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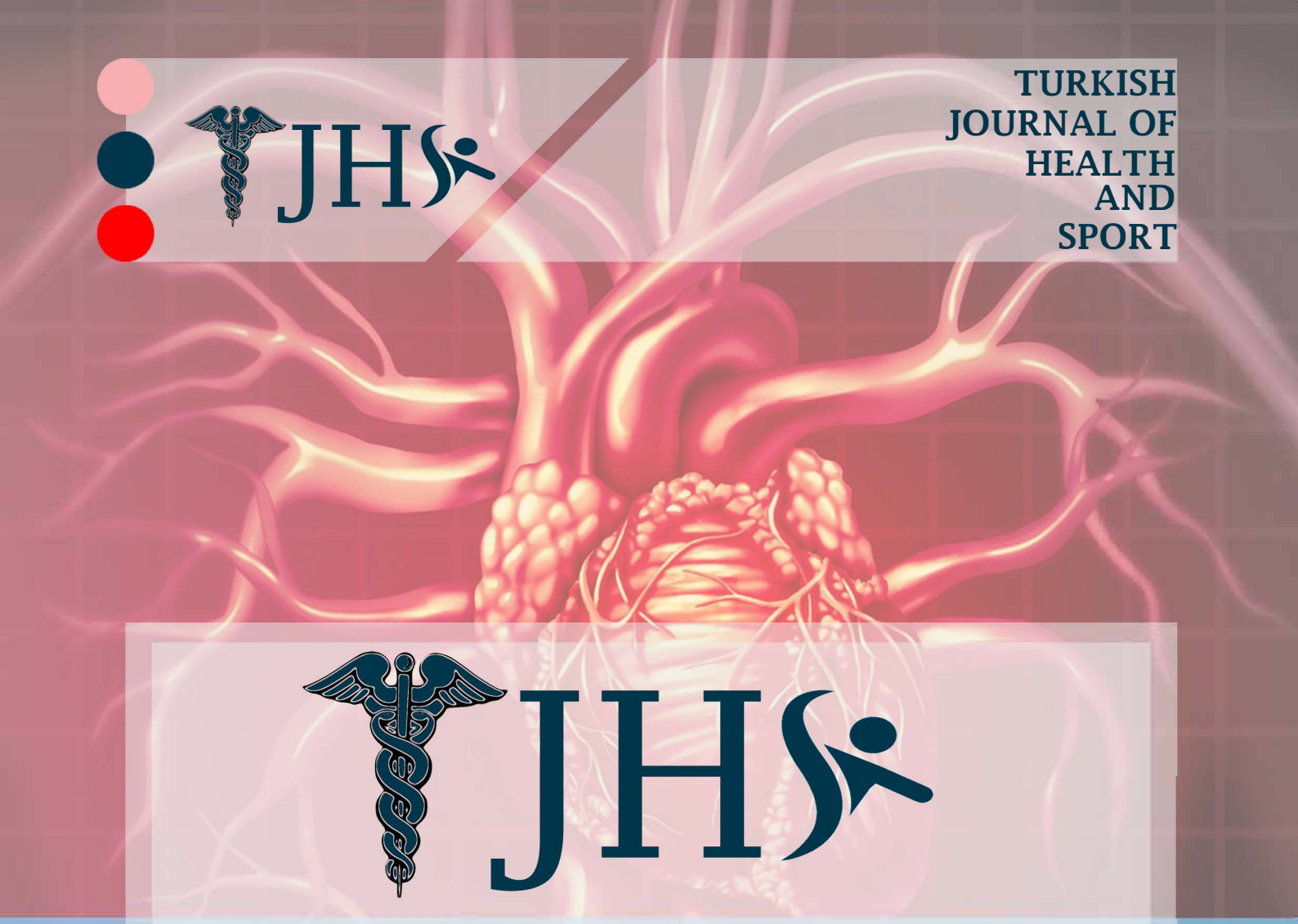

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Experience of Middle Cerebral Artery Occlusion with Monofilament Technique and Investigation of Ischemia-Reperfusion-Related Structural Changes in Brain Tissue

Monofilament Tekniği ile Orta Serebral Arter Oklüzyonu Deneyimi ve Beyin Dokusundaki İskemi-Reperfüzyon İlişkili Yapısal Değişimlerin İncelenmesi

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Abstract

Aim: In this experiment, we aimed to convey our experience of the temporary stroke model created with the monofilament technique in the middle cerebral artery and to characterize the histopathological changes in the brain tissue caused by ischemia-reperfusion at the microscopic level.

Material-Method: Eleven adult (27 weeks-old) Wistar-Albino female rats were used in the experiments. Experimental animals were randomly divided into 2 groups. The first group tagged as SHAM underwent the mock operation. The other group, which underwent 3 days of reperfusion after 1 hour of ischemia, was labeled as IR. Histological examinations were performed from the isolated brain tissues at the end of the reperfusion period.

Results: Our histological examinations showed that the cortical structure was highly irregular, vacuolization in the molecular layer and homogeneous cytoplasm and paler nuclei were observed in some Purkinje cells in the images belonging to the IR group. In addition, our experience with surgical procedures has shown that animal deaths in studies with the monofilament technique are mostly due to vagus nerve damage.

Conclusion: In this study, we reported the histopathological changes in the brain tissue caused by stroke at the microscopic level. We also presented our experience in the surgical processes of an animal model used in the study of stroke. As a result, we concluded that it is important to gain manual dexterity by making preliminary work due to the difficulty of creating a stroke model with the monofilament technique.

Keywords: Animal Model, Brain Diseases, Ischemia-Reperfusion, Ischemic Stroke, Middle Cerebral Artery

Özet

Amaç: Bu deneyde orta serebral arterde monofilament tekniği ile oluşturulan geçici inme modeline ait tecrübelerimizi aktarmayı ve beyin dokusunda iskemi-reperfüzyonun neden olduğu histopatolojik değişiklikleri mikroskobik düzeyde karakterize etmeyi amaçladık.

Materyal-Metot: Deneylerde 11 yetişkin (27 haftalık) Wistar-Albino dişi sıçan kullanıldı. Deney hayvanları rastgele 2 gruba ayrıldı. İlk grup, sahte operasyon geçiren SHAM olarak etiketlendi. 1 saat iskemi ve ardından 3 gün reperfüzyon uygulanan diğer grup IR olarak etiketlendi. Reperfüzyon süresi sonunda izole edilen beyin dokularından histolojik incelemeler yapıldı.

Bulgular: Histolojik incelemelerimiz IR grubuna ait görüntülerde kortikal yapının oldukça düzensiz, moleküler tabakada vakuolizasyon ve bazı Purkinje hücrelerinde homojen sitoplazma ve soluk nükleusların gözlendiğini gösterdi. Ayrıca cerrahi işlemlerle ilgili tecrübelerimiz, monofilament tekniği ile yapılan çalışmalarda hayvan ölümlerinin çoğunlukla vagus siniri hasarına bağlı olduğunu göstermiştir.

Sonuç: Bu çalışmada beyin dokusunda inmenin neden olduğu histopatolojik değişiklikleri mikroskobik düzeyde bildirdik. Ayrıca inme çalışmasında kullanılan bir hayvan modelinin cerrahi süreçlerindeki deneyimlerimizi de sunduk. Sonuç olarak monofilament tekniği ile inme modeli oluşturmanın zorluğundan dolayı ön çalışma yaparak el becerisi kazandırmanın önemli olduğu kanaatine vardık.

Keywords: Beyin Hastalıkları, Hayvan Modeli, İskemik İnme, İskemi-Reperfüzyon, Orta Serebral Arter

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INTRODUCTION

Stroke is one of the most important problems that threaten human health. World Stroke Organization (WSO) shows that one of the most important causes of neurological morbidity and mortality is ischemic stroke (1). According to the World Health Organization (WHO) data, approximately 15 million people worldwide have a stroke each year, one-third of whom are permanently disabled and one-third die. Therefore, researchers strive to imitate stroke on animal models to reveal the pathophysiology of human stroke and to develop new treatment methods (2,3).

Among the stroke cases that may occur due to different reasons, the number of ischemia-related ones is considerably higher than the ones that occur due to hemorrhage and atherosclerosis (4). Stroke may occur due to ischemia in different arteries and may cause different complaints depending on the artery in which the blood flow is blocked. The middle cerebral artery (MCA), the largest branch of the internal carotid artery (ICA), provides blood flow to most of the cerebral hemispheres such as the frontal, parietal, temporal, and occipital lobes, and the lateral sulcus, insula cortex, and inner capsule. Therefore, a blood flow disorder that may occur in MCA can have serious consequences (5,6). When examining the patients who visited the clinic, it was found that stroke most occurred in the MCA (7).

Studies show that the pathological outcomes of experimental MCA-ischemia in rats and stroke in humans are similar (8). For this reason, animal model studies are carried out to reveal the pathophysiological consequences of MCA-ischemia and to develop new treatment methods for human stroke. The method, in which MCA occlusion (MCAO) is created with a monofilament insertion from the ICA branch of the common carotid artery (CCA), allows a successful stroke model to be created without craniotomy (9). Thanks to the restoration of blood flow after ischemia, free radical production from ischemia-reperfusion occurs (10). It is known that increased free radicals cause damage not only in the ischemic region but also in the blood circulation, causing distant tissue/organ damage (11). Therefore, the focal points of stroke studies are ischemia-reperfusion injury as well as ischemia. An advantage of the mentioned MCAO model is that it can be investigated for ischemia-reperfusion injuries after the removal of the inserted monofilament (12,13).

When MCAO studies are examined, results showing that some experimental animals die during or after surgical procedures are seen (12,14). However, it has not been sufficiently clarified at which stage of the surgical processes of the model caused the death of experimental animals as a result of mistakes. Therefore, it would not be right to plan a study without gaining experience with this model. In this study, it was aimed to determine the causes of animal losses encountered during the application of the MCAO animal model with the monofilament technique, to convey the experiences during the surgical procedures, and to report the histopathological changes at the microscopic level in the rat brain tissue after ischemia-reperfusion.

MATERIALS and METHODS

Experimental Animals

In all experiments, eleven adult (27 weeks-old) Wistar-Albino female rats weighing between 300-350 grams were used. Experimental animals were randomly divided into 2 groups. The first group was labeled as SHAM which had the sham operation. The second group was labeled as IR which had MCAO for an hour and then reperfusion for 3 days. Since one of the aims of our study is to determine the causes of animal death during and after MCAO procedures, the number of animals in the IR group was initially determined as 6 against possible losses. After our first studies, our application to the ethics committee was approved to reinforce our experience in this group and 3 more animals were added. In the sham group, the number of animals was determined as 2, since more than one micrometer-thick and consecutive preparation could be obtained from isolated brain tissue for histological examinations. Throughout the reperfusion period, animals were housed one per cage under temperatures of 22-24 oC and 12-h light / 12-h dark conditions and all animals received food and water ad libitum. The protocol that is used is approved by the Selçuk University Experimental Medicine Research and Application Center, Local Ethics Committee for Animal Experiments which is subject to the principles of the Declaration of Helsinki (Appr. no: 2021-53).

Monofilament Preparation

In the ischemic stroke model studies performed with the monofilament insertion technique, researchers use self-developed monofilaments. We also made monofilament from nylon suture as in the literature (15-17). Sterile 4-0 nylon suture (Ethilon Nylon Monofilament Suture, Ethicon Inc.) was cut 4-5 cm long and blunted by bringing the tip close to the heat source (Figure 1-A) (12). It is known that there is an MCA bifurcation of the ICA at a distance of about 1.8 cm from the arteriotomy site shown in Figure 1-E. So the monofilament was marked at 1.8 cm to confirm the correct amount of insertion.

Surgical Procedures

The animals were anesthetized with 80 mg/kg Ketamine and 7 mg/kg Xylazine given intraperitoneally (i.p.), and the animals' body temperatures were stabilized with the heat-controlled table during all surgical procedures. Operations were performed under the surgical microscope (PSMT5N, World Precision Instruments, Inc., USA). After shaving the neck of all experimental animals, a skin incision of approximately

2 cm in length was first made in the longitudinal axis along the inter-mandibular line to reach the CCA (Figure 1-B). As the anatomical details were explained in previous studies, the mandibular glands that were accessed after passing through the fatty tissue were separated in the right and left directions. It was noted in previous studies that the points where the sternohyoid muscle contacts the omohyoid muscles clearly mark the CCA on the longitudinal axis (18). From this point of contact, the CCA was reached by blunt entry using surgical equipment. (Figure 1-C). Care was taken to ensure that the CCA reaches the external and internal carotid artery bifurcation and makes the ICA visible, making the ICA thinner and less visible than the external carotid artery (ECA) and extending slightly lower. At this stage, the ICA was made visible by pulling the 6-0 silk suture thread attached to the CCA and ECA. (Fig. 1-D, E). When attaching the silk thread to the CCA, it was necessary to isolate the CCA from the vagus nerve running parallel to it. Otherwise, this nerve, which is functional for the cardiovascular, respiratory, and nutritional systems, will be damaged and rats may die during the operation (19). A knot is loosely tied to the visible ICA with the same suture thread and a vascular clamp was placed just behind this knot to prevent bleeding during arteriotomy (Figure 1-D). An incision was made from the region shown in the figure to the CCA with the help of microsurgical scissors, and the monofilament insertion was directed into the ICA from there (Figure 1F). During insertion, the knot on the ICA was tightened slightly when the monofilament came to the vessel clamp and the vessel clamp was lifted and the suture was fully tied when the monofilament was advanced up to the pre-marked 1.8 cm. As a result, occlusion was achieved in the MCA bifurcation of the ICA. In this study, the monofilament was removed after 1 hour of ischemia in the animals of the IR group and the rats were taken to a three-day reperfusion period by suturing the opened incisions. In SHAM group animals, after reaching the CCA, ECA, and ICA bifurcation, the incisions were sutured. Brain tissue samples were collected three days later from all rats in the SHAM and IR groups. All rats were housed in a cage for each rat after surgical procedures, and immediately after the operation, their wounds were dressed and fluid replacement (5 ml, saline – i.p.) was administered.

Histological Experiments

The brain tissues taken from each group were firstly fixed 10% formaldehyde fixation solution for 24 hours, and then they were washed under tap water for 24 hours. Afterward, brain tissues were subjected to routine histological follow-up series. In the next step, brain tissues were embedded in paraffin blocks. Then, sections of 5-6 µm thickness were taken from these paraffin blocks. SHAM and IR group brain tissue preparations were stained with Hematoxylin-Eosin (H&E) and Toluidine-Blue (TB) for structural evaluations. After the preparations were prepared, they were examined under a light microscope (Olympus BH-2), and photographed.

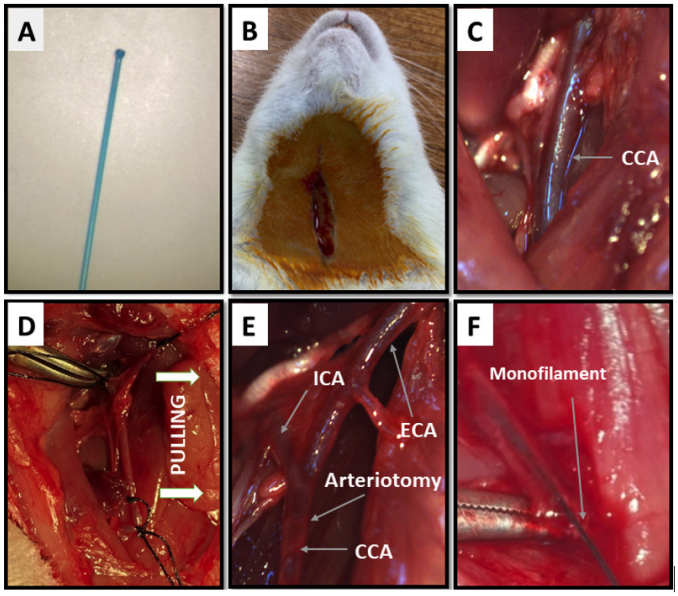

Figure 1: Creating an ischemic stroke model with the monofilament technique step by step. A: Monofilament blunted by fire. B: Skin incision C: Access to CCA (Common Carotid Artery). D: Exposure of ICA (Internal Carotid Artery) E: Display of CCA, ECA (External Carotid Artery), ICA arteries, and arteriotomy site F: Monofilament insertion.

RESULTS

11 rats with the stated features were employed in our investigation, and 2 of them were placed in the SHAM group for histological analysis. The model was effectively established in the following 6 experimental animals after the first 3 experimental animals in the IR group passed away during the procedure. It was recognized that the vagus nerve injury in these 3 experimental animals was the cause of their demise. On the first day of the post-operative reperfusion period, one of the modeled rats passed away. Since the rest 6 animals were operated on later and avoided contact with the vagus nerve, there was no animal loss.

The cortical structure and cerebral cortex were visualized in the outermost molecular layer, followed by the Purkinje cells layer and the innermost granular layer from the preparations (Figure 2-A1). In SHAM group images, cells were neatly located in the cortex, the cell membrane was intact, and the nucleus was large and rounded. Purkinje cells were arranged in a single row and had a paler basophilic cytoplasm and a central vesicular nucleus. Tightly packed granule cells were present in the granular cell layer. Neurons were observed with normal appearance and membrane integrity. In addition, capillary endothelial and surrounding tissue integrity were normal. The number of glial cells was as expected in light microscopic observations (Figure 2-B1). In the sections stained with Toluidine Blue, Purkinje cells with Nissl granules in their cytoplasm were selected as darker stained (Figure 3-A1, B1).

The cortical structure in the IR group was completely disorganized compared to the SHAM group (Figure 2-A2). Vacuolization within the molecular layer, homogenized cytoplasm, and paler nuclei were observed in some Purkinje cells. The granular layer had an appearance with more cavities compared to the SHAM group (Figure 2-B2). Cytoplasmic shrinkage, irregularity in the cell and nuclear borders, and an increase in the density of the neuron cytoplasm were observed in the pyramidal neurons of the IR group. Many neurons were observed to be triangular, with a pycnotic nucleus and wide perinuclear space, and shrunken. Significant atrophy was observed in many neurons, structurally triangular, with pycnotic nuclei and wide perinuclear spaces. In addition, perineuronal and pericapillary edema was evident. Histomorphometric red neurons, indicative of damaged neurons were observed (Figure 2-C1, C2). Sections stained with Toluidine Blue showed a decrease in Nissl granule content and a paler stain compared to the SHAM group (Figure 3-A2, B2).

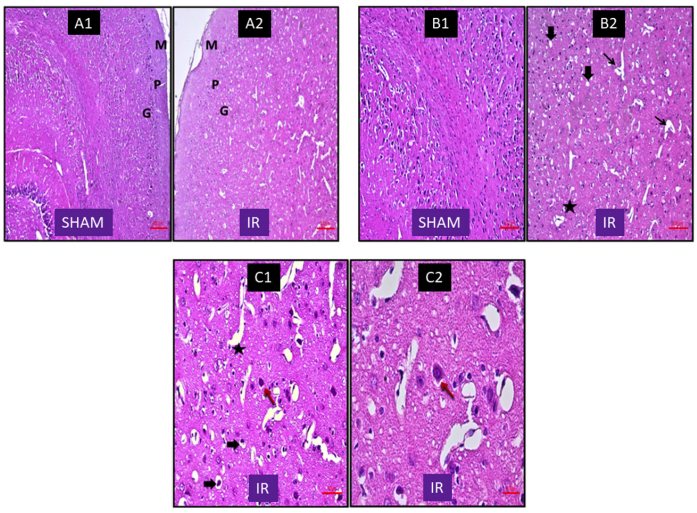

Figure 2: Images of rat brain tissues after Hematoxylin & Eosine (H&E) staining. A1 and A2: SHAM and IR rats, respectively; general view, Molecular layer (M), Purkinje layer (P), and Granular layer (G). B1: SHAM rats; normal tissue image. B2: IR rats; pericapillary (thin arrow) and perineuronal edema (star), vacuolation (thick arrow). C (C1 and C2): IR rats; pericapillary (star) and perineuronal edema (thick arrow) and red neuron (red arrow). Scale bar A: 200 µm, B: 100 µm, C: 50 µm and 20 µm, respectively.

DISCUSSIONS

In our study, we demonstrated that the MCAO model with the monofilament technique successfully established the transient stroke model and that the model's success depends on avoiding contact with the vagus nerve. This finding contributed to the success of subsequent studies with high survival rates in animals during and after MCAO surgical procedures.

In our histological evaluations, structural differences between SHAM and IR groups were detected in parallel with the literature. In a study evaluating the morphological changes of brain cells after stroke, it was summarized that there were structural changes in the stem and axons of neurons in the ischemic area, vacuolization occurred in their plasms, and their nuclear structures were disrupted. They also noted the disintegration of Nissl granules, acidophilic cytoplasm, and red neuron formations disrupting protein structure (8,20). As a result of H&E staining in our samples, degenerated Purkinje cells, darkly stained cytoplasm, and spaces between nuclei and granular layer cells were visualized (Figure 2-B2). In another histological study performed with the stroke model, shrunken and triangular cells were detected similar to ours (Figure 2-C1, C2) (21). In addition, our findings were similar to a study stating that the amount of Nissl granules was reduced as a result of disintegration (chromatolysis) due to ischemia (Figure 3-B2) (22).

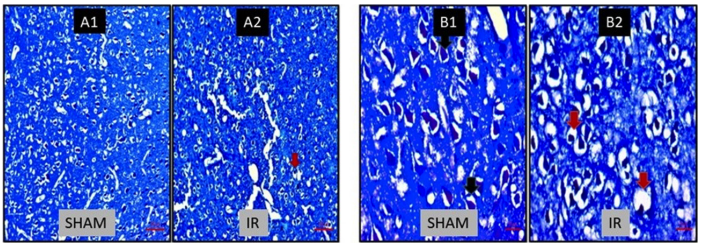

Figure 3: Images of rat brain tissues after Toluidine Blue (TB) staining. A (A1 and A2): SHAM and IR rats; general view. B1: SHAM rats; normal neuron image (black arrows), B2: IR group; paler in color, shrunken neurons, and increased density in their cytoplasm (red arrows). Scale bar A: 100 µm, B: 20 µm.

When the studies with the MCAO model are examined, it is mentioned that experimental animals can die during or after surgical procedures. Intracerebral hemorrhage, anesthesia, and compression on the trachea and neuronal structures have been reported as the cause of these deaths (12,19). On the other hand, Veltkamp et al. reported that experimental animals lived with stroke for 14 days after successfully completing surgical procedures in the MCAO study (23). Due to this different information in the literature, we investigated the details that enabled the survival of the animals and the successful formation of the model in MCAO surgery. To convey our experience of surgical procedures, a total of 11 rats were used in these experiments, which were designed as a pilot study, and 2 of them were assigned to the SHAM group for histological evaluations. The first 3 of the IR group experimental animals died during the operation, and the model was successfully created in the next 6 animals. As we understood from our later experience, these 3 experimental animals died from vagus nerve damage. One of the modeled rats died on the first day of the post-operative reperfusion period. Contact with the vagus nerve was avoided as much as possible during the surgical procedures of the rats for which the model was successfully created. As a result, if the vagus nerve is damaged in MCAO model studies, rats may die either during or after surgery. Researchers working with the same model also mentioned the difficulty of the model and animal deaths (14,24).

As a result, studies on the treatment and prevention of ischemic stroke, which threatens people's life and health, are very important. Our experience with this study and our histological findings shows that structural changes occur in the brain tissue as a result of an ischemic stroke model that allows reperfusion with the monofilament technique. In addition, as our most important result compared to similar studies, contact with the vagus nerve should be avoided as much as possible during the application of this technique.

CONCLUSION

In this study, we demonstrated that the monofilament MCAO model established the transient stroke model successfully. We also reported the histopathological changes of the stroke-induced brain tissue at the microscopic level. Thus, the structure of the brain tissue after stroke was evaluated and structural changes were emphasized. As a result, we concluded that it is very important to gain manual dexterity by making a preliminary study in creating an ischemic stroke model with the monofilament technique due to the difficulty of the procedure. The ability to separate the vagus nerve with such finesse is crucial.

Limitations of the Study

This study is a pilot study on the ischemic stroke model with the monofilament technique and was studied with a limited number of animals. The damage caused by the model in the brain tissue was tried to be explained by histological techniques. Due to the limited number of animals studied, histopathological scoring could not be performed. Revealing the results of ischemia-reperfusion injury and the causes of animal deaths encountered during experiments with more concrete evidence requires the support of different techniques.

Conflict of Interests

None of the authors have a conflict of interest.

Financial Support

There is no financial support.

Ethical Approval

The protocol that is used is approved by the Selçuk University Experimental Medicine Research and Application Center, Local Ethics Committee for Animal Experiments which is subject to the principles of the Declaration of Helsinki (Appr. no: 2021-53).

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Research Article

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Relationship between premature ventricular contractions and cognitive functions

Erken ventriküler kasılmalar ve bilişsel işlevler arasındaki ilişki

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Abstract

Objective: Arrhythmias impair cerebral blood flow. Cognitive impairment (CI) caused by cerebral hypoperfusion may be aggravated by premature ventricular contractions (PVCs). We aimed to study the relationship between PVC counts on 24-hour ambulatory ECG monitoring (Holter) and cognitive impairment.

Methods: A total of 340 individuals, comprising 244 patients and 96 healthy controls were enrolled in the research. All patients had routine blood testing conducted. ECG was conducted. Each subject had a 24-hour Holter ECG performed. ECG parameters and Holter ECG were analyzed. The minimal mental state examination (mini-MSE) test was administered, a validated global cognitive evaluation for detecting CI.

Results: The mean age of the study group was 44.84±12.89 years and the 44.56±13.58 years control group. There was no appreciable difference among the groups for hematological and biochemistry blood tests ($p>0.05$). There was a significant difference between the PVC frequency groups (minimal, occasional, and frequent) and the control group in terms of mini-MSE components (except copying, $p=0.145$) ($p<0.001$). There was a significant negative correlation between orientation, registration, attention and calculation, recall, and language from mini-MSE components and both ECG parameters and HRV (except RMSSD and LF/HF) ($p<0.001$). There was a substantial correlation between LF/HF and language ($p<0.05$). A significant negative correlation was between copying with QTcd and TpTe/QTc ($r = -,139$, $p<0.05$ and $r = -150$, $p<0.001$). In univariate and multivariate analysis, there was no statistically significant predictive factor of mini-MSE scores ($p>0.05$).

