ethical compliance (protocol number: 2023/55, date: 10.05.2023). In addition, the participants were informed about the study and signed the informed consent form.

Results

The study was completed with the participation of 288 students. Of the participants 62.5% were female and 55.6% of them were studying in the fourth grade. The mean age of the students was 23.40±2.33 years and 80.9% were between 18-24 years (Table 1).

Of the participants 78.8% had not been exposed to any disaster before and 51.4% of them had individuals with a history of disaster in their environment and family. Of the students 71.2% had participated in disaster exercises, 93.7% had not intervened in disasters and 84% had not received any disaster training other than medical education. Newspapers, magazines and internet sources were preferred by 81.3% of the participants to access information on disaster medicine. Of the participants 67.7% wanted to work voluntarily in disasters. After the 6 February earthquake, 86.8% of the students' perspectives on disasters changed, 85.4% of the students' level of concern about disasters increased and 69.8% of the students' interest in disaster medicine increased. Of the students 63.2% did not think of changing the region they lived in after the earthquake. Of the participants 53.5% considered themselves prepared for disasters at the level of 1-2 and the mean level of self-preparedness against disasters was 2.45±0.95. In addition, 72.9% of the participants considered the disaster risk of the region they lived in at the level of 3-5. The mean level of the participants' perception of the disaster risk of their region was found to be 3.17±1.06 (Table 2).

Table 3 shows the mean scores of the DPPSPWPEHS Scale sub-dimensions and the mean total score of the participants.

Table 4 shows the mean scores of the scale sub-dimensions and the total scores of the participants.

There was no difference between the mean total scores of the students participating in the study in terms of gender. When the mean scores of the sub-dimensions were compared, it was determined that the mean score of the willing sub-dimension of females was significantly higher than that of males (p=0.016). On the other hand, when analysed in terms of the mean score of the self-efficacy sub-dimension, it was determined that the mean score of males was significantly higher than the mean score of females (p=0.018).

A statistically significant difference was found between the mean total scores of the scale according to the grade level of the participants (p=0.000). In the analyses, the scale total mean score of the fourth grade students was significantly lower than the fifth and sixth grade students (p=0.003 and p=0.000, respectively). The mean score of the willing sub-dimension showed a significant difference between the classes (p=0.011). In pairwise comparisons, it was found that the mean scores of the sixth grade students in the willing sub-dimension were higher than the mean scores of the fourth grade students (p=0.008). The intervention skill sub-dimension mean score showed a significant difference between

Table 1. Sociodemographic characteristics of the participants

Sociodemographic characteristics	n	%
Gender		
Female	180	62.5
Male	108	37.5
Classroom		
Fourth	160	55.6
Fifth	73	25.3
Sixth	55	19.1
Age (mean ± standard deviation: 23.40±2.33)		
18-24	233	80.9
25 and above	55	19.1

Table 2. Characteristics of the participants regarding disasters

Feature related to disasters	n	%
Exposure to disaster		
Disaster exposure (+)	61	21.2
Disaster exposure (-)	227	78.8
Exposure to disaster in family and environment		
Disaster exposure (+)	148	51.4
Disaster exposure (-)	140	48.6
Participation in disaster excercises		
Participated in the exercise	205	71.2
Did not participate in the exercise	83	28.8
Disaster response status		
Responded to the disaster	18	6.3
No disaster response	270	93.7
Disaster training other than medical education		
Received training	46	16.0
Received no training	242	84.0
Sources used in access to disaster medicine information*		
Newspaper-magazine-internet	234	81.3
TV-radio	111	38.5
Conference-seminar-course	52	18.1
People in the environment	93	32.3
Medical education	147	51.0
Willing to volunteer work in case of disaster		
Willing to work	195	67.7
Not willing to work	13	4.5
Undecided	80	27.8
Change in perspective on disasters after the 6 February earthquake		
Changed	250	86.8
Unchanged	29	10.1
Undecided	9	3.1
Increase in post-earthquake disaster concern		
Increased	246	85.4
No increase	31	10.8
Undecided	11	3.8
Increased interest in disaster medicine after the 6 February earthquake		
Increased	201	69.8
No increase	42	14.6
Undecided	45	15.6
Willingness to change the region of residence after the 6 February earthquake		
Wanted	84	29.2
Didn't want	182	63.2
Undecided	22	7.6
Level of self-preparedness for disasters (mean ± SD: 2.45±0.95)		
1-2	154	53.5
3-5	134	46.5
Level of perception of disaster risk of the region of residence (mean ± SD: 3.17±1.06)		
1-2	78	27.1
3-5	210	72.9

*Since participants gave more than one answer, n and percentage were multiplied, SD: Standard deviation

Table 3. Participants' DPPSPWPEHS scale sub-dimensions and total score averages

Sub-dimensions	Mean ± standard deviation
Willing	10.22±2.89
Importance	23.15±2.76
Self-efficacy	20.61±6.08
Intervention skill	27.44±5.90
Benefit	12.59±3.38
Total	**94.03±13.65**

DPPSPWPEHS: Disaster Preparedness Perception Scale of Personnel Working in Prehospital Emergency Health Services

the classes (p=0.000). The intervention skill sub-dimension mean score of the fourth grade students was significantly lower than the fifth and sixth grade students (p=0.000 and p=0.000, respectively). There was also a difference between the mean scores of the benefit sub-dimension according to the grades of the students (p=0.000). The mean scores of fourth grade students were significantly lower than those of fifth and sixth grade students (p=0.006 and p=0.000, respectively).

Age factor was found to be effective on the scale total mean score and the mean scores of self-efficacy, intervention skill and benefit sub-dimensions. Participants aged 25 years and older had significantly higher mean scale total scores and mean scores of self-efficacy, intervention skill and benefit sub-dimensions compared to participants aged 18-24 years (p=0.000, p=0.018, p=0.000 and p=0.008, respectively).

