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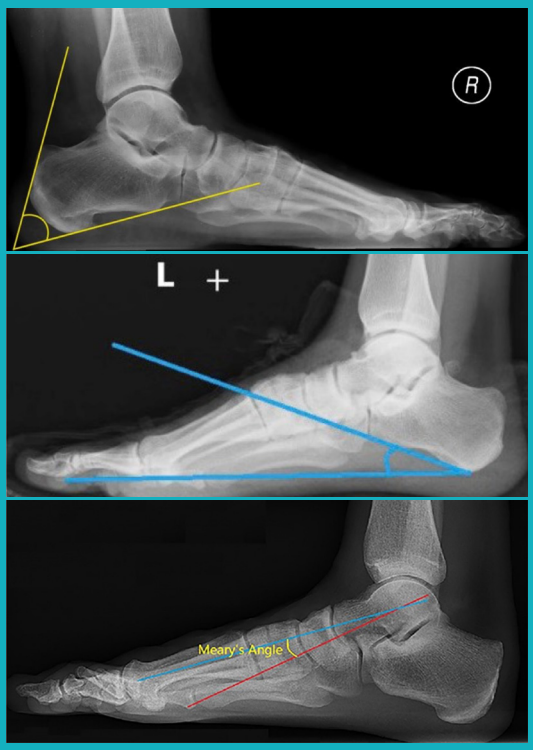

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1. Video articles should include a brief introduction on case, surgery technique or a content of the video material.

2. Presentations in image / static image format: JPG, GIF, TIFF, BMP

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4. The file size should be a maximum of 100 MB.

5. The file size should be no more than 10 minutes.

6. Main text should not exceed 500 words.

7. References are welcomed and should not be more than 5.

8. Video and images must be cited within main text.

9. There should be no identification of patient / physician / institution / city / country in pictures and especially video images.

The tables, figures and illustrations included in your article should be declared as original. When received from sources other than the original,the source must be cited, and hardcopy format or electronic versions of the source should be submitted to the Editor-in-Chief office with permission from the copyright holder (publisher, journal or author). The rules for references, figures and tables apply to all article types.

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Editor(s) as author: Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

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Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

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EDITORIAL

Dear Readers;

We are with you again in the 4th issue of 2022. I thought that the Bezmialem Science team, working with a very intense and tiring pace, deserved some rest this summer. But the increase in the number of our publications did not allow us to reduce our working speed in this summer. I would like to thank each of my teammates for their support and effort, who took the time to evaluate the high demand for our journal last spring season, which was full of scientific studies, academic activities and partly congresses.

We continue to publish our remaining articles from the previous system. Unfortunately, due to the accepted articles until the first issues of 2023, we have to delay the publication of newly accepted articles. We have decided to announce the estimated publication time of the accepted articles on our website in order to respond to the many messages we receive from the authors about the time of publication. I hope this will partially remove the ambiguities.

We are very happy to be together once again with beautiful topics in this issue.

For this issue, we have chosen the cover art from the study of Büyükdoğan et al. titled

“Assessment of Calcaneal Morphology and Radiological Parameters in Haglund’s Syndrome”.

Other notable articles are; 2- Prognostic Significance of Flow Cytometric Immunophenotyping in Patients with Acute Myeloid Leukemia, 3- Determining the Amount of Iodine in Edible Salts Obtained from Markets and District Bazaars in İstanbul, 4-Management of Acute Biliary Pancreatitis in Cholecystectomized Patients, 5- Attitude, Knowledge and Donor Cardiac Volunteering of Nursing Students Regarding Organ Donation

With this issue, we are starting another innovation in the editorial.

For us, your every work is valuable and important. However, we would like to select a study on public health and current issues in each issue and share a brief evaluation with you. In this issue, we have chosen the article titled “Determining the Amount of Iodine in Edible Salts Obtained from Markets and District Bazaars in Istanbul” by Mr. Bilgin et al. Iodine is really one of the very important minerals for us. Both its deficiency and excess use cause thyroid diseases. As a requirement of the policies of the Ministry of Health, the practice of adding iodine to salts had been started years ago. What is not known by the society is that the excess of iodine is as harmful as its deficiency. Another problem is that iodine solutions have been widely presented as a panacea recently and their uncontrolled sale on the internet due to the abuse of iodine deficiency has reached a level that threatens public health. It is essential that the Ministry of Health and Ministry of Agriculture officials should conduct more extensive studies based on the study of Bilgin et al.. Although the sample size was small in the study, only 50% of the iodine amount was found suitable, while 50% was found to be less or more. This is a rate that should not be underestimated. Society needs a balanced intake of iodine for the prevention of thyroid diseases. I would like to thank Bilgin and his colleagues for bringing this issue to the fore.

In this issue, we are with you with new and different topics. I am sure you will find an article about your own branch. I would like to thank my associate editors, referees, publishing house and our valued readers for their continuous support. Everything you get your heart desires…

Kind regards

Prof. Dr. Adem AKÇAKAYA
Chief Editor

Comentary

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Bezmialem Science 2022;10(4):398-401

Surgeon's Neoadjuvan Therapy Approach in Gastric Cancer

Mide Kanserinde Cerrahın Neoadjuvan Tedaviye Yaklaşımı

Adem AKÇAKAYA

Bezmialem Vakıf University Faculty of Medicine, Department of General Surgery, İstanbul, Turkey

Dear Readers,

I want to share with you, from a surgeon's point of view, the subject of recommending neoadjuvant treatment to patients with gastric cancer (GC), which is mostly obtained as a result of multidisciplinary meetings.

As is known, neoadjuvant therapy is the administration of therapeutic agents before the main therapy. Our main treatment for GC is surgery. In locally advanced gastric cancers, the standard treatment method is to administer neoadjuvant chemotherapy before surgery. The aim of neoadjuvant therapy is to facilitate surgery by reducing the primary mass, to test whether the tumor is sensitive to chemotherapy, and most importantly to contribute to survival. Neoadjuvant therapy reduces the tumor mass, allowing us to perform surgery without leaving any tumor behind. It facilitates the differentiation of cancerous tissue from normal tissue. With this, it can improve post-operative recovery and reduce the chance of cancer recurrence in the long run.

Despite advances in surgery and adjuvant therapy, local recurrence and distant metastasis are the main causes of death due GC. Increased R0 resection rates, prevention of local recurrence and distant metastases have become the main goal of treatment, which has led to the development of neoadjuvant therapy in GC (1).

The reasons for performing neoadjuvant therapy in gastric surgery include the patient's higher tolerance to the chemotherapy administered before surgery, the increased chance of R0 resection and the possibility of obtaining a pathological complete response, the possibility to plan the postoperative regimen by seeing the pathological and biological response to the treatment, early treatment of micrometastases, and prevention of development of distant metastasis development in the early period. Also, theoretically, a higher potential effect can be obtained by administering drugs to the intact tumor bed after surgery (2,3).

According to The National Comprehensive Cancer Network (NCCN) guideline, neoadjuvant therapy is recommended for clinically ≥T2 tumors or node positivity (4). In patients with gastroesophageal junction (GEJ) stage II-III adenocarcinoma and GC, perioperative chemotherapy (CT) and combined neoadjuvant chemoradiotherapy (CTRT) have been shown to improve overall survival (OS) compared to surgery alone (2,5).

Many studies have been conducted to date in the treatment of gastric cancer. However, it is still controversial which neoadjuvant

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therapy is best. I have mainly benefited from the publication of Dirikoç et al., which summarizes the medical oncology course of neoadjuvant therapy, and I thank the authors for their good work (2). With this article, I wanted to share the work done so far and finally my opinion as a surgeon.

One of the important steps is the MAGIC study. In this study, in which the perioperative ECF protocol was compared with surgery, the 5-year OS increased significantly (36% vs 23%, p=0.009, respectively) (5). In the subsequent EORTC 40954 study, no survival advantage could be demonstrated with the neoadjuvant 48-day cisplatin-fluorouracil regimen, and R0 resection rates were found to be higher only in the neoadjuvant treatment arm (7). In the French FNLCC-FFCD study, a statistically significant increase was observed in R0 resection rates, disease-free survival (DFS), and OS with the perioperative cisplatin-fluorouracil (CF) regimen in gastroesophageal junction tumors (8). In addition to neoadjuvant CT studies, the role of chemoradiotherapy was also investigated, and the primary goal in these studies was to increase pathological complete response (PCR) rates. In the phase 2 RTOG 9904 study, after 2 cycles of CF induction CT, infusional fluorouracil and weekly paclitaxel-guided CRT were administered, and a PCR rate of 26% and an R0 resection rate of 77% were achieved. The number of cycles of neoadjuvant therapy was another matter of debate, and an answer to this question was sought in the COMPASS study. In a 4-arm study consisting of 2 or 4 courses of S-1-cisplatin (SC) and paclitaxel-cisplatin (PC) regimens, PCR was obtained only in the combination arms and was independent of the agent. Different studies on neoadjuvant therapy in GC were conducted by the Japanese Clinical Oncology Group. In the phase 2, JCOG 0210 study, the efficacy of preoperative SC treatment in type 3 and type 4 GC was investigated and the mean survival was 17.3 months, while the 3-year survival rate was found to be 24.5%, and it was suggested that this combination could be effective and safe. In the JCOG 1002 study, the efficacy of preoperative docetaxel-cisplatin-S-1 treatment and adjuvant 1-year S-1 treatment were investigated in patients with extensive lymph node metastasis (ELM) who underwent D2 dissection and paraaortic lymph node dissection, and a response rate of 50% was obtained, which was inadequate (2).