Conclusion: This study revealed that global cognitive functions decreased as the frequency of PVC increased, especially in orientation and language scores of the mini-MSE.

Keywords: Premature ventricular contraction, cognitive impairment, arrhythmia, Holter monitoring, heart rate variability.

Özet

Giriş/Amaç: Aritmiler serebral kan akımını bozar. Serebral hipoperfüzyonun neden olduğu bilişsel bozukluk (CI), erken ventriküler kasılmalar (PVC'ler) ile şiddetlenebilir. 24 saatlik ambulatuar EKG monitorizasyonunda (Holter) PVC sayımları ile kognitif bozukluk arasındaki ilişkiyi incelemeyi amaçladık.

Yöntem: Araştırmaya 244 hasta ve 96 sağlıklı kontrol olmak üzere toplam 340 kişi alındı. Tüm hastalara rutin kan testi yapıldı. EKG yapıldı. Her deneğe 24 saatlik Holter EKG uygulandı. EKG parametreleri ve Holter EKG'si analiz edildi. CI'yi tespit etmek için geçerliliği kanıtlanmış bir genel bilişsel değerlendirme olan minimal mental durum muayenesi (mini-MSE) testi uygulandı.

Bulgular: Çalışma grubunun yaş ortalaması 44,84±12,89, kontrol grubunun ise 44,56±13,58 idi. Hematolojik ve biyokimya kan testleri açısından gruplar arasında fark yoktu ($p>0,05$). Mini-MSE bileşenleri (kopyalama hariç, $p=0,145$) açısından PVC frekans grupları (minimum, ara sıra ve sık) ile kontrol grubu arasında anlamlı fark vardı ($p<0,001$). Mini MSE bileşenlerinden oryantasyon, kayıt, dikkat ve hesaplama, hatırlama ve dil ile hem EKG parametreleri hem de KHD (RMSSD ve LF/HF hariç) arasında anlamlı bir negatif korelasyon vardı ($p<0.001$). LF/HF ile dil arasında önemli bir korelasyon vardı ($p<0.05$). QTcd ile kopyalama ile TpTe/QTc arasında anlamlı bir negatif korelasyon vardı ($r = -,139$, $p<0.05$ ve $r = -150$, $p<0.001$). Tek değişkenli ve çok değişkenli analizde, mini-MSE puanlarının istatistiksel olarak anlamlı bir yordayıcı faktörü yoktu ($p>0.05$).

Sonuç: Bu çalışma, özellikle mini-MSE'nin oryantasyon ve dil puanlarında, PVC sıklığı arttıkça genel bilişsel işlevlerin azaldığını ortaya koydu.

Anahtar kelimeler: Prematüre ventriküler kasılma, kognitif bozukluk, aritmi, Holter izleme, kalp atış hızı değişkenliği

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INTRODUCTION

Premature ventricular contractions (PVCs) are a frequent occurrence in daily practice (1). It is typically considered benign and not causing any symptoms in most individuals (2). It has been linked to age, male gender, hypertension, smoking, caffeine use, and reduced physical activity (3). Although it is considered benign, recent studies have reported that low or high PVCs burden is associated with atrial fibrillation (AF), heart failure, and stroke-like symptoms such as numbness, weakness, loss of vision, conversation, comprehension, and stroke (4).

Cognitive function encompasses the mental activities involved in information acquisition, data processing, and reasoning (5). Perception, attention, learning, decision-making, memory, and language ability are the content of cognitive performance tests. Cognitive impairment (CI) has been linked to arrhythmias in previous studies (6).

Arrhythmias impair cerebral blood flow (7). Cerebral hypoperfusion caused by hemodynamic factors may be aggravated by heart rate variability (HRV) (8). The ability of microvascular endothelial cells to produce structures resembling capillaries does not emerge at an early period (9). Compared to other organs, hypoperfusion has an early and profound effect on the brain's cells. The development of arrhythmia was related to a quicker deterioration in cognitive function as judged by mental state examinations (MSE) throughout follow-up periods (10). According to reports, AF is connected with a 40% greater risk of cognitive impairment in patients without stroke and bradycardia causes a decrease in intelligence (11). But, there is a lack of literature on the relationship between PVCs and CI.

Our study aimed to investigate whether PVC burden at 24-hour Holter ECG monitoring is associated with CI using a mini-MSE test.

MATERIALS AND METHODS

Study design and subject

The study was conducted prospectively between May 2022 and February 2023. During a 24-hour Holter ECG recording, patients with no notable arrhythmia other than PVC were included. Individuals with structural heart disease (except heart failure with preserved ejection fraction), pre-existing AF, anti-arrhythmic drug use, bundle branch block, cerebrovascular or depressive disorders and neurologic diseases, electrolyte imbalance, hypertension, diabetes, history of brain injury trauma, and age 65 were removed from the study. The control group included 96 volunteers without arrhythmias. The Gazi Yasargil Training and Research Hospital approved the research (date: 20/05/2022, permit number: 89). It met the ethical requirements of the Helsinki Declaration for human experimentation (2013).

Study Protocol

All patients had routine blood testing conducted. Demographic details were collected from the database. Using an electrocardiograph (ECG-1350K Nihon-Kohden), an ECG was conducted. Each subject had a 24-hour Holter ECG (Northeast Monitoring, MA) performed. A cardiologist with no knowledge of the research analyzed the ECG parameters and Holter ECG. The mini-MSE test was administered, a validated global cognitive evaluation for detecting CI. An expert psychiatrist determined CI based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).

Definitions

PVC was defined as the early appearance of an aberrant QRS complex (length usually 120 ms, T wave typically wide and opposite to primary QRS deflection, no preceding P wave) (12).

The mini-MSE (30 points) measures several cognitive areas, including orientation, recall, attention and calculation, registration, language, and coping. Normal scores were evaluated 25-30 out of 30, moderate impairment 21-24, significant impairment 10-20, and serious impairment below 10 (13).

The frontal QRS-T angle was computed using the distance between the QRS and T axes. Subtracting the shortest QT duration from the maximum QT duration yielded QT dispersion. Likewise, Pd, QTcd, TeTp, and TeTpc were yielded.

The time-domain analysis assessed the root mean square of successive differences (RMSSD), the percentage of the total number of R-R periods (pNN50), and the standard deviation of R-R periods (SDNN). High frequency (HF) and low frequency (LF) components were distinguished from the overall power.

Statistical analysis

For the analysis, the IBM SPSS 26.0 package application was used. The individuals were separated into two groups as study and control according to the presence of PVC in the Holter results. In addition, according to the frequency of PVC in the 24-hour Holter study group; individuals were divided into three groups minimal (<5%), occasional (5-10%), and frequent (>10%). Continuous data were expressed as means, standard deviations, or medians (interquartile range). Categorical data were described employing frequency and percentage, and the chi-square test or Fisher's exact test was applied. The normal distribution of the variables was examined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The T-test or the Mann–Whitney U-test, as appropriate, was employed to compare continuous data. One Way ANOVA Test (Post-Hoc; Tukey) was used to determine the variance among more

than two groups. Pearson correlation test was employed to determine mini-MSE components with ECG parameters and HRV. Univariate and multivariate regression analysis was performed for ECG parameters and HRV that may contribute to decreasing mini-MSE scores. P-values below 0.05 indicated statistical significance.

RESULTS

A total of 340 individuals, comprising 147 women (43.2%) were included in the research. The mean age of the study group was 44.84±12.89 years and 44.56±13.58 years control group. No clinically significant distinctions among the groups for dyslipidemia, thyroid dysfunction, or body-mass index (p>0.05, Table 1).

Table 1. Clinical characteristics and laboratory parameters of patients

Parameters	Study group (n=244)	Control group (n=96)	P-value
Age (Years)	44.84±12.89	44.56±13.58	0.860
Gender, female, n (%)	110 (45.1)	37 (38.5)	0.273
BMI (kg/m2)	23.64±3.96	23.08±3.40	0.218
SBP (mmHg)	123.61±13.46	114.67±5.26	p<0.001
DBP (mmHg)	80.04±8.35	72.67±4.56	p<0.001
Thyroid dysfunction, n (%)	4 (1.6)	4 (4.2)	0.166
DL, n (%)	18 (7.3)	10 (10.4)	0.496
Heart failure, n (%)	15 (6.1)	-	-
Smoking, n (%)	57 (23.4)	18 (18.7)	p<0.001
Iron deficiency anemia, n (%)	15 (6.1)	10 (10.4)	0.175
Hemoglobin (g/dl)	14.98±1.10	14.92±1.19	0.618
Glucose (mg/dl) [IQR]	99 (18)	94.5 (25)	0.259
Fe (ml/ng)	90.03±12.58	87.55±16.66	0.138
Sodium, meq/L	139.59±3.12	139.28±3.27	0.419
Potassium, meq/L	4.11±0.42	4.05±0.44	0.237
TSH (mIU/L)	1.01 (1.02)	1.02 (1.17)	0.787
fT3 (pg/mL)	4.71±1.45	4.76±1.42	0.814
LDL (mg/dl)	116.68±32.68	109.58±31.65	0.070
HDL (mg/dl)	42.45±10.72	43.27±9.67	0.515
ALT (U/L)	16 (13)	16 (10.5)	0.439
AST (U/L)	17 (8)	15 (6.5)	0.531
EF %	52.86±7.45	65.60±2.77	p<0.001
QTd (ms)	49.92±4.58	40.76±2.98	p<0.001
QTcd (ms)	68.04±3.30	56.16±3.47	p<0.001
TpTe (ms)	79.43±2.33	75.13±1.60	p<0.001
TpTec (ms)	87.15±3.92	85.29±2.18	p<0.001
TpTe/QT	0.20±0.04	0.19±0.02	0.407
TpTe/QTc	0.19±0.03	0.18±0.01	0.122
Heart rate (beats/min)	82.10±10.96	87.04±10.92	p<0.001
Frontal QRS-T angle	45.87±6.56	33.82±5.04	p<0.001
SDNN (ms)	121.10±25.58	134.58±16.88	p<0.001
RMSSD (ms)	38.19±11.21	28.13±5.44	p<0.001
pNN50 (%)	7.43±3.94	10.81±2.42	p<0.001
LF* (nu)	63.68±7.77	53.05±6.00	p<0.001
HF* (nu)	30.92±4.46	29.45±4.48	0.007
LF/HF	2.08±0.27	1.83±0.29	p<0.001

Data are expressed as appropriate as mean ± SD and median [interquartile range]. BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, DL: Dyslipidemia, TSH: Thyroid-stimulating hormone, fT3: free triiodothyronine, LDL: Low-density lipoprotein, HDL: High-density lipoprotein: ALT: alanine aminotransferase, AST: aspartate aminotransferase, EF: Ejection fraction.

*Frequency domain measures of heart rate variability were log-transformed

There was a clinically substantial disparity among the groups in terms of systolic and diastolic blood pressure, and smoking (p<0.001). Heart failure with preserved ejection fraction was present in 15 (6.1%) individuals in the study group. There were no appreciable differences among the groups for hematological and biochemistry blood tests (p>0.05). There was a substantial difference in ejection fractions across groups (p<0.001). There were significant distinctions across the PVC frequency groups (minimal, occasional, and frequent) and the control group in terms of mini-MSE components (except coping, p=0.145) (p<0.001, Table 2).

There was no statistically significant correlation between RMSSD and attention or calculation (p>0.05). There was a significant negative correlation between LF/HF and language (p<0.05). A significant negative correlation was between copying with QTcd and TpTe/QTc (r = -139, p<0.05 and r = -150, p<0.001). Univariate and multivariate regression analysis was performed for ECG parameters and HRV that may contribute to decreasing mini-MSE scores. There was no statistically significant predictive factor of mini-MSE scores (p>0.05). On the other hand, the independent factors accounted for 35% of the variance in the mini-MSE scores (adjusted r2 =0.358). In the subgroup analyses, the occasional and frequent groups differed significantly in heart rate, QTd, QTcd, TpTe, TpTec, and the frontal QRS-T angle than the minimal group (p<0.001). The ratios of TpTe/QT and TpTe/QTc did not vary substantially between the control and minimal groups (p>0.05).

There was a significant negative correlation between orientation, registration, attention and calculation, recall, and language from mini-MSE components and both ECG parameters and HRV (except RMSSD and LF/HF) (p<0.001, Table 3).

DISCUSSION

This study showed that global cognitive functions decreased as the frequency of PVC increased, especially in orientation and language scores of the mini-MSE. QTd, QTcd, TpTe, TpTec durations, TpTe/QT, TpTe/QTc ratios, and QRS-T angle were higher in PVC patients than controls. Additionally, PVC patients had autonomic nervous system disorders.

PVCs cause heart failure. The decrease in cardiac output leads to a decrease in cerebral blood flow (14). Xie et al. found an increased risk of mild CI and dementia in patients

Table 2. Mini-mental state examination score of the study group

CATEGORY	Control (n=96)	PVC frequency/24 hour			P-Value
		Minimal, <5% (n=139)	Occasional, 5% to<10% (n=57)	Frequent, ≥ 10% (n=48)	
		8.37±0.65	7.78±0.88	7.25±0.83	P<0.001
Registration	2.99±0.3	2.98±0.11	2.96±0.18	2.81±0.39	P<0.001
Attention and Calculation	4.63±0.30	4.61±0.50	4.42±0.49	4.25±0.48	P<0.001
Recall	2.99±0.2	2.96±0.22	2.87±0.38	2.72±0.44	P<0.001
Language	7.9±0.45	7.76±0.45	7.45±0.80	6.79±0.89	P<0.001
Copying	0.97±0.8	0.96±0.18	0.94±0.22	0.93±0.24	0.145
Total score	29.74±1.12	27.74±1.44	26.49±2.23	24.77±2.49	P<0.001

Table 3. Correlation of mini-MSE components with ECG and heart rate variability parameters of the study group

	Mini-MSE components					
	Orientation	Registration	Attention and Calculation	Recall	Language	Copying
Pd (ms)	-,317**	-,223**	-,275**	-,218**	-,441**	-,090
QTd (ms)	-,353**	-,227**	-,355**	-,209**	-,399**	-,053
QTcd (ms)	-,345**	-,181**	-,397**	-,218**	-,408**	-,139*
TpTe (ms)	-,407**	-,238**	-,368**	-,222**	-,436**	-.085
TpTec (ms)	-,332**	-,194**	-,227**	-,220**	-,357**	-,051
TpTe/QT	-,331**	-,151**	-,199**	-,169**	-,324**	-,026
TpTe/QTc	-,435**	-,231**	-,281**	-,183**	-,432**	-,150**
Heart rate (beats/min)	-,321**	-,230**	-,204**	-,199**	-,394**	-,045
fQRS-T°	-,426**	-,286**	-,375**	-,252**	-,461**	-,101
SDNN (ms)	-,347**	-,196**	-,290**	-,209**	-,340**	-,039
RMSSD (ms)	-,219**	-,156**	0,44	-,144**	-,151**	0,42
pNN50 (%)	-,311**	-,121**	-,125**	-,172**	-,231**	0,98
LF (nu)	-,390**	-,246**	-,339**	-,210**	-,464**	-,094
HF (nu)	-,281**	-,162**	-,213**	-,156**	-,254**	-,066
LF/HF	-,069	-,066	-,035	-,044	-,172*	-,025

Values are presented as mean ± SD. Pmax: P-wave maximum duration, Pmin: P-wave minimum duration, Pd: P-wave dispersion, QTd: QT dispersion, QTcd: Corrected QT dispersion, TpTe: T-peak to T-end, TpTec: Corrected TpTe, fQRS-T°: Frontal QRS-T angle, HRVI: Heart rate variability triangular index, SDNN: Standard deviation of the NN (R-R) intervals, RMSSD: Root mean square of the successive differences, pNN50: The proportion of NN50 divided by the total number of NN (R-R) intervals, LF: Low frequency, HF: High frequency., *p <0.05 **p <0.001

with heart failure (15). In the study of Ginty et al., middle cerebral artery mean blood velocity decreased during PVCs in all groups and demonstrated reducing in peak velocities by transcranial doppler ultrasonography (16). Witt et al. reported a decrease in memory and executive functions in patients with heart failure during their 15-year follow-up (17). PVCs also induce remodeling of the left ventricle and increased thromboembolism (18). Patients who get thrombotic strokes experience a decline in cognitive abilities. Furthermore, ventriculoatrial activity may act as a premature atrial contraction and initiate AF by causing a structural change in the atrium (19). Måneheim et al. stated that increased PVCs independently double the likelihood of developing AF (20). In our research, mild CI was more prevalent in the group with more frequent PVCs.

Hypertension stage and exposure time are associated with cognitive functions. CI aggravates by causing vascular remodeling and microinfarctions (21). Zhou et al. reported that the change in SBP was associated with low memory functions, while DBP was associated with low executive function (22). In our study, patients with hypertension were excluded and their systolic and diastolic blood pressures were evaluated as normal. Although there was a statistically substantial distinction among the groups, it was not clinically significant.

Cardiovascular disease is more likely to impact ventricular repolarization than ventricular excitement (23). Zonneveld et al. noted that longer QT, JT, JTc, and QRS intervals were linked to mild CI (24). Lucas et al. revealed that TpTe length decreased global cognitive performance, especially in the elderly population (25). Coppola et al. emphasized that mild cognitive impairment was common in those with high QT dispersion (26). Mehinrad et al. stated that there was a rapid decline in all cognitive functions in patients with wide QRS-T

angle (27). Our study found a significant negative correlation between the mini-MSE score, which evaluates cognitive functions, and ECG parameters.

HRV causes cognitive dysfunction with stimulated brain damage due to an increase in isovolumetric contraction and an increase in mean arterial pressure (28). Kallen et al. found a correlation between RMSSD, LF, HF, SDNN, and CI in individuals with frequent PVCs. (29). Howell et al. stated that high HRV caused a decrease in verbal or visuospatial memory (30). Conversely, Frewen et al. noticed that reduced HRV was linked to decreased linguistic and attention performance (31). Solernó et al. noted that LF/HF ratio was associated with worse cognitive performance in memory and language (32). In this study, there was no correlation between RMSSD and attention or calculation but, a significant correlation was between LF/HF and language.

Limitations

The number of patients in the study was small and consisted of single-center data. PVCs were not differentiated from right or left ventricular origin. Post-treatment patients were not evaluated and only mini-MSE was applied.

CONCLUSION

Our research revealed a correlation between cognitive impairment and more than 5% PVCs in Holter. Nevertheless, long-term follow-up studies are required to determine if HRV and ECG parameters can identify PVC patients at risk of cognitive impairment in the future and whether PVC treatment may prevent cognitive impairment.

Conflict of interest

None.

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None

Author Contributions

Conceptualization; Serhat Günlü (SG), Elif Merve Kurt Tunagur (EMKT), Muhammed Raşit Tanircan (MRT), Adem Aktan (AA), Fethullah Kayhan (FK), Mehmet Zülkif Karahan (MZK); Data curation; SG, AA, MZK, MRT; Formal analysis; SG, FK, MRT, AA; Funding acquisition; SG, AA, MZK; Investigation; SG, FK, MZK; Methodology; SG, EMKT, FK, MRT; Project administration; SG, AA, EMKT, FK; Resources; SG, MZK; Software; SG, MZK; Supervision; SG, MZK, EMKT; Validation; SG, AA; Visualization; SG, MRT; Roles/Writing - original draft; SG, FK, AA, MZK; Writing - review & editing; SG, EMKT, MZK

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Traditional And Complementary Treatment Practices Used In The Covid-19 Pandemic In Turkey: A Cross-Sectional Study

Türkiye'de Covid-19 Pandemisinde Kullanılan Geleneksel ve Tamamlayıcı Tedavi Uygulamaları: Kesitsel Bir Araştırma

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Abstract

Introduction: Traditional and Complementary Medicine interventions can be used for reasons such as strengthening the immune system, preventing diseases and controlling the disease. It was aimed to determine the herbs and supplements that individuals use to prevent COVID-19 infection, strengthen immunity or alleviate symptoms.

Materials and Methods: This descriptive study was carried out with 836 individuals by using an online survey with the virtual snowball sampling method between December 2021 and March 2022. "Sociodemographic Questionnaire" and "The usage of Traditional and Complementary Treatment Practices in the COVID-19 Pandemic" were used to collect the data. The data were collected by sending the online link prepared using "Google Forms" via e-mail or WhatsApp. Mean, standard deviation, percentage and chi-square analysis were used to evaluate the data.

Results: The individuals' mean age was 37.73±12.106 and 76.9% were women. It was determined that 68.9% had knowledge about Traditional and Complementary Medicine, 17.3% used vitamin C, 15.0% ginger, 13.9% had a fruit and vegetable diet, 7.1% were walking to protect themselves from COVID-19 during the pandemic; 3.5% started using thyme, 3.3% ginger, 4.2% vitamin C, 4.2% zinc, and 2.6% received intravenous vitamins and supplements after they became COVID-19.

There was a statistically significant difference between the knowledge of the individuals about Traditional and complementary medicine and their individual characteristics (gender, education level, having a chronical disease, and having elderly/chronically ill person/persons at home) ($p< 0.05$).

Conclusion: In our study, the individuals in the community used various Traditional and Complementary Medicine methods during the COVID-19 pandemic and most of them had knowledge about these methods. In order to ensure the appropriate use of Traditional and complementary medicine in Turkey, accurate information and planned training programs are recommended.

Key words: COVID-19; Prevention; Therapy; Traditional Medicine; Complementary Medicine

Özet

Giriş: Geleneksel ve tamamlayıcı tıp uygulamaları bağışıklık sisteminin güçlendirilmesi, hastalıkların önlenmesi ve hastalığın kontrol altına alınması gibi nedenlerle kullanılabilmektedir. bu çalışmada bireylerin COVID-19 enfeksiyonunu önlemek, bağışıklığı güçlendirmek veya semptomları hafifletmek için kullandıkları bitki ve takviyelerin belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Tanımlayıcı nitelikteki bu çalışma, Aralık 2021 - Mart 2022 tarihleri arasında sanal kartopu örnekleme yöntemi ile çevrimiçi anket kullanılarak 836 kişi ile gerçekleştirilmiştir. Veri toplamak için "Sosyodemografik form" ve "COVID-19 Pandemisinde Geleneksel ve Tamamlayıcı Tedavi Uygulamalarının Kullanımı Formu" kullanılmıştır. Veriler, "Google Forms" kullanılarak hazırlanan çevrimiçi bağlantının e-posta veya WhatsApp aracılığıyla gönderilmesiyle toplanmıştır. Verilerin değerlendirilmesinde ortalama, standart sapma, yüzde ve ki-kare analizi kullanılmıştır.

Bulgular: Bireylerin yaş ortalaması 37,73±12,106 olup %76,9'u kadındır. Bireylerin %68,9'unun Geleneksel ve Tamamlayıcı Tıp hakkında bilgi sahibi olduğu, %17,3'ünün C vitamini, %15,0'ının zencefil kullandığı, %13,9'unun meyve ve sebze diyeti yaptığı, %7,1'inin pandemi sırasında COVID-19'dan korunmak için yürüyüş yaptığı; %3,5'inin kekik, %3,3'ünün zencefil, %4,2'sinin C vitamini, %4,2'sinin çinko kullanmaya başladığı ve %2,6'sının COVID-19 olduktan sonra damar içi vitamin ve takviye aldığı belirlenmiştir.

Bireylerin Geleneksel ve Tamamlayıcı Tıp hakkındaki bilgileri ile bireysel özellikleri (cinsiyet, eğitim düzeyi, kronik bir hastalığa sahip olma ve evde yaşlı/kronik hasta kişi/kişiler olması) arasında istatistiksel olarak anlamlı bir fark bulunmuştur ($p< 0,05$).

Sonuç: Çalışmamızda, toplumdaki bireylerin COVID-19 pandemisi sırasında çeşitli TCM yöntemlerini kullandıkları ve çoğunun bu yöntemler hakkında bilgi sahibi olduğu görülmüştür. Türkiye'de Geleneksel ve Tamamlayıcı Tıp uygulamalarının uygun kullanımını sağlamak için doğru bilgilendirme ve planlı eğitim programları önerilmektedir.

Anahtar kelimeler: COVID-19; Önleme; Tedavi; Geleneksel Tıp; Tamamlayıcı Tıp

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GİRİŞ

Aralık 2019'un sonunda, Çin'in Wuhan kentinde ortaya çıkan yeni bir SARS-CoV-2 (COVID-19)'nin neden olduğu koronavirüs enfeksiyonu, yüksek hava yoluyla bulaşma potansiyeli nedeniyle birkaç hafta içinde hızla dünyaya yayılarak küresel sağlık krizine neden oldu. Dünya Sağlık Örgütü (DSÖ) tarafından Mayıs 2020'de dünya çapında yaklaşık 5 milyon COVID-19 hastası ve 30 binden fazla ölüm olduğu bildirilmiştir (1,2). Hastalık COVID-19 ile enfekte kişilerden damlacık enfeksiyonu yoluyla göz, burun veya ağız mukozasından bulaşabilmektedir. Koronavirüs ile enfekte vakaların bazılarında semptom gözlenmemekte (yaklaşık %80), bazılarında ağrı, burun tıkanıklığı, burun akıntısı, boğaz ağrısı ve diyare görülebilmekte, bazı hastalarda ise ciddi solunum yolu sıkıntıları nedeni ile entübasyon ve mekanik ventilasyon gerekmektedir (2,3). COVID-19' un halihazırda mevcut olan ilaçların başka bir amaçla kullanılması ve aşı dışında kanıtlanmış tedavisinin olmaması nedeniyle zengin bir geleneksel tıp geçmişine sahip Çin ve Hindistan gibi ülkeler, COVID-19'u tedavi etmek için geleneksel ilaçların etkinliğini araştırmaktadırlar (3,4). Charan ve arkadaşlarının Hindistan'da bir izolasyon merkezinde COVID-19 hastaları tarafından Tamamlayıcı ve Alternatif Tıp (TAT) ve Evde Tedavi Yöntemlerinin kullanımını araştırdıkları bir çalışmada araştırmaya katılan 495 katılımcının 367'si (%74,1) herhangi bir TAT ürünü kullanmadığını, 128 (%25,8)'i tedavi sırasında ve sonrasında 161 TAT ürünü kullandığını belirtmiştir (5).