Table 5 shows the mean scores of the scale sub-dimensions and total scores according to the status of the participants regarding disasters. As a result of the analyses, it was seen that exposure to disasters, having individuals exposed to disasters in the family and environment, participation in disaster exercises, receiving disaster training, willingness to volunteer in disasters, participants' level of seeing themselves as prepared for disasters and the level of perceiving the disaster risk of the region where they lived were effective on the mean total score of the DPPSPWPEHS scale.

The mean total scale score of the participants who were exposed to disasters was significantly higher than the participants who were not exposed to disasters, the mean total scale score of the participants who had a history of disaster in their family and environment was significantly higher than the participants who had no disaster experience in their family and environment, and

the mean total scale score of the participants who participated in disaster exercises was significantly higher than the participants who did not participate in exercises (p=0.031, p=0.041, p=0.005). The mean scale scores of the participants who received disaster training other than medical education were significantly higher than those who did not receive disaster training other than medical education, and those who wanted to work voluntarily in case of a disaster were significantly higher than those who did not want to work voluntarily and those who were undecided (p=0.001 and p=0.001, respectively). The scale total mean score of the participants who considered themselves prepared for disasters at the level of 3-5 was significantly higher than those who considered themselves prepared for disasters at the level of 1-2 (p=0.000). In addition, the scale total mean score of those who considered the disaster risk of the region they lived in between 3-5 was significantly higher than those who considered the disaster risk of the region they lived in between 1-2 (p=0.035).

In the analyses related to the mean scores of the sub-dimensions of the scale, it was found that the mean scores of the self-efficacy and benefit sub-dimension of the participants who had been exposed to disaster in their family and environment were significantly higher than those who had no history of disaster in their family and environment (p=0.017 and p=0.005, respectively). The mean scores of self-efficacy and intervention skill sub-dimension of the students who participated in disaster exercises were significantly higher than those who did not participate in disaster drills (p=0.030, p=0.015). The mean ranks of willingness, self-efficacy and intervention skill sub-dimension scores of the students who received disaster education other than medical education were significantly higher than those who did not receive disaster education other than medical education (p=0.007, p=0.015 and p=0.001, respectively). Willingness to volunteer in disasters created a statistically significant difference in willingness and importance sub-dimensions. The mean ranks of the willing sub-dimension of those who wanted to volunteer were significantly higher than those who were undecided, and the mean scores of the importance sub-dimension of those who wanted to volunteer were significantly higher than those who did not want to volunteer and those who were undecided (p=0.001 and p=0.000, respectively). The mean ranks of the self-efficacy and intervention skill sub-dimensions of the students whose perspective on disasters did not change after the earthquake were significantly higher than those whose perspective changed (p=0.020 and p=0.029, respectively). The mean ranks of intervention skill sub-dimension of the participants who said that the level of concern about disasters increased after the earthquake and the participants who said that it did not increase were higher than the students who remained undecided (p=0.015). The mean rank of the participants whose level of concern about disasters did not increase was significantly higher than those who were undecided (p=0.016).

When the level of interest in disaster medicine after the earthquake was analysed, it was found that the mean ranks of the willing and importance sub-dimensions of those whose interest in disaster medicine increased were significantly higher than those whose interest level did not increase (p=0.001 and p=0.000, respectively). When the level of participants' perceiving

Table 4. DPPSPWPEHS scale sub-dimensions and total score averages according to sociodemographic characteristics of the participants

Sociodemographic characteristics	Willing mean ± SD	Importance mean ± SD	Self-efficacy mean ± SD	Intervention skill mean ± SD	Benefit mean ± SD	Scale total score mean ± SD
Gender						
Female	10.53±2.77	23.31±2.75	19.96±6.03	26.99±5.53	12.62±3.26	93.43±13.42
Male	9.69±3.02	22.87±2.77	21.71±6.04	28.18±6.43	12.55±3.57	95.02±14.02
	t=-2.413	t=-1.300	t=2.383	t=1.661	t=-0.162	t=0.959
	p=0.016	p=0.195	**p=0.018**	p=0.098	p=0.872	p=0.338
Classroom						
Fourth	9.82±3.03	23.10±2.84	20.21±6.31	25.42±5.61	11.83±3.26	90.40±13.45
Fifth	10.38±2.56	22.95±2.68	20.63±5.60	29.20±5.45	13.27±3.22	96.45±11.48
Sixth	11.16±2.71	23.56±2.63	21.76±6.00	30.96±4.87	13.90±3.37	101.36±13.41
	F=4.630	F=0.814	F=1.321	F=26.306	F=10.269	F=16.291
	p=0.011	p=0.444	p=0.269	**p=0.000**	**p=0.000**	**p=0.000**
	post-hoc: 6>4			5>4 and 6>4	5>4 and 6>4	5>4 and 6>4
Age						
18-24	10.18±2.86	23.05±2.94	20.20±6.17	26.78±5.92	12.34±3.39	92.57±13.43
25 and above	10.38±3.05	23.58±1.78	22.36±5.43	30.20±5.00	13.67±3.13	100.20±12.93
	t=-0.453	t=-1.280	t=-2.383	t=-3.948	t=-2.651	t=-3.813
	p=0.651	p=0.201	**p=0.018**	**p=0.000**	**p=0.008**	**p=0.000**

SD: Standard deviation, DPPSPWPEHS: Disaster Preparedness Perception Scale of Personnel Working in Prehospital Emergency Health Services

Table 5. DPPSPWPEHS scale sub-dimensions and total score averages according to the disaster related characteristics of the participants