The CROSS study compared long-term follow-up results of surgery alone and CTRT combined with surgery. CTRT combined with surgery was shown to have more significant OS benefits in patients with squamous cell carcinoma than in patients with GEJ adenocarcinoma (9).

Although local DFS was significantly increased with CRT in the POET study, no difference in OS was detected. In the results of the TOPGEAR study, no difference was found between the CT and CRT arms in terms of toxicity and complications. In the CRITICS study, epirubicin-cisplatin or oxaliplatin capecitabine was used as CT, and a cisplatin-capecitabine regimen was applied with RT. At the end of the 61.4-month follow-up period, OS was found to be 43 months in the CT arm and 37 months in the CRT arm, with no additional contribution of adjuvant CRT. The effectiveness of targeted therapies in neoadjuvant therapy was investigated in two separate studies. In the ST03 study, bevacizumab was added to the perioperative treatment of gastroesophageal junction tumors, and the survival benefit of adding panitumumab was investigated in the NEOPECX study. Both studies showed no improvement in histological response or OS, but increased toxicity.

At the end of all these studies, when we came to 2019, the FLOT4 study, which would change our practice, was published and in this study, the Magic regimen and the FLOT (50mg/m2 docetaxel, 85 mg/m2 oxaliplatin, 200mg/m2 leucoverin and 2600 mg/m2 fluorouracil 24-hour infusion) protocol were compared and a 5-year OS contribution (36 months vs 23 months, respectively, p<0.001) was determined. For resectable GC or GEJ adenocarcinomas in the FLOT4 study, perioperative CT showed a significant OS benefit of docetaxel-based triple (fuorouracil plus leucovorin, oxaliplatin, and docetaxel) plus surgery compared to the ECF/ECX-MAGIC regimen. This study changed our point of view on neoadjuvant therapy, which we, surgeons, were previously skeptical of because of patients in whom treatment was previously unsuccessful or who relapsed with progression. In the end, perioperative FLOT treatment took its place as the first line recommendation in the NCCN guideline.

The efficacy of preoperative regimens containing S-1 was investigated in the PRODIGY and RESOLVE studies published in 2021. In the PRODIGY study, the addition of the neoadjuvant docetaxel oxaliplatin-S-1 regimen to the 1-year post-surgical adjuvant S-1 treatment improved DFS, while the RESOLVE study compared the adjuvant CapOx with the adjuvant and perioperative S-1-oxaliplatin (SOX) regimen. Perioperative SOX was superior to the adjuvant CapOx regimen based on 3-year DFS. The RESONANCE II study, which investigates how many cycles of the neoadjuvant SOX regimen should be administered, is ongoing (10). Following the positive results in the ToGA study of trastuzumab CT combination in Her-2 positive advanced GC, trastuzumab and other anti-Her-2 agents were also tested in neoadjuvant therapy, along with studies on the metastatic disease with other anti-Her-2 agents. The primary endpoint of the HER-FLOT study,

in which trastuzumab was applied in combination with the perioperative FLOT regimen, was reached, with a DFS of 42.5 months and a 3-year OS of 82.1% (11). In the PETRARCA study, it was observed that the addition of trastuzumab and pertuzumab to the periperative FLOT regimen increased the rates of PCR (35% vs 12%, respectively, p=0.02) (12).

Immunotherapy was also brought to the agenda with the addition of pembrolizumab to perioperative CT in the Keynote-585 study. Studies are continuing in which the combination of another immunotherapy agent, camrelizumab, and a tyrosine kinase inhibitor, apatinib, and the combination of an antiVEGFR monoclonal antibody, ramucirumab, and FLOT are tested (13).

One area where we experience confusion as surgeons is gastroesophageal tumors. While the perioperative strategy to be applied in the treatment of GEJ cancer Siewert III and GC was more or less determined as we mentioned above, the questions regarding the ideal treatment of Siewert 1 and 2 cancers could not be fully answered. Some published meta-analyses have failed to clarify this dilemma. It is not easy to reach clear results due to patient characteristics and regional differences in surgical treatment (14,15). I think that the completion of studies in this area will allow surgeons to clarify their approach.

In conclusion, developments in neoadjuvant therapy and successful results have led us as surgeons to change our approach to GC. Better OS and DFS with neoadjuvant therapy, replacing the previous unsuccessful treatments, has increased the confidence in this approach. Neoadjuvant CT provides the opportunity to treat both the main tumor and invisible micrometastases, especially in tumors that have a high metastasis potential and have spread locally. Although there are some fuzzy points in patient and drug selection, treatment standards become clearer day by day after the FLOT 4 study. Another contribution that pleases us, the surgeons, is the increase in our R0 resection rates. The fact that the differences arising from the demographic characteristics of the patients, such as the location of the tumor and the type of tumor are overcome by immunotherapy and newly developed treatment models, seems to increase the usage area of neoadjuvant therapy even more.

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Original Article

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Prognostic Significance of Flow Cytometric Immunophenotyping in Patients with Acute Myeloid Leukemia

Akut Miyeloid Lösemili Hastalarda Akış Sitometrik İmmünofenotiplemenin Prognostik Önemi

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ABSTRACT

Objective: Chromosomal abnormalities are one of the most important prognostic factors in acute myeloid leukemia (AML). However, not all patients may have such informative chromosomal abnormalities. Although there are many studies on the prognostic value of immunophenotyping in AML, it is still not used as a prognostic marker. In this study, we aimed to investigate the effects of CD13, CD33, CD34, CD117, MPO and HLADR expressions on prognosis of non-acute promyelocytic leukemia AML.

Methods: One hundred thirteen patients diagnosed as having non-acute promyelocytic leukemia AML and followed up between 2010 and 2018 were included in this study. The associations of CD13, CD33, CD34, CD117, MPO and HLA DR expressions with chemotherapy response, progression free survival (PFS) and overall survival (OS) were statistically analyzed.

Results: It was seen that response to chemotherapy was achieved in 67.3% of the patients. Median PFS duration was 9 months and median OS duration was found as 13 months. Of the immunophenotypic characteristics, only MPO expression was determined to be an independent risk factor for PFS and OS.

Conclusion: Immunophenotypic features may be helpful in the diagnosis of AML as well as give an idea about prognosis. In this

ÖZ

Amaç: Kromozomal anormallikler, akut miyeloid lösemide (AML) en önemli prognostik faktörlerden biridir. Bununla birlikte, tüm hastalarda bu tür bilgilendirici kromozomal anormallikler olmayabilir. AML'de immünofenotiplemenin prognostik değeri üzerine birçok çalışma olmasına rağmen, immünofenotipleme halen prognostik belirteç olarak kullanılmamaktadır. Bu çalışmada akut promiyelositik lösemi dışı AML'de CD13, CD33, CD34, CD117, MPO ve HLADR ekspresyonlarının prognoz üzerindeki etkilerini araştırmayı amaçladık.

Yöntemler: Bu çalışmaya 2010-2018 yılları arasında tanı konup tedavi edilen 113 akut promiyelositik lösemi dışı AML'li hasta dahil edilmiştir. CD13, CD33, CD34, CD117, MPO ve HLA DR ekspresyonlarının kemoterapi yanıtı ve progresyonsuz (PFS) ve genel sağkalım (OS) ile ilişkisi istatistiksel olarak analiz edildi.

Bulgular: Hastaların %67,3'ünde kemoterapiye yanıt alındığı görüldü. Medyan PFS süresi 9 aydı ve medyan OS süresi 13 ay olarak bulundu. İmmünofenotipik özelliklerden yalnızca MPO ekspresyonunun, PFS ve OS için bağımsız bir risk faktörü olduğu belirlendi.

Sonuç: İmmünofenotipik özellikler AML tanısında yardımcı olabileceği gibi prognoz hakkında da fikir verebilir. Bu çalışmada,

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study, MPO expression was shown to be an independent risk factor for PFS and OS in our own patient population.

Keywords: Acute myeloid leukemia, immunophenotyping, prognosis, survival

MPO ekspresyonunun kendi hasta popülasyonumuzda PFS ve OS için bağımsız bir risk faktörü olduğu gösterilmiştir.

Anahtar Sözcükler: Akut miyeloid lösemi, immünofenotipleme, prognoz, sağkalım

Introduction

Acute myeloid leukemia (AML) is a malignant disorder of hematopoietic stem cells characterized by clonal proliferation of abnormally differentiated myeloid series blasts. AML is one of the most common types of leukemia in adults. There are approximately 19,940 new cases of AML and approximately 11,180 deaths from AML annually in the United States (1). Treatment response and overall survival (OS) in AML are heterogeneous. A number of prognostic factors have been identified for patient and tumor characteristics for AML, including age, performance status, and karyotype (2-4). Advanced age (>60), poor performance status, treatment-related AML, myelodysplasia, or AML after myeloproliferative neoplasms are known to be poor prognostic factors (5-7). Today, AML risk classification is performed based on cytogenetic and molecular properties. Criteria published by European LeukemiaNet (ELN) group in 2017 is used for risk classification (8).

In recent years, immunophenotyping has become a standard practice in the diagnosis of hematologic neoplasms and in the definition of the origin of cells. The clinical significance of surface antigen expression has been investigated for more than 20 years, but so far several consistent results have been achieved (9). The markers with significant prognostic associations demonstrated in multiple studies were CD13, CD14, and CD15 (10-13). Many other markers claimed to have prognostic significance, alone or in combination, have not been supported in other studies. In this study, we aimed to investigate the effects of CD13, CD33, CD34, CD117, MPO and HLADR expressions on prognosis of non-acute promyelocytic leukemia AML.