Geleneksel ve Tamamlayıcı Tıp (GETAT-Traditional and Complemantary Medicine) da özellikle geleneksel Çin tıbbında veya Hindistan'da Ayurveda tıp sisteminde kullanılan bitkisel ilaçlar, yüzyıllar boyunca yaygın kullanımları nedeniyle genellikle "güvenli" olarak kabul edilmektedirler (6). Yalnız uzun süreli kullanımda bu müdahalelerin güvenliğini sağlamak oldukça önemlidir. Çünkü literatürde başka komorbiditeleri olan hastaların bu ürünlerin devam eden ilaçları ile ilaç-bitki etkileşimi nedeniyle zararlı bir etkiye sahip olabileceği belirtilmektedir (5). Ayrıca, literatürde hastaların bağışıklık sistemi COVID-19 enfeksiyonunda önemli bir rol oynadığından, immünomodülatör etkiye sahip olan bitkisel bir ilacın, COVID-19 enfeksiyonunu önleyici ve hatta tedavi edici potansiyele sahip olabileceği de belirtilmektedir. Örneğin, zerdeçal, antioksidan, antienflamatuar, antimutajenik, antikanser ve antimikrobiyal etkileri nedeniyle Asya'daki birçok ülke tarafından geleneksel olarak bir ilaç veya takviye olarak kullanılmaktadır (7). Bu bağışıklık düzenleyici gıdaları ve bitkileri kullanmak bağışıklık sistemini güçlendirebilir ve vücudu COVID-19'a karşı koruyabilir (1). Bağışıklık sistemi ile egzersiz, stres azaltma, sağlıklı beslenme, doğada zaman geçirme, olumlu içsel tutumlar ve esenlik gibi çeşitli yaşam tarzı faktörleri arasındaki karmaşık ilişkiler zaten gösterilmiştir (8). Ancak, bu gözlemler bilimsel veya klinik çalışmalarla doğrulanmalıdır (1).

GETAT önlemleri, dünya çapında bireyler ve toplumlar arasında stres, korku, endişe ve depresyona neden olan COVID-19 salgını gibi olağanüstü bir durumda yaşam kalitesini iyileştirmek için tamamlayıcı bir tedavi olarak kullanılabilir. Bu çalışma, ülkemizde COVID-19 sürecinde bireylerin COVID-19'a yönelik GETAT yöntemlerini kullanım durumunun belirlenmesi amacıyla yapılmıştır.

GEREÇ VE YÖNTEMLER

Tanımlayıcı olarak planlanan bu araştırmanın evrenini Türkiye'de yaşayan ve 18 yaşından büyük bireyler oluşturdu. Çalışmada, COVID-19 pandemisinde uygulanan kısıtlamalar nedeniyle olasılıksız örnekleme yöntemlerinden "Kartopu Örneklem Seçim Tekniği" kullanılmıştır. Kartopu örneklem yöntemi, evren hakkındaki bilgilerin eksik olduğu ve evreni oluşturan bireylere ulaşmanın zor olduğu durumlarda kullanılan bir tekniktir (9).

Araştırmanın veri toplama formu hazırlanırken "Google Forms" uygulaması kullanılmıştır. Araştırmacılar kendi sosyal medya hesaplarındaki kişilere ve katılmayı gönüllü olarak kabul eden bireylere anket formunu yönlendirerek, bu kişilerin de kendi hesaplarında kayıtlı olan kişilere ulaşması istenerek katılımcılara ulaşılmıştır. Online anket linki e-posta veya WhatsApp yolu ile gönderilmiştir. Anketin başlangıcında katılımcıların çalışmaya katılmak için onamı alınmıştır. Kabul etmeyenlere anket açılmamıştır.

Örneklem büyüklüğü, G*Power 3.1.9.7 (Franz Faul, Universitat Kiel, Germany) programında, Dişsiz ve Yılmaz'ın (2016) çalışmasında GETAT kullanan ve kullanmayan yetişkinlerin Sağlık Okuryazarlığı Ölçek puan ortalamaları kullanılarak 0.05 anlamlılık düzeyi, düşük etki büyüklüğü (0.19) ve %80 güç baz alınarak hesaplanmış ve 834 kişi olarak belirlenmiştir (10). %10 kayıp oranı dikkate alınarak hesaplanan örneklem büyüklüğü 918 kişidir. Araştırmanın örneklemini, Aralık 2021- Mart 2022 tarihleri arasında çalışmaya katılmayı kabul eden 836 birey oluşturmuştur.

Dahil edilme kriterleri:

-18 yaş ve üzeri olmak

-Türkçe konuşmak ve okuyabilmek

Araştırmanın verileri araştırmacılar tarafından oluşturulan katılımcıların yaş, cinsiyet, ekonomik durumu, COVID-19 geçirme durumu, COVID-19 aşısı olma durumu ve kronik hastalığının olup olmadığını belirleyen 13 sorudan oluşan 'Sosyodemografik Soru Formu' ile 7 sorudan oluşan 'COVID-19 Pandemisinde Geleneksel ve Tamamlayıcı Tedavi Uygulamalarının kullanımı'nı gösteren form ile toplandı. Bu formda katılımcıların COVID-19 pandemi öncesinde ve pandemi süresince kullandığı bitki ve destekleyici ürünlerin belirlendiği sorular yer almaktadır. Sorular literatür

doğrultusunda araştırmacı tarafından hazırlanmıştır (11-13).

Araştırmanın bağımlı değişkenleri arasında bireylerin tamamlayıcı tedavi kullanım durumu yer alırken; bağımsız değişkenleri arasında ise bireylerin yaş, cinsiyet, meslek, eğitim düzeyi, gelir düzeyi, çalışma durumu, COVID-19 geçirme durumu, COVID-19 aşısı olma durumu, kronik hastalığa sahip olma durumu gibi özellikler yer almıştır.

Verilerin analizi SPSS 22.0 programı kullanılarak yapılmıştır. Tanımlayıcı özelliklerin değerlendirilmesinde ortalama, standart sapma, sayı ve yüzde kullanılmıştır. Bireylerin bazı sosyo-demografik özellikleri ile Covid-19 pandemi öncesinde ve pandemi süresince kullandıkları bitki ve destekleyici ürünler arasında fark olup olmadığını belirlemek için de ki kare analizi kullanılmıştır. İstatistiksel anlamlılık sınırı $p < .05$ olarak alınmıştır.

Bu araştırma, Helsinki Bildirgesi'nde belirtilen ilkelere uygun olarak gerçekleştirilmiş olup araştırma ve yayın etiği ilkelerine uyulmuştur. Araştırmacılar tarafından araştırmanın yürütülmesi için bir üniversitenin etik kurulundan (27/10/2021 tarihinde yapılan 10 No.lu toplantıda, sayı: 61351342/EKİM 2021-26) izin alınmıştır. Bireylere ulaştırılan online anketin ilk bölümüne aydınlatılmış onam formu eklenmiş ve çalışmaya gönüllü olarak katıldığını beyan eden kişiler araştırmaya dahil edilmiştir.

BULGULAR

Bu çalışmaya katılan bireylerin %76.9'u kadın, %64.7'si lisans mezunu, %19.9'u öğrenci, ve yaş ortalaması 37.73±12.106'dır. Katılımcıların %52.8'inin gelirlerinin giderlerine eşit, %60.9'unun çalışmadığı, %14.1'inin kronik bir hastalığının bulunduğu, %35.5'inin evde yaşlı/kronik hastalığa sahip kişi ve/veya kişilerin olduğu, %31.8'inin COVID-19 geçirdiği, %94.1'inin COVID-19 aşısı yaptırdığı ve %68.9'unun GETAT uygulamaları hakkında bilgi sahibi olduğu belirlenmiştir (Tablo 1).

Araştırmaya katılan bireyler COVID-19 pandemi öncesinde devamlı kullandıkları baharatın nane (%80.1), bitki çayının ıhlamur (%74.2), yemek ve çorbalarda kullandıkları ürünün limon (%90.9) ve ağızdan aldıkları vitamin takviyesinin ise D vitamini (%38.5) olduğunu ifade etmiştir.

COVID-19'dan korunmak için pandemi döneminde bireylerin %15.0'inin baharat olarak zencefili, %12.2'sinin bitki çayı olarak yine zencefili, %9.6'sının yemek/çorbalarda üzüm sirkesini kullandıkları ve %17.3'sinin ise ağızdan C vitamini takviyesini aldıkları görülmüştür.

Bireyler COVID-19 olduktan sonra en sık kullanmaya başladıkları baharatın zencefil (%3.1), bitki çayı olarak kekik (%3.5), yemek ve çorba olarak kelle paça çorbası (%3.7) ve ağızdan alınan vitamin takviyesi olarak C vitamini (%4.2) aldıklarını belirtmişlerdir (Tablo 2).

Araştırmaya katılan bireylerin %82.52inin COVID-19 pandemi öncesinde devamlı olarak protein ağırlıklı beslendiği, pandemi döneminde ise COVID-19'dan korunmak için %13.9'unun daha fazla meyve/sebze tükettiği saptanmıştır. Bireylerin %74.6'sının COVID-19 pandemi öncesinde, %7.1'inin ise pandemi döneminde yürüyüş yaptığı, COVID-19 geçirenlerin ise hastalıktan sonra %2.6'sının voleybol, %2.42ünün ise yoga yaptıkları görülmüştür. Bireylerin COVID-19 pandemi öncesinde sıklıkla yaptırdıkları girişimsel uygulamaların hacamat (%12.4) olduğu, pandemi döneminde COVID-19'dan korunmak için damardan vitamin ve destek uygulamaları (%4.2) yaptırdığı, COVID-19 olan katılımcıların da hastalık sonrasında en çok damardan vitamin ve destek uygulamaları (%2.6) yaptırdığı belirlenmiştir (Tablo 3).

Bu çalışmaya katılan bireylerin bireysel özellikleri (cinsiyet, eğitim düzeyi, kronik hastalığa sahip olma ve evde yaşlı/kronik hastalığa sahip kişi ve/veya kişilerin olma durumları) ile GETAT konusunda bilgi sahibi olma durumları karşılaştırıldığında istatistiksel olarak anlamlı farklılık olduğu belirlenmiştir ($p<0.05$) (Tablo 4).

Araştırmaya katılan bireylerin yaş, cinsiyet, gelir düzeyi, eğitim düzeyi, çalışma durumu, kronik hastalığa sahip olma durumu, evde yaşlı/kronik hastalığa sahip kişi ve/veya kişilerin olma durumu, COVID-19 aşısı olma durumu, GETAT uygulamaları hakkında bilgi sahibi olma durumu ile COVID-19 geçirip-geçirmeme durumları karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık bulunmamıştır ($p > 0.05$).

TARTIŞMA

Bu araştırma, COVID-19 nedeni ile nüfusun çoğunluğunun aşılı olduğu ve ölümlerin oldukça azaldığı dönemde, bireylerin GETAT uygulamaları olarak kullandıkları bitki ve destekleyici ürünlerin belirlenmesi için yapılmıştır.

Featherstone ve ark. nın yaptıkları çalışmada kadınların erkeklere oranla GETAT uygulamalarını daha fazla kullandıkları belirlenmiştir (14). Karataş ve ark. nın yaptığı çalışmada da GETAT kullanan katılımcılar arasında kadınların GETAT kullanma oranının (%64) daha yüksek olduğu saptanmış (11). Çalışmada COVID-19 pandemi öncesinde kadınların %79.7'si nane, %79.42'ü ıhlamur, %78.9'u D vitaminini devamlı olarak kullanırken, erkeklerin %20.3'ünün nane, %22.8'inin limon ve %21.1'inin D vitaminini devamlı olarak kullandığı görüldü.

Kılıç ve ark. nın çalışmasında meditasyon, Tai Chi, ayuverdik tıp, masaj, reiki, maneviyat (dua) ve bitkisel terapi bilgisi erkek ve kız öğrenciler arasında istatistiksel olarak farklı bulunmuştur. Kız öğrenciler Tai Chi, ayuverdik tıp, reiki hakkında daha fazla bilgi sahibiyken, erkekler meditasyon, masaj, maneviyat (dua) ve bitkisel terapi hakkında daha fazla bilgiye sahip olduğu görülmüştür (15). Bu çalışmada da kadınların (%72.0) GETAT konusunda anlamlı derecede daha fazla bilgiye sahibi olduğu saptanmıştır.

Tablo 1. Katılımcıların Tanıtıcı Verileri (n=836)

Tanıtıcı Veriler	n	%
Yaş X±SS = 37.73±12.106		
19-25	139	16.6
26-45	430	51.4
46 ve üzeri	267	31.9
Cinsiyet		
Kadın	643	76.9
Erkek	193	23.1
Gelir düzeyi		
Geliri Giderinden Fazla	148	17.7
Geliri Giderine Eşit	441	52.8
Geliri Giderinden Az	247	29.5
Eğitim düzeyi		
İlköğretim	77	9.2
Lise	142	17.0
Lisans	541	64.7
Lisansüstü	76	9.1
Çalışma durumu		
Çalışıyor	327	39.1
Çalışmıyor	509	60.9
Meslek		
Sağlık çalışanı	126	15.1
İşçi	115	13.8
Memur	76	9.1
Emekli	34	4.1
Öğrenci	166	19.9
Ev hanımı	120	14.4
Eğtimci	98	11.7
Diğer	101	12.1
Kronik hastalığa sahip olma durumu		
Var	118	14.1
Yok	718	85.9
Evde yaşlı/kronik hastalığa sahip kişi ve/veya kişilerin olma durumu		
Var	297	35.5
Yok	539	64.5
COVID-19 geçirme durumu		
Evet	266	31.8
Hayır	570	68.2
COVID-19 aşısı olma durumu		
Evet	787	94.1
Hayır	49	5.9
COVID-19 aşısı olmadıysa nedeni (n=44)		
Güvenmeme	18	40.9
Etkili/ gerekli olmadığını düşünme	4	9.1
Alerji/ kronik hastalığı olma	4	9.1
Korku	3	6.8
Nedeni yok	3	6.8
Yan etkilerinin olması	2	4.5
Hamile olma/ emziriyor olma	2	4.5
Diğer	8	18.3
GETAT uygulamaları hakkında bilgi sahibi olma durumu		
Evet	576	68.9
Hayır	260	31.1
GETAT uygulamaları hakkında bilgiyi nereden aldığı*(n=1602)		
Aile/Arkadaş	414	25.8
Sağlık personeli	244	15.2
Aktarlar	104	6.5
Sosyal medya	572	35.7
Diğer	268	16.8

Tablo 2. Katılımcıların Kullandığı Ürünler (n=836)

Ürünler	COVID-19 pandemi öncesinde devamlı olarak kullanıyordum	COVID-19'dan korunmak için pandemi döneminde kullandım	COVID-19 olduktan sonra kullanmaya başladım	Kullanmadım
Baharatlar	n (%)	n (%)	n (%)	n (%)
Nane	670 (80.1)	49 (5.9)	8 (1.0)	109 (13.0)
Kekik	607 (72.6)	76 (9.1)	20 (2.4)	133 (15.9)
Zencefil	374 (44.7)	125 (15.0)	26 (3.1)	311 (37.2)
Zerdeçal	326 (39.0)	84 (10.0)	18 (2.2)	408 (48.8)
Susam	493 (59.0)	34 (4.1)	15 (1.8)	294 (35.2)
Çörek otu	494 (59.1)	54 (6.5)	17 (2.0)	271 (32.4)
Üzüm çekirdeği	214 (25.6)	37 (4.4)	25 (3.0)	560 (67.0)
Bitki çayları				
Zencefil	265 (31.7)	102 (12.2)	19 (2.3)	450 (53.8)
Zerdeçal	181 (21.7)	79 (9.4)	19 (2.3)	557 (66.6)
Kekik	288 (34.4)	94 (11.2)	29 (3.5)	425 (50.8)
Yeşil çay	499 (59.7)	68 (8.1)	14 (1.7)	255 (30.5)
Adaçayı	387 (46.3)	68 (8.1)	20 (2.4)	361 (43.2)
Ginseng	73 (8.7)	50 (6.0)	23 (2.8)	690 (82.5)
Kuşburnu	406 (48.6)	56 (6.7)	16 (1.9)	358 (42.8)
Ihlamur	620 (74.2)	72 (8.6)	14 (1.7)	130 (15.6)
Ekinezya	88 (10.5)	38 (4.5)	20 (2.4)	690 (82.5)
Yemek ve çorbalarda kullanılan ürünler				
Limon	760 (90.9)	46 (5.5)	6 (0.7)	24 (2.9)
Soğan	719 (86.0)	44 (5.3)	10 (1.2)	63 (7.5)
Sarımsak	684 (81.8)	51 (6.1)	13 (1.6)	88 (10.5)
Elma sirkesi	398 (47.6)	78 (9.3)	26 (3.1)	334 (40.0)
Üzüm sirkesi	375 (44.9)	64 (7.7)	27 (3.2)	370 (44.3)
Zencefil	265 (31.7)	80 (9.6)	28 (3.3)	463 (55.4)
Zerdeçal	266 (31.8)	73 (8.7)	25 (3.0)	472 (56.5)
Kelle paça çorbası	329 (39.4)	61 (7.3)	31 (3.7)	415 (49.6)
Kimyon	527 (63.0)	32 (3.8)	16 (1.9)	261 (31.2)
Kişniş	197 (23.6)	32 (3.8)	26 (3.1)	581 (69.5)
Fesleğen	300 (35.9)	25 (3.0)	29 (3.5)	482 (57.7)
Tarçın	480 (57.4)	37 (4.4)	19 (2.3)	300 (35.9)
Karabiber	684 (81.8)	34 (4.1)	14 (1.7)	104 (12.4)
Ağızdan alınan vitamin takviyesi				
D vitamini	322 (38.5)			
C vitamini	281 (33.6)	132 (15.8)	34 (4.1)	348 (41.6)
B12 vitamini	304 (36.4)	145 (17.3)	35 (4.2)	375 (44.9)
Bakır	70 (8.4)	75 (9.0)	28 (3.3)	429 (51.3)
Çinko	108 (12.9)	48 (5.7)	24 (2.9)	694 (83.0)
Balık yağı	154 (18.4)	84 (10.0)	35 (4.2)	609 (72.8)
Magnezyum	145 (17.3)	56 (6.7)	27 (3.2)	599 (71.7)
Kalsiyum	163 (19.5)	56 (6.7)	27 (3.2)	608 (72.7)
		52 (6.2)	27 (3.2)	594 (71.1)

Tablo 3. Katılımcıların Beslenme, Egzersiz Türü ve Yaptıkları Girişimsel Uygulamalar (n=836)

Beslenme/ Ergzersiz/ Girişimsel Uygulamalar	COVID-19 pandemi öncesinde devamlı olarak kullanıyordum	COVID-19'dan korunmak için pandemi döneminde kullandım	COVID-19 olduktan sonra kullanmaya başladım	Kullanmadım/ Yapmıyorum/ Yaptırmıyorum
	n (%)	n (%)	n (%)	n (%)
Beslenme				
Meyve ve sebze ağırlıklı	657 (78.6)	116 (13.9)	16 (1.9)	47 (5.6)
Protein ağırlıklı	690 (82.5)	88 (10.5)	15 (1.8)	43 (5.1)
Karbonhidrat ağırlıklı	572 (68.4)	41 (4.9)	14 (1.7)	209 (25.0)
Yağ ağırlıklı	382 (45.7)	45 (5.4)	16 (1.9)	393 (47.0)
Kuruyemiş ağırlıklı	635 (76.0)	107 (12.8)	16 (1.9)	78 (9.3)
Egzersiz				
Yoga	98 (11.7)	35 (4.2)	20 (2.4)	683 (81.7)
Yürüyüş	624 (74.6)	59 (7.1)	11 (1.3)	142 (17.0)
Reiki	37 (4.4)	27 (3.2)	12 (1.4)	760 (90.9)
Basketbol	102 (12.2)	29 (3.5)	10 (1.2)	695 (83.1)
Futbol	137 (16.4)	23 (2.8)	16 (1.9)	660 (78.9)
Voleybol	152 (18.2)	21 (2.5)	22 (2.6)	641 (76.7)
Girişimsel Uygulamalar				
Hacamat	104 (12.4)	26 (3.1)	11 (1.3)	695 (83.1)
Ozon	26 (3.1)	26 (3.1)	9 (1.1)	775 (92.7)
Sülük	51 (6.1)	24 (2.9)	9 (1.1)	752 (90.0)
Damardan vitamin ve destek uygulamaları	80 (9.6)	35 (4.2)	22 (2.6)	699 (83.6)

Tablo 4. Katılımcıların GETAT Konusunda Bilgi Sahibi Olma Durumları ile İlişkili Özellikler (n=836)

Özellik	GETAT Bilgisi Olan		GETAT Bilgisi Olmayan		Değer**
	n	%	n	%	p*
Yaş					0.135
19-25	87	62.6	52	37.4	
26-45	296	68.8	134	31.2	
46 ve üzeri	193	72.3	74	27.7	
Cinsiyet					0.001
Kadın	463	72.0	180	28.0	
Erkek	113	58.5	80	41.5	
Gelir düzeyi					0.233
Geliri Giderinden Fazla	103	69.6	45	30.4	
Geliri Giderine Eşit	313	71.0	128	29.0	
Geliri Giderinden Az	160	64.8	87	35.2	
Eğitim düzeyi					0.022
İlköğretim	54	70.1	23	29.9	
Lise	87	61.3	55	38.7	
Lisans	373	68.9	168	31.1	
Lisansüstü	62	81.6	14	18.4	
Çalışma durumu					0.078
Çalışıyor	237	72.5	90	27.5	
Çalışmıyor	339	66.6	170	33.4	
Kronik hastalığa sahip olma durumu					0.002
Var	96	81.4	22	18.6	
Yok	480	66.9	238	33.1	
Evde yaşlı / kronik hastalığa sahip kişi ve/veya kişilerin olma durumu					0.012
Var	221	74.4	76	25.6	
Yok	355	65.9	184	34.1	
COVID-19 aşısı olma durumu					0.265
Evet	546	69.4	241	30.6	
Hayır	30	61.2	19	38.8	
COVID-19 geçirme durumu					0.471
Evet	188	70.7	78	29.3	
Hayır	388	68.1	182	31.9	

*p<0.05 anlamlılık düzeyi; **Pearson ki kare testi

Literatürde Türkiye'de GETAT uygulamaları konusunda sağlık profesyonelleri ve tıp öğrencilerinin bilgi düzeylerini ve tutumlarını irdeleyen çalışma sayılarının giderek artmakta olduğu, gelecek yıllarda GETAT'ın yaygınlaşması ve bireylerin doğru bilgilenmesi açısından araştırmaların önemli olduğu belirtilmektedir (16). Yine, Gardiner ve ark. nın çalışmalarında sağlık okuryazarlığı ile GETAT deneyimi arasında pozitif yönlü bir ilişkili olduğu gösterilmiştir (17). Karataş ve ark. nın çalışmasında da öğrenim düzeyi yüksek ve daha önce GETAT kullanan katılımcıların GETAT kullanma oranlarının daha yüksek olduğu belirlenmiştir (11). Yapılan başka bir araştırma ise ilkokul mezunu olan kadınların GETAT kullanımlarının daha yaygın olduğu ortaya konmuştur (18). Ibrahim ve ark. nın yaptığı çalışmada da GETAT kullanımının eğitim düzeyi yükseldikçe kullanım oranının anlamlı olarak azaldığı bildirilmiştir (12). Bu çalışmada eğitimi lisans düzeyinde olan katılımcıların GETAT konusunda anlamlı derecede daha fazla bilgiye sahibi olduğu bulunmuştur. Bulgulardaki farklılığın çalışmaların örneklem büyüklüğü, popülasyon ve bireylerin hastalık özelliklerindeki farklılıklardan kaynaklandığı düşünülmektedir.

Kanser, diyabet ve kardiyovasküler hastalık gibi kronik hastalıklar dünyada olduğu gibi ülkemizde de yaşlı nüfusun çoğalması ile birlikte giderek artmadır. Literatürde de, kronik hastalığı olan bireylerin birçok tedaviyi bir arada almak ve hastalıklarını kendileri yönetmek durumunda kalmaları nedeniyle, hastalıklarının tedavi ve semptomlarının hafifletilmesinde GETAT yöntemlerine başvurulduğu belirtilmektedir (16,19). Ayraler ve ark. nın yapmış olduğu çalışmada, kronik hastalık varlığı ve GETAT bilgi ve tutum düzeyleri arasında fark görülmediği belirtilmektedir (16). Çalışmada kronik hastalığa sahip olan bireylerin olmayanlara göre GETAT konusunda anlamlı derecede daha fazla bilgiye sahibi olduğu bulundu. Bu bize kronik hastalığı olanların sağlıkla ilgili sorunlarını en aza indirmek ve kontrol altına almak için bitkisel ürün ve besin takviyeleri kullandıkları, çareyi daha çok geleneksel yaklaşımlarda aradıklarını ve bu nedenle daha fazla araştırdıklarını düşündürmektedir.