Feature related to disasters	Willing mean ± SD	Importance mean ± SD	Self-efficacy mean ± SD	Intervention skill mean ± SD	Benefit mean ± SD	Scale total score mean ± SD
Exposure to disaster						
Disaster exposure (+)	10.50±2.98	23.16±3.15	21.91±6.20	28.60±6.17	13.18±3.62	97.37±14.59
Disaster exposure (-)	10.14±2.87	23.14±2.65	20.26±6.02	27.12±5.80	12.44±3.30	93.13±13.27
	t=0.867	t=0.035	t=1.887	t=1.742 p=0.083	t=1.521	t=2.170
	p=0.386	p=0.972	p=0.060		p=0.129	**p=0.031**
Exposure to disaster in family and environment						
Disaster exposure (+)	10.46±2.92	23.08±3.30	21.45±6.21	27.48±6.11	13.14±3.33	95.62±14.05
Disaster exposure (-)	9.96±2.85	23.22±2.05	19.73±5.84	27.39±5.70	12.02.3.34	92.34±13.04 t=2.053
	t=1.472	t=-0.452	t=2.412	t=0.134 p=0.893	t=2.846	**p=0.041**
	p=0.142	p=0.652	**p=0.017**		**p=0.005**	
Participation in disaster exercises						
Participated in the exercises	10.31±2.79	23.22±2.72	21.11±6.16	27.98±5.92	12.81±3.34	95.45±13.93
Did not participate in the exercises	9.98±3.15	22.96±2.87	19.39±5.76	26.10±5.66	12.06±3.42	90.51±12.31 t=2.812
	t=0.872	t=0.737	t=2.179	t=2.458	t=1.721	**p=0.005**
	p=0.384	p=0.462	**p=0.030**	**p=0.015**	p=0.086	
Disaster response status*						
Responded to the disaster	165.36	134.33	127.64	155.19	142.86	142.67
No disaster response	143.11	145.18	145.62	143.79	144.61	144.62
	Z=-1.111	Z=-0.558	Z=-0.888	Z=-0.564	Z=-0.087	Z=-0.096
	U=2054.500	U=2247.000	U=2126.500	U=2237.500	U=2400.500	U=2397.000
	p=0.266	p=0.374	p=0.374	p=0.573	p=0.931	p=0.923
Receiving disaster training other than medical education*						
Received training	174.41	149.33	171.72	181.59	161.12	181.54
Received no training	138.81	143.58	139.33	137.45	141.34	137.46
	Z=-2.691	Z=-0.448	Z=-2.422	Z=-3.302	Z=-1.483	Z=-3.292
	U=4190.000	U=5344.000	U=4314.000	U=3860.000	U=4801.500	U=3862.000
	p=0.007	p=0.654	**p=0.015**	**p=0.001**	p=0.138	**p=0.001**
Willingness to volunteer in case of disaster*						
Willing to work	156.45	162.08	148.35	149.03	152.47	156.55
Not willing to work	106.08	74.88	117.77	120.46	121.73	96.15
Undecided	121.61	112.95	139.45	137.36	128.77	122.99
	X^2 =13.147	X^2 =31,896	X^2 =2.057	X^2 =2.259	X^2 =5.664	X^2 =13.804
	p=0.001	**p=0.000**	p=0.358	p=0.323	p=0.059	**p=0.001**
	works > undecided	works > does not work and works > undecided				works > does not work and works > undecided
Change in perspective on disasters after the earthquake*						
Changed	143.55	148.14	139.14	139.79	142.66	140.61
Unchanged	146.93	115.36	179.48	183.10	154.29	171.98
Undecided	163.11	137.33	180.67	150.89	163.94	163.89
	X^2 =0.520	X^2 =4.463	X^2 =7.872	X^2 =7.113	X^2 =1.022	X^2 =4.193
	p=0.771	p=0.107	**p=0.020**	**p=0.029**	p=0.600	p=0.123
			unchanged > changed	**unchanged > changed**		

Table 5. Continued

Feature related to disasters	Willing mean ± SD	Importance mean ± SD	Self-efficacy mean ± SD	Intervention skill mean ± SD	Benefit mean ± SD	Scale total score mean ± SD
Increase in concern about disaster after the earthquake*						
Increased	144.55	145.70	142.98	144.91	143.88	145.27
Not increased	150.63	149.92	157.66	164.19	170.00	158.52
Undecided	126.14	102.45	141.50	79.82	86.59	87.77
	X^2 =0.720	X^2 =3.255	X^2 =0.873	X^2 =8.410	X^2 =8.313	X^2 =6.007
	p=0.698	p=0.196	p=0.646	**p=0.015** Increased > undecided and not increase > undecided	**p=0.016** **Not increased > undecided**	p=0.05
Increased interest in disaster medicine after the earthquake*						
Increased	153.81	155.66	146.88	143.29	143.74	148.87
Not increased	103.06	101.17	130.90	145.18	142.48	120.56
Undecided	141.61	135.09	146.57	149.29	149.77	147.31
	X^2 =13.289	X^2 =16,958	X^2 =1.315	$X^{2=}$ 0.195	X^2 =0.223	X^2 =4.079
	p=0.001 **Increased > not increased**	**p=0.000** **Increased > not increased**	p=0.518	p=0.907	p=0.894	p=0.130
Willingness to change the region of residence after the earthquake*						
Wanted	157.45	151.71	147.16	143.93	146.36	151.24
Didn't want to	136.00	141.50	142.27	146.59	145.10	141.78
Undecided	165.39	141.80	152.80	129.41	132.45	141.25
	X^2 =5.443	X^2 =0.970	X^2 =0.436	X^2 =0.844	X^2 =0.516	X^2 =0.778
	p=0.066	p=0.616	p=0.804	p=0.656	p=0.773	p=0.678
Level of self-preparedness for disasters*						
1-2	9.96±3.07	22.92±2.73	18.56±5.18	25.58±5.64	11.69±3.30	88.73±12.33
3-5	10.52±2.66	23.41±2.78	22.97±6.21	29.57±5.47	13.63±3.16	100.11±12.54
	t=-1.644	t=-1.478	t=-6.570	t=-6.065	t=-5.061	t=-.754
	p=0.101	p=0.141	**p=0.000**	**p=0.000**	**p=0.000**	**p=0.000**
Level of perception of disaster risk of the region*						
1-2	9.69±2.94	22.51±3.64	20.16±6.74	27.03±6.58	11.84±3.74	91.25±13.60
3-5	10.41±2.86	23.39±2.32	20.78±5.83	27.59±5.64	12.87±3.19	95.06±13.55
	t=-1.899	t=-2.413	t=-0.766	t=-0.704	t=-2.315	t=-2.115
	p=0.059	**p=0.016**	p=0.444	p=0.482	**p=0.021**	**p=0.035**