Methods

Patients

One hundred thirteen patients diagnosed as having non acute promyelocytic leukemia AML and followed up between 2010 and 2018 were included in this study. The gender, age at diagnosis, complete blood count at the time of diagnosis, flow cytometry results, risk groups, treatment responses, PFS, OS, and final status were obtained retrospectively from the patient files. Approval was received from the Non-invasive Clinical Research Ethics Board at Van Yüzüncü Yıl with the date 07.12.2018 and approval number 10.

Risk groups were determined according to ELN 2017. t(8; 21), t(15; 17), inv16, NPM1 mutation without FLT3-ITD mutation, and those with biallelic CEBPA mutation were considered as good risk groups. Co-mutation in NPM1 and FLT3-ITD, t(9; 11) and presence of cytogenetic abnormalities not classified as positive or negative were accepted as standard risk groups. t(6; 9), t(v; 11q23.3), t(9; 22), inv(3), -5 or del(5q), -7; -17/abn(17p), complex karyotype, monosomal karyotype, presence of FLT3-ITD mutation without NPM1 mutation, and presence of RUNX1, ASXL1, TP53 mutations were considered as bad risk group (8). ELN 2017 risk classification was evaluated with conventional cytogenetics, FISH and next-generation sequencer. RUNX1, ASXL1, TP53 could not be studied due to technical impossibilities.

OS was calculated from diagnosis to death. Progression free survival (PFS) was defined as the time from start of treatment to disease progression or death from any cause. Survivals were expressed in months.

As induction chemotherapy, 73% of the patients received idarabucine (2 or 3 days) + cytarabine (5 or 7 days) chemotherapy, 27% received azacitidine (7 days) or desitabine (5 days). Those who did not remitter after idarabucine + cytrabine CT were given reinduction chemotherapy with the same chemotherapy regimen. After remission was achieved, patients in the high-risk group had allogeneic stem cell transplantation if donor was present. Patients without high risk were consalidated with 3-4 cycles of high-dose cytarabine. Patients receiving azacitidine or decitabine were evaluated for response after 4-6 cycles. Treatment was continued with responders until progression.

Immunophenotypic Analysis

Flow cytometric immunophenotyping was performed on bone marrow aspirate. Specimen processing was performed according to a routine red cell lysis protocol. Single cell suspensions were stained with various 4 fluorochrome-conjugated antibody combinations and analyzed in reference to isotype-matched fluorochrome-conjugated control antibodies. Immunophenotypic properties were evaluated with Multiparametric Flow Cytometry (Facs Canto II, BD, Brussels, Belgium). CD3, CD5, CD7, CD19, CD22, CD13, CD33, CD14, CD15, CD16, CD34, CD41, CD56, CD64, CD117, MPO, Tdt, and HLADR expressions were studied in the acute leukemia panel. While expressions >20% were considered positive, <20% was considered negative.

Statistical Analysis

While our study expressed the continuous variables with their descriptive characteristics as median, minimum and maximum values, the categorical variables were expressed in terms of frequencies and percentages. The chi-square test was performed to detect the relationship between response rates to the treatments and immunophenotypic features; and also detect the relationship between the risk groups and the survival rates. The PFS and OS curves were constructed by Kaplan-Meier method and differences among groups were calculated by using log-rank

test. The Cox proportional hazard regression model was used to examine the potential prognostic factors for outcome. A two-sided p value of <0.05 was accepted to be statistically significant. All analyses were performed using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Fifty four (47.8%) of the patients were female and 59 (52.2%) were male. The median age at diagnosis was 51 (18-81) years. Other descriptive properties are given in Table 1.

The mean PFS duration was 9 (0-113) months and the mean OS duration was found as 13 (1-115) months. There was no statistical difference between response to chemotherapy and the expression of CD13 (p=0.356), CD33 (p=0.676), CD34 (p=0.256), CD117 (p=0.108), MPO (p=0.246), and HLADR (p=0.154).

The OS and PFS differences were observed between the risk groups (Table 2). PFS was longer in favorable risk group than intermediate and adverse risk groups (p=0.036 and p=0.007, respectively). No difference was observed in terms of PFS between the intermediate risk group and adverse risk group (p=0.16). In favorable risk group, OS was longer than intermediate and adverse risk groups (p=0.001 and p=0.00, respectively). There was no difference in terms of OS between the intermediate risk group and the adverse risk group (p=0.09) (Table 2).

The MPO expression was found to be an independent risk factor on PFS and OS (p=0.008 and p=0.015, respectively) (Table 3). In the survival analysis, PFS and OS were longer in the group expressing MPO (p=0.009 and p=0.004, respectively) (Figure 1). Although patients with HLADR negativity had higher PFS and OS advantage (p=0.045 and p=0.025, respectively) (Figure 1) and also was found to have significant impact on PFS- OS in univariate regresyon analysis, we did not found HLADR negativity as an independent predictor on PFS-OS in multivariate cox regression analysis (Table 3).

Discussion

In this study, we investigated the relationship between cell surface expressions and treatment response status, PFS and OS in our own patient population. PFS and OS were found to be longer in patients expressing MPO and not expressing HLADR. However, it was shown that while MPO expression was independent of PFS and OS, HLADR was not an independent factor. Expression of CD13, CD33, CD34 and CD117 was also found to have no effect on PFS and OS. Cytogenetic risk groups were also found to be associated with PFS and OS, consistent with the literature.

The high rate of MPO expression in patients with AML has been associated with better PFS and OS with better complete response

Table 1. Descriptive features

*Age	51 (18-81)
Sex	
Female	47.8%
Male	52.2%
Risk category	
Favorable	14.2%
Intermadiate	61.1%
Adverse	24.7%
CD13 status	
Positive	90.2%
Negative	9.8%
CD33 status	
Positive	83.2%
Negative	16.8%
CD34 status	
Positive	57.6%
Negative	42.4%
CD117 status	
Positive	81.7%
Negative	18.3%
MPO status	
Positive	43.9%
Negative	56.1%
HLADR status	
Positive	69.2%
Negative	30.8%
Response rate	
CR	67.3%
NR	32.7%
Survival status	
Alive	45.1%
Ex	54.9%

*median (min-max)
min: Minimum, max: Maximum, CR: Complete response, NR: Normal response, Ex: Exitus

Table 2. Relationship of risk groups between PFS and OS

	Progression free survival						Overall survival					
Risk category	Favorable		Intermediate		Adverse		Favorable		Intermediate		Adverse	
	x^2	p	x^2	p	x^2	p	x^2	p	x^2	p	x^2	p
Favorable			4.38	.036	7.34	.007			11.13	.001	20.95	.00
Intermediate	4.38	.036			1.97	.16	11.13	.001			2.18	.09
Adverse	7.34	.007	1.97	.16			20.95	.000	2.81	0.09		

X^2: Chi-square, PFS: Progression free survival, OS: Overall survival

(CR) rates in many studies (14-17). In a study of 233 patients with AML, Dong et al. (18) demonstrated low MPO expression (<70%) as an independent risk factor for CR, OS, and PFS. They found over 70% MPO expression associated with favorable risk group, high CR rates, and longer PFS and OS (18). Kamijo et al. (19) found high MPO expression (>50%) associated with RUNX1-RUNX1T1, the KIT mutation and CEBPA double mutations, and low MPO expression (<50%) associated with DNMT3A mutation, FLT3 tyrosine kinase domain mutation and TP53 mutations. Similar to the literature, in our study, the presence of MPO expression was found to be associated with prolonged PFS and OS, and it was shown to be a favorable prognostic factor. In our study, unlike other studies, statistical significance was obtained with MPO above 20%. As mentioned above in other studies, the threshold value was determined between 50-70%. Based on these results, MPO expression at the time of diagnosis can be used as a good prognostic marker. In some places, the cytogenetics and especially the next-generation sequencer are still not easy to be reached. MPO expression can be a guide for centers that cannot reach these examinations.

Table 3. Relationship between immunophonotypic characteristics and PFS and OS, multivariate Cox regression analysis

	Progression free survival		Overall survival	
	p	Exp (B)	p	Exp (B)
CD13	0.487	0.537	0.609	0.737
CD33	0.248	0.295	0.550	1.325
CD34	0.156	0.408	0.851	0.938
CD117	0.135	2.526	0.605	0.798
MPO	0.008	7.223	0.015	2.481
HLADR	0.429	0.513	0.712	0.852

PFS: Progression free survival, OS: Overall survival

Chang et al. (20) found that CD34 positivity was associated with low CR in patients with de novo AML. And they showed that co-expression of CD34 and HLADR was a negatively independent risk factor for achieving CR (20). Other studies have shown poor response to induction chemotherapy in the presence of CD34 expression (21,22). Yang et al. (23) found that CD34 positivity was associated with low CR rates, but did not find a significant difference between CR status and CD13 and CD33 positivity.