Literatürde GETAT kullanımının ülkenin coğrafi konumuna, etnik kökene, eğitime, sosyoekonomik faktörlere ve dini inanışlara göre farklılık göstermekte olduğu; batı ülkelerinde en sık multivitaminler, meditasyon, hipnoterapi, gevşeme egzersizleri ve aromaterapi kulanırken; doğu ülkelerinde bitkisel karışımların kullanıldığı belirtilmektedir (20). Çalışmada COVID-19 pandemi öncesinde, bireylerin en sık olarak nane, ıhlamur, limon ve D vitamini kullandığı, protein ağırlıklı beslendiği, yürüyüş yaptığı ve girişimsel olarakta hacamat yaptırdığı görülmüştür. Pandemi döneminde ise, bireylerin COVID-19'dan korunmak için en sık olarak zencefil, C vitamini aldığı, meyve ve sebze ağırlıklı beslendiği, damardan vitamin ve destek uygulamaları yaptırdığı saptanmıştır. Gülgün ve Kaya'nın yapmış oldukları çalışmada katılımcıların en çok sırasıyla ıhlamur çayı, yeşil çay, kekik, adaçayı ve üzüm çekirdeği kullandığı, meyve-sebze-balık-tavuk-yoğurt, havuç, sarımsak, nar, hamur, sütlü tatlı ve kırmızı et tercih ettikleri belirlenmiştir (20). Çalışmanın literatürle kısmen uyumlu olduğu görülmektedir.

Literatürde GETAT kullanımının tıbbi, ekonomik ve sosyal yönü olduğu, GETAT uygulamalarının bireyler tarafından genellikle bilinçsiz şekilde kullanıldığı, bireylerin bu konuda eğitim almadan, çevrelerinden ya da yazılı ve görsel medyadan edindikleri bilgiler ile bu yöntemlere başvurduğu belirtilmektedir (21). Altan ve ark. nın tıp fakültesi öğrencileri arasında yaptıkları çalışmada, GETAT konusunda en sık bilgi edinilen kaynağın medya olduğu belirtilmiştir (22). Birçok çalışmada medyanın, özellikle de internetin en sık yararlanılan kaynak olduğu tespit edilmiştir (15,16,23). Çalışmaya katılan bireylerin GETAT uygulamaları konusunda benzer şekilde en sık bilgi kaynağı olarak sosyal medya olduğu karşımıza çıkmış, daha sonra aile ve arkadaşların geldiği saptanmıştır. Günümüzde internet erişimi ve teknoloji kullanımının insanların yaşamında yaygınlaşması ile bu yoldan bilgiye erişimin kolaylaştığı düşünülmektedir.

SONUÇ

Çalışma sonuçlarına göre katılımcıların COVID-19 öncesi dönem ve COVID-19 döneminde çeşitli GETAT uygulamalarını kullandıkları ve çoğunluğunun GETAT uygulamaları hakkında bilgi sahibi oldukları saptanmıştır. Günümüzde COVID-19 olmayı önleyecek herhangi bir besin veya besin takviyesi yoktur. Ancak destekleyici olarak kullanılmaktadır. Bireylerin hastalıklardan korunmak ve sağlıklı bir yaşam sürdürmeleri için bağışıklık sisteminin desteklenmesi gerekmektedir. COVID-19'da GETAT kullanımı ile ilgili sınırlı sayıda çalışma bulunmaktadır. Bu çalışma COVID-19 döneminde Türk nüfusu arasında yaygın olarak kullanılan GETAT yöntemleri hakkında bilgi vermektedir. GETAT yöntemleri ile ilgili daha spesifik çalışmalar yapılması önerilmektedir.

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Research Article

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The effectiveness of two different treatment approaches in individuals with chronic non-specific neck pain: a randomized control trial

Kronik non-spesifik boyun ağrısı olan bireylerde iki farklı tedavi yaklaşımının etkinliği: randomize kontrollü çalışma

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Abstract

Abstract

The aim of this study is to examine the effectiveness of conventional treatment and mobilization exercises in individuals with chronic nonspecific neck pain (CNNP). A total of 28 patients enrolled in the study. The Mobilization group (MG) completed a 4-week combined conservative physiotherapy and cervical mobilization program, whereas the control group (CG) received only the 4 weeks of conservative physiotherapy. Pain severity according to the Visual Analogue Scale (VAS) was used as primary outcome. Secondary outcomes were included the Bourdon Attention Test (BAT), Beck Anxsiety Scale (BAS), range of motion (ROM), muscle strength. All outcomes were assessed both prior to and following the treatment. In 2-way mixed-design repeated-measures ANOVA analysis, when the change in time was analyzed between the groups (Group*Time [interaction]), a statistical difference was found for the VAS (p = .000, ηp2 = .007), BAT score (p = .001, ηp2 = .082), BAS (p= .000, ηp2 = .001), ROM flexion (p= .000, ηp2 = .104), ROM extansion (p= .000, ηp2 = .076), ROM right rotation (p= .006, ηp2 = .033), ROM left rotation (p= .05, ηp2 = .006), ROM right lateral flexion (p= .000, ηp2 = .060), ROM left lateral flexion (p= .002, ηp2 = .019), muscle strength flexion (p= .000, ηp2 = .008), muscle strength extansion (p= .000, ηp2 = .019), muscle strength right rotation (p= .000, ηp2 = .012), muscle strength left rotation (p= .000, ηp2 = .001), muscle strength right lateral flexion (p= .000, ηp2 = .001) and muscle strength left lateral flexion (p= .000, ηp2 = .011) parameters in favour of MG. Cervical mobilization produced a significant benefit to recovery of pain, ROM, muscle strength, attention and anxiety outcomes of patients with CNNP when added to a conventional CNNP physical therapy program.

Keywords: Chronic non-specific neck pain, cervical mobilization, pain

The study was registered on the Clinical Trials Registry (registration number: NCT05377645)

Özet

Bu çalışmanın amacı kronik nonspesifik boyun ağrılı (KNBA) bireylerde mobilizasyon egzersizleri ile geleneksel tedavinin etkinliğinin karşılaştırılmasıdır. Çalışmaya 28 hasta dahil edildi. İki gruba da 4 hafta boyunca haftada 3 gün geleneksel tedavi uygulandı. Mobilizasyon grubuna (MG) geleneksel tedaviye ek olarak servikal mobilizasyon egzersizleri yapıldı. Primer sonuç ölçeği olarak ağrı, Vizüel Analog Skala (VAS) ile ölçüldü. Sekonder sonuç ölçeği olarak Burdon Dikkat Testi (BDT), Beck Anksiyete Skalası (BAS), eklem hareket açıklığı (ROM), kas gücü ölçümü yapıldı. Tüm ölçümler tedavi başlangıcında ve sonunda yapıldı. Gruplar arası iki yönlü tekrarlı ANOVA analizi sonucunda VAS (p = .000, ηp2 = .007), BDT skoru (p = .001, ηp2 = .082), BAS skoru (p= .000, ηp2 = .001), ROM fleksiyon (p= .000, ηp2 = .104), ROM ekstansiyon (p= .000, ηp2 = .076), ROM sağ rotasyon (p= .006, ηp2 = .033), ROM sol rotasyon (p= .05, ηp2 = .006), ROM sağ lateral fleksiyon (p= .000, ηp2 = .060), ROM sol lateral fleksiyon (p= .002, ηp2 = .019), fleksör kas gücü (p= .000, ηp2 = .008), ekstansör kas gücü (p= .000, ηp2 = .019), sağ rotasyon kas gücü (p= .000, ηp2 = .012), sol rotasyon kas gücü (p= .000, ηp2 = .001), sağ lateral fleksiyon kas gücü (p= .000, ηp2 = .001) ve sol lateral fleksiyon kas gücü (p= .000, ηp2 = .011) parametreleri mobilizasyon grubu lehine anlamlı bulunmuştur. KNBA'lı hastalarda konvansiyonel tedavi programına ek olarak uygulanan servikal mobilizasyon ağrı, ROM, kas gücü, dikkat ve anksiyete parametrelerinde sadece geleneksel tedavi uygulanan gruba göre anlamlı düzeyde iyileşme sağlamıştır.

Anahtar Kelimeler: Kronik non-spesifik boyun ağrısı, servikal mobilizasyon, ağrı

Bu çalışma klinik denemeler listesine kaydedilmiştir (kayıt numarası NTC05377645).

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Introduction

Chronic non-specific neck pain (CNNP) is known as a common public health problem in the modern world, (1) and although its lifetime prevalence is approaching 50% (2) it is frequently seen in adolescents (3). CNNP is considered severe discomfort in the lateral and posterior of the neck lasting more than 3 months (4) resulting from neck cancer, infection, poor posture, degenerative and mechanical changes (5, 6). CNNP causes disability, limitation of activities of daily living, job dissatisfaction, and increased economic and social costs (7, 8).

Various applications such as physiotherapy, exercise, massage, chiropractic, spinal mobilization and manipulation are used in the treatment (9, 10). Manual therapy (MT) is an increasingly popular treatment for people with CNNP, and many countries include it in their national guidelines for the treatment of musculoskeletal disorders (11-13). Overall, this treatment is considered to be more beneficial than non-invasive or placebo treatments(14-17). MT includes both passive techniques and active techniques. Palmgren et al. concluded that chiropractic practices positively affect prorioception and pain in patients with chronic neck pain (13). In another randomized controlled trial, Zaproudina et al. demonstrated that mobilization techniques reduce the level of disability and pain in patients with chronic neck pain (17). Also, Cleland et al. reported that thoracic spine manipulation had analgesic results in individuals with mechanical neck pain (18).

When the literature was examined, we could not find any comprehensive study examining the effects of conventional treatment and cervical mobilization exercises on pain, muscle strength, neck joint range of motion, anxiety and attention in individuals with CNNP. Therefore, the aim of this study is to examine the effectiveness of conventional treatment and mobilization exercises in individuals with CNNP.

Methods

Trial Design

The study design was a randomized, single-blind 1:1 parallel-group study and it was held at Kırşehir Ahi Evran University School of Physical Therapy and Rehabilitation between December 2021 and June 2022. The study proposal was approved by the local ethics committee, and conducted in accordance with the Declaration of Helsinki principles (12/24/2021). Prior to the study, written and oral consent was given by all participants and their families. The authors confirm that all ongoing and related trials for this study were registered. Due to an error of omission, the trial was registered retrospectively on May 17, 2022, before the data was analyzed (ClinicalTrials.gov Identifier: NCT05377645). We hereby state that all future trials will be registered prospectively.

Participants

The participants of the study were people who applied to Kırşehir Ahi Evran University Physical Therapy Hospital with chronc non-specific neck pain. Inclusion criteria for the study: 18-65 years of age, current neck pain lasting at least 3 months. Exclusion criteria: previous neck surgery, spinal fractures and tumors, people with visual and mental impairments that would affect the assessment.

Interventions

Participants received only the treatment determined by the investigators; they did not combine treatment with medications or other physiotherapy practices. Any additional intervention to the treatment was grounds for exclusion and they were warned about it. The treatment was applied in 12 sessions for 4 weeks.

Group 1: Conventional Group (CG)

Conventional Transcutaneous Electrical Nerve Stimulation (TENS- Elettronica Pagani Class1 type BF brand device with a frequency of 100 Hz, pulse duration 200 μsec and current strength between 20-35 mA) was applied to the neck area for 20 minutes along with hot application for 20 minutes in the patients in the CG (Figure 1) (19). In addition, neck isometric exercises were applied.

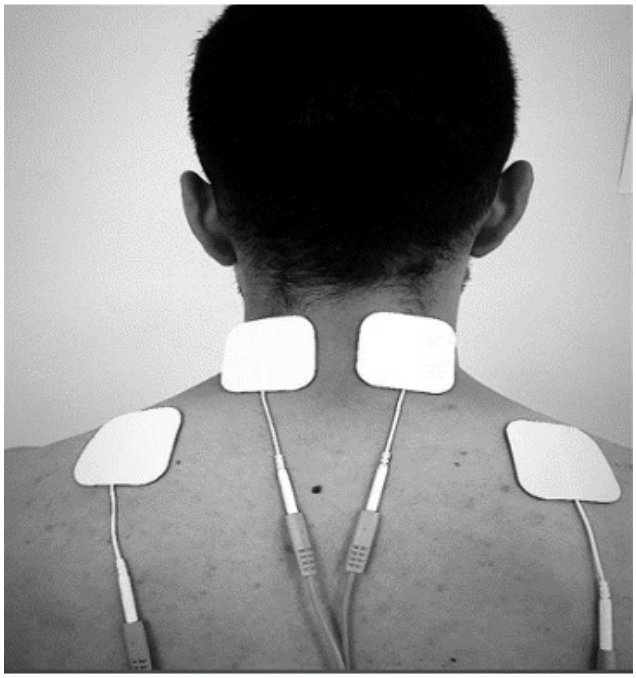

Figure 1. TENS application electrode placement

Group 2: Mobilization Group (MG)

In addition to the conventional physiotherapy program, cervical region mobilization was applied to the patients in MG. Cervical mobilization bridging technique (Figure 2), manual traction (MT) (Figure 3) with MT rotation (Figure 4), anterior-posterior with MT gliding (Figure 5), lateral gliding (Figure 6) was applied (20).

Figure 2. Bridge technique

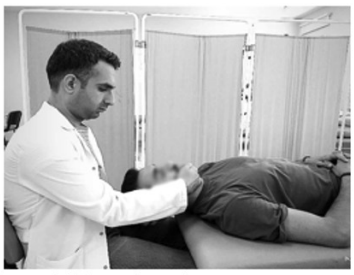

Figure 3. Manuel traction

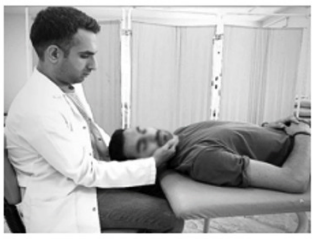

Figure 4. Rotation with manual traction

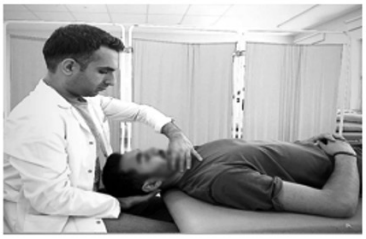

Figure 5. Anterior-posterior with MT gliding

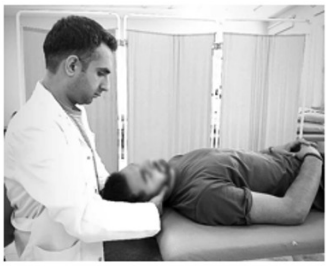

Figure 6. Lateral gliding

Outcomes

All measurements were repeated in the same way after the treatment.

Primary Outcome

Pain

Pain intensity was evaluated by marking the Visual Analogue Scale (VAS) on a 10 cm horizontal line (21).

Secondary Outcome

Anxiety

Anxiety levels of the patients were evaluated using the Beck Anxiety Scale (BAS). BAS individual lives evaluate the frequency of anxiety symptoms. Consisting of twenty-one items, between 0-3 a scored self-assessment is the scale. Trouble with questions asked to the patient how much has your feeling been in him for the past week? disturbing is questioned. Score Range is 0-63. The high score obtained from the scale, the severity of the anxiety experienced by the individual shows (22).

Cognitive assessment

The Bourdon Attention Test was used to assess the cognitive levels of the participants. The test developed in 1955 by Benjamin Bourdon. Turkish validity and reliability studies were carried out by Karaduman (23).

Range of Motion

The neck is active and passive range of motion (ROM) using the universal goniometer evaluated. Pivot of the goniometer with the patient in the sitting position designated point, fixed arm and movable arm placed in reference regions. The patient is active was asked to do the movements and actively passive after the end of ROM passively by continuing the movement joint ROM was measured (24).

Muscle Strength

Muscle strength of patients Lovett's levels were assessed using a manual muscle test scale ranging from 0 to 5. Muscle strength was evaluated bilaterally and the averages were recorded (24).

Sample Size

To determine the sample of the study, version 3.1.9.4 of the G*Power program (HeinrichHeine-Universita¨t Dusseldorf, Germany) was used (25). According to previous studies, it was determined that the effects of mobilization exercises on neck pain were determined to be from small to moderate (0.16–0.38)(26, 27). To obtain 80% statistical power (1 – β error probability) with an α error level probability of 0.05, we performed repeated measure analysis of variance (ANOVA) within and between interactions, used a medium effect size of 0.30 to consider the two groups, and used two measurements for the primary outcome, generating a sample size of 28

participants. Considering the drop-out rate of 15% and aiming to increase the statistical power of the results, a total of 28 participants (14 for each group) were recruited into the study.

Randomization

A randomization process was performed to divide the 28 CNNP patients randomly between the two study groups (MG and CG), using matched-pairs randomization based on their age and sex. Matched-pairs randomization was performed with numbers sorted using the Research Randomizer program on the www.randomizer.org website (28).

Blinding

At the baseline and after application of the 4-week treatment period, all assessments were evaluated by the investigator, who was blinded to the groups throughout the study (İ.C.).

Statistical Methods

Statistical analyzes were performed using SPSS (IBM Corporation, Armonk, NY) version 24 software. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Shapiro-Wilk tests). Descriptive analyzes were given using the mean and standard deviation for normally distributed variables. Number and % were given for nominal variables. Student's T test was used to test the significance of the difference between the two means in the comparison of the measured values of the mobilization and control groups. Chi-square test (Pearson chi-square) was used to examine the relationship between categorical variables. Two-way analysis of variance (Mixed design repeated measures ANOVA) was used in repeated measurements to evaluate the changes in the variables determined by measurement in the mobilization and control groups over time and the group-time interactions. For statistical significance, the total type-1 error level was determined as 5%.

RESULTS

Thirty-three volunteers applied for the study, and 28 satisfied the inclusion criteria. The patients distributions were n=14 for the MG and n=14 for the CG after randomization. The flow chart of the study is shown in Figure 7.

Demographic characteristics of the MG and CG are shown in Table 1. There was no statistically significant difference between the MG and CG in terms of demographic characteristics ($p>0.05$). This result shows that the groups are similar in terms of demographic characteristics distribution.

Baseline, after treatment and score changes for BAS, BAT, VAS, ROM, and muscle strength parameters of MG and CG are given in Table 2. According to the 2-way mixed design repeated-measures ANOVA analysis, when the change in time was analyzed between the groups (Group*Time (interaction)), no statistical difference was found for all parameters ($p>0.05$). In other words, when the mean change scores of the groups were examined, similar score changes occurred in the mobilization and control groups for all parameters (Table 2). This result shows that the treatment methods applied to individuals with neck pain have a similar effect. When the changes within the groups (within group (Time-Main effect)) were examined, a statistical difference was found for all parameters except the left rotation range of motion subparameter ($p<0.05$). In other words, the treatment methods applied to individuals with neck pain are effective for both groups.

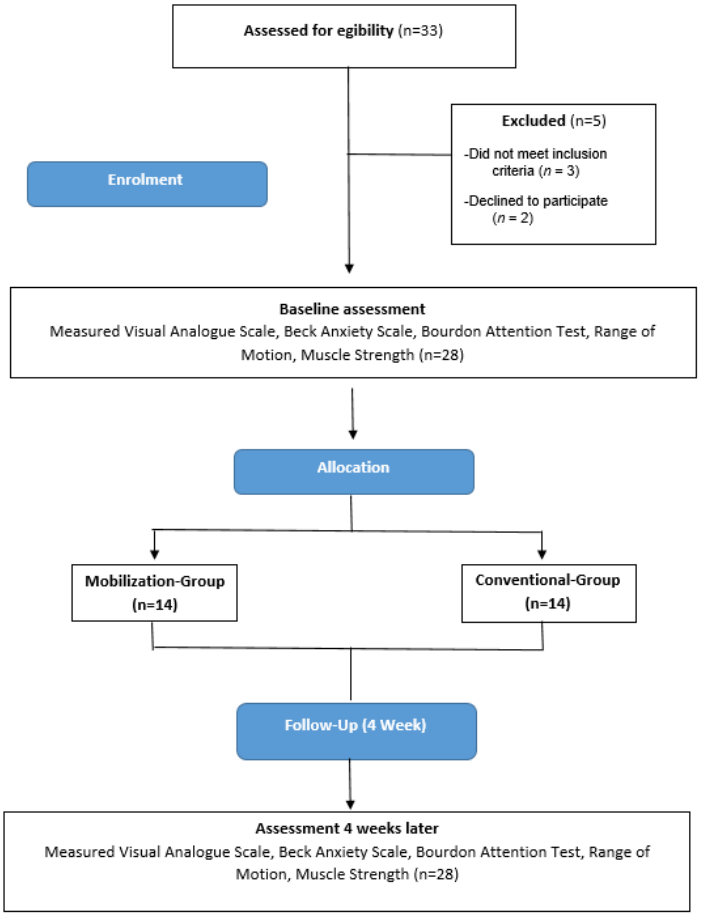

Figure 7. Study flowchart

DISCUSSION

The most important result of our work; we concluded that both conventional and cervical mobilization treatment approaches decrease the pain severity level in patients with CNNP. In addition, both treatment approaches were found to have positive results on muscle strength, ROM, cognitive level and anxiety. However, it turned out that the groups did not have superiority over each other.

There are many risk factors for neck pain such as physical problems, work-load, psycho-social factors and health-related behaviors have been identified in the literature (29). Ganesh et

Table 1. Demographic characteristics of mobilization and control groups

		MG(n=14)		CG(n=14)		t	p
		X	SD	X	SD		
Age (years)		38.4	15.2	41.7	12.2	-0.63	0.531
BMI (kg/m2)		27.6	5.0	27.1	4.8	0.29	0.772
		n	(%)	n	(%)	X2	p
Gender	Female	8	57.1	8	60	0.24	0.876
	Male	6	42.9	6	40		

BMI; Body Mass Index, t, Student T Test, X^2, Chi-square Analysis, X; mean, SD; Standart Deviation

Table 2: Baseline, after treatment and score changes for BAS, BAT, VAS, ROM and muscle strength parameters of the groups included in the study

			MG (n=14)		CG (n=14)		Between-group difference in change scores	Time (Main effect)	Group*Time (Interaction)	η2
			in change scores	Time (Main effect)	Group* Time (Interaction)	SD	Mean	p	F/ p value	
BAS		Baseline	14.1	7.3	22.8	13.8	0.4	0.000	0.03/0.863	0.001
		After treatment	8.0	4.2	16.3	12.1				
BAT		Baseline	77.4	21.5	80.6	26.5	8.9	0.001	2.39/0.133	0.082
		After treatment	92.2	15.3	86.5	27.2				
VAS		Baseline	5.6	1.7	7.5	1.8	0.3	0.000	0.20/0.657	0.007
			2.4	1.5	4.0	2.6				
ROM	Flexion	Baseline	41.2	9.6	39.3	7.8	4.7	0.000	3.30/0.080	0.109
		After treatment	51.1	6.8	44.5	5.4				
	Extansion	Baseline	32.9	7.8	36.3	7.7	3.2	0.000	2.21/0.148	0.076
		After treatment	40.7	6.1	41.0	7.1				
	Right Rotation	Baseline	46.6	9.8	43.7	11.9	2.5	0.006	0.92/0.344	0.033
		After treatment	51.8	8.7	46.3	10.6				
	Left Rotation	Baseline	50.0	9.1	45.3	10.9	0.9	0.050	0.15/0.696	0.006
		After treatment	52.9	8.7	47.3	11.4				
	Right Lateral Flexion	Baseline	33.1	10.7	30.5	7.4	-3.8	0.000	1.71/0.201	0.060
		After treatment	37.1	8.4	38.3	6.4				
	Left Lateral Flexion	Baseline	34.5	10.3	33.1	7.0	-2.0	0.002	0.510/0.481	0.019
		After treatment	38.2	10.6	38.9	6.2				
Muscle Strength	Flexion	Baseline	4.4	0.8	4.0	0.7	-0.1	0.000	0.21/0.647	0.008
		After treatment	4.9	0.3	4.6	0.6				
	Extension	Baseline	4.4	0.7	4.3	0.7	0.2	0.000	0.52/0.477	0.019
		After treatment	4.9	0.3	4.7	0.5				
	Right Rotation	Baseline	4.2	0.8	4.1	0.7	-0.1	0.000	0.33/0.566	0.012
		After treatment	4.6	0.5	4.5	0.6				
	Left Rotation	Baseline	4.1	0.8	4.1	0.7	0.0	0.000	0.02/0.879	0.001
		After treatment	4.6	0.5	4.5	0.6				
	Right Lateral Flexion	Baseline	4.3	0.7	4.0	0.7	0.0	0.000	0.03/0.862	0.001
		After treatment	4.7	0.5	4.5	0.6				
	Left Lateral Flexion	Baseline	4.2	0.7	4.1	0.6	-0.1	0.000	0.30/0.589	0.011
			4.6	0.5	4.6	0.5				

BAS: Beck anxiety scale, BAT: Bourdon attention test VAS: Visual analogue scale, ROM: Range of Motion, 2-way mixed design repeated-measures analysis of variance, SD: Standard deviation, η2: Effect size

al. divided the patients with chronic neck pain into 3 groups. Maitland mobilization technique and exercise were given to the 1st group; Mulligan applied the mobilization technique and exercise to the 2nd group, and only exercise to the 3rd group. They did not find a significant difference between the 3 groups in terms of pain parameters after the treatment and at the control at the end of the 12th week (30). Palmgren et al. concluded that chiropractic practices in patients with nontraumatic chronic neck pain caused an improvement in pain and proprioception (13). In another study, Acet et al. concluded that manual therapy approaches are more effective than traditional physiotherapy programs in terms of pain, range of motion and disability in patients with non-specific neck pain (19). In our study, it was revealed that the exercises given to both groups decreased the level of pain.

Cervical suboccipital muscles have been shown to have 36 muscle spindles per gram of muscle tissue; the gluteus maximus, by contrast, has 0.7 spindles per gram (31). The high number of stretch receptors in these tissues, and their essential link from the eye movements to coordination of the rest of the back musculature, ensure their central role for cognitive performance (32). Neuroimaging studies have demonstrated structural and functional changes in regions of the brain responsible for cognitive and emotional modulation of pain in individuals with chronic neck pain. Some studies have revealed that compared to healthy volunteers, patients with chronic neck pain exhibit worse cognitive performance, especially in areas such as attention, concentration, working memory and processing speed abilities (33, 34). In the current study The BAT was used to assess the attention levels of the participants. When the test data were examined, there was an increase seen in the post-treatment measurements for both groups, although there was no significant difference found between the groups.