*In the evaluation of two-group data that do not fit the normal distribution, mean rank information was entered, DPPSPWPEHS: Disaster Preparedness Perception Scale of Personnel Working in Prehospital Emergency Health Services, SD: Standard deviation

themselves as prepared for disasters was analysed, it was seen that the mean scores of self-efficacy, intervention skill and benefit sub-dimension of those who perceived themselves as prepared at the 3-5 level were significantly higher than those who perceived themselves as prepared at the 1-2 level (p=0.000, p=0.000 and p=0.000, respectively). In addition, the mean scores of the importance and benefit sub-dimensions of the participants who perceived the disaster risk of the region at 3-5 level were significantly higher than those who perceived the disaster risk of the region at 1-2 level (p=0.016, p=0.021).

Discussion

In the study, the mean DPPSPWPEHS scale total score of the medical students was 94.03±13.65. The mean score of fourth year students who did not receive any training on disasters during medical education was significantly low (90.40±13.45) considering the mean scores of fifth and sixth year students who received training on disasters during medical education (96.45±11.48 and 101.36±13.41) with p<0.000 in both comparisons. Okan et al. (14) found that the mean DPPSPWPEHS scale total score was found to be 117.95±13.82 in emergency health service workers. The reason for this higher mean score can be explained by the participant characteristics differences. In our study, only fourth, fifth, and sixth year

medical students participated. On the other hand, in the study conducted by Okan et al. (14) emergency health service workers were the participants.

When the scale total score and sub-dimension mean scores according to sociodemographic characteristics were examined, it was found that the mean score of the self-efficacy sub- dimension of male students in our study was higher than that of females, while the mean score of the willing sub-dimension of female students was higher than that of males. Okan et al. (14) found that the mean scores of self-efficacy and intervention skill sub-dimension scores of male 112 workers were significantly higher than females in their study. In different studies, it has been shown that men have higher knowledge scores in terms of disaster preparedness than women (8,16,17) and have higher levels of disaster preparedness (15). Our findings are consistent with the literature. It is thought that the advantage of men over women in terms of physical strength is effective in the higher mean self-efficacy score in men (16). In addition, women may be more emotional than men and women may be more willing in disasters (16,18).

It was found that the grade level was effective in the perception of disaster preparedness. As the grade level increased, it was observed that the mean total score of the scale and the mean scores of the subscales of willingness, intervention skill and benefit increased. In the study of Durmuş Sarıkahya and Yorulmaz (16), it was determined that the level of disaster preparedness of nursing students did not change according to the grade level. In a different study, similar to our findings, it was shown that the level of preparedness of students studying for health care specialisation for intervention in disasters increased with the increase in class level (19). It is thought that the fact that students are more experienced in a theoretical and practical sense with the increase in class level brings with it an increase in self-confidence and has an effect on the perception of preparedness for disasters (20). In our context, the disaster training module is in the fifth year. So, the difference between the fourth and fifth + sixth year students can be explained by the effect of this module.

In our study, it was determined that the mean total score of the scale and the mean scores of self-efficacy, intervention skill and benefit sub-dimensions of the students aged 25 years and above were significantly higher than the students aged 18-24 years. In the study in which 112 employees' perceptions of disaster preparedness were examined, the mean scores of the intervention skill sub-dimension of the 22-32 age group were found to be significantly higher than those of the higher age groups. In addition, although it was not significant, the highest level of scale total mean scores was found in the 22-32 age group (14). In a different study, it was found that age had no effect on the perception of preparedness for disasters (21). Our findings are partially compatible with the literature. Reaching a maturity level is effective in increasing the perception of preparedness for disasters. However, it is thought that the perception of preparedness for disasters may decrease with the advancement of age, especially in terms of physical losses.

In our study, the mean total scale score of those who had a history of disaster was found to be significantly higher than those who had not experienced a disaster. In the study conducted by Durmuş Sarıkahya and Yorulmaz (16) with nursing students and Ünal et al. (8) with NMRT employees, it was found that individuals who had experienced disasters before were more prepared for disasters. Similarly, in a different study, it was determined that disaster experience increased nurses' perception of disaster preparedness (22). Being exposed to disasters may have increased the level of awareness of individuals about what needs to be done in the disaster management process and increased their perception of preparedness for disasters (23). The mean total score of the scale and the mean scores of self-efficacy and benefit sub-dimensions of the students whose family and friends had experienced disasters were significantly higher than the other students. Similar to having a disaster history, it could be expected that the students who were affected by disasters in their environment would increase their perception of preparedness for disasters thanks to the information they obtained from the relatives of disaster victims.

Participation in disaster exercises was found to be another factor affecting the perception of disaster preparedness in our study. It was observed that the mean total scale score and the mean scores of self-efficacy and intervention skill sub-dimensions of those who participated in disaster exercises were higher than those who did not participate in the exercises. Okan et al. (14) found that those who received in-service training on disasters had significantly higher DPPSPWPEHS scale total score and sub-dimension score averages than those who did not receive training. Similarly, Dinçer and Kumru (24) found that those who participated in disaster exercises had higher perceptions of preparedness. Our findings are in parallel with the literature. Since information sharing and practical applications are included in the exercises, people who participate in the exercises are better equipped about what they should do in case of a disaster. It is thought that being equipped may be effective in increasing individuals' perceptions of disaster preparedness.