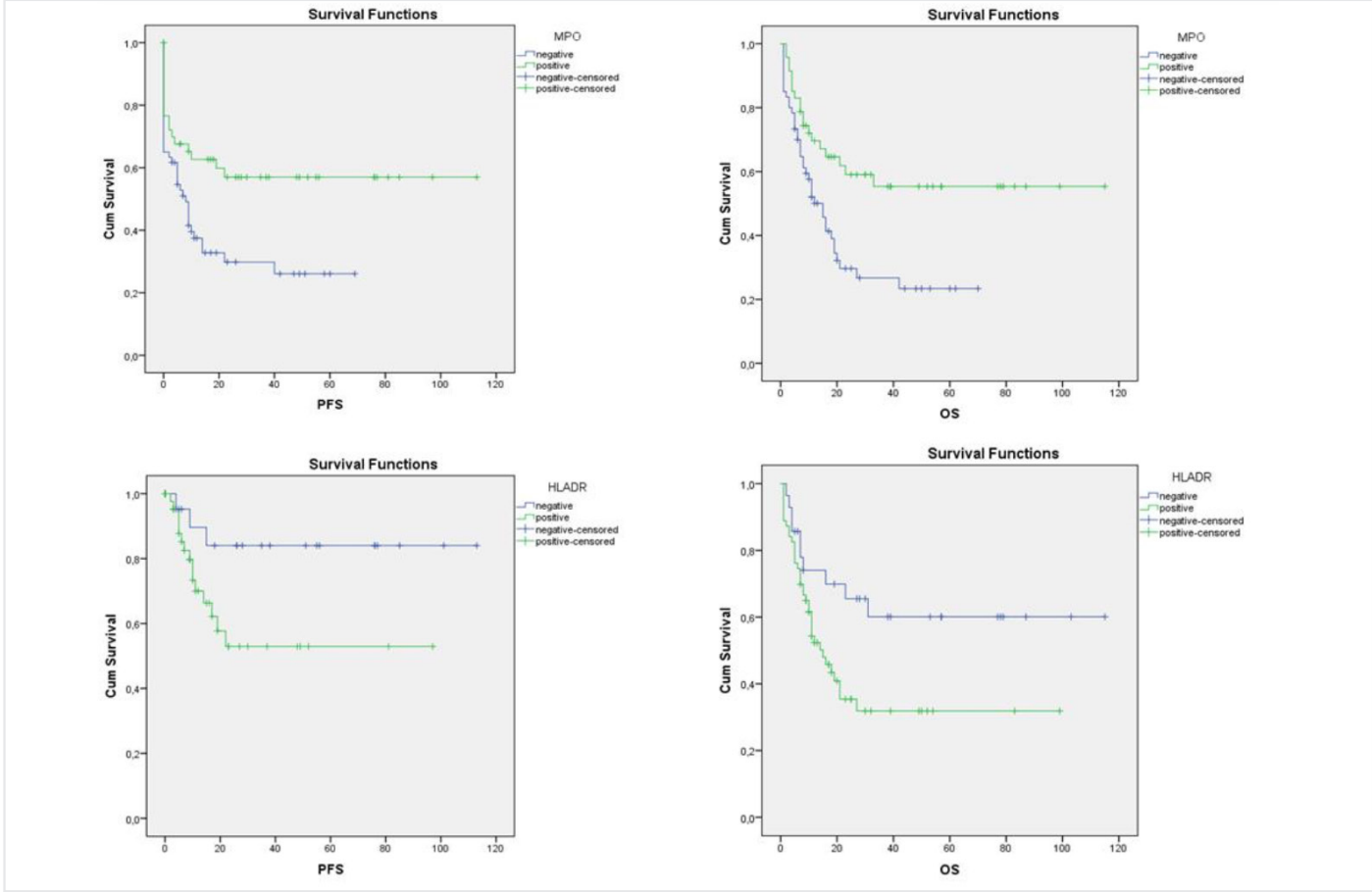

Figure 1. MPO positive patients have longer PFS and OS, HLADR negative patients have longer PFS and OS
PFS: Progression free survival, OS: Overall survival

Contrary to these data, CD34 expression did not have an effect on treatment response and survival in our study.

The absence of expression of CD9, CD11b, CD13, CD34 and CD41 in patients with AML, or the presence of CD15, CD33, CD38, CD64, and MPO expression was associated with longer OS. CD9, CD13, CD34 and CD64 have been shown to be independent risk factors on survival (24). In another study, it was found that patients in whom MPO, CD13, CD33, CD65 and CD117 were co-expressed had a better prognosis (25). Plesa et al. (26) showed that CD34-positive/CD33-positive or CD34-negative/CD33-negative was poor prognostic, CD34-positive/CD33-negative was intermediate prognostic, and CD34-negative/CD33-positive was favorable prognostic.

While several studies have shown that CD13 has no prognostic significance (9,25), another study has shown a significant correlation between the low CR rate of CD13 positivity (27). In another study, CD13 positivity was reported to be associated with low CR and short OS, whereas CD33 was not associated with CR and OS (28). CD117 was associated with decreased CR rates (29). In our study, expression of CD13, CD33 and CD117 was not related to CR, PFS and OS.

About 40-50% of patients with AML display a normal karyotype at diagnosis (30). As prognosis of AML with a normal karyotype is heterogeneous, efforts have been made to stratify patients into different risk categories and to guide therapeutic decisions. Mutations in a number of genes have been found in AML with a normal karyotype; the most commonly affected are nucleophosmin1 gene (NPM1), the FMS-like tyrosine kinase 3 gene (FLT3), and the *DNA-methyltransferase 3a* gene (DNMT3A). There are two main types of FLT3 mutations. The most common are internal tandem duplications (ITD) of different length that result in ligand-independent activation of the FLT3 receptor and a proliferative signal (31,32). The prognostic impact of FLT3-ITD is influenced by its mutational context, including the absence of the wild-type FLT3 allele (ie, homozygous or hemizygous FLT3-ITD), the concurrent mutation status of NPM1, and the FLT3 mutant/wild-type allelic ratio. Homozygous or hemizygous FLT3 and higher AR are associated with poorer outcomes (33-35). Munoz et al. (36) found that AML with FLT3-ITD often had a myelomonocytic immunophenotype expressing CD33 and CD15, with rather low CD34 expression. In addition, CD11b and CD4 were often expressed in these patients. If FLT3-ITD cannot be measured, the status of FLT3-ITD can be predicted by immunophenotypic expressions.

Study Limitations

In several studies, HLADR expression has been associated with reduced CR rates and shorter CR duration (20,37,38). In our study, it was determined that the absence of HLDR expression, which supported the literature, was associated with longer PFS and OS. Unlike the literature, no correlation was found between HLDR and response status.

Conclusion

Our aim in this study was to investigate the prognostic value of immunophenotypic markers that were routinely checked at the time of diagnosis. Previous studies have shown the prognostic significance of MPO and CD34 expression. In our study, MPO positivity was found to be consistent with longer PFS and OS, similar to the literature, but unlike the literature, no correlation was found between CD13, CD33, CD34, CD117 and PFS and OS. Randomized prospective controlled studies are needed to determine the prognostic significance of immunophenotypic features.

Ethics

Ethics Committee Approval: Van Yüzüncü Yıl University Non-Invasive Clinical Research Ethics Committee (date: 07.12.2018/ decision no: 10).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: S.D., T.U., Design: S.D., T.U., Data Collection or Processing: Ö.E., A.D., Analysis or Interpretation: S.D., T.U., Literature Search: S.D., Writing: S.D., T.U.

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Original Article

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Comparison of Single and Double Incision Repair Techniques in Distal Biceps Tendon Rupture

Distal Biseps Tendon Rüptüründe Tek İnsizyon ile Çift İnsizyon Tamir Tekniklerinin Karşılaştırılması

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ABSTRACT

Objective: The purpose of this study was to compare the complications and functional outcomes of single versus double-incision repair techniques for the treatment of distal biceps brachii tendon rupture

Methods: Between 2012 and 2018, patients with distal biceps brachii tendon rupture who were treated with a single or double-incision repair technique were included in this retrospective study. Range of motion (ROM) and Mayo elbow performance scores (MEPS) were evaluated.

Results: Seventeen patients with a mean age of 45.6±6.4 years (range: 34-58 years) who underwent single (n=9) and double (n=8) incision techniques were included in this study. The mean follow-up was 33±10.5 months (range: 24-62 months). Preoperative and postoperative ROM and MEPS were similar between two groups (p>0.05). In the single incision repair technique group, 3 patients had lateral antebrachial cutaneous nerve (LACN) palsy and 1 patient was re-operated due to re-rupture. In the double incision repair technique group, 1 patient had posterior interosseous nerve (PIN) palsy and 1 patient had hematoma that did not require surgical drainage. No significant differences were detected in terms of complications (p=0.62).

Conclusion: Good functional results were obtained after both single and double incision techniques for the treatment of distal biceps brachii tendon rupture. Both single and double incision techniques were reliable however LACN was at risk in single incision technique and PIN in double incision technique.

Keywords: Distal biceps tendon, tendon rupture, surgical repair, single incision, double incision

ÖZ

Amaç: Bu çalışmanın amacı, distal biseps brakii tendon rüptürünün tedavisinde tek ve çift insizyon tamir tekniklerinin komplikasyonlarını ve fonksiyonel sonuçlarını karşılaştırmaktı.

Yöntemler: Bu retrospektif çalışmaya 2012-2018 yılları arasında distal biseps brachii tendon rüptürü olan ve tek veya çift insizyon tamir tekniği ile tedavi edilen hastalar dahil edildi. Hareket açıklığı (HA) ve Mayo dirsek performans skorları (MEPS) değerlendirildi.

Bulgular: Çalışmaya tek (n=9) ve çift (n=8) insizyon tekniği uygulanan, yaş ortalaması 45,6±6,4 yıl (dağılım: 34-58 yıl) olan 17 hasta dahil edildi. Ortalama takip süresi 33±10,5 aydı (dağılım: 24-62 ay). Preoperatif ve postoperatif HA ve MEPS iki grup arasında benzerdi (p>0,05). Tek insizyon tamir tekniği grubunda 3 hastada lateral antebrakial kutanöz sinir (LACN) felci görüldü, 1 hasta ise tekrar rüptür nedeniyle tekrar ameliyat edildi. Çift insizyon tamir tekniği grubunda 1 hastada posterior interosseöz sinir (PIN) felci ve 1 hastada cerrahi drenaj gerektirmeyen hematom gelişti. Komplikasyonlar açısından anlamlı farklılık tespit edilmedi (p=0,62).