Farooq et al. concluded that both the traditional physiotherapy program and cervical mobilization exercises applied in addition to the traditional physiotherapy program in patients with chronic mechanical neck pain resulted in improvements in pain, disability and cervical ROM (35). Ganesh et al. Maitland and Mulligan compared the effectiveness of mobilization exercises in patients with mechanical neck pain. According to the results of the study, they concluded that both mobilization techniques were effective in reducing pain, improving ROM and disability (30). Snodgrass et al found no change immediately after mobilization, but a reduction in stiffness by day 4. The mobilization group in the painful area was 17% less stiff compared to the placebo group (36). In our study, improvement in cervical region ROMs was observed in both treatment groups, but neither treatment was superior to each other.

Lee et al. applied Maitland mobilization of the thoracic and cervical region to patients with chronic neck pain and stated that there was a significant improvement in the muscle strength of the upper trapezius muscle after the treatment (37). Copurgensli et al. investigated the effect of kinesiotaping application and Mulligan mobilization in patients with cervical spondylosis and concluded that Mulligan mobilization increased cervical region flexor muscle strength (38). Our study is compatible with the literature, and cervical region muscle strength increased in both MG and CG, but there was no statistically significant difference between the groups.

In a 2015 study, Lopez et al. stated that cervical mobilization techniques reduce anxiety (39). In another study, Santos et al. stated that there is a decrease in the rates of anxiety and depression in personnel who have undergone spinal mobilization at work (40). Yıldırım et al. stated that mobilization techniques had a positive effect on anxiety in the acute period. (41). In this study Beck Anxiety Scale (BAS) was used to determine the risk of anxiety in patients and/or to measure the level of anxiety symptoms and the change in its severity (22). As a result of this study, both groups showed a decrease in anxiety levels, and this decrease was found to be higher in the mobilization group.

CONCLUSION

This study showed that mobilization exercises and conventional therapy used in the treatment of CNNP are effective in improving pain level, muscle strength, cognitive level, ROM and anxiety level.

Limitations

This study has a limitations. In our study, we evaluated pain with VAS based on patient statement, but a more objective evaluation such as algometry could be made.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Research Article

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Spexin Modulates the Glucose Homeostasis in Streptozotocin (STZ)-Induced Diabetes in Rat

Spexin, Sıçanlarda Streptozotosin (STZ) Kaynaklı Diyabette Glukoz Homeostazisini Düzenler

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Abstract

Background; Despite the various pharmacological agents and insulin treatment, still, there is lack of an effective and therapeutic treatment for diabetes. The present study investigated the possible therapeutic role of spexin (SPX) in glucose homeostasis both functionally and structurally in streptozotocin (STZ)-induced diabetic rats.

Methods; Male Wistar albino rats(n=28) were randomly divided into the control(n=4), diabetes mellitus (DM+saline;1mL/kg, n=6), DM+S10(SPX;10 µg/kg/Ml, n=6), DM+S30(SPX; 30 µg/kg/mL, n=6) and DM+S100 (SPX; 100 µg/kg/mL, n=6). Diabetes was induced by administering a single dose of STZ (35mg/kg, i.p. Blood and pancreatic tissue samples were taken for insulin level measurements by ELISA. Liver and muscle tissue samples were taken for glycogen measurement. Histopathological changes in liver, muscle and pancreas tissues were examined. Data were analyzed by ANOVA, and the Mann-Whitney-U test was used for multiple comparisons.

Results; STZ-induced diabetes increased blood and urine glucose levels in the DM group as compared to the control group and significantly decreased with 10 mg/kg dose of SPX treatment. Glucose loss rate (K value) in insulin sensitivity measurements was lowered by STZ-induced diabetes (8.030) vs the control group (9.973) witha weak negative correlation (R=-0.1786). Glycogen content in liver and muscle tissues has declined, and SPX limited the decrease in hepatic glycogen levels in the DM+S10 group. SPX treatment reduced the degeneration levels and tissue damage in all tissues.

Conclusions; Our findings indicated that SPX has a regulatory role in glucose homeostasis via insulin secretion, glycogen metabolism, and blood glucose regulation in a dose-dependent manner.

Keywords: Spexin, diabetes, glucose, insulin, glycogen.

Özet

Amaç: Çeşitli farmakolojik ajanlar ve insülin tedavisine rağmen halen diyabetin etkili ve tedavi edici bir tedavisi bulunamamıştır. Bu çalışma, streptozotosin (STZ) ile indüklenen diyabetik sıçanlarda spexinin (SPX) glukoz homeostazisindeki olası terapötik rolünü hem fonksiyonel hem de yapısal olarak araştırdı.

Gereç ve Yöntem: Erkek Wistar albino sıçanlar (n=28) rastgele olarak, kontrol (n=4), diyabet (DM+salin;1mL/kg, n=6), DM+S10(SPX;10 µg/kg/Ml, n=6), DM+S30(SPX; 30 µg/kg/mL, n=6) ve DM+S100 (SPX; 100 µg/kg/mL, n=6) gruplarına ayrıldı. Tek doz STZ (35mg/kg, i.p.) uygulanarak diyabet oluşturuldu. Kan ve pankreas dokusu örnekleri insülin düzeyinin ELISA ile ölçümü için alındı. Glikojen ölçümü için karaciğer ve kas dokusu örnekleri alındı. Karaciğer, kas ve pankreas dokularında histopatolojik değişiklikler incelendi. Veriler ANOVA ile analiz edilmiş ve çoklu karşılaştırmalarda Mann-Whitney-U testi kullanıldı.

Bulgular: STZ ile indükelenen diyabet, DM+salin grubunda kontrol grubuyla karşılaştırıldığında kan ve idrar glukoz düzeyleri arttı ve 10 mg/kg SPX tedavisi ile anlamlı düzeyde azaldı. İnsülin duyarlılığı ölçümlerinde hesaplanan glikoz kaybolma oranı (K değeri), zayıf bir negatif korelasyonla (R=-0,1786) kontrol grubuna (9,973) kıyasla STZ kaynaklı diyabet (8,030) ile azaldı. Karaciğer ve kas dokularındaki glikojen içeriği azaldı ve SPX, DM+S10 grubunda hepatik glikojen seviyelerindeki düşüşü sınırladı. SPX tedavisi tüm dokulardaki dejenerasyon düzeylerini ve doku hasarını azalttı.

Sonuç: Bulgularımız SPX'in insülin sekresyonu, glikojen metabolizması ve kan şekeri regülasyonu yoluyla glukoz homeostazisinde doza bağlı olarak düzenleyici bir role sahip olduğunu gösterdi.

Anahtar Kelimeler: Spexin, diyabet, glukoz, insülin, glikojen.

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INTRODUCTION

Diabetes does characterize by chronic hyperglycemia resulting from decreased circulating insulin concentration (insulin deficiency), the diminished response of peripheral tissues to insulin (insulin resistance), or both [1]. The development of diabetes is caused by several pathological processes, such as insulin deficiency resulting from the destruction of β cells in the pancreas or abnormalities arising from developing resistance to insulin action. Impairment of insulin effectiveness does cause by one or more of the pathways involved in the mechanism of action of the hormone insulin. Decreased insulin secretion or impaired cellular response to insulin underlies these disorders [2].

Spexin (SPX), known as neuropeptide Q, was first discovered in 2007 with Markov modelling [3]. SPX is expressed in many tissues in rats, including gastrointestinal tract, kidney, bladder, heart, uterus, lung, skeletal muscle, thymus, spleen, brain, hypothalamus, adenohypophysis, thyroid, adrenal, testis, and ovary [4]. Recent studies showed that SPX might improve glucose tolerance and insulin sensitivity, reduce HbAlc levels, and reduce the possibility of type 2 diabetes [5, 6]. Long-term SPX treatment has been found to improve liver function and increase glycogen storage in the liver in type-2 diabetic mice [7]. In addition, a recent study showed an association between SPX and increment in body weight which correlates positively with diabetes [8]. Researchers identified that SPX, galanin and kisspeptin genes are localized on the close ancestral chromosomes, and SPX is more related to galanin. According to Mirabeau et al. these peptides are natural ligands for GALR2/3 [3]. Galanin has a significant contribution by accelerating GLUT-4 to the plasma membrane of diverse insulin-sensitive cells, regulating glucose homeostasis, and reducing insulin resistance [9]. SPX increases glucose utilization by inhibiting galanin binding to GALR2. By these effects, SPX has been shown to regulate biological processes that occur in obesity, diabetes, and hepatic steatosis [10].

Well-established that diabetes is a global disease that significantly affects health and mortality [1] [2]. However, the influence of SPX on diabetes has remained unclear. According to recent studies, SPX may play a regulatory role in glucose metabolism. The experimental work presented here provides one of the first investigations into how SPX treatment impacts glucose homeostasis in diabetes in a dose-dependent manner. The present study lays the groundwork for future research to elucidate the tissue crosstalk of diabetes-associated hormones and newly discovered neuropeptides.

MATERIAL & METHODS

Animals

Male Wistar Albino rats (6-8 weeks old, 180-220 g) were supplied by the Trakya University Animal Center. All experimental procedures were applied according to Universal Declaration on Animal Welfare and approved by the Trakya University Animal Research and Ethics Committee, Edirne, Turkey (Approved date 27.12.2019, number:2019-12-03). Rats were housed in standard environmental conditions and had free access to food and water. The clinical signs constituted in all experimental procedures.

Rats (n=28) were randomly divided into the control (n=4), diabetes mellitus (DM, saline; 1mL/kg, i.p, n=6), DM+ SPX10 (Spexin; 10 µg/kg/mL, i.p, n=6), DM+SPX30 (Spexin; 30 µg/kg/mL, i.p, n=6) and DM+SPX100 (Spexin; 100 µg/kg/mL, i.p, n=6) groups. Spexin peptide was purchased from PolyPeptide (Cat.no: SC1547)). Diabetes induced with a single dose of streptozotocin (Santa Cruz Biotechnology, USA). (STZ 35 mg/kg, prepared in 0.1 M citrate buffer (pH 4.4, i.p.). Following 72 hours of STZ injection, blood glucose levels were measured from the tail vein of the rats after night fasting. Rats with a fasting plasma glucose level of 250 mg/dl or higher were considered diabetic. These values were acquired in all rats injected with STZ. Following the diagnosis of diabetes, saline or SPX was administered intraperitoneally at the above-mentioned doses once a day for five days. At the end of the study, rats were placed in a 24-hour metabolic cage, and water intake and urine output were monitored. Following metabolic measurements, rats were sacrificed under ketamine (75 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia following an insulin sensitivity test. Blood samples were collected in a serum clot activator tube and centrifuged at 3000g (15 min at 4 °C). Pancreas, skeletal muscle (gastrocnemius), and liver tissues of each animal were removed and stored at −80 °C for glycogen and insulin measurements. The liver, gastrocnemius muscle, and pancreas tissue samples were fixed in formaldehyde for histopathological assessment (The experimental design showed in Figure 1).

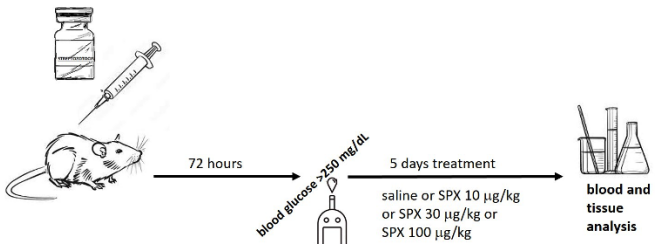


Figure 1. Graphical demonstration of the experiment

Insulin sensitivity test

The insulin sensitivity test was conducted according to Panigrahi et al. study [11]. The insulin sensitivity test stood carried out on the 5th day of the treatment. After 3 hours fasted periods the sensitivity test was performed under anaesthesia. Animals received human insulin (the dose of 0.1 U/kg) which has a short effect intravenously through the tail veins. Blood samples were collected from the tail veins at five times, 0 (just before insulin injection), 4, 8, 12, and 16 min of insulin

administration for glucose estimation. Insulin sensitivity showed glucose disappearance rate, which is calculated from the average slope of in the fitting curve by linear regression calculation. Then, multiplying the slope by −1, the -value (mg/dL/min) was calculated.

Blood samples

Blood samples were taken at the end of the experimental procedure, and glucose levels were measured by counter plus one (Ascensia Diabetes Care, Basel, Switzerland). In addition, insulin levels from the sacrificed rats at the end of experiments were measured by rat insulin ELISA kit (SUNRED 201-11-0708) according to the kit procedure.

Tissue glycogen content analyses

Tissue glycogen contents were measured in liver and gastrocnemius muscle according to Murat et al. [12]. Briefly, 1-1.5 g tissue was homogenized in 30% potassium hydroxide solution and then boiled at 100 °C for 30 minutes. Samples were then cooled in an iced cold solution with sodium sulfate. The glycogen was precipitated by adding ethanol, and the supernatant was discarded. The precipitate was brought into the solution by adding distilled water again. Glycogen content was determined spectrophotometrically (Shimadzu UV 1280) at a wavelength of 620 nm by adding the chemical anthrone reagent.

Histopathological analysis

Liver, gastrocnemius muscle, and pancreas tissues from rats in each group were gently dissected and immediately fixed in a 10% neutral buffered formalin solution for 72 hours. The samples were dehydrated in ascending (70%, 80%, 90%, 96%) ethanol series, cleared with xylene, and embedded in paraffin. The paraffin blocks were cut into 4-5 µm thick sections with a rotary microtome (Medite M530). Dewaxed cells were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation, and for histochemical reaction, Periodic Acid Schiff (PAS) was used to demonstrate glycogen deposition. Morphological and histopathological changes in the liver, muscle, and pancreas tissues were examined using a computer-equipped (LasV 4.10 program), the camera attached photo-light microscope (Leica DM 2500, Germany), and micrographs were taken from sections. Pathological changes were assessed with vacuolization, intensely stained pyknotic nuclei, congested sinusoidal walls, lymphocyte cell infiltration, lipid droplet, and adipocyte accumulation. Semi-quantitative scoring criteria (0: no damage; 1: mild; 2: moderate; 3: severe) were used to detect tissue degeneration and PAS-positive cell distribution. The average scores were calculated for each group, and statistical analysis was performed.

Statistics

The results are expressed as the mean ± SD. To evaluate the level of statistical difference, One-way analysis of variance (ANOVA) and Mann-Whitney-U test were used for multiple comparisons (GraphPad Prism 6.0, San Diego, CA, USA). "p" values 0.05 have been accepted to be statistically significant.

RESULTS

Plasma glucose levels in the diabetic group (330 ± 66.28) were significantly higher than the control group (116,80 ± 8.73) at the beginning of SPX treatment ($p<0.05$) (Figure 2a). SPX at a dose of 10 ug/kg (290.1 ± 27.29) prevented the increase of plasma glucose level compared to the DM group (321.2±23.41) till the end of the experiment ($p<0.01$). At a dose of 100 ug/kg (454.8 ± 58.6), SPX has elevated plasma glucose levels significantly compared to DM and the control group ($p<0.01$). Urine glucose output was significantly elevated in the DM group (221.5 ± 32.09) as compared to the control group (250.9 ± 109.0, $p<0.05$). This elevation was determined in the DM+SPX10 group (285.3 ± 80.02) on the 1st day of treatment as compared to the DM group (333.6 ± 62.74, $p<0.05$). Within days, the urine glucose levels of the DM+SPX100 group gradually increased except on 4th day of the treatment (Figure 2b). The insulin sensitivity test measured glucose disappearance rates (K value). Glucose levels in the control group dropped within minutes, with a significant negative correlation (R=-0.8018) with insulin therapy. However, this strong negative correlation was not seen in the DM group (R= -0.1786). When comparing the K values, the control group was 9.973, while the DM group was 8.030. Treatments of SPX showed different effects in a dose-dependent manner. According to these data, the K value of the DM+SPX30 group was the most similar to the control group.

The weight changes showed a positive correlation with STZ injection which was R= 0.769 in the name of the control group as a sign of weight gain, while was R= - 0.405 for the DM group (Figure 2d) which means affects weight gain during the experimental days. Therefore, SPX treatments did not significantly affect weight changes on all the experimental days.

Plasma and pancreatic tissue insulin levels were examined (Table 1). We did not find any significant difference between the DM and the control group. However, there was a substantial increase in plasma insulin levels in the DM+S10 group compared to the DM group ($p<0.05$). In addition, liver and muscle tissue glycogen contents were measured, and STZ injection caused a significant decrease in a large amount of glycogen from both tissues compared to the control group ($p<0.05$). Liver glycogen content was not improved by SPX treatment in any doses. However, muscle glycogen content recoveries at 100 ug doses of SPX compared to the saline-treated DM group ($p<0.01$).

Light microscopic evaluation of the H&E stained liver tissue

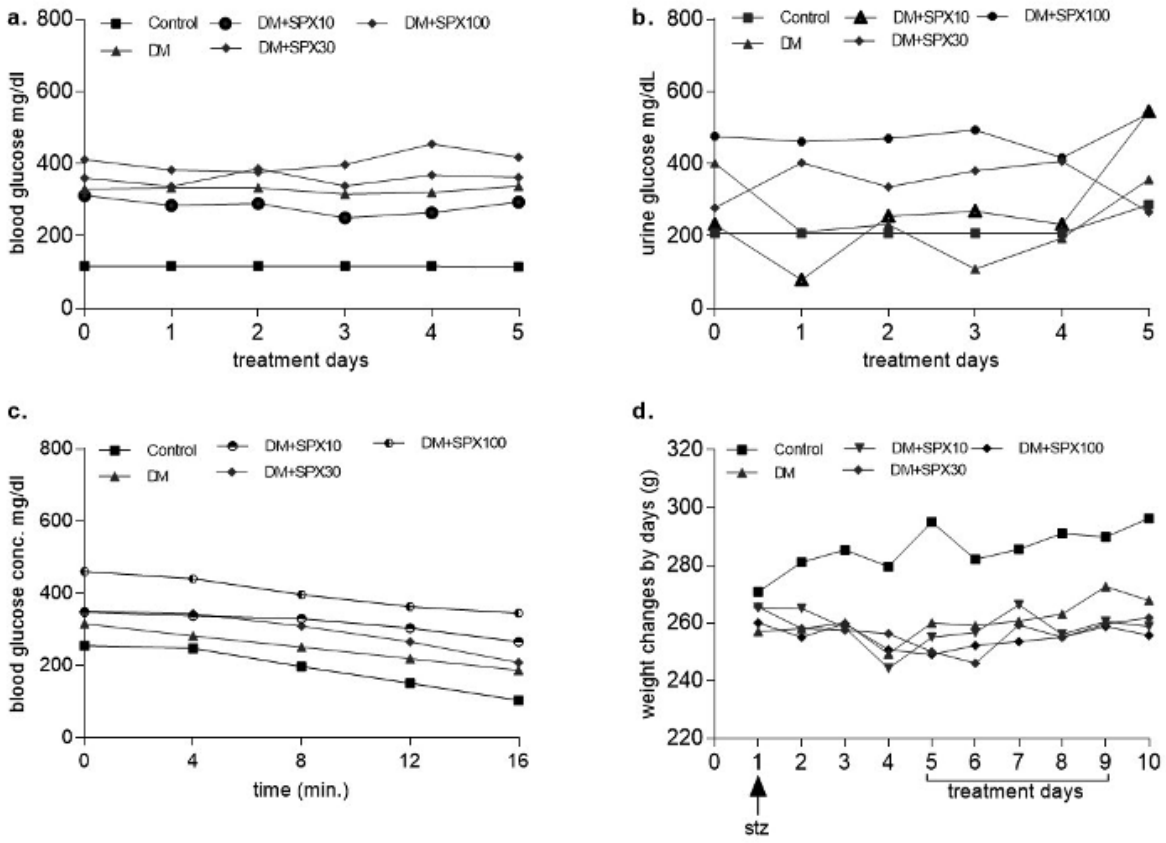
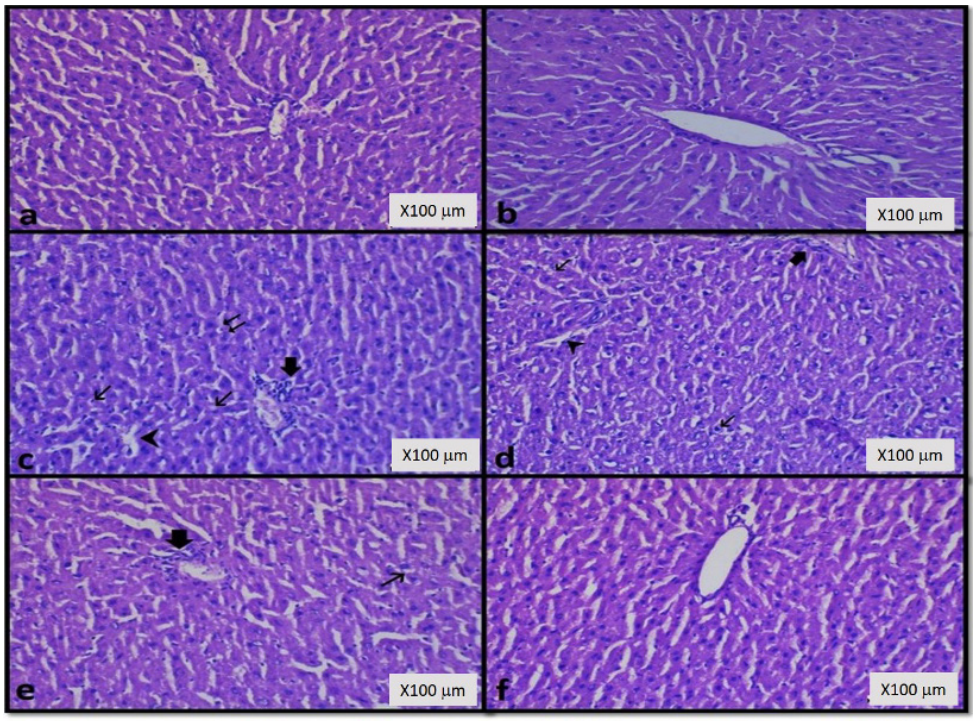


Figure 2. The figure shows plasma glucose levels (a), urine glucose levels (b), plasma glucose disappearance slope with insulin (c), and weight changes by days (d) in experimental groups.

Table 1. The table shows plasma insulin levels, pancreatic tissue insulin content, liver glycogen content, and skeletal muscle glycogen content in experimental groups. (*p<0.05 vs control group. +p<0.05 vs the DM group).

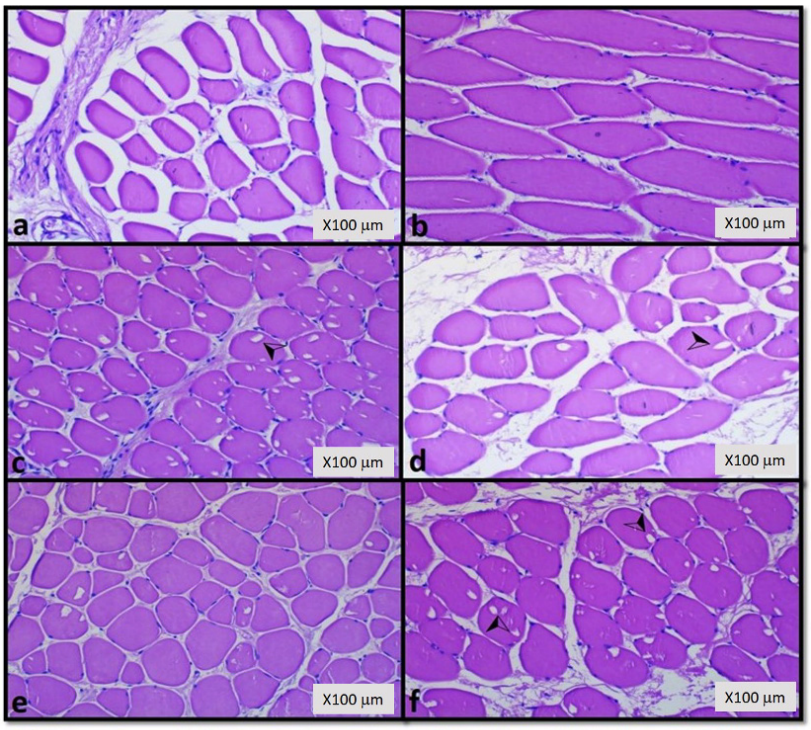
Table 1.	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Plasma insulin IU/mL	6.21±0.67	6.28±0.49	8.41±1.5^{+}	7.12±0.81	9.02±3.28
Pancreatic insulin IU/mg tissue	9.90±3.40	17.29±7.35	6.04±1.86	6.18±1.78	7.21±2.12
Liver glycogen (mg/100 mg tissue)	8218±4051	2905±1040*	2991±1839	2923±1285	2705±847.1
Muscle glycogen (mg/100 mg tissue)	1413±122.6	324 ±162.2*	528 ±254.2	454±299.4	953±103.4^{++}

of the control group rats revealed hepatocyte cords radiating from the central vein, polygonal hepatocytes with eosinophilic cytoplasm, central round nucleus, some binucleated cells, hepatic sinusoids between cords, and portal triads (Figure 3a-b). Skeletal muscle tissue investigation of the control group revealed a regular fascicle structure with perimysium, endomysium, skeletal muscle fibers, and their peripherally located nuclei (Figure 4a,b). Pancreas tissue of the same group showed intact Langerhans Islets and exocrine area with serous acini and ducts (Figure 5a-b). However, lymphocytic infiltration around the central vein, congested liver sinusoids, some hepatocytes with pyknotic nuclei, and diffuse vacuolar changes in hepatocytes were observed in the liver tissue of the Diabetes (DM) group (Figure 4c). Distinct and abundant lipid droplets were detected in skeletal muscle fibers of all Diabetes group animals (Figure 4c). In addition, enlarged Langerhans Islets and vacuolization in serous acini were seen in the pancreas tissue of the same group (Figure 5c). The degenerative changes seen in the diabetic group were also detected in the liver, muscle, and pancreatic tissues of the DM+SPX10



Groups	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Vacuolization	0.33±0.51	2.66±0.51*	2.66±0.51	2.33±0.51	2.33±0.51
Pyknotic nuclei	0.33±0.51	2.66±0.51*	2.66±0.51	2.33±0.51	2.68±0.51
Congested sinusoids	0.33±0.51	1.5±0.55*	1.5±0.54	1.33±0.52	1.33±0.52
Lymphocyte cell infiltration	0	2.67±0.52**	2.66±0.51	2.33±0.52	2.33±0.51

Figure 3. Photomicrographs and histological scores showing liver tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Lymphocytic infiltration around the central vein (⬇), congested sinusoids (⮜), pyknotic nucleus (⇆), and vacuolar changes (↙) in hepatocytes are seen (Hematoxylin and eosin, X100 µm)(*p<0.05, **p<0.01 vs the control group).