The mean ranks of the students who received disaster training other than medical education were found to be significantly higher than those who did not receive education in the mean ranks of willing, self-efficacy and intervention skill sub-dimensions. In a study examining the effects of disaster training on disaster nurses, it was found that the level of disaster preparedness increased significantly after disaster training compared to before disaster training (25). Similarly, Mirzaei et al. (26) found that the knowledge, attitudes and performances of hospital personnel who received disaster training significantly increased after the training. In a study in our country, the level of disaster preparedness of 112 employees who received in-service training on disasters was found to be significantly higher than those who did not receive training (14). Our findings are supported by the literature. Students receiving medical education have a certain level of knowledge and skills related to disasters. However, improving the curriculum on disasters should be targeted. Simulation-based training is one of the recommended

methods to improve students' knowledge and skills in disasters (27,28). In a study conducted on nursing students in Türkiye, a role-play scenario based method was used to examine the challenges students faced with disaster triage. It is shown that students need to receive structured programs in undergraduate education (29). The simulation-based training should be well-structured to avoid any harm to students (30) and low quality of training was shown as a negative predictor for willingness (31). To avoid harm, training should be prepared by experts in disaster management and simulation-based training.

In our study, the level of disaster preparedness of those who wanted to volunteer to work in disasters was found to be significantly higher than those who did not want to work and those who were undecided. In a study conducted in 2021, the willingness levels of the participants were found related to high levels of preparedness (31). It is thought that a high perception of preparedness for disasters triggers students' willingness to volunteer in disasters.

The level of perception of the disaster risk of the region where the students lived affected their disaster preparedness perceptions. The mean total score of the scale and the mean scores of importance and benefit sub-dimensions of those who perceived the disaster risk of the region where they lived between 3-5 were significantly higher than those who perceived the disaster risk of the region where they lived between 1-2. In a study, it was reported that the participants in Montalban city of the Philippines, where urban settlement is common and disaster risk is high, had a higher level of preparedness than the participants in Batasan city, which is less risky in terms of disaster. In the same study, it was shown that those living closer than one kilometer to the coastline of Thailand had a higher level of disaster preparedness (23). The fact that the region of residence carries a high risk in terms of disasters increases the possibility of people living in the region encountering disasters and being harmed by disasters. These possibilities require people to be more prepared before, during and after disasters in order to protect their lives. This situation is thought to be effective in the higher perception of disaster preparedness among those who perceive the disaster risk of the region they live in as high.

Study Limitations

The study was conducted only with the participation of students studying in the fourth, fifth and sixth grades of the medical faculty of a state university. Being a single-centred study was a limitation of the study. Another limitation was that the entire population could not be reached because some of the students were not willing to participate in the study and were absent during the data collection process.

Conclusion

As a result of the research, it was determined that the majority of the students studying at the faculty of medicine had individuals with disaster experience in their family and environment and that information on disaster medicine was mostly accessed from newspapers, magazines and internet sources. The earthquake on 6 February caused a change in the perspective on disasters in the majority of the students. In addition, concern about disasters and interest in disaster medicine increased. Students' self-efficacy in disaster preparedness increased with the effect of participation in disaster exercises. In general, the perception of disaster preparedness was affected by modifiable and non-modifiable factors. It is recommended to conduct well-structured simulation-based training modules to improve students' perceptions of disaster preparedness and ensure students' learning.

Ethics

Ethics Committee Approval: Ethical permission was obtained from Karadeniz Technical University Faculty of Medicine Scientific Research Ethics Committee for ethical compliance (protocol number: 2023/55, date: 10.05.2023).

Informed Consent: Participants were informed about the study and signed the informed consent form.

Footnotes

Authorship Contributions

Concept: B.D., B.Y., A.G., Design: B.D., B.Y., A.G., Data Collection or Processing: A.S.Ş., B.Y., Analysis or Interpretation: E.B., Literature Search: B.D., A.S.Ş., E.B., B.Y., A.G., Writing: A.S.Ş., E.B., A.G.

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Case Report

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Behçet's Disease Diagnosed with a Multidisciplinary Approach in Pregnancy

Gebelikte Multidisipliner Yaklaşımla Tanı Konan Behçet Hastalığı

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ABSTRACT

Behçet's disease (BD) is a disease that is characterized by recurrent oral aphthae and has various involvements especially in eye, skin and vascular structures. It was presented to the modern medical community by Hulusi Behçet in 1937. Although the prevalence of BD varies according to the population, it is mostly diagnosed in Türkiye. BD in patients with recurrent oral aphthae (at least three times in one year) plus two of the following clinical features: recurrent genital aphthae, eye lesions, skin lesions and a positive pathergy test. In the literature, data on BD diagnosed during pregnancy could not be reached. Our case was referred to the dentist at the 17th week of her pregnancy because of the wounds in her mouth. Then, after evaluation with a multidisciplinary approach, BD was diagnosed and treatment was started. In the literature, the effect of pregnancy on the disease or the effect of the disease on pregnancy has been investigated. Our case is the first in the literature to be diagnosed during pregnancy, a period when the disease is generally in remission.