Sonuç: Distal biseps brachii tendon rüptürünün tedavisinde hem tek hem de çift insizyon tekniklerinden sonra iyi fonksiyonel sonuçlar elde edilmektedir. Her iki teknik güvenilirdir, ancak tek insizyon tekniğinde LACN, çift insizyon tekniğinde ise PİN risk altındadır.

Anahtar Sözcükler: Distal biseps tendonu, tendon rüptürü, cerrahi tamir, tek insizyon, çift insizyon

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Introduction

The frequency of distal biceps brachii tendon rupture (DBBTR) is 1-5/100,000 per year, and 96% of all traumatic DBBTRs originate from the long head, 1% from the short head, and 3% from the distal insertion of the biceps tendon (1). DBBTR usually occurs during weight lifting in men in their fourth or fifth decade of life and occurs in the dominant extremity as a result of eccentric loading of the muscle tendon unit (2). Smoking, anabolic steroid use, and intratendinal degeneration or hypovascularity are factors that increase the likelihood of this injury (3).

Surgical repair is applied as the first-line treatment in DBBTR, and tendon repair provides more successful and satisfactory results compared to conservative treatment (3-5). Therefore, surgical repair is recommended in active patients with DBBTR to restore elbow flexion and supination strength and to ensure limb endurance. Conventional single-incision or double-incision methods are used in distal biceps tendon repair (3).

The aim of this study was to compare the results of patients who underwent single incision cortical suspension system and tenodesis screw combination repair technique (6) and double incision bone tunnel repair technique (7) for distal biceps tendon repair. Our hypothesis was that the functional outcomes of treatments with different surgical techniques would be similar.

Methods

Before the start of the study, academic committee approval was obtained from the Bezmialem Vakıf University Orthopedics Department (no: 45446446-010.99-3655). The data of patients who underwent distal biceps tendon repair due to DBBTR between December 2012 and January 2018 were reviewed retrospectively. Patients with a follow-up of less than two years were excluded from the study. Acute injuries in active patients with a traumatic full-thickness tear on magnetic resonance imaging (MRI) and clinically accompanied by a decrease in supination muscle strength were considered as the indication for distal biceps tendon repair. Demographic information, extremity dominance, background and occupation of the patients participating in the study were documented. Consent was obtained from all patients for inclusion in the study. One of two different repair techniques was applied to all patients by a single surgeon (K.B.).

Preoperative Evaluation

Preoperative hook test (8) was performed on all patients with suspected distal biceps injury and MRI was requested for radiological evaluation of possible injury. Range of motions (ROM) of elbow flexion and extension, and forearm pronation and supination of the patients were measured preoperatively with a universal goniometer, and Mayo elbow performance scores (MEPS) were recorded. Patients without these assessments were not included in the study.

Surgical Technique

Informed surgical consent forms were obtained from all patients before surgery. All patients were operated in the supine position after full muscle relaxation was achieved under general anesthesia. Following the IV administration of 1 gram of cefazolin, the biceps muscle was stroked to the distal and a tourniquet was applied by extending the muscle length. All surgical procedures were performed on the swimsuit table after sterile staining and dressing. In patients with excessive biceps tendon retraction on MRI, a double incision technique was preferred, and in patients without tendon retraction, a single incision technique was preferred.

Anterior Single Incision

The skin and subcutaneous tissue were passed through a transverse 3-4 cm incision 3 cm distal to the elbow crease. After the skin incision, the lateral cutaneous antebrachial nerve adjacent to the cubital veins was dissected and suspended. The distal biceps tendon, which migrated proximally by blunt dissection, was found and the tendon tip was debrided and thinned. The tendon was prepared using the 4 cm Krackow suture technique with 2.0 Etibond (Ethicon, Somerville, NJ) suture. Then, to prepare the radial tuberositas, it was deepened by blunt dissection between the brachioradialis and pronator teres muscles. The bone was reached with the elbow extended and the forearm hypersupinated. The tendon stump was removed from the bone with the aid of a curette and a rongeur. After proper retraction, bone double cortex was drilled with a 3.2 mm drill bit for bone tunnel preparation. Then, the proximal single cortex was drilled 7-8 mm according to the tendon thickness. Tendon sutures were loaded onto the cortical suspension system (Smith & Nephew, Memphis, TN) and double cortex was bypassed. The sutures were stretched and the tendon was placed in the bone tunnel and tightened with a 1 mm thin tenodesis screw (Smith & Nephew, Memphis, TN) from the tendon at appropriate tension. Then, the tendon was sutured with the threads coming out of the cortical suspension system (Figure 1) (6).

Double Incision

With a 2-3 cm transverse incision made from the elbow bend, the skin and subcutaneous tissue were passed. After LACN dissection and tendon preparation, soft tissue resection was performed with blunt dissection. The location of the second 3-4 cm longitudinal incision to be made posterolaterally between the extensor carpi ulnaris and the extensor digitorum communis muscle interval with a blunt curved surgical instrument was determined using the inside-out method. The forearm was pronated and the posterior interosseous nerve (PIN) was removed. The radial tuberositas cortex was debrided with the aid of a rongeur and curette. The tunnel into which the tendon would enter was prepared with Burr. Three holes were drilled on the sides of the tunnel through which the sutures were to be passed, and 4 sutures, through which the tendon was sutured, were taken and passed through the posterolateral incision with the inside-out method. The tendon was fixed on the prepared place on the bone (Figure 2) (7).

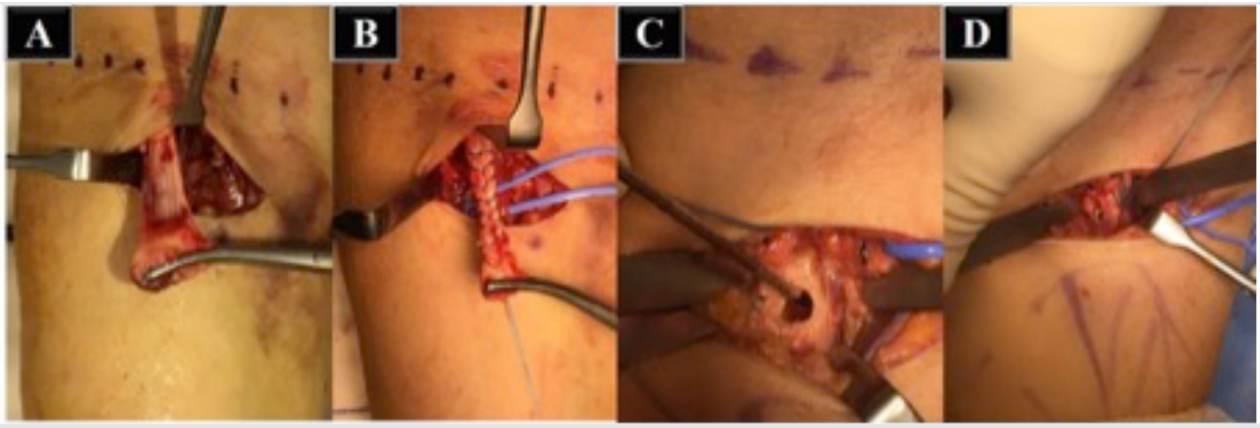

Figure 1. A) Finding the ruptured biceps tendon from the anterior incision, B) Preparing the tendon with Krackow suture technique while preserving the LACN, C) Preparing the radial tuberositas before tenodesis, D) Applying the tenodesis screw

Postoperative Rehabilitation

Postoperatively, the upper extremity of all patients was placed in a long arm plaster splint with the elbow in 90° flexion and the forearm in supination. Wound dressing was recommended every three days as a standard to the patients. The plaster splint was removed 2 weeks after the operation. Exercises to increase ROM with the support of gravity were started and the patients were recommended to use a shoulder arm sling for 2 weeks. Thirty degrees of active extension until the sixth week and full elbow extension at the second month were aimed. Active flexion and strengthening exercises were started in the second month. The patients returned to their daily lives after 3 months and were released for sports activities at 6 months.

Postoperative Functional Evaluation

Postoperative ROM and MEPS of all patients at the latest clinical follow-up were evaluated by a single physiotherapist. The two surgical techniques were compared with each other in terms of complications of heterotropic ossification (HO), radioulnar synostosis, infection, nerve damage, fracture, dislocation of the cortical suspension system, and re-rupture of the distal biceps tendon during clinical follow-ups.