Groups	Control	DM	DM+SPX10	DM+SPX30	DM+SPX100
Vacuolization	0.33±0.52	0.16±0.4	0.16±0.4	0	0
Pyknotic nuclei	0.16±0.4	2.66±0.52**	2.66±0.52	2.33±0.52	2.68±0.52

Figure 4. Photomicrographs and histological scores showing skeletal muscle tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Arrowheads (⮟) represent lipid droplets in skeletal muscle fiber sarcoplasm (Hematoxylin and eosin, X100 µm). (**p<0.01 vs the control group).

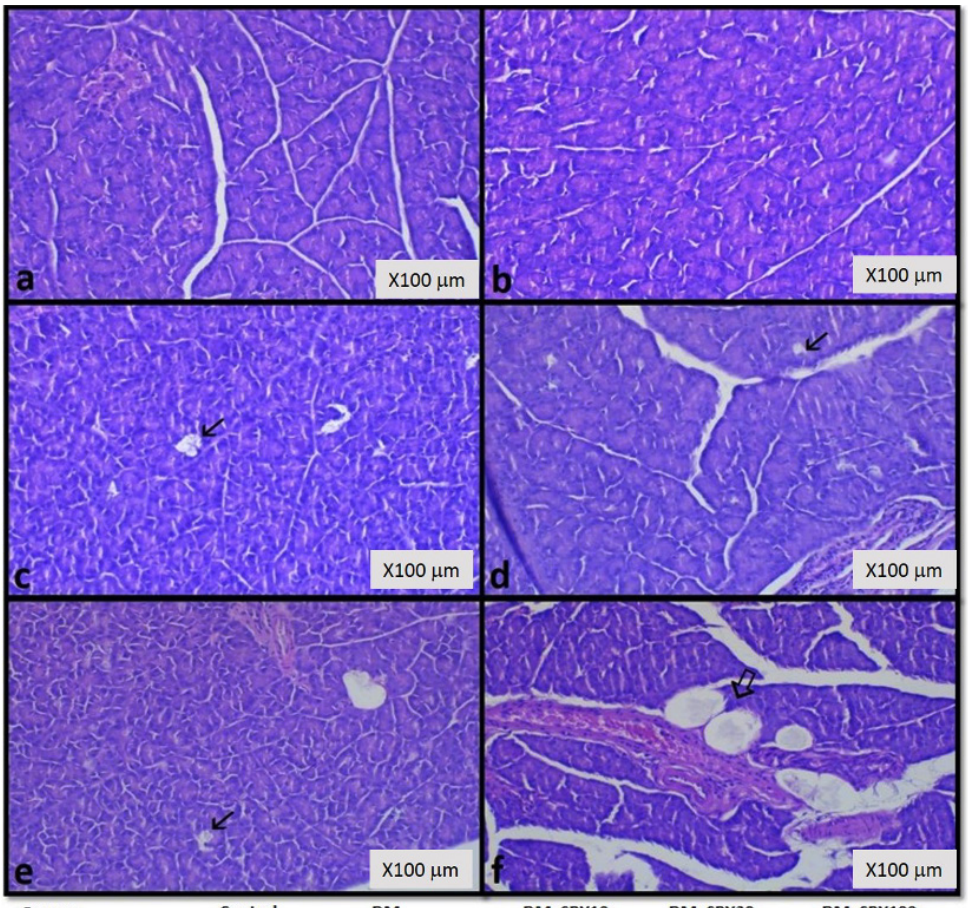


Figure 5. Photomicrographs and histological scores showing pancreatic tissue of the control (a,b), diabetes mellitus (c), DM+SPX10 (d), DM+SPX30 (e), and DM+SPX100 (f) groups. Vacuolization in serous acini (↙) and interlobular adipocytes (⇨) are clearly seen (Hematoxylin and eosin, X100 µm) (*p<0.05 vs the control group).

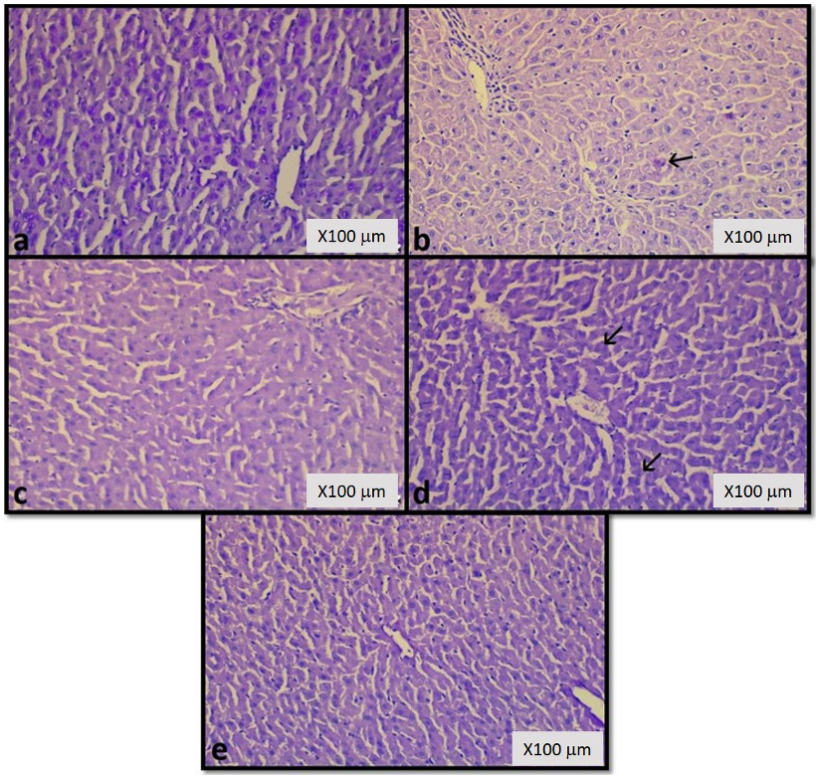


Figure 6. Photomicrographs demonstrating PAS histochemistry in liver tissue of the control (a), diabetes mellitus (b), DM+SPX10 (c), DM+SPX30 (d), and DM+SPX100 (e) groups. Arrows (↑) represent PAS-positive cells (Periodic Acid-Schiff, X100 µm).

group (Figures 3d, 4d, 5d), but they slightly declined in the DM+SPX30 group (Figures 3e,4e,5e). Although the liver and pancreatic tissues of the DM+SPX100 group have significantly resembled the DM+SPX30 group, interlobular adipocyte formation was observed in pancreatic tissue (Figure 5f), and an increased number of lipid droplets were still detected in the skeletal muscle (Figure 4f). Light microscopic evaluation of the PAS histochemistry revealed a strong positive reaction in hepatocyte cytoplasms of the control group (Figure 6a). In contrast, the reaction was weak and uneven in DM (Figure 6b) and DM+SPX10 (Figure 6c) treated groups. A moderate response was observed in the DM+S30 group (Figure 6d), and decreased weak reaction was detected in the DM+SPX100 group (Figure 6e). Besides the histological appearance when we compared the histological score between the groups, while STZ showed degenerative changes in DM groups, we did not observe significant changes by spexin treatment. As mentioned by the histological score in the DM group hepatic tissue showed degenerative changes with vacuolization, pyknotic nuclei, congested sinusoids, and lymphocyte cell infiltration (Figure 3.). Meanwhile, muscle tissue pyknotic nuclei levels were considerably higher in the DM group as compared to the control group (Figure 4, $p<0.05$). The pancreatic tissue histological score showed high levels of vacuolization in the DM group (Figure 5, $p<0.05$).

DISCUSSION

DM is a common condition which is needed to be a detailed understanding of its pathophysiology by identifying key agents that are effective in metabolic changes. It is important to elucidate these mechanisms in order to find possible effective treatments. Recently defined substances that enable tissue crosstalk to gain importance. Spexin, a peptide hormone, has gained considerable attention for its role in regulating glucose levels, energy homeostasis, and metabolism.

To maintain normal physiological functions, the human body must control plasma glucose levels within a narrow range of 4–6 mM [13]. Along with the continuous carbohydrate taken to the body with meals throughout the day, plasma glucose homeostasis is maintained using glycogen sources. Miscellaneous hormones affect the cellular use of glucose and control plasma glucose levels though there are two most significant hormones; insulin and glucagon. Glucagon delivers glucose from hepatic glycogen between meals and sleeps [14]. Insulin has opposite effects on plasma glucose and lowers plasma glucose for using cellular uptake during meals [15]. This study showed increased plasma glucose levels by STZ, and SPX showed its beneficial effect in the 10 ug/mL dose on plasma glucose and plasma insulin levels. Recent studies indicated that SPX levels decreased in patients with Type I and Type II diabetes or obesity. These results consider that SPX may influence glycemic control in diabetes and metabolic diseases [9, 16, 17]. Our result suggests that SPX endeavors decrease plasma glucose levels by increasing the plasma insulin level. The slight decrease in insulin content in pancreatic tissue, actually not statistically significant, in groups treated with SPX confirms our hypothesis partially.

Cellular insulin resistance due to impaired insulin sensitivity is observed in diabetes, mainly developing with a metabolic disease background in type-2 diabetes[18]. In this study, we observed insulin sensitivity development by STZ treatment, and SPX helps to recover from its effects on plasma glucose on a 10 ug/kg dose. These results suggest that SPX may have therapeutic effects on plasma glucose levels in an insulin-dependent manner. Sassek et al. study also supports our results, which is that SPX treatment reduced insulin secretion from cultured pancreatic islet cells when fronted with high glucose [19]. The SPX at 30 mg/mL and 100 mg/mL is beyond the beneficial effects, showing elevated plasma glucose levels. We think these doses are in supraphysiological quantities, so we predict it has such an effect. Sherman et al. previously showed that at supraphysiological doses, spexin lost its effect on thermogenic and weight compared to standard doses [20]. Urine glucose output also increased in DM groups, and the spexin at 10 mg/kg dose limited this increase on the first day of treatment. However, high-dose spexin treatment increased urine glucose output, such as plasma glucose levels. We had difficulty explaining fluctuations in urinary glucose, but we attributed this to changes in urine output.

Glycogenolysis is also crucial for fasting periods and is promoted by insulin [13]. The muscle and liver have buffer activity for plasma glucose changes after meals by storing the osmotically active metabolite in an inert form, such as glycogen [21]. However, glycogen storage is impaired and is one of the typical pathological causes seen in diabetes. As shown in this study, muscle and liver glycogen content was decreased by STZ treatment. Two main steps in glucose metabolism are essential here; the transport of glucose to tissue and the conversion of glucose to glycogen by glycogen synthase [22]. Interfering with glycogen metabolism with various agents, such as reducing glycogen breakdown with glycogen phosphorylase inhibitors, has beneficial effects on plasma glucose levels in diabetes [23]. Other interventions on glycogen metabolism, like inhibition of glycogen synthase kinase-3, have also shown beneficial effects on plasma glucose control [22, 24]. Muscle glycogen synthase is not stimulated in type-2 diabetes under euglycemic hyperinsulinemia [25] but the studies are conducted under the ambient hyperglycemia of type 2 diabetes, the defect in muscle glucose uptake is less apparent [25, 26]. The study about diurnal glycogen uptake in type-2 DM, find out that with a lack of insulin stimulation glycogen synthesis liver has a normal capacity for store of glycogen. This is probably the difference in the rate-limiting of cellular uptake of glucose via GLUT4

[21]. Suggesting these results with our study, SPX recovered the glycogen content of the muscle with the 100 ug/kg dose. Also, the liver glycogen content decreased by STZ treatment but we couldn't see recovery with spexin treatment. There is a lack of studies about muscle glycogen storage in diabetes. Further studies should be carried on to clarify the effects of glucose transporters on muscle glycogen synthesis in diabetes.

It has been known for years that diabetes causes focal sclerosis, inflammation, fat accumulation, and arteriosclerosis in the pancreas [27]. In addition, studies showed that patients with type 1 diabetes have a smaller pancreas than those with healthy and type 2 diabetes patients [28]. And it has been demonstrated that the exocrine pancreas is smaller in patients with both type 1 and type 2 diabetes and is more tending to fibrosis, fatty degeneration, infiltration of inflammatory cells, and atherosclerosis [29, 30]. The histopathological findings of the present study are supported by the biochemical results that STZ-induced diabetes has significant degenerative effects on the liver and muscle tissue besides the pancreas. Furthermore, for the first time in the literature, we indicated the therapeutic impact of SPX treatment on cellular damage in DM.

CONCLUSION

The present study set out to explore the potential influence of SPX in DM. The findings of our study complement those of earlier studies that point out plasma SPX levels were low in diabetic patient groups, making us think that SPX may play a crucial role in modulating glucose homeostasis. This paper contributes to recent concerns about the effects of SPX on insulin secretion. We have revealed the regulatory role of SPX on plasma glucose levels and pancreatic and hepatic cellular damage, glycogen metabolism, and insulin secretion. Moreover, we demonstrated the effectiveness of SPX tested at different doses. Further studies need to indicate the in vitro and in vivo effects of SPX by the increased number of subjects, especially by working on intracellular mechanisms with specialized cell lines.

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Evaluatıon of The Role of Mast Cells in Gallbladder Dysplasia

Safra Kesesi Displazisinde Mast Hücrelerinin Rolünün Değerlendirilmesi

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Abstract

Aim: Although the gallbladder is a frequently evaluated material, our knowledge in this area is limited, except for our predictions about some histological features in gallbladder dysplasia. In addition, cellular behaviors and expression profiles associated with Mast Cells (MC) in gallbladder dysplasia remain unclear.

Material-Method: In our study, gallbladder tissues with mild and severe dysplasia obtained by open cholecystectomy and laparoscopy were compared with control tissues and examined in terms of MCs markers Fc epsilon (ε) RI Alpha (FcεRIα) and Fc epsilon (ε) RI Gamma (FcεRIγ), Anti-Mast Chymase (McC) and Mast Cell Tryptase (McT). The status of these specific markers, associated with inflammatory processes and whose behavior in dysplasia cannot be entirely determined, were evaluated in tissues immunohistochemically and terms of general tissue structure with Hematoxylin&Eosin and Masson Trichrome staining. The tissue protein expression level was measured with Image J, and the difference between the groups was compared with One-way ANOVA, and $p<0.05$ was accepted as a significant difference.

Results: Our results show that FcεRIα, FcεRIγ, McT, and McC levels increase in dysplasia compared to the control. However, there is a decrease as the severity of dysplasia increases, especially McC and McT, suggesting that MC activity is impaired ($p<0.05$).

Conclusion: Although it is thought that there is a close relationship between dysplasias and MC, this situation needs to be investigated in more detail in terms of its clinical consequences.

Keywords: Gallbladder Dysplasia, Mast Cells, Mast Cell Tryptase, Mast Cell Chymase, FcεRIα, FcεRIγ

Özet

Amaç: Sıklıkla değerlendirilen bir materyal olmasına rağmen safra kesesi displazisindeki bazı histolojik özelliklere ilişkin öngörülerimiz dışında bu alandaki bilgimiz sınırlıdır. Ayrıca safra kesesi displazisinde Mast hücreleri (MC) ile ilişkili hücresel davranışlar ve ekspresyon profilleri hala belirsizliğini korumaktadır.

Gereç-Yöntem: Çalışmamızda açık kolesistektomi ve laparoskopi yöntemiyle elde edilen hafif ve şiddetli displazili safra kesesi dokuları kontrol dokuları ile Fc epsilon (ε) RI Alpha (FcεRIα) ve Fc epsilon (ε) RI Gamma (FcεRIγ), Anti-Mast Kimaz (McC) ve Mast Hücre Triptaz (McT) belirteçleri açısından incelendi. İnflamatuar süreçlerle ilişkili olan ve displazideki davranışı tam olarak belirlenemeyen bu spesifik belirteçlerin durumu dokularda immünohistokimyasal olarak değerlendirilmekle birlikte ayrıca Hematoxylin&Eosin ve Masson Trichrome boyanması ile genel doku yapısı yönünden de değerlendirildi. Dokudaki protein ekspresyonu Image J ile ölçüldü ve gruplar arasındaki değişiklik One-way ANOVA ile karşılaştırıldı ve $p<0,05$ anlamlı fark olarak kabul edildi.

Bulgular: Sonuçlarımız displazide FcεRIα, FcεRIγ, McT ve McC düzeylerinin kontrole göre arttığını göstermektedir. Ancak displazinin şiddeti arttıkça özellikle McC ve McT'de azalma olması displazilerde MC aktivitesinin bozulduğunu düşündürmektedir ($p<0.05$).

Sonuç: Displaziler ile MC arasında yakın bir ilişki olduğu görülmekle birlikte, bu durumun klinik sonuçları açısından daha detaylı araştırılması gerektiği düşünülmektedir.

Anahtar Kelimeler: Safra Kesesi Displazisi, Mast Hücreleri, Mast Hücre Triptazı, Mast Hücre Kimazı, FcεRIα, FcεRIγ

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INTRODUCTION

Epithelial metaplasias in the gallbladder are primarily located in two large groups: gastric epithelial (pseudo-pyloric and antral) and intestinal epithelial types. Aschoff describes gastric antral metaplasia as small glands lined with basal nucleated columnar epithelium, which the cytoplasm filled with mucin (and acid mucins containing small amounts of sulfate) within the lamina propria of the gallbladder (1, 2). It is an important detail that should be remembered during the pathological examination that reactive changes resulting from mucosal injury in the gallbladder can cause significant cytomorphological changes in epithelial cells that should be differentiated from accurate neoplastic transformation processes (dysplasia) (3). Generally speaking, gallbladder dysplasia is characterized by atypical columnar, cubic, or elongated cells proliferating unevenly (4). Although dysplastic epithelial transformation is thought to be associated with inflammation, there is no evidence for this (3). There are two types of MC found in the mucosa and connective tissue, and literature reports that connective tissue MC is related to the inflammatory process (5). MC is stimulated via Fc epsilon (ε) RI Alpha (FcεRIα,) and it leads to their degranulation (6). In addition, MC functions are associated with Fc epsilon RI Gamma (FcεRIγ) (7). In our study, we aimed to immunohistochemically examine the alteration of these MC markers in gallbladder dysplasia.

MATERIAL and METHODS

Experimental Studies

Three groups were formed, each containing six tissues: control, mild, and severe dysplasia. 5 µm thick sections were taken from these tissues, which were diagnosed with laparoscopic and open cholecystectomy materials in Bozok University Research Hospital and Konya Numune Hospital between 2012-2022. After using 1X PBS solution to wash the sections, they were treated with Citric Acid buffer before staining. 3% H2O2 solution was used to abolish endogenous peroxidase activity, followed by Ultra V Block. Primary antibodies: Anti-Fc epsilon RI/FcεRIα (My Biosource, 1/500), Anti-Fc epsilon RI/FcεRIγ, (NBP3-04822, 1/200), Anti-Mast Cell Tryriptase (ARC2328, 1/100) and Anti-Mast Cell Chymase (Invitrogen, PA5-106362, 1/100) were used. The Goat Anti-Rabbit was the secondary antibody. The 3,3'Diaminobenzidine tablet was used as chromogen, and Mayer's Hemalum Solution/ Hematoxylin&Eosin (109249, Merck) was used for contrast staining. The general structure staining was evaluated with Masson's trichrome staining kit (GBL-5022) and Hematoxylin&Eosin.

Evaluation

Protein expression levels were measured with Image J (1.52 R, National Institutes of Health, USA) software, and statistical analysis was performed. In the statistical evaluations, images of 3 different areas from 3 randomly selected sections. The tissue expression level was measured with Image J, and the difference between the groups was compared with One-way ANOVA, and $p<0.05$ was accepted as a significant difference. Abundant formerly stored in secretory granules in MC, the enzyme tryptase is present (8), and tryptase levels may represent MC count and/or activation (9). For this reason, especially tryptase levels were considered in interpreting MC number and activation.

RESULTS

When gallbladder control tissues and mild and severe dysplasia tissues are evaluated in terms of Hematoxylin&Eosin and Masson Trichrome Staining, the shape change in the epithelial structure is remarkable in Hematoxylin&Eosin staining (Fig.1/ blue arrows). Vascular changes are prominent in severe dysplasia (Fig.1/orange arrows). Multiple mitotic hyperchromatic and atypically arranged pseudostratified epithelium were observed in dysplasia (Fig.1). Masson trichrome staining showed that as the severity of dysplasia increased, collagen (blue), muscle, and elastic fiber (red) structures deteriorated. Separating cellular and fibrous components in stroma areas has become difficult (Fig.2).

FcεRIα and FcεRIγ expression in our study is especially intense in the epithelial line (Fig.3 and Fig.4/blue star) and increased with dysplasia (Fig.3 and Fig 4). The expression of McC and McT were increased in dysplasia compared to the control tissue. Expression was especially intense in connective tissue areas (Fig.5 and Fig.6/ blue star). However, when the severity of dysplasia increased, it was observed that the expression intensity decreased (Fig.5 and Fig.6) ($p<0.05$).

DISCUSSION

The gallbladder is an organ located just below the liver, and its role is to store bile. There is a complex functioning between the gallbladder and cystic duct in response to cholecystokinin (10). Cholecystectomy is one of the most common surgical procedures, and the gallbladder is removed either as part of a complex resection or for symptomatic, inflammatory gallbladder disease. Sometimes, gallbladder dysplasia is encountered in these tissues' histological sections (11).

Metaplasia can transform into dysplasia and adenocarcinoma following a multistep process, including genetic disorders (12). Although dysplasia is thought to be a precursor of invasive gallbladder carcinoma, the relationship of dysplasia with different lesions is unclear. The literature states that patients with dysplastic gallbladder may develop biliary tract malignancies (13). MC infiltration is significantly increased in the muscular structures of the gallbladder in cases with functional gallbladder disorders (14). The increase we observed, especially in dysplasias, is consistent with the literature and is intense in the stroma of our tissues.

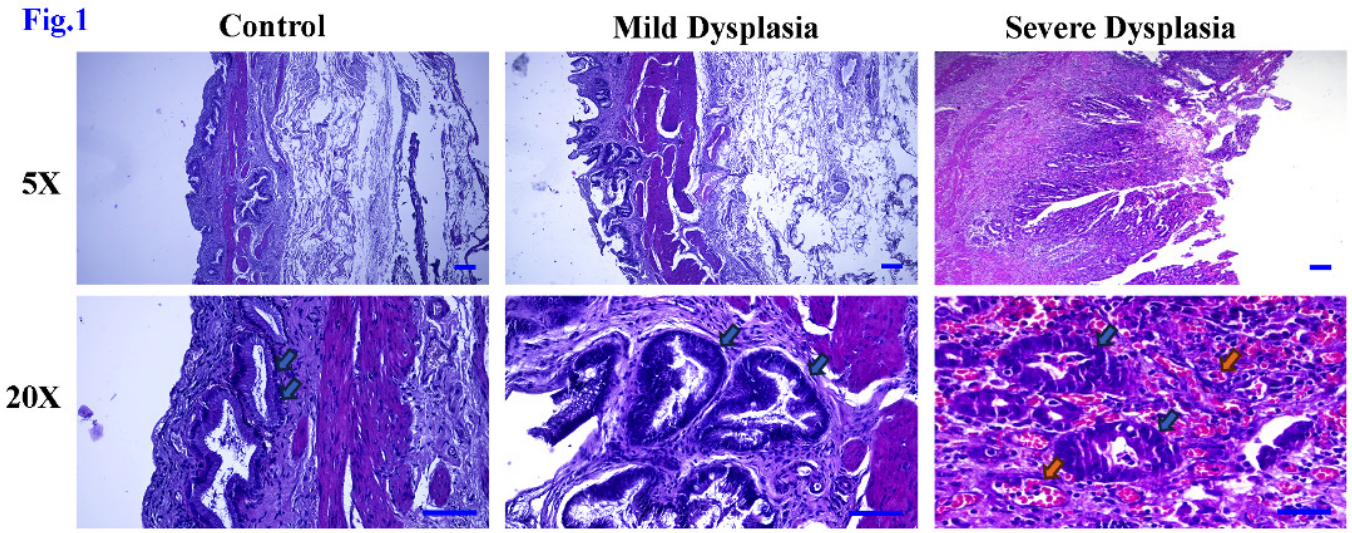

Fig.1: Hematoxylin&Eosin Staining in Control and Dysplasia Tissues. Blue arrows: epithelial structure, orange arrows: blood vessels, scale bar, 50 µm, 5X,20X

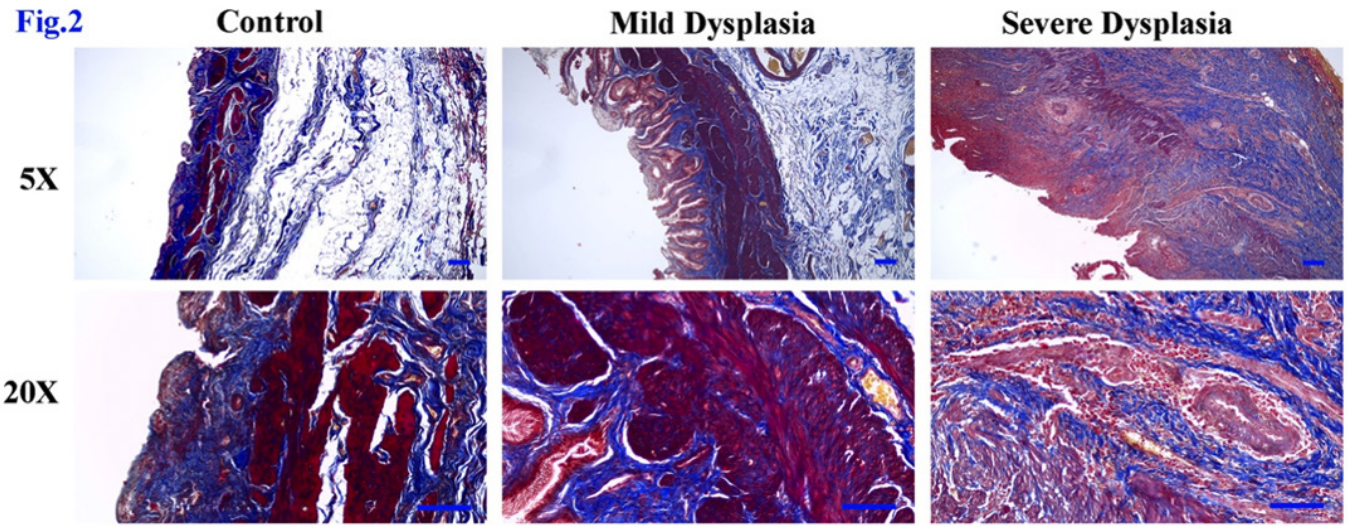

Fig.2: Masson Trichrome Staining in Control and Dysplasia Tissues. Collagen fiber: blue, muscle-elastic fiber: red stained, scale bar, 50 µm, 5X,20X.