Keywords: Behçet's disease, multidisciplinary approach, pregnancy

ÖZ

Behçet hastalığı (BH), tekrarlayan oral aftlar ile karakterize olan ve özellikle göz, deri ve vasküler yapılarda çeşitli tutulumlar yapabilen ve modern tıp camiasına 1937 yılında Hulusi Behçet tarafından sunulan bir hastalıktır. BH'nin prevalansı topluma göre değişmekle birlikte en fazla oranda Türkiye'de tanı konmaktadır. Tekrarlayan oral aft (yılda en az 3 kez) ve yanında tekrarlayan genital aft, göz lezyonları, deri lezyonları ve pozitif paterji testinden en az ikisinin varlığı tanı koydurucudur. Literatürde gebelikte tanı alan BH verilerine ulaşılamamıştır. Bizim olgumuz gebeliğinin 17. haftasında ağız içindeki yaralar sebebi ile tarafımıza başvurmuş olup diş hekimine yönlendirilmiş idi. BH düşünülerek multidisipliner yaklaşımla değerlendirilmesinin ardından tanısı konup tedavisi başlandı. Literatürde genelde tanısı olan ve gebeliğin hastalık üzerindeki etkisi ya da hastalığın gebelik üzerine etkisi araştırıldığı görülmüştür. Bizim olgumuz genelde hastalığın remisyonda olduğu bir dönem olan gebelikte tanı alması bakımından literatürde ilk olma özelliği taşımaktadır.

Anahtar Kelimeler: Behçet hastalığı, gebelik, multidisipliner yaklaşım

Introduction

Behçet's disease (BD) is a disease that is characterized by recurrent oral aphthae and can have various involvements especially in the eye, skin and vascular structures.It was reported to be first described by Hippocrates, but was introduced to the modern medical community by Hulusi Behçet in 1937 (1,2).

The prevalence of BD varies according to the population (3). The prevalence of the disease, which is mostly diagnosed in Türkiye, varies between 80 and 370 cases per 100,000 (3).

The diagnostic criteria of BD are as follows; patients with recurrent oral aphthae (at least three times in one year) plus two of the following clinical features: recurrent genital aphthae, eye lesions, skin lesions and a positive pathergy test (4).

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Although the etiology is not known exactly, it is histopathologically characterized by vasculitis and vasculitis is seen in all affected organs. In addition, pathophysiologies associated with autoimmunity, microbiological agents and HLA-B51 have been suggested (5,6).

Data on BD diagnosed during pregnancy could not be reached in the literature. In general, the effect of pregnancy on the disease or the effect of the disease on pregnancy was investigated and the results were given.

However, we wanted to present this case in order to show that such an important diagnosis can be made during pregnancy with a detailed medical history and to show that we can possibly prevent any complications during pregnancy with treatment.

Case Report

A 26-year-old G3P2A0 patient was admitted to our department at her 17th gestational week with complaints of intraoral sores.

The patient, whose medical and surgical history was unremarkable, did not have any obstetric complaints. Only, it was learned that the other two pregnancies occurred as preterm delivery at 36 weeks of gestation.

In the obstetric evaluation, a fetus compatible with the gestational week was observed in the intrauterine regular gestational sac, and no major structural anomaly was observed in the fetus.

The patient was first evaluated in detail by the periodontologist with her current complaints, and in the medical history taken, it was learned that the ulcerated lesions in the mouth recurred 4-5 times during the year. When the anamnesis was deepened, it was learned that the patient had new-onset joint swelling, as well as low back pain that started in the pre-pregnancy period but continued to increase in severity. It was learned that the patient also had pustular lesions similar to the lesions in the mouth from time to time in the genital area. In previous dermatology examinations, it was documented that papulopustular and acneiform lesions persisted on her skin.

Upon the suggestion of the periodontologist, BD was suspected and the patient was consulted to the immunology department, and as a result of the examinations and exclusion of other rheumatological diseases, especially systemic lupus erythematosus and rheumatoid arthritis, BD was diagnosed - based on other clinical findings even though pathergy test is negative - and the patient was started on colchicine 2*0.5 mg orally. At the 2-week follow-up evaluation after treatment, the symptoms of low back pain and swelling in the joints improved dramatically.

The pregnancy of the patient, who is at 28 weeks of gestation, continues with colchicine treatment and without any symptoms and problems. Informed consent was obtained.

Discussion

In the literature, many studies have shown that BD goes into remission during pregnancy, but it has been reported that the risk of complications is higher than those who do not have the disease (7-10).

In the study of Noel et al., (8) exacerbation was observed in 36% of pregnancies, and the annual incidence of exacerbations per patient was shown to be lower than before and after pregnancy. In the same study, the overall complication rate was reported as 16%, among which miscarriages, HELLP syndrome, and preterm birth were reported. Complications were reported to be significantly less common in patients receiving colchicine.

A case-control study of 31 patients during 135 pregnancies demonstrated higher rates of miscarriage, pregnancy complications, and cesarean sections in patients with BD compared with controls (7).

In a retrospective analysis conducted in Türkiye in 2018, it was shown that there was a history of 24.2% miscarriage, 3% intrauterine death, and 24% preterm birth (10).

Preterm delivery occurred in the previous 2 pregnancies of our case, and both babies of the patient were delivered at 36 weeks of gestation.

Due to the existence of cases of BD associated with preterm birth in the literature, the following question comes to mind. Perhaps the patient also had this disease in her previous pregnancies, and if she had been diagnosed at that time and treatment had been started, the delivery would not have occurred at 36 weeks of gestation.

Therefore, it is very important to take a medical history and work multidisciplinary. We understand this even more in this case.

BD is an immunological disease that can be recognized and treated with a proper medical history, even during pregnancy, when it is generally in remission, and the complication rate is very low when treatment is started.

Ethics

Informed Consent: Informed consent was obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.D., G.Ö.D., Concept: Ö.D., G.Ö.D., Design: Ö.D., G.Ö.D., Data Collection or Processing: Ö.D., G.Ö.D., Analysis or Interpretation: Ö.D., Literature Search: Ö.D., Writing: Ö.D. G.Ö.D.