Statistical Analysis

All statistical analyzes were performed using the SPSS statistical software package (IBM Corp.© Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Conformity of continuous data to normal distribution was

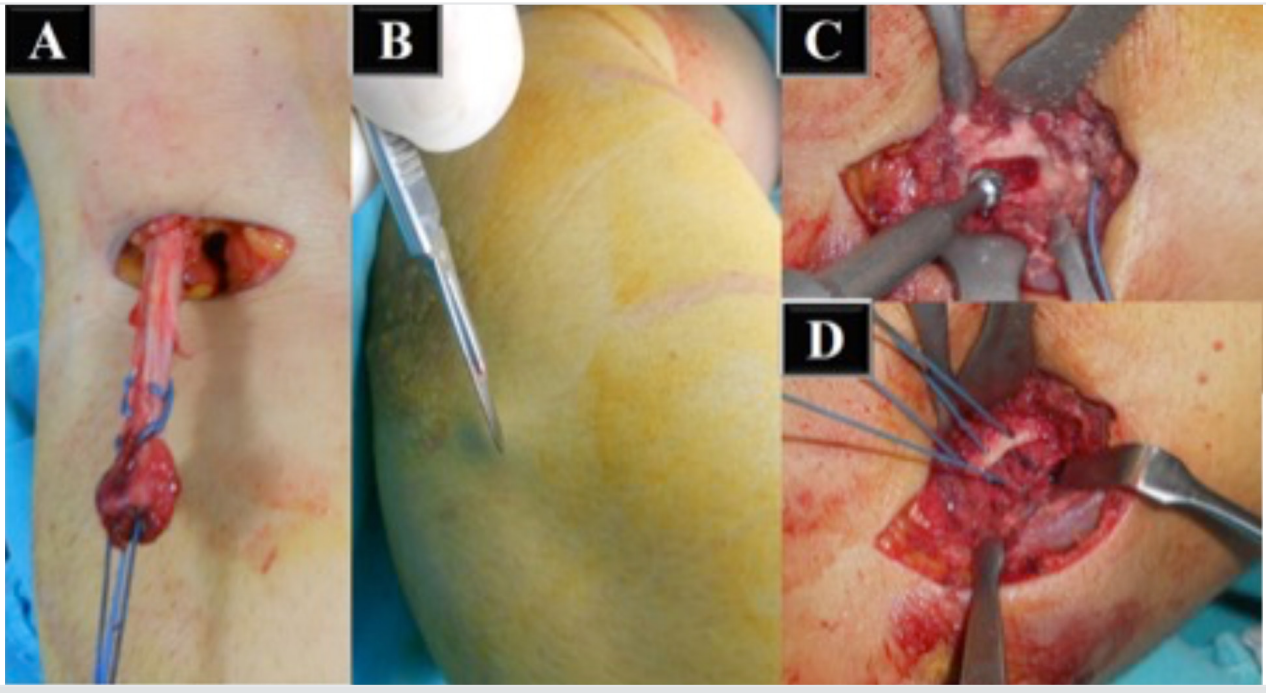

Figure 2. A) Preparing the biceps tendon, B) Determining the location of the second incision using the inside-out method, C) Preparing the radial tuberosity with a burr, D) Passing the threads through the holes

evaluated by Kolmogorov-Smirnov test. Continuous variables were expressed as median (minimum-maximum) and mean ± standard deviation. Categorical variables were expressed as frequency (percentage) values. Continuous comparisons were made using the Mann-Whitney U test, and categorical comparisons were made using the Fisher-exact test. The results were reported as the respective p-values. P<0.05 was considered statistically significant.

Results

Demographic Results

The data of 28 patients who underwent surgery for DBBTR were reviewed retrospectively. Four patients with a follow-up period of less than 24 months, 3 patients who did not comply with the standard postoperative rehabilitation protocol, 2 patients who were operated for chronic tears, and 2 patients whose postoperative follow-up was not documented were excluded from the study. A total of 17 patients, 9 of whom were treated with the single incision technique and 8 with the double incision technique, with a follow-up period of at least 24 months were included in the study. The mean age of the patients was 45.6±6.4 (34-58), and the mean follow-up time was 33±10.5 (24-62). All patients with DBBTR were male. 13/18 (72%) of the patients were physically active working or doing sports. The dominant arms of 10 and the non-dominant arms of 7 of the patients participating in the study were injured. The mean time from injury to surgery was 2.1±1 (1-4) weeks. There was no difference between the two groups in terms of demographic characteristics (Table 1).

Functional Results

The mean MEPS of the patients increased from 78.2±12.2 preoperatively to 97.6±5.3 postoperatively. There was no significant difference between the groups in terms of pre- and postoperative ROM and MEPS (p>0.05) (Table 2).

Complications

No complications such as fracture, wound problem, infection, stiff elbow, HO, and synostosis occurred in any of the patients who underwent surgical repair due to DBBTR. Transient LACN palsy developed in 3 patients who were operated with a single incision technique and were followed up with conservative treatment. Revision distal biceps tendon repair was performed in 1 patient due to re-rupture in the second month after surgery. Transient PIN palsy developed in 1 patient who was operated with the double incision technique and recovered in three months with conservative treatment. One patient had a hematoma that did not require surgical drainage. There was no significant difference between the two groups in terms of complications (p=0.62).

Discussion

The important finding of our study is that both single incision and double incision techniques are reliable in the surgical treatment of DBBTR and these two techniques have similar results in terms of ROM, functional results and complication rates. The complication rate of both surgical techniques is low, and patients treated with the single incision technique are at risk for LACN and those treated with the double incision technique are at risk for PIN. In the case of neuropraxia due to injury to

Table 1. Demographic data of patients

	Single incision (n=9)	Double incision (n=8)	P value
Age	45.3±7.8	45.9±4.8	0.868[a]
Dominance (dominant/non-dominant)	6/3	4/4	0.637[b]
Gender (male/female)	9/0	8/0	1[b]
Sportsman and worker (n)	7	6	1[b]
Preoperative time (weeks)	2.4±1	1.8±0.9	0.194[a]
Post-operative follow-up (weeks)	30.9±6.1	35.4±14.1	0.398[a]

[a]: Mann-Whitney U test; [b]: Fisher Exact test

Table 2. Functional results

	Single incision (n=9)		Double incision (n=8)		P value	
	Preoperative value	Postoperative value	Preoperative value	Postoperative value	Preoperative value	Postoperative value
Flexion (°)	140.6±5.3	141.7±2.5	143.1±2.6	143.8±2.3	0.963	0.743
Extension (°)	1.1±3.3	2.2±6.7	1.3±3.5	0±0	0.37	0.167
Pronation (°)	84.5±3.9	86.1±3.3	78.1±3.7	78.8±3.5	0.084	0.112
Supination (°)	84.4±4.6	85±4.3	88.1±3.7	88.8±2.3	0.136	0.93
MEPS	80.6±15.7	98.3±5	75.6±6.8	96.9±5.9	0.815	0.673

MEPS: Mayo Elbow Performance score

these nerves, successful results are obtained with conservative follow-up.

In cadaveric studies comparing the single and double incision technique in the treatment of DBBTR, it was claimed that the footprint restoration of the tendon with a single incision tended to be made more forward and this led to supination weakness. In addition, it was reported that footprint restoration with double incision repair was more anatomical (9,10). In contrast, Grewal et al. (11) found no difference in terms of ROM, functional score and supination strength between the two techniques, but flexion strength was found to be significantly higher in the double incision group in a randomized clinical study. Castioni et al. (12) showed that more flexion and pronation were obtained with the single incision technique in a meta-analysis comparing single and double incisions in which they included 2,622 patients. We found no significant difference in terms of postoperative ROM (pronation: 86.1°-88.8°, supination 85°-88.8°, flexion 141.7°-143.8°) and functional MEPS scores (98.3-96.9) between the two techniques.

The PIN injury is one of the most important complications of distal biceps repair (13,14). Amarasooriya et al. (15) reported that post-repair motor nerve injuries were found around 2% and most of them were PIN injuries in their systematic review of acute distal biceps tendon repair. However, they showed that 92% of PIN injuries healed with conservative follow-up. There was no difference between single incision (2/233) and double incision (6/411) in terms of PIN injury. Luthringer et al. (16) showed that the PIN injury due to single anterior incision in the repair of the distal biceps tendon might result from placing an improperly distal retractor, and that forearm supination during instrumentation was protective against this injury in an MRI study investigating the relationship between altered forearm rotation and the distance of the PIN from the radial tuberositace. Similarly, Dunphy et al. (17) reported that PIN damage was significantly higher in the double incision technique (3.4% vs 0.8%). In contrast, Amin et al. (14) found that PIN damage was more common in patients with single anterior incision compared to double incision (2.7-0.2%). In our study, although we did not find any postoperative PIN damage in 9 patients in whom we made a single anterior incision, transient PIN palsy developed in 1 of 8 patients who were repaired with a double incision. The patient recovered within 3 months with conservative follow-up. Similar to the literature, we think that a retractor should not be used towards the distal radius of the radius neck in order to protect from PIN damage and that long-term use of retractors should be avoided.

Although care is taken to preserve the LACN during distal biceps repair, the most common postoperative complication is related to this nerve. Although LACN injury is usually in the form of neuropraxia, the frequency of persistent LACN palsy ranges between 0.6% and 26% in the literature (18-21). Castioni et al. (12) found significantly less LACN injuries in patients who underwent double incision than in patients who underwent single incision. Amarasooriya et al. (15) found significantly more LACN damage in patients who underwent repair with a single incision compared to those who underwent double incision (9.3-5.8%). In our study, in parallel with the current literature, LACN damage was found in 3 of the patients who underwent repair with a single incision, and the nerve damage was healed within 3 months in all of these patients. LACN damage was not observed in any of the patients who underwent double incision. We think that double incision prevents traction damage.

Finally, regarding the method of fixing the tendon to the bone, the cost of materials such as screws, anchors (22,23) or cortical suspension system (24,25), which are more expensive than standard sutures, should be considered. In our study, the cortical suspension system and tenodesis screw used in the single incision technique caused additional costs. In this regard, Grant et al. (26) investigated whether the use of implants in DBBTR provided a shortening of the operation time enough to cover the additional cost of the implants. They found that there was no significant difference between the transosseous group and the anchored group in terms of operation time. However, they emphasized that the cost of the anchor should be taken into account.

Study Limitations

There are many limitations of our study. First of all, a limited number of patients were included in the study and the data were reviewed retrospectively. The fact that all surgeries were performed by a single surgeon could also be considered as a limitation. Another limitation was the inability to measure muscle strength before and after surgery. Another limitation was that double incision was preferred in injuries with excessive tendon retraction and single incision repair in injuries without tendon retraction. Finally, the physiotherapist's inability to be blinded to the treatment performed in the postoperative functional evaluation due to the existing incision scars could be considered as a limitation.