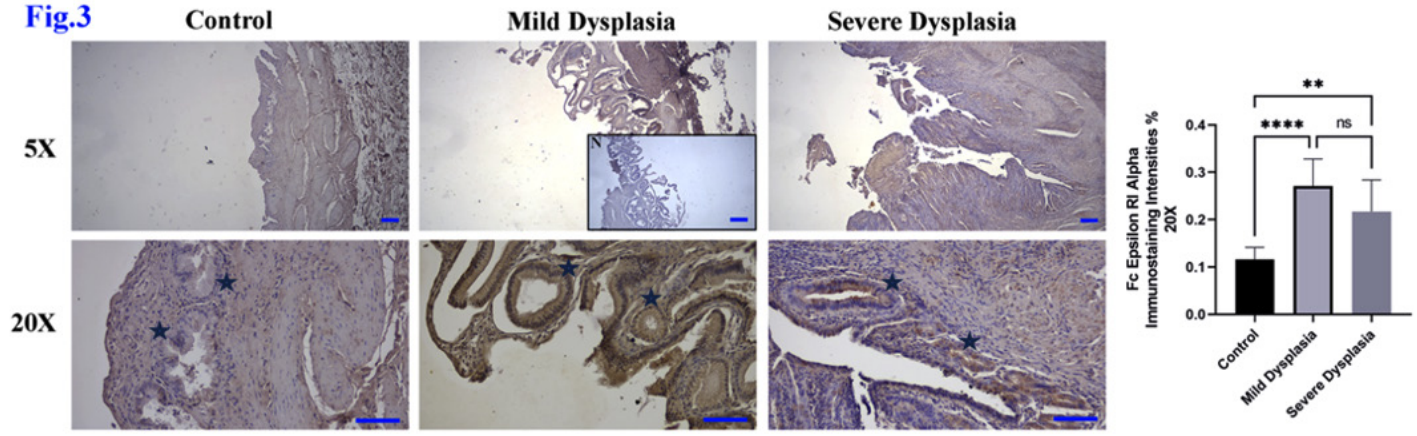

Fig.3: Anti-Fc epsilon RI Alpha (FcεRIα) Staining in Control and Dysplasia Tissues. Blue stars: epithelial structure, scale bar, 50 µm, 5X,20X.

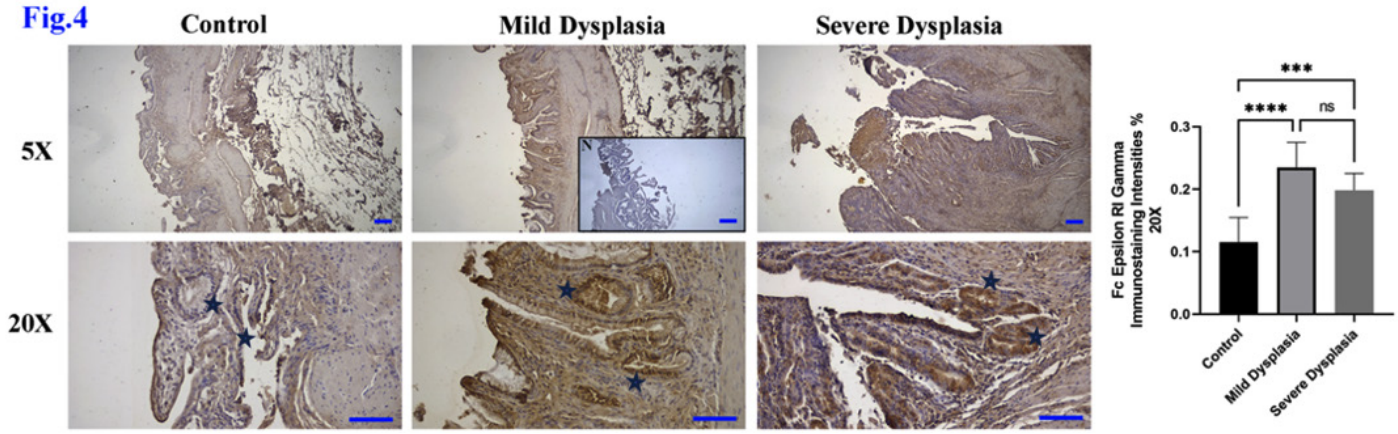

Fig.4: Anti-Fc epsilon RI Gamma (FcεRIγ) Staining in Control and Dysplasia Tissues. Blue stars: epithelial structure, scale bar, 50 µm, 5X,20X

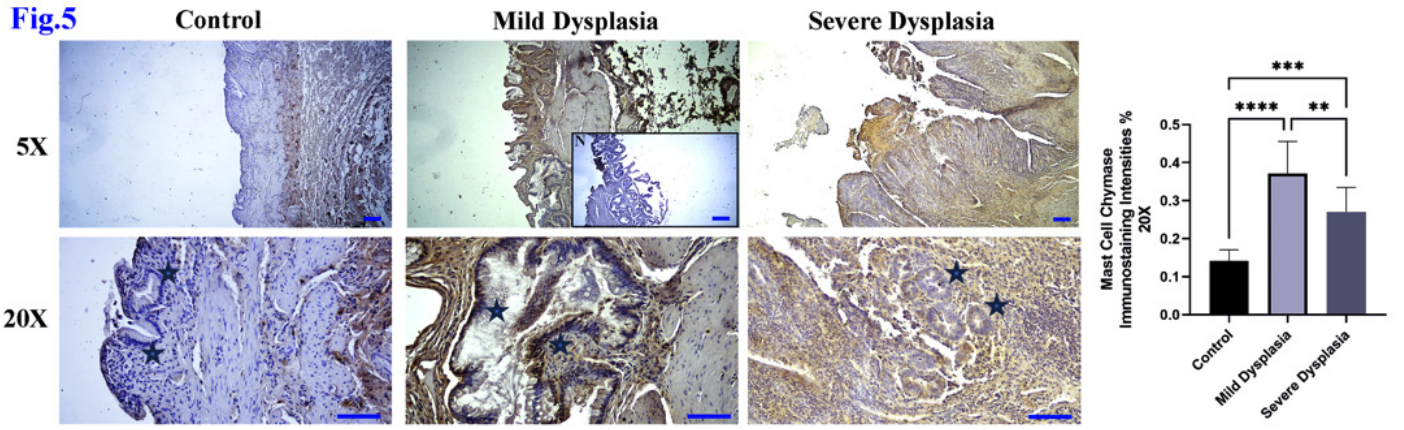

Fig.5: Anti-Mast Cell Chymase (McC) Staining in Control and Dysplasia Tissues. Blue stars: epithelial structure, scale bar, 50 µm, 5X,20X.

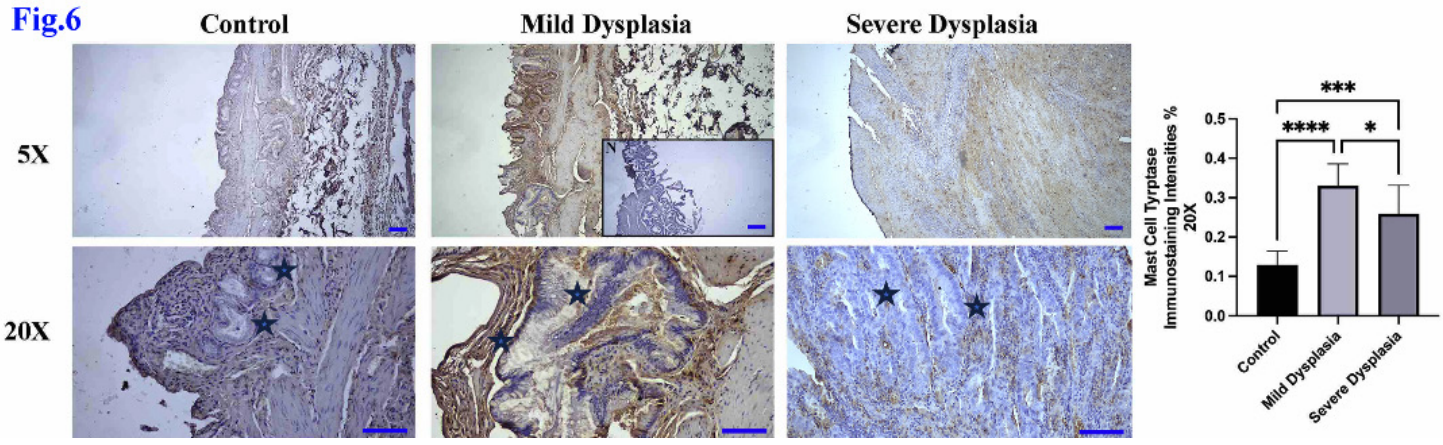

Fig.6: Anti-Mast Cell Tyriptase (McT) Staining in Control and Dysplasia Tissues. Blue stars: epithelial structure, scale bar, 50 µm, 5X,20X

McC and McT are major serine proteases released by activated MC (14). The types and amounts of tryptases and chymases expressed in different tissue microenvironments can vary. These proteases are essential in spreading or limiting inflammation (15, 16).

In the MC, the enzyme tryptase is present in granules (8), and tryptase levels may represent MC count and/or activation (9). McC degrades fibronectin and collagen by activating metalloproteinases, and McT likewise activates metalloproteases in the extracellular matrix (17). Also, some studies mention the relationship between MC and especially the surrounding nerves and fibers (18). In our study, Masson trichrome staining results show that fiber organization deteriorates as the severity of dysplasia increases in connective tissue areas. In our study, the increase in serine proteases observed in dysplasia and inducing metalloprotease activity was thought to be related to this. These results are in line with the literature.

FcεRI is selectively expressed at various steps during the development of the MC lineage (19). FcεRI, a high-affinity activating IgE receptor, contains an alpha (FcεRIα, IgE binding chain), a β-subunit (FcεRIβ), and two gamma (γ)-subunits. FcεRIα plays a role in IgE-mediated allergic diseases (20). Stimulation via FcεRIα causes degranulation of MCs (6), and FcεRIγ is associated with the anti-inflammatory response (7). MC activity is linked to degranulation and products released after degranulation (21). Activation of MCs causes the release of chemokines and cytokines, which are associated with chronic inflammation (22). In our study, MC markers related to inflammation were increased. This may specifically explain the stromal damage and deterioration and indicate the worsening of the process. The findings are in line with the literature.

CONCLUSION

When the relationship between MC markers and gallbladder dysplasia was evaluated, our results showed that the markers of these cells, which also play a role in inflammatory processes, provide hemostasis, and restore the disturbed balance, tend to increase in dysplastic tissues. However, as the severity of dysplasia increased, it was suggested that the decreased expression was associated with the deterioration of MC functions over time.

Declarations: Ethics Statement: Approval was obtained from the ethics committee of Akdeniz University KAEK: 08.03.2023/195.

Conflicts Of Interest: The authors declare no conflicts of interest.

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Research Article

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Apigenin 7-O-Glicoside Induces Cell Death Through Apoptosis and Autophagic Pathways in PANC-1 Cell Line

Apigenin 7-O-Glikozit, PANC-1 Hücre Hattında Apoptoz ve Otofajik Yollarla Hücre Ölümüne Neden Olur

Yazarlar

Kurumlar

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Abstract

Aim: Apigenin-7-O-glucoside (AP7Glu) is a glycoside derivative of a flavonoid called apigenin and is found in various fruits and vegetables. This compound has the same basic structure as apigenin, which exhibits anti-inflammatory, antioxidant, and antitumor activities in various cancer cell lines. However, the exact workings and which cellular pathways are affected by the apoptotic effects of apigenin-7-O-glucoside remain not fully understood. Therefore, this study aimed to elucidate the anticancer effects of AP7Glu against PANC-1 cells through apoptotic and autophagic pathways.

Materials and Methods: The XTT test was used to determine the cytotoxic dose of AP7Glu in PANC-1 cell lines. Apoptosis was determined both by the colorimetric TUNEL test and the ELISA-based caspase-3 activity test. Expression changes in apoptosis and autophagy pathway genes were analyzed by RT-qPCR. Finally, autophagic activity was tested with the colorimetric LC3 activity test.

Results: It was determined that AP7Glu had a cytotoxic effect at a concentration of 15 µM over 48 hours against PANC-1 cell lines. AP7Glu treatment led to caspase-3 mediated apoptosis in PANC-1 cells. Moreover, the increase in Bax and cleaved PARP gene expressions and the decrease in Bcl-2 gene expression support mitochondrial-mediated apoptosis. The significant decrease in genes involved in the autophagy pathway, ATF4 and XBP1, and the significant increase in the CHOP gene indicate that AP-7Glu also affects cellular death through autophagic mechanisms.

Conclusion: While AP-7Glu exhibits antiproliferative and apoptotic effects against PANC-1 cells, further studies are intriguing.

Keywords: AP-7Glu, Apoptosis, Autophagy, PANC-1, Cytotoxicity.

Özet

Amaç: Apigenin-7-O-glukozit (AP7Glu), apigenin adlı bir flavonoidin glikozit türevidir ve çeşitli meyve ve sebzelerde bulunmaktadır. Bu bileşik, çeşitli kanser hücre hatlarında anti-inflamatuar, antioksidan ve antitümör aktiviteler gösteren apigenin ile aynı temel yapıya sahiptir. Ancak, apigenin-7-O-glukozitin apoptotik etkilerinin tam olarak nasıl çalıştığı ve hangi hücresel yolların etkilendiği henüz tam anlamıyla anlaşılmamıştır. Bu nedenle bu çalışma, PANC-1 hücreleri üzerindeki AP7Glu'nun antikanser etkilerini apoptotik ve otofajik yollar aracılığıyla açıklamayı amaçlamaktadır.

Gereç ve Yöntem: XTT testi, PANC-1 hücre hatlarında AP7Glu'nun sitotoksik dozunu belirlemek için kullanılmıştır. Apoptoz hem colorimetrik TUNEL testi ile hem de ELISA temelli kaspaz-3 aktivite testi ile belirlenmiştir. Apoptoz ve otofaji yolu genlerindeki ekspresyon değişiklikleri RT-qPCR ile analiz edilmiştir. Son olarak, otofajik aktivite colorimetrik LC3 aktivite testi ile test edilmiştir.

Bulgular: AP7Glu'nun 48 saat boyunca PANC-1 hücre hatlarına karşı 15 µM konsantrasyonda sitotoksik etkisi olduğu belirlenmiştir. AP7Glu tedavisi, PANC-1 hücrelerinde kaspaz-3 aracılı apoptoza neden olmuştur. Ayrıca, Bax ve cleaved PARP gen ekspresyonlarındaki artış ve Bcl-2 gen ekspresyonundaki azalış mitokondri aracılı apoptoza işaret etmektedir. Otofaji yoluyla ilgili genlerde, ATF4 ve XBP1'deki anlamlı azalma ve CHOP genindeki anlamlı artış, AP-7Glu'nun aynı zamanda otofajik mekanizmalar aracılığıyla hücresel ölüme etki ettiğini göstermektedir.

Sonuç: AP-7Glu, PANC-1 hücrelerine karşı antiproliferatif ve apoptotik etkiler gösterirken, ileri çalışmalar ilgi çekicidir.

Anahtar Kelimeler: AP-7Glu, Apoptoz, Otofaji, PANC-1, Sitotoksisite.

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INTRODUCTION

Pancreatic cancer is one of the most deadly types of cancer worldwide, with high mortality rates (1). It is approximately the 7th most deadly cancer type in terms of death rates worldwide (2). Treatment is particularly challenging for this type of cancer due to its high level of resistance to current chemotherapeutic agents. There are several drugs used clinically. Among them, Gemcitabine (Gemzar), 5-fluorouracil (5-FU), irinotecan (Camptosar), oxaliplatin (Eloxatin), and paclitaxel (Abraxane) are used as chemotherapy drugs; erlotinib (Tarceva) and nab-paclitaxel (Abraxane) are employed as targeted treatments, while pembrolizumab can be utilized in immunotherapy (3). A combination of these treatment options generally provides the most effective results. However, the treatment of pancreatic cancer is more challenging compared to other types of cancer and is generally less successful (4). Therefore, it is essential to identify new components with potential anti-cancer activities.

Plant-derived compounds are gaining attention as potential anti-cancer agents due to their relatively low toxicities and side effects. Apigenin-7-O-glucoside (AP7Glu) is a glycoside derivative of a flavonoid compound called apigenin, found in many fruits and vegetables (5). It naturally occurs in various plants, especially in citrus fruits, parsley, celery, fennel, and chamomile (6). Data regarding AP7Glu is not as extensive as that for apigenin. Therefore, its current ethnobotanical and pharmacological effects have been evaluated together. From an ethnobotanical perspective, plants containing apigenin and AP7Glu have been used in traditional medicine for treating various ailments, including anxiety, sleep disorders, gastroprotective effects, hepatoprotective effects, and pain relief (7). Pharmacologically, apigenin and AP7Glu possess antioxidant (8), anti-inflammatory (9), apoptotic (10), anti-cancer (11), anti-anxiety, and antidiabetic effects (12). Notably, AP7Glu has been shown to regulate a series of signaling pathways leading to apoptotic cell death, including mitochondrial membrane potential loss and caspase activation (13). Moreover, this compound inhibits cell proliferation by halting the cell cycle in the G2/M phase and increasing the expression of cell cycle regulatory proteins such as p53 and p21 (14). AP7Glu can also elevate the levels of reactive oxygen species (ROS) in cancer cells, which can induce cell death by leading to DNA damage, protein oxidation, and lipid peroxidation (15). Literature reviews reveal that information about AP7Glu is very limited. Specifically, there is limited research on how it exerts its anti-tumor properties, particularly in pancreatic cancer cells. Therefore, this study aims to investigate how AP7Glu induces cell death in PANC-1 cells, a human pancreatic cancer cell line, through apoptotic and autophagic pathways.

MATERIAL and METHODS

Cell Culture and Treatment

The PANC-1 human pancreatic cell line (ATCC® CRL-2266™) was grown in a mixture of DMEM: F12 (Sigma-Aldrich, Cat. no: D6421), enriched with 10% fetal bovine serum, 2 mM L-glutamine, and 1% Penicillin-Streptomycin. The cells were stored at 37°C in a humidified incubator under a 5% CO2 environment. Moreover, AP7Glu (Sigma-Aldrich, 578-74-5) was made soluble by dissolving in a 0.1% DMSO solution.

Cell Viability

The XTT cell viability assay was employed to assess the impact of AP7Glu on cell proliferation. Cells were seeded in 96-well plates at a density of 2x104 cells/well and were given 24 hours to adhere. To evaluate the impact of AP7Glu on cell viability, cells were treated with different concentrations of AP7Glu (5, 10, 15, 20, 30, 40, 50, 75, and 100 µM) for time periods of 24 or 48 hours. Following this treatment, 100 µl of XTT solution (Biological Industries - Cat. No.: 20-3001000) was added to every well. Four hours post this addition, the absorbance in each well was measured at 450 nm using a plate reader. Cell viability was calculated as a percentage by taking the ratio of the absorbance of the treated cells to that of the control cells and then multiplying the quotient by 100.

Assessment of Apoptosis and Autophagy Pathway Gene Expression Using RT-qPCR

Gene expression alterations associated with apoptosis and autophagy pathways were examined using RT-qPCR. PANC-1 cells were placed in 6-well plates at a concentration of 2.5x104 cells for each well and were permitted to grow for 24 hours at 37°C in a 5% CO2 environment. Post-incubation, the cells were exposed to the IC50 concentration of AP7Glu. Thereafter, total RNA was isolated employing the RiboEx solution (GeneAll, 301–001). A nanodrop spectrophotometer (Thermo Scientific, USA) was used to assess the RNA samples' quality and concentration by observing the optical density at 260 and 280 nm. The DNase I enzyme (Thermo Scientific, USA) was used to remove any possible DNA impurities. The purified RNAs were then transcribed into cDNA utilizing the iScriptTM cDNA Synthesis Kit (Bio-Rad, 170–8891). The BrightGreen 2x qPCR MasterMix (abm, Canada) was used for the quantitative evaluation of mRNA expression, as per the manufacturer's protocol. In the apoptosis pathway, the expression patterns of CASP3, PARP, BAX, and BCL-2 genes, as well as CHOP, XBP1, ATF4, ATF6, and PERK genes in the autophagy pathway were analyzed using SYBR in the RT-qPCR assay on an Applied Biosystems (USA) machine. The primer sequences for these genes were sourced from IDT PrimerQuest (https://eu.idtdna.com/Primerquest/Home/Index). The RT-qPCR protocol was as follows: an initial step at 95°C for 4 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 60 seconds, and a final

step at 72°C for 4 minutes.

Caspase-3 Analysis by Enzyme-linked immunosorbent assay (ELISA) for Detection of Apoptosis

To determine the extent of apoptosis, a colorimetric assay kit specific for caspase-3 from BioVision, CA, USA was employed as per the provided instructions. This method identifies DNA fragmentation present in the cytoplasm of apoptotic cells. For the analysis, PANC-1 cells were seeded in 96-well plates with a density of 3x104 cells per well and incubated for 24 hours. After this period, the cells underwent treatment with inhibitory concentrations of rhein. Subsequently, cells were collected and combined with 50 µL of lysis buffer, then set aside on ice for a cooling period of 10 minutes. Each cytoplasmic extract was then supplemented with 50 µL of a 2X reaction buffer. As the final step, 5 µL each of the caspase-3 substrate Asp-Glu-Val-Asp (DEVD)-p-nitroaniline (pNA) was integrated into the protein lysate in the wells. After a 37˚C incubation for 2 hours, absorbance readings were taken at 405 nm using a microplate reader (Bio Rad Laboratories, CA, USA). The relative changes in caspase-3 activitiy was determined by comparing the readings from AP7Glu-treated samples against the baseline readings from untreated controls.

TUNEL Assay

The TUNEL assay, pivotal for identifying cell apoptosis, was performed in line with the manufacturer's recommendations. PANC-1 cells, after treatment with AP7Glu, were first washed with PBS, then re-suspended and spread on poly-L-lysine coated slides. Next, the slides were immersed in a 4% paraformaldehyde solution for 25 minutes at room temperature, followed by two 5-minute rinses with PBS. For permeabilization, the slides were briefly treated with a 0.2% Triton®X-100 solution in PBS for 5 minutes at ambient temperature. The slides then received a 100 µl aliquot of the rTdT reaction mix, comprising 98% equilibration buffer, 1% biotinylated nucleotide mix, and 1% rTdT enzyme (all from the DeadEnd colorimetric TUNEL system). They were subsequently incubated in a humidity-controlled chamber at 37°C for 60 minutes. After incubation, the coverslips were gently detached, and the reaction was terminated by immersing the slides in 2xSSC (saline-sodium citrate). The slides were finally sealed with 100% glycerol. Using the Zen software on a light microscope (Carl Zeiss, Oberkochen, Germany), images from five different areas for each sample group were recorded.

To determine the proportion of apoptotic cells, apoptotic nuclei displaying a dark coloration were tallied in five arbitrary sections on each slide. The average from these sections was then computed as [(number of apoptotic cells/total cell count) × 100].

LC3 ELISA Assay to Identify Cellular Autophagy

The LC3 ELISA test, centered on the Microtubule-associated protein 1A/1B-light chain 3, is frequently employed to track autophagy in cancerous cells given that LC3 serves as a primary marker of autophagy. The Autophagy ELISA Kit (specifically LC3-II Quantitation) (CBA-5116) was used as per the guidelines provided by the manufacturer. Initially, PANC-1 cells (with a cell density of 3x104) at about 80% confluence were rinsed with PBS and then exposed to the IC50 concentration of AP7Glu to stimulate autophagy. The cells were then kept at 37°C for a specified period. After this incubation, 1.5 mL of 1X Cytosolic LC3 Removal Reagent was introduced and the mixture was left for 5 minutes. This was followed by a 10-minute treatment with lysis buffer. Afterward, the cells underwent lysis, were relocated to tubes, centrifuged at 12,000 x g for 10 minutes, and preserved at -80°C until needed for analysis. During the experimental procedure, both the lysate and a standard sample (each 100 µL) from the treated set were loaded onto a plate coated with the anti-LC3 antibody. This setup was incubated at 37ºC for 4 hours. After the initial incubation, 250 µL of 1X Wash Buffer was added, and this washing step was repeated thrice. 100 µL of anti-LC3 antibody was subsequently poured into every well, followed by a 2-hour incubation. Later, 100 µL of HRP-linked secondary antibody was added and left for another hour. In the concluding steps, 100 µL of Substrate Solution was introduced and allowed to react for 20 minutes. The enzymatic reaction was halted by adding 100 µL of Stop Solution. The optical density of each sample was measured at 450 nm using an ELISA plate reader.

Statistical analysis

All outcomes are depicted as the average value with the inclusion of the standard deviation (SD). Statistical evaluations between the control and experimental groups were conducted using GraphPad Prism software (version 10.0.2, GraphPad Software, La Jolla, CA) through the application of the Student's t-test and one-way ANOVA test. $P<0.05$.