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Review

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Labial Fusion in Childhood: Management and Treatment Strategies

Pediatrik Çağda Labial Füzyon: Yaklaşım ve Tedavi Seçenekleri

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ABSTRACT

Labial fusion (LF) is frequently an asymptomatic condition and hypoestrogenism plays an important role in the pathopysiology. In the presence of a hypoestrogenic environment friction i.e. in the form of vigorous perineal cleaning leads to inflamation and desquamation of the epithelium which results in LF. Expectant management and reassurance of the family is key in asymptomatic patient. However if the condition results in complications such as urinary retention, recurrent urinary tract infection or vaginitis topical therapy with estrogen or bethametazone is the first treatment option. For patients who do not respond to a course of medical treatment or for cases in which recurrence occurs manuel or surgical seperation may be offered. Regardless of the choice of treatment the family should be counselled of the possibility of recurrence. Recurrence rates decrease with increasing age and the commence of endogenous estrogen production. Factors that contribute to recurrence are poor genital hygiene, recurrent vulvovaginitis and vulvar dermatoses.

Keywords: Labial fusion, labial adhesion, labial synachiae, labial agglutination

ÖZ

Labial füzyon (LF) çoğunlukla asemptomatiktir ve herhangi bir klinik şikayete yol açmamaktadır. LF patofizyolojisinde "hipoöstrojenemi"nin önemli rol oynadığı düşünülmüştür. Hipoöstrojenize ortamda sık ve aşındırırcasına yapılan perine temizliği lokal inflamasyon, epitelin deskuamasyonu ve doku iyileşmesi sırasında füzyonla sonuçlanmaktadır. Asemptomatik olan hastalarda herhangi bir müdahale gerekmemekle beraber aileyi telarş zamanı geldiğinde kanda östrojen miktarının yükselmesiyle beraber LF'nun kendiliğinden çözüleceği konusunda rahatlatmak yeterlidir. Ancak enflamasyon bulguları, vajinit, idrar yolu enfeksiyonu gelişirse geleneksel olarak kullanılan ilk tedavi yaklaşımı krem şeklinde topikal düşük doz östrojen tedavisidir. Topikal betametazon tedavisi de etkin bir alternatiftir. Medikal tedavi ile LF gerilemezse veya tekrar eden uygulamalarda rekürrens gelişirse labiumların manuel seperasyonu veya keskin insizyonla cerrahi ayrıştırılması gerekebilir. Tedavide hangi yöntem kullanılırsa kullanılsın aileye rekürrens olasılığı hakkında bilgi verilmelidir. Rekürrens riski artan yaşla ve endojen östrojen üretiminin artmasıyla azalır. Rekürrense katkıda bulunan faktörler arasında kötü genital hijyen, tekrarlayan vulvovajinitler ve vulvar dermatozlar yer almaktadır.

Anahtar Kelimeler: Labial füzyon, labial adezyon, labial sineşi, labial aglütinasyon

Introduction

Labial fusion (LF) or as it is also called in the literature, "labial adhesion", "labial synechia", "labial agglutination" is the state of the labia minora sticking together on the vestibule in the midline (Figure 1). In the area where there is adhesion, there is a white-gray translucent and fibrotic tissue with varying degrees of fibrosis called "raphe". The presence of raphe allows differential diagnosis between congenital anomalies and LF. In a study conducted in our country in 2020, Huseynov and Hakalmaz (1) classified LF

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according to the degree of fibrosis. In type 1 LF, the fusion tissue is translucent and very thin. In type 2 LF, the fused tissue is fibrotic and thick. The degree of fusion may vary from partial fusion in the anterior or posterior to complete fusion. Partial adhesions mostly occur posteriorly but can close the vaginal introitus. Although the exact etiology of LF is not fully known, various risk factors related to its development will be discussed below. LF is most frequently observed in premenarche and mostly between the ages of 2-7 (2). According to an epidemiological study conducted in the USA, the age distribution in which it is observed is given as 6-24 months (3). As diaper use decreases and the physical ambulation of the child increases, the probability of LF decreases (4). The incidence of LF in girls in childhood is stated as 0.6-5% in different sources (5,6).

LF is mostly asymptomatic and does not cause any clinical complaints. In symptomatic cases, since it prevents urine flow in infants or prepubertal girls, complaints related to urination occur. Difficulty in urination, post-urination dripping or frequently recurring urinary tract infections are the most common symptoms we encounter in symptomatic patients. In addition, vaginitis may develop when synechiae close the vaginal opening and prevent vaginal secretions from draining. According to the study published by Mayoglou et al. (2) in 2009, 50% of the patients with LF were asymptomatic, while 19.9% had urinary tract infection, 12.6% had post-micturition drip, 8.6% had vaginitis, and 7.3% had frequent urination.

Risk Factors for the Development of Labial Fusion

It has been thought that "hypoestrogenemia" plays an important role in the pathophysiology of LF, which occurs with low estrogen levels at the two extremes of a woman's life; prepuberty and postmenopause. Since maternal estrogen, which passes from mother to fetus, persists in the newborn, LF is not observed in the first 3 months postnatally. In the literature, it has been determined that among the etiological risk factors, in addition to hypoestrogenism, perineal cleaning is also performed excessively by families (7). Frequent and abrasive perineal cleaning in a hypoestrogenized environment results in local inflammation, desquamation of the epithelium and fusion during tissue healing (8,9). Possible factors that may lead to local inflammation are given in Table 1. As a result of tissue trauma following infection or inflammation, the labial epithelium may erode and fuse. Simultaneous fecal contamination may also contribute to inflammation and result in vulvovaginitis. Some chronic inflammatory diseases, especially lichen sclerosus, have been associated with LF (10,11). Patients with poorly controlled diabetes, especially those with a history of recent antibiotic use, who develop candida vulvovaginitis, or infants who have frequent diaper dermatitis are also at risk for developing LF. Other infectious agents that may cause LF, although not very common, include N. gonorrhae, C. trochomatis, and T. vaginalis. It should be kept in mind here that if there is a history of sexually transmitted diseases in this age group, sexual abuse should definitely be excluded.