Conclusion

Both single and double incision techniques are reliable in DBBTR. The results of both techniques are similar in terms of functional score, ROM and complications, but PIN in double incision technique and LACN in single incision technique are at risk. In the future, prospective, randomized studies with more patients will shed light on which technique is more advantageous.

Ethics

Ethics Committee Approval: Before the start of the study, academic committee approval was obtained from the Bezmialem Vakıf University Orthopedics Department (no: 45446446-010.99-3655).

Informed Consent: Informed surgical consent forms were obtained from all patients before surgery.

Informed Consent:

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.K., A.P., V.U., O.T., K.B., Design: M.K., A.P., V.U., O.T., K.B., Data Collection or Processing: M.K., A.P., V.U., O.T., K.B., Analysis or Interpretation: M.K., A.P., V.U., O.T., K.B., Literature Search: M.K., A.P., V.U., O.T., K.B., Writing: M.K., A.P., V.U., O.T., K.B.

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

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Original Article

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Assessment of Exercise Stress Test Parameters in Patients with Erectile Dysfunction

Erektil Disfonksiyonlu Hastalarda Treadmill Stres Testi Parametrelerinin Değerlendirilmesi

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ABSTRACT

Objective: Erectile dysfunction (ED) has a significant impact on quality of life, given its high prevalence and association with cardiovascular diseases. In the present study, we aimed to evaluate exercise treadmill parameters in patients with ED.

Methods: A total of 178 patients who were admitted to the cardiology clinic was enrolled in the study. Patients were divided into two groups according to their International Index of Erectile Function-5 (IIEF-5) score. Exercise time, maximum heart rate (HR), resting HR, chronotropic index (CI), HR recovery at one minute (HRR1) and two minutes (HRR2) were evaluated for each patient.

Results: Subjects with ED were older, had similar rates of diabetes mellitus and hypertension compared to control group. Exercise time was longer and peak HR was higher in patients with ED compared to the healthy counterparts [556.00 (61) sec. vs 575.5 (84) sec. p=0.025, and 156.00 (13) bpm vs 160.50 (11) bpm p=0.001, respectively]. We did not find statistically significant differences with respect to resting HR, HRR1, HRR2, CI, maximum systolic and diastolic blood pressure, or rate pressure product between two groups. IIEF-5 score was negatively correlated with age (r=-0.54, p=<0.001), TC (r=-0.32, p=<0.001), LDL-C (r=-0.34, p=<0.001), TG (r=-0.17, p=0.02) and positively correlated with exercise time (r=0.19, p=0.01) and maximal HR (r=0.24, p=0.001). Prevalence of abnormal HRR1 values was significantly higher in study group (n=29, 23.6%) compared to control group (n=6, 10.9 %) (p=004). According to Quade's ANCOVA results, exercise time and maximal

ÖZ

Amaç: Bu çalışmada erektil disfonksiyonlu (ED) hastalarda treadmill egzersiz testi parametrelerini incelemeyi amaçladık.

Yöntemler: Kardiyoloji polikliniğine başvuran 178 hastaya Uluslararası Erektil İşlev Formu-5 (UAEİF-5) doldurtuldu. Sonuçlara göre hastalar iki gruba ayrıldı. Hastaların egzersiz zamanı, maksimum kalp hızı (KH), istirahat KH, kronotropik indeksi (Kİ), birinci ve ikinci dakikadaki KH toparlanma indeksleri (KHTİ 1 ve KHTİ 2) hesaplandı.

Bulgular: ED'si olan hastalar daha yaşlı idi, diyabet ve hipertansiyon açısından her iki grup arasında fark gözlenmedi. Egzersiz zamanı ED'li hastalarda kontrol grubuna göre daha uzundu ve maksimum KH daha yüksekti [556,00 (61) dk ile 575,5 (84) dk p=0,025 ve 156,00 (13) vuru/dk ile 160,50 (11) vuru/dk p=0,001]. İstirahat KH, KHTİ 1, KHTİ 2 ve Kİ açısından gruplar arasında fark saptanmadı. UAEİF-5 skoru yaş (r=-0,54, p=<0,001), total kolesterol (r=-0,32, p=<0,001), LDL-K (r=-0,34, p=<0,001) ve TG (r=-0,17, p=0,02) ile pozitif, egzersiz süresi (r=0,19, p=0,01) ve maksimum KH (r=0,24, p=0,001) ile negatif korrelasyon gösterdi. Anormal KHTİ 1 değerleri çalışma grubunda kontrol grubuna göre anlamlı olarak yüksek bulundu (sırası ile, n=29, %23,6 ile n=6, %10,9, p=0,04). Quade ANCOVA sonuçlarına göre yaş kovaryant olarak alındığında egzersiz zamanı ve maksimum KH iki grup arasında farklı bulunmadı (sırası ile, F=1,032 p=0,311 and F=1,264, p=0,262).

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HR were not different between two groups when age was used as a covariate (F=1.032 p=0.311 and F=1.264, p=0.262, respectively).

Conclusion: Abnormalities of autonomic system activity is more prevalent in patients with ED. Exercise treadmill testing can be used to assess autonomic activity in that group of patients.

Keywords: Erectile dysfunction, heart rate recovery, stress

Sonuç: ED'li hastalarda anormal değerdeki KHTİ 1 daha fazla gözlenmektedir. Egzersiz treadmill testi bu hasta grubunda otonom aktiviteyi değerlendirmek için kullanılabilir.

Anahtar Sözcükler: Erektil disfonksiyon, kalp hızı toparlanma indeksi, stres

Introduction

Erectile dysfunction (ED), failure to maintain an adequate erection for satisfactory intercourse, has a high prevalence ranging from 9% to 54% depending on age and cardiovascular risk factors of the subjects (1). It is considered as a reflective of vascular disorders and predictive of cardiovascular mortality (2). Various conditions may cause ED including advanced age, hypogonadism, hypertension, diabetes mellitus, hyperlipidemia and depression (3). Normal erectile function requires proper interaction of the vascular, autonomic, and somatic nervous systems. Erection is mediated through parasympathetic mediated vascular dilatation, whereas ejaculation is mainly under the control of sympathetic nervous system (4). Hence, functional abnormalities of the autonomic nervous system may be exhibited as erectile dysfunction.

An intact heart rate (HR) response during and after exercise is essential for good cardiovascular performance. Exercise induced increase in sympathetic activity, together with the withdrawal of the parasympathetic influence, results in increased HR, cardiac output and restriction of blood flow to the skeletal muscles (5). An inability to raise HR during exercise, (i.e., chronotropic incompetence) is a major contributor of exercise intolerance and has prognostic value in cardiovascular diseases. Chronotropic response is usually assessed by chronotropic index (CI) which is defined as percentage of the HR reserve achieved by the patient (6). HR begins to decelerate soon after the termination of exercise, which is directly related to parasympathetic efferent nerve activity. The decrease of HR shows bimodal pattern: a slow decline after a steep reduction in the first 30 seconds, which is blunted by atropine (7,8). As with chronotropic incompetence, delayed HR recovery (HRR) is a predictor of cardiovascular disease and death (9). Therefore, abnormalities of sympathetic and/or parasympathetic systems can emerge through HR responses during treadmill testing.

Since both exercise testing and sexual performance could give information about autonomic nervous system activity, we aimed to investigate treadmill exercise indices in patients with ED and compare them with controls.

Methods

This was a cross-sectional, single center study that included 178 patients who were admitted to treadmill laboratory between November 2019 and December 2020. Patients with ischemic heart disease, valvular disease, rhythm-conduction disorder, left bundle branch block, hypo-hyperthyroidism, renal and/or hepatic failure were excluded from the study. The study was approved by local ethical committee and informed consents were obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki.

For evaluation of ED within past 6 months, international index of erectile function-5 (IIEF-5) questionnaire was used. It is composed of 5 Likert-type questions scored from 1 (severe dysfunction) to 5 (no dysfunction), with lower scores indicating greater disability. Severity of ED is classified into four groups: severe (5-7 points), moderate (8-11 points), mild-moderate (11-16 points), mild (17-21 points) and normal erectile function (22-25 points). Turkish validation study of IIEF-5 questionnaire was conducted by Turunç et al (10). Participants were divided in to two groups according to their IIEF-5 score; patients with a score of 1-21 (n=123) and 22-25 (n=55) constituted study and control group, respectively.

Venous blood samples of the subjects were obtained from antecubital vein after an overnight fast. Biochemical analysis was done to measure total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol and triglyceride (TG) levels. Bruce protocol was used for exercise testing (Schiller CS-200, Switzerland). B-blockers and Ca-channel blockers were stopped 48 hour before the test. Blood pressure of the participants was measured in every stage and recovery period. Exercise testing was stopped if the patient developed chest pain, dyspnea, ischemic electrocardiogram changes, hypo/hypertensive response or when 85% of the maximum predicted HR was achieved. Exercise time was calculated in seconds. Maximal predicted HR was calculated using the formula "220-age". HRR at one minute (HRR1) and two minutes (HRR2) were estimated by subtracting first and second minute post-exercise HR from maximal HR, respectively. In our treadmill laboratory, cessation of the exercise testing was performed with either in standing or sitting position without cool-down period. Since studies performed previously showed that HRR1 values of ≤12 bpm and ≤18 bpm were abnormal in protocols with and without cool-down period, respectively, we accepted HRR1 value of ≤18 bpm as abnormal (11,12). CI was calculated by dividing the difference of maximum HR and resting HR to the difference of maximum predicted HR and resting HR. Values greater than 0.80 were accepted as normal. Multiplication of HR with BP gave us the result of rate-pressure product.