RESULTS and DISCUSSION

AP7Glu is a glucoside form of the flavonoid apigenin, commonly found in various vegetables and fruits. In this study, the potential of AP7Glu to induce cell death through apoptotic and autophagic pathways in PANC-1 cells was examined. Chen et al. (2014) reported a significant increase in apoptosis and cytotoxic effects in gastric cancer cell lines HGC-27 and SGC-7901 after 48 hours of 10 µM apigenin treatment. Another study on the SK-MEL-24 cell line demonstrated significant antiproliferative, cytotoxic, and antimigratory effects at both 30 µM and 60 µM (16). Lastly, AP7Glu has been shown to inhibit the proliferation of HeLa cells in 48 hours (IC50, 47.26 µM) (17). In the presented study, the cytotoxic activity of AP7Glu in the PANC-1 cell line was determined to be 15 µM in 48 hours (Figure 1).

Both the mitochondria and death receptor pathways can initiate apoptosis. Each represents a distinct mechanism for triggering cell death. Caspase 3 is a pivotal caspase involved in initiating and executing apoptosis. Studies have shown that apigenin stimulates PARP (poly (ADP-ribose) polymerase), an enzyme that regulates DNA repair and cell death processes, and activates p53 (18). Moreover, following AP7Glu treatment, the level of p53 protein, that has a vital function in regulating the cell cycle, repairing DNA, and other cellular processes, increased, activating the p53-dependent apoptotic pathway (19). Additionally, AP7Glu was reported to promote apoptosis by increasing the expression of apoptotic proteins such as Bax and Caspase-3 through the p53 pathway (15). Oishi et al. (2013) found that apigenin treatment raised p53 levels in PANC-1 cells, leading to cell cycle arrest and the induction of apoptosis (20). Consistent with these findings, in the current study using the same cell line, treatment of PANC-1 cells with AP7Glu increased the expression levels of proapoptotic genes. Furthermore, Liu et al. (2020) reported that AGL treatment halts the cell cycle in the G0/G1 phase, stimulates cytochrome c release by regulating Bcl-2 family proteins, activates caspase 9/3 to induce cell apoptosis, and reduces mitochondrial membrane potential (MMP) (17). In parallel with these studies, the TUNEL test have also confirmed these findings (Figure 2). Additionally, the presented work observed an increase in the expression of CASP3, BAX, and cleaved PARP after treatment with the effective dose of AP7Glu (Figure 3).

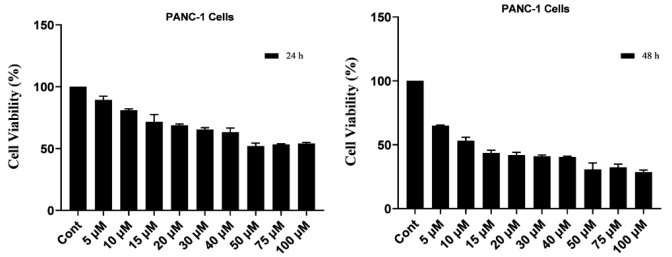

Figure 1. The cytotoxic effect of AP7Glu on PANC-1 cell line. Cells were treated with control and AP7Glu (5-10-15-20-30-40-50-60-75-100 µM) for 24 and 48h. XTT cell proliferation assay was used for the detection of IC50 values. The dose and control group was subjected to least three independent experiments

utophagy is a cellular cleaning process that helps cells survive under stress. However, excessively induced autophagy can trigger programmed cell death in cancer cells (21). Lee et al. (2014) demonstrated that AP7Glu can enhance the formation of autophagic vacuoles in colon cancer cells and induce autophagic flux (22). Another study reported that AP7Glu modulates the levels of autophagic markers, LC3-II and p62/SQSTM1, thereby regulating cellular pathways involved in autophagy (19). In the present study, treatment of PANC-1 cells with AP7Glu resulted in a statistically significant decrease in the levels of the autophagic genes ATG4 and XBP1, while no significant change was observed in the ATG6 gene (Figure 3). Additionally, an increase in CHOP levels was observed, suggesting that it contributes to cell death and cell inhibition in PANC-1 cells. Lastly, the significant change observed in the PERK level indicates that PERK induces the translation of ATF4 and triggers cell death via upregulation of CHOP. In the presented study, a significant increase in the levels of LC3, a critical indicator of the autophagic process, was observed (Figure 4).

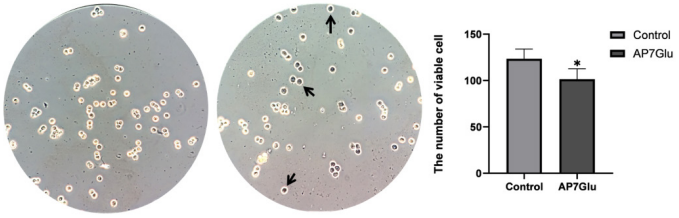

Figure 2. The effect of AP7Glu on apoptosis. After culturing, the cells were treated to the concentration of the IC50 value and incubated for 48 hours. The analysis was carried out using the colorimetric TUNEL assay. Apoptotic cell nuclei are visualized as dark blue dots. Control cells (A) and AP7Glu-treated cells (B) were examined and counted in five random fields under a microscope. In the control group, the percentage of live cells was 86.73%, and the percentage of apoptotic cells was 13.27%. In the group treated with AP7Glu, the percentage of live cells was 70.52%, and the percentage of apoptotic cells was 29.4%. The bar graph shows the average value ± standard deviation, with p*<0.05.

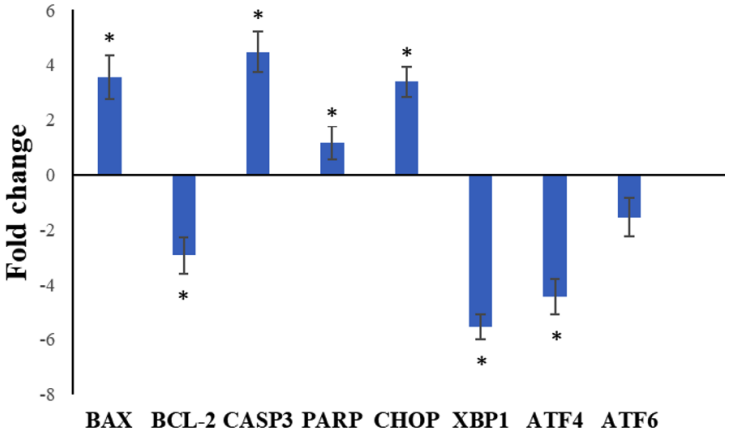

Figure 3. The effect of AP7Glu on expression levels of apoptosis and autophagy-related genes in PANC-1 cells. Ct values of each gene were normalized with Ct values of the GAPDH housekeeping gene. (*p< 0.05)

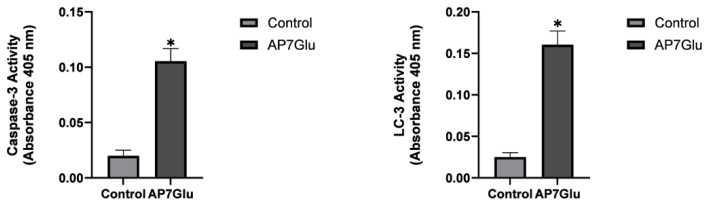

Figure 4. Activity of caspase-3 and LC-3 activity after AP7Glu treatment. The colorimetric ELISA assay was employed to measure the activities of caspase-3 and LC-3. These activities were then normalized to control cells and represented as a fold change. Consequently, there was an elevation in the activities of caspase-3 and LC3 post treatment of AP7Glu when compared to the control. Data is presented as mean ± standard deviation (std) with a sample size of 3 (n=3) and a significance level of p*<0.05.

CONCLUSION

The findings from this study suggest that within the tested dose range, AP7Glu inhibits the growth of the PANC-1 cell line by displaying cytotoxic effects and enhancing the levels of apoptotic genes while decreasing the level of the anti-apoptotic protein, exerting both apoptotic and autophagic effects. However, more detailed data are needed to understand the underlying molecular mechanisms of these effects. This is especially crucial for evaluating apigenin-7-O-glucoside as a potentially more potent therapeutic agent compared to apigenin, especially in cell lines like PANC-1 that are often resistant to chemotherapy and radiation.

Conflicts of Interest

The author declare no conflicts of interest.

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Research Article

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Determine The Effects of Fermented and Probiotic Suplemented Dairy Products on Dental Health Parameters and Quality of Health

Fermente ve Probiyotik Eklenen Süt Ürünlerinin Diş Sağliği Parametreleri ve Sağlik Kalitesi Üzerindeki Etkilerinin Belirlenmesi

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Abstract

Objective: Fermented dairy products have recently begun to attract attention on oral health. The aim of this study was to determine the effects of fermented and probiotic added dairy products on oral and dental health parameters and quality of health.

Methods: Forty adult participants were divided into four groups and consumed different fermented dairy products, including 240 mL of kefir, 200 mL of probiotic yogurt, and 200 mL of industrial plain yogurt every day for 15 days. The control group did not consume any of these fermented dairy products. The values obtained as a result of the analysis of saliva samples and the scores obtained from the quality of life scales were analyzed with the SPSS version 15.0 package program.

Results: There was no significant change in pH of saliva after consumption of fermented dairy products ($p > 0.05$). However, there was an increase in the buffering capacity after kefir and probiotic yogurt consumption (4.1 ± 0.41 and 3.7 ± 0.66, $p < 0.05$, respectively), and a slight increase was determined in the participants consumed plain yogurt (3 ± 0.61, $p > 0.05$) but there was a decrease in the control group (3 ± 0.61, $p < 0.05$). The greatest decrease in OHIP-14 (Oral Health Impact Profile-14) score was seen after kefir consumption.

Conclusions: The short-term consumption of fermented dairy products increases the buffering capacity and contributes to the improvement of oral health-related quality of life by supporting oral health.

Keywords: Buffering capacity, Fermented dairy products, Oral and dental health, OHIP-14

Özet

Amaç: Fermente süt ürünleri son yıllarda ağız sağlığı konusunda dikkat çekmeye başlamıştır. Bu çalışmanın amacı, fermente ve probiyotik katkılı süt ürünlerinin ağız ve diş sağlığı parametreleri ve sağlık kalitesi üzerine etkilerini belirlemektir.

Yöntemler: Kırk yetişkin katılımcı dört gruba ayrıldı ve 15 gün boyunca her gün 240 mL kefir, 200 mL probiyotik yoğurt ve 200 mL endüstriyel sade yoğurt olmak üzere farklı fermente süt ürünleri tüketti. Kontrol grubu, bu fermente süt ürünlerinden hiçbirini tüketmedi. Tükürük örneklerinin analizi sonucunda elde edilen değerler ve yaşam kalitesi ölçeklerinden alınan puanlar SPSS versiyon 15.0 paket programı ile analiz edildi.

Bulgular: Fermente süt ürünleri tüketiminden sonra tükürüğün pH değerinde önemli bir değişiklik olmamıştır ($p > 0.05$). Ancak kefir ve probiyotik yoğurt tüketiminden sonra tamponlama kapasitesinde anlamlı artış (sırasıyla $4,1 \pm 0,41$ ve $3,7 \pm 0,66$, $p < 0,05$) sade yoğurt tüketen katılımcılarda hafif bir artış belirlendi ($3 \pm 0,61$, $p > 0,05$), fakat kontrol grubunda anlamlı düşüş belirlendi ($3 \pm 0,61$, $p < 0,05$). OHIP-14 (Ağız Sağlığı Etki Profili-14) skorundaki en büyük düşüş ise kefir tüketiminden sonra görüldü.

Sonuç: Fermente süt ürünlerinin kısa süreli tüketimi tamponlama kapasitesini artırmakta ve ağız sağlığını destekleyerek ağız sağlığına bağlı yaşam kalitesinin iyileştirilmesine katkıda bulunmaktadır.

Anahtar Kelimeler: Tamponlama kapasitesi, Fermente süt ürünleri, Ağız ve diş sağlığı, OHIP-14

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Introduction

Adequate and balanced nutrition is of great importance for growth, development and the maintenance of general health throughout life as well as for maintaining optimal oral and dental functions. Nutrition can cause changes in oral health by changing oral and dental health parameters. Particularly, consumption of foods that buffer oral cavity pH can have a protective effect on oral and dental health.

Milk and dairy products are known to be protective against dental caries because they contain anti-cardiogenic components such as calcium, phosphate, casein and lipids (1). Some previous studies have shown that the consumption of milk and dairy products can reduce the risk of caries by increasing the saliva buffering capacity (2, 3).

Recent studies investigating the effects of consumption of different fermented and probiotic added dairy products on oral-dental health parameters have drawn attention (4, 5).

Yogurt, a fermented dairy product originated from Central Asian Turks, is widely preferred due to its nutritional quality and sensory properties. It is obtained by fermenting milk with lactic acid bacteria. Despite its high acid content, yogurt has low cariogenicity due to its calcium, phosphate, casein, lipids and whey proteins (1). Kefir, originating from Tibet and the Caucasus, is a dairy product that has positive effects on health due to lactic acid and acetic acid bacteria, yeast and filamentous fungi and various bioactive components produced by these microorganisms during fermentation (6). Probiotics, which are usually added to fermented dairy products as additional cultures, are live microorganisms that provide health benefits to the host when given in sufficient quantities (7).

It is known that fermented and probiotic containing dairy products have significant effects on general health and well-being due to their rich protein, vitamin and mineral content. However, it is a matter of interest that these positive effects can also be seen in oral health and the quality of life associated with it, and we have limited evidence to say that they all have the same effect.

Considering that the research focuses especially on infants and children, more studies are needed investigating the effects of consumption of fermented dairy products and probiotic containing foods on oral health and quality of life in adults.

Therefore, this study was performed to determine the effects of fermented products such as yogurt and kefir and yogurt supplemented with probiotics on oral health parameters and quality of life regarding oral and dental health.

Methods

This study was a prospective type of intervention study conducted with 40 people who applied to an oral and dental health clinic in İstanbul between February and April in 2020.

Participants

The patients who applied to the outpatient clinic were examined by the dentist and then adult volunteer participants without active caries were included in the study. Those who used antibiotics in the last month, smokers, have any systemic disease and active caries were excluded from the study. In order to reduce the effect of confounding factors, the food consumption frequency of the participants was recorded, and the frequency of sugar use with tea-coffee consumption and tooth brushing frequency were questioned. Considering these information, similar study groups were formed.

Study design

In the practice, which lasted for 15 days, participants were divided into four groups with 10 people in each. The participants in control group consumed 60 g of white cheese and 240 mL of milk per day for 15 days. In addition to these foods, the participants in the remaining groups consumed the following foods;

Group 1: 240 mL of industrial kefir daily (at least 1.0 * 106 cfu / g live probiotic microorganisms),

Group 2: 200 g industrial probiotic yogurt (Lactobacillus bulgaricus, Streptococcus thermophilus, Lactococcus lactis and Bifidobacterium lactis DN 173 010/CNCM I-2494),

Group 3: 200 mL industrial plain yogurt (at least 1.0 * 107 cfu / g symbiotic cultures of Streptococcus thermophilus and Lactobacillus delbrueckii subsp.bulgaricus),

Group 4 (Control group): 60 g of white cheese and 240 mL of milk daily, without any additional supplement.

Within the scope of the study, all dairy products, which were produced in accordance with the relevant communiqué of the Ministry of Agriculture and Forestry Food Codex, consumed by the participants were purchased from the markets by considering their expiration dates.

Participants consumed yogurt and kefir at noon, all at once and without any other food, and did not brush their teeth for an hour.

Collection of Saliva Samples

Unstimulated saliva samples were collected from all participants two hours after breakfast. Participants had their meals and brushed their teeth at least two hours before the collection of saliva samples, and did not eat or drink anything until the collection of saliva samples. In addition, they did not consume cariogenic foods such as jam, honey, candy and chocolate on the day of sampling. Participants were asked to sit comfortably with their mouths open and their heads bent downwards and collect their saliva in their mouths for

five minutes and then spit out the accumulated saliva into a wide-mouthed and lidded sterile disposable box. Samples were analyzed immediately (8).

Saliva pH and Buffering Capacity

The pH measurement of the saliva samples was carried out with pH indicator paper (Universal Test Paper). The indicator paper was dipped directly into the saliva sample and removed, and the pH value was determined by matching the color change on the paper with the color scale given in the table on the indicator box.

Saliva buffering capacity was determined with Ericsson method. A 1 mL of unstimulated saliva sample was withdrawn with the help of a 3 mL of disposable pipette and transferred to a different container, and 3 mL of 0.0033 N HCl was added to it. Carbon dioxide was removed by applying gentle vibrational movements to the container. Approximately of 15 minutes later, measurements were made with a pH-009 (I) A brand pocket pen type pH meter. According to the measurement result, the buffering capacity was classified as high when the pH was between 7.5-6, normal between 6-4, and low when 4 (8).

Evaluation of Quality of Life

The Oral Health Impact Profile-14 (OHIP-14) scale, which was developed by Slade and Spencer in 1994, and revised in 1997 by Slade, was used to evaluate the oral and dental health-related quality of life of the participants. Scoring of the scale is five-point Likert type that evaluates the quality of life over seven categories as functional limitations, physical pain, physical disability, mental distress, social disability, mental disability and handicap (9). The score obtained from the scale is in the range of 0-56 points, and if the score closer to high values it indicates the poor quality of life related to oral and dental health whereas the total score closer to zero means the higher oral and dental health-related life quality.

Statistical analysis

The values obtained as a result of the analysis of saliva samples and the scores obtained from the quality of life scales were analyzed with the SPSS version 15.0 package program. Differences between independent groups were evaluated using the Kruskal Wallis test for parameters that were not normally distributed. The group that caused the difference was determined by Bonferroni test. The Wilcoxon test was used for nonparametric variables to compare the data within the group. An overall 5% type-I error level was used to infer statistical significance.

Ethic

Ethical approval was given by the Research Ethics Committee at Biruni University (approval number: 2020/37-12). This study was carried out following the latest version of the Helsinki Declaration. All participants were informed about research and practice, and the "Enlightened Written Consent Form" was received from them.

Limitations of the Study

In this study, the short-term effects of fermented dairy products were investigated by limiting the consumption to 15 days. However, longer-term consumption of fermented dairy products may result in more permanent and different effects on oral microbiota and oral and dental health. In addition, the products containing the above-mentioned bacteria were used in this study. Different types of probiotic bacteria may have different effects on oral and dental health.

Results

The mean age of the individuals participated in the study was 24.9 ±7.79 years and 90 % of them graduated from university and were living in İstanbul. Istanbul is a metropolitan city located in the western region of Turkey, connecting the continents of Asia and Europe.

There was no significant change in saliva pH values of the participants after consumption of fermented dairy products (p>0.05) (Table 1).

Table 1: Mean Saliva pH Values of the Participants

	Before Intervention	After Intervention	++p	Z
Saliva pH	$\overline{X}$ ±SS	$\overline{X}$ ±SS		
Group 1	6.8±0.40	6.9±0.39	0.414	-0.816
Group2	6.8±0.24	6.8±0.24	0.180	-1.342
Group3	7±0.33	7±0.36	0.317	-1.000
Group4	6.7±0.58	6.7±0.55	0.317	-1.000
+p	0.502	0.438		

+Kruskal Wallis Test ++Wilcoxon Test Z: Test statistic value

However, there was an increase in the buffering capacity of the participants consuming kefir and probiotic yogurt at the end of the intervention, and a decrease was observed in the control group (p<0.05). The highest saliva buffering capacity was 4.1 ± 0.41 after kefir consumption (p<0.05), and the lowest was 3±0.61 after plain yogurt consumption (p>0.05) (Table 2).

Table 2: Mean Saliva Buffering Capacity of Participants

	Before Intervention	After Intervention	++p	Z
Buffering Capacity	$\overline{X}$ ±SS	$\overline{X}$ ±SS		
Group 1	3.1▯0.81	4.1▯0.41a	0.021*	-2.310
Group2	3.1▯0.55	3.7▯0.66ab	0.005*	-2.805
Group3	2.9▯0.72	3.2▯0.57b	0.075	-1.781
Group4	3.4▯0.77	3▯0.61bc	0.027*	-2.214
+p	0.502	0.438		

Similarly, it was determined that the mean OHIP-14 score decreased after kefir and probiotic yogurt consumption, but this was found to be statistically significant only in the

kefir consumed group (p <0.05). Although there were slight increases in the mean OHIP-14 score of the plain yogurt group and the control group, no statistically significant difference was found (p>0.05) (Table 3).

Table 3: Mean Scores of the OHIP (Oral Health Impact Profile)-14 Scale of Participants

	Before Intervention	After Intervention	++p	Z
OHIP-14 Score	$\overline{X}$ ±SS	$\overline{X}$ ±SS		
Group 1	12.6 4.81	9.4 3.77	0.040*	-2.055
Group2	12.6 6.70	10.8 6.05	0.234	-1.190
Group3	11.5 7.66	11.8 7.11	0.932	-0.085

Discussion

Saliva is an exocrine secretion that has an important role in protection and maintaining the health of oral tissues. Due to its buffering ability, saliva prevents enamel demineralization by neutralizing the acids produced by acidogenic microorganisms, and plays a protective role against formation of caries by providing the necessary minerals in the remineralization process with the appropriate pH (6-7) (10). The pH and buffering capacity of saliva, which are important parameters of oral health, can be affected by the consumed foods. For example, high consumption of acidic foods and beverages can affect the natural buffering capacity of saliva that causes a decrease in saliva pH and may lead to dental erosion (11). On the contrary, when anticariogenic foods such as milk and dairy products are consumed, the pH of saliva increases to an alkaline level and its buffering capacity develops thus protection is provided against tooth decay (12).

In this study, consumption of fermented and probiotic bacteria-containing dairy products, kefir, probiotic yogurt and industrial plain yogurt, by adults for 15 days did not cause a significant change in saliva pH. However, consumption of kefir containing probiotic bacteria and yogurt with probiotics increased the mean saliva buffering capacity (p <0.05). Such an effect was not observed in those who consumed industrial plain yogurt without probiotic bacteria.

It was suggested that probiotics can increase saliva pH and buffering capacity by working as antagonists of cariogenic bacteria and preventing their proliferation as well as neutralizing acids formed as a result of fermented carbohydrate metabolism in the oral environment (13-15).

However, when the recent studies in the literature are reviewed, it can be seen that the effect of fermented and probiotic-containing dairy products on salivary pH and buffering capacity has been mostly investigated in children and adolescents.

Eden et al. (2019) investigated the effect of short-term consumption of probiotic yogurt on caries risk factors in six to eight-month-old babies, and they found no significant difference in saliva pH and in the levels of Streptococcus mutans and Lactobacilli after consumption of probiotic yogurt containing Bifidobacterium Longum BB536, Bifidobacterium Bifidum Bb12 and Lactobacillus Rhamnosus HN001 for three weeks; however, they found a significant increase in saliva buffering capacity. Alp and Baka (2018), in their study on adolescents aged 12-17 years, reported that 200 mL of kefir consumption per day for three weeks caused a decrease in the level of Streptococcus mutans in saliva.

One of the longer applications was performed by Villavicencio et al. (2018) on preschool children, and they found that consumption of probiotic milk supplemented with Lactobacillus rhamnosus and Bifidobacteruim longum for nine months significantly increased the saliva buffering capacity but caused no change in saliva pH. Rodriguez et al. (2016) also reported that consumption of probiotic supplemented milk for ten months can reduce the development of caries compared to standard milk consumption in preschool children with high caries incidence.

The results of our study conducted on adults were similar to the results of these studies (2, 4) conducted on young age groups who consumed yogurt containing probiotic bacteria for short or long terms. The use of dairy products containing probiotic bacteria in a short period of 15 days has shown an effect that increases the saliva buffering capacity as in the long term. The use of dairy products containing probiotic bacteria in a short period (15 days) also increased the saliva buffering capacity similar to the long term use. However, Streptococcus mutans levels were not investigated in the present study.

Oral and dental health can affect physical, psychological and social status of a person and is accepted as a fundamental component of quality of life and general health (16).

Oral and dental health-related quality of life is a concept that reflects how a person perceives the effects of oral health on general health status and social activities (17). In previous studies, it has been shown that oral cavity diseases such as periodontitis, tooth decay, tooth loss and pain / discomfort in this region cause deterioration in quality of life by affecting negatively the general well-being of individuals, as well as their external appearance and self-esteem (18,19). However, in the literature review, it has been observed that the effect of the consumption of fermented and probiotic dairy products on the quality of life have not been adequately investigated.

In this study, OHIP-14, one of the comprehensive and widely used scales, was used to evaluate oral and dental health-related quality of life (9). At the end of the study, a decrease was determined only in the OHIP-14 scores of individuals who consumed kefir (p 0.05). This positive effect on quality of life was thought to be due to the positive effect of kefir

consumption on oral health. In addition, consumption of probiotic yogurt also showed a positive effect on quality of life, but this change was not statistically significant. Probiotic bacteria protect the oral biofilm from environmental stress and maintain the health-related symbiosis as well as repair the dysbiotic biofilm, thus modulating the oral ecosystem with both its protective and therapeutic properties (20).

In this study, the consumption of dairy products containing fermented and probiotic bacteria was limited to 15 days due to the COVID-19 pandemic. It is thought that with longer consumption of fermented dairy products, more positive effects can be seen on the quality of life related to oral and dental health.

As a result, it can be said that short-term consumption of fermented dairy products such as kefir and probiotic yogurt increases the saliva buffering capacity and can positively affect oral and dental health and quality of life. However, it would be beneficial to conduct long-term studies aimed at different groups in the society in order to reveal the effects of these products on oral and dental health more clearly. The consumption of dairy products containing fermented and probiotic bacteria should not be ignored while developing community nutrition interventions for the prevention of oral and dental diseases.

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Conflict of Interest Disclosure

For the authors of this study, there were no conflicts of interest; including but not limited to; memberships or relationships with members of the scientific and medical committees, consulting, expertise, employment in any firm, and shareholding, et cetera.

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