Figure 1. A partial labial fusion patient accompanied by vulvovaginitis. The patient was 20 months old and was brought to the hospital after being noticed by her family

Recommended Clinical Examination for Labial Fusion

If the patient is under 8 years of age, it is a rational approach to examine her in the mother's lap with the legs in the "butterfly" position (12). This approach is a basic principle in pediatric gynecology, especially in terms of gaining the trust of young children and families who have received training that strangers should not touch the genital area. After being positioned in the supine position on the mother's lap or with the hip joint abducted and the heels touching each other, the examination consent is obtained with a calm voice. The urethral meatus and vaginal intrauterine occlusion should normally be visualized with gentle posterolateral traction of the vulva. Differential diagnosis of adhesions covering the entrance to the vaginal orifice, imperforate hymen and vaginal agenesis should be made. If this

Table 1. Etiological risk factors that may lead to labial fusion

Poor genital hygiene
Trauma
Excessive perineal cleaning
Exual abuse
Female circumcision
Infection
Candida
Group A streptococcus
Gardnerella vaginalis
Chlamydia trachomatis
Trichomonas vaginalis
Vulvar dermatoses
Lichen sclerosus
Diaper dermatitis

distinction cannot be made at the first visit, the vaginal orifice should definitely be inspected with a thin cotton-tipped swab at the post-treatment control visits.

First-stage Approach in Treatment

Although no intervention is required in asymptomatic patients, the applied approach can be expressed as "wait and see". It is sufficient to reassure the family that LF will resolve spontaneously with the increase in the amount of estrogen in the blood when thelarche time comes. Indeed, 80% of LF cases resolve without any treatment (13). In the presence of serious anatomical concerns, in cases of recurrent febrile urinary tract infection or vaginitis due to failure to drain vaginal secretions, the first-stage treatment methods are either topical estrogen-containing or topical betamethasone-containing preparations.

Topical Estrogen Treatment

Although most cases are asymptomatic and will not require treatment other than the "wait and see" approach, if signs of inflammation, vaginitis, or urinary tract infection develop, the traditional first-stage treatment approach is topical low-dose estrogen treatment in the form of a cream. Estrogen treatment protocols are usually applied as a thin layer to the fused area once a day for 4-8 weeks (14-17). Although thinner and more transparent adhesions are more likely to open, a success rate of 50-89% has been reported after estrogen treatment (18). No direct relationship has been reported between the duration of treatment and the resolution of labial adhesions. Recurrence rates ranging from 11-41% have been reported with this method (2,17,19-21). It is also necessary to mention the theoretical risks of long-term estrogen treatment. Increased labial blood flow and labial hyperpigmentation may be observed during treatment (22). Premature breast budding or breast tenderness may be observed as side effects in higher dose applications.

Topical Steroid Therapy

Topical betamethasone (0.05-0.01% concentration) treatment is the most commonly preferred agent (5,23). Betamethasone treatment was first used successfully in the treatment of phimosis in boys (24). With application twice daily with light traction, 67-95% of cases were successfully treated (25). In one study, in cases who were treated primarily with estrogen cream and then had recurrence, 68% of LF was reported to open after 4-6 weeks of twice daily betamethasone treatment (14). Betamethasone side effects include erythema, itching, folliculitis, skin atrophy or growth of fine villi hairs in the application area (26).

Approach in Cases Resistant to Medical Treatment

If LF does not regress with medical treatment or recurrence develops with repeated applications, manual separation of the labia or surgical separation with a sharp incision may be required. In addition, if there is a serious complication such as urinary retention, manual separation under sedation/local anesthesia or surgical separation is indicated. Among the techniques reported in the literature, there are studies in which manual separation is performed with the help of local sedatives and/or anxiolytics in the office environment (27-29). Local anesthetic creams with a combination of 2.5% prilocaine and 2.5% lidocaine are suitable for use in pediatric patients. Topical anesthetics can be used in surgical separation, and sedation can also be given in the operating room (30). Topical estrogen or betamethasone preparations have been described to be used during or after separation techniques (17,29). It is recommended that topical treatments be continued for 2-4 weeks to prevent reepithelialization after surgical treatment, followed by the use of vaseline-like preparations to moisturize the perineum for an additional 3-6 weeks to reduce recurrence (5,17). As with any treatment, compliance and maintenance of genital hygiene are essential after surgical treatment. After the surgical incision, the healing epithelium should be prevented from adhering to the midline again. For this purpose, creams containing antimicrobial agents such as local vaseline or polymyxin were applied after the procedure (30).

Recurrence

Regardless of the method used in treatment, the family should be informed about the possibility of recurrence. The risk of recurrence decreases with increasing age and increased endogenous estrogen production. Factors contributing to recurrence include poor genital hygiene, recurrent vulvovaginitis, and vulvar dermatoses. The method used in primary treatment does not determine the possibility of recurrence (18). When recurrence occurs, treatment approaches are the same as in the primary case. Observation without treatment can be done, topical treatment can be given again, or surgery can be tried. There are not many studies in the literature on recurrent LF cases. According to one study, repeated use of topical estrogen in recurrent cases was found to be effective in 35% of cases (21).

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.F.G.K., Ç.Ç., Concept: A.F.G.K., Ç.Ç., Design: A.F.G.K., Ç.Ç., Data Collection or Processing: A.F.G.K., Ç.Ç., Analysis or Interpretation: A.F.G.K., Ç.Ç., Literature Search: A.F.G.K., Ç.Ç., Writing: A.F.G.K., Ç.Ç.

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