Statistical Analysis

Parametric data were expressed as mean standard deviation, non-parametric data were expressed as median and interquartile

range [IQR (IQR1-IQR3)]. For comparison of two groups; independent sample t-test (normally distributed data) or Mann-Whitney U test (not normally distributed data) was used. Correlation between parameters was assessed by Spearman's correlation analysis. Quade's ANCOVA was used to test the differences of exercise time and maximal HR between groups where age served as covariate.

Result

Subjects with ED were older, had similar rates of DM and HT compared to control group. Frequency of smoking and alcohol consumption were higher in the control group than in the study group. Exercise time was longer and peak HR was higher in patients with ED compared to the healthy counterparts [556.00 (61) sec. vs 575.5 (84) sec. p=0.025, and 156.00 (13) bpm vs 160.50 (11) bpm p=0.001, respectively]. We did not find statistically significant differences with respect to resting HR, HRR1, HRR2, CI, maximum systolic and diastolic blood pressure, or rate pressure product between two groups. Baseline demographic, biochemical and exercise stress values of the groups is presented in Table 1.

According to their IIEF-5 scores, 3 (1.7%) subjects had severe-moderate disease, 35 subjects (19.6%) had mild-moderate disease, 85 (47.8%) had mild disease and the rest (n=55, 30.9%) had normal erectile function. Frequency distribution of IIEF-5 score is shown in Figure 1.

The IIEF-5 score was negatively correlated with age (r=-0.54, p=<0.001), TC (r=-0.32, p=<0.001), LDL-C (r=-0.34, p=<0.001), TG (r=-0.17, p=0.02) and positively correlated with exercise time (r=0.19, p=0.01) and maximal HR (r=0.24, p=0.001) (Table 2).

We further investigated the prevalence of abnormal HRR1 values between two groups. Twenty-nine subjects (23.6%) had abnormal HHR1 values in the study group, whereas 6 subjects (10.9 %) had abnormal HRR1 values in the control group (p=004).

According to Quade's ANCOVA results, exercise time and maximal HR were not different between two groups when age was used as a covariate (F=1.032 p=0.311 and F=1.264, p=0.262, respectively).

Discussion

In the present study we found that patients with ED had lower exercise capacity and HR response to exercise. IIEF-5 score was negatively correlated with age, exercise duration, maximal HR, TC, LDL-C and TG levels. These correlations imply common pathophysiological mechanisms between ED and cardiovascular diseases. Although we did not find any statistically significant differences in the HRR1 and HRR2 values of the two groups, the prevalence of abnormal HRR1 values was statistically significantly higher in patients with ED.

Table 1. Demographic, biochemical and treadmill parameters of the patients

	Study group (n=123)	Control group (n=55)	p
Age (years)	46.83±6.752	40.38±6.072	<0.001
Exercise time (second)	556.00 (61)	575.5 (84)	0.025
Resting HR (bpm)	84.91±12.827	86.10±13.696	0.332
Max HR (bpm)	156.00 (13)	160.50 (11)	0.001
HRR1 (bpm)	26.00 (8.00)	28.00 (13.00)	0.111
HRV2 (bpm)	42.00 (11)	45.00 (18)	0.297
CI	0.77 (0.14)	0.815 (0.15)	0.059
HRR1 (≤18 bpm) (n, %)	29 (23.6)	6 (10.9)	0.040
Max SBP (mmHg)	150.00 (25.00)	150.00(30.00)	0.254
Max DBP (mmHg)	80.00 (10.00)	85.00 (10.00)	0.617
RPP (mmHg*bpm)	23400.00 (5660)	23885.00 (5108)	0.190
TC (mg/dL)	199.77±43.832	195.75±39.125	0.761
TG (mg/dL)	121.00 (100.00)	125.00 (113.00)	0.517
HDL -C(mg/dL)	43.00 (12.0)	42.00 (10.5)	0.143
LDL-C(mg/dL)	125.00 (53.0)	123.55 (56.5)	0.336
Smoking (n, %)	49 (39.8)	32 (58.2)	0.023
Alcohol (n, %)	24 (19.5)	21 (38.2)	0.010
DM (n, %)	7 (5.7)	2 (3.6)	0.552
HT (n, %)	11 (9.1)	4 (7.3)	0.685

CI: Chronotropic index, DBP: Diastolic blood pressure, DM: Diabetes mellitus, HDL-C: High density lipoprotein cholesterol, HR: Heart rate, HRR1: Heart rate recovery 1 minute, HRR2: Heart rate recovery 2 minutes, HT: Hypertensiton, LDL-C: Low density lipoprotein cholesterol, RPP: Rate pressure product, SBP: Systolic blood pressure, TC: Total cholesterol, TG: Triglyceride

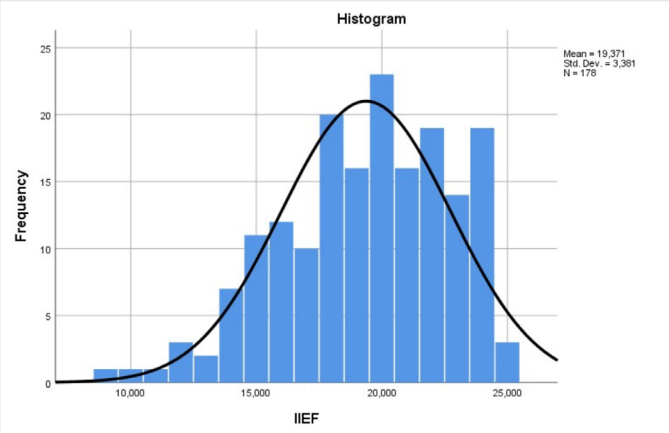

Figure 1. Frequency distribution of IIEF-5 score among subjects.

ED is the most common sexual abnormality in males. Although vascular pathologies are the leading cause of ED, abnormalities of autonomic function are among the well-recognized etiologic factors in its development (13). Thus, there has been increasing interest in evaluating autonomic functions in patients with ED. Because exercise stress testing could provide sufficient information regarding dynamic changes in autonomic function, investigation of exercise stress test parameters in males with ED has attracted attention of many researchers.

Hemodynamic changes during exercise mainly reflects the complex interaction between the sympathetic and parasympathetic nervous systems. Increase in HR during exercise is mainly related to the stimulation of adrenoceptors by catecholamines (14). Immediately after exercise with the activation of baroreflex arch and other mechanisms, parasympathetic activation and sympathetic withdrawal occur and the HR gradually slows (8). Many studies have shown the prognostic value of exercise test indices in various diseases, including coronary artery disease, hypertension, and myocardial infarction (15, 16).

Dogru et al.(17) showed that males with ED had an attenuated HR response to exercise and had an abnormal decline in HR during postexercise period. In their study, the HRR1 values of subjects with and without ED were 14.3±8.2 bpm and 20.8±8.4, respectively. Cardiac rehabilitation in patients with ischemic heart disease has been reported to result in significant improvement in erectile quality and HRR, indicating enhanced autonomic balance in this group (18). Ulucan et al. (19) investigated HRR indices of 90 patients with ED and 50 healthy subjects and found that patients with ED had lower values of HRR1, HRR2, and HRR3 and decreased effort capacity than their healthy counterparts. The HRR1 values of the two groups were 34.8±11.2 bpm and 41.7±15.5 bpm, respectively ($p<0.001$). HRR1 and HRR3 were independent predictors of ED. Abnormal values of HRR may differ according to the protocol used during treadmill test which can be stopped abruptly (no cool down period) or gradually with a predetermined speed and slope. In the former case ≤18 bpm is considered abnormal, and in the latter case ≤12 bpm is considered as abnormal (11,12). When we carefully looked at the results of the study by Ulucan et al. (19), 95% of the values fell into normal range. Kucukdurmaz et al. (20) compared exercise duration, CI, and first, second, and third minute HRR values in patients with or without ED. In their study, total exercise duration was statistically significantly shorter and HRR indices were lower in patients with ED. Specifically, HRR1 values of the study and control groups were 30.6±11.9 and 36.9±9.9, respectively ($p=0.01$). They also evaluated the number of patients who had abnormal HRR1 values. The percentage of patients with HRR1 below 18 bpm were 12% and 3% in the study and control groups, respectively ($p=0.03$). Ioakeimidis et al. (21) studied endothelial function and exercise test parameters in patients with ED. Exercise capacity and CI were found to be decreased in patients with ED whereas they could not find any differences with respect to HRR1 and HRR2 indices after exercise. When they divided patients with ED into three groups according to their IIEF-5 score, severity of ED was correlated with exercise time, HRR2 and CI. On post-hoc analysis, patients with severe ED had statistically significantly lower CI and HRR2 values compared to patients with mild and moderate disease.

Table 2. Correlation analysis of IIEF-5 score

	R value	P value
Age	-0.54	**<0.001**
TC	-0.32	**<0.001**
LDL -C	-0.34	**<0.001**
HDL-C	0.14	0.06
TG	-0.17	**0.02**
Exercise time	0.19	**0.01**
Resting HR	0.05	0.49
Max HR	0.24	**0.001**
HRR1	0.12	0.09
HRR2	0.08	0.29
CI	-0.11	0.14
Max SBP	-0.11	0.11
Max DBP	-0.02	0.79
RPP	-0.02	0.71

CI: Chronotropic index, DBP: Diastolic blood pressure, DM: Diabetes mellitus, HDL-C: High density lipoprotein cholesterol, HR: Heart rate, HRR1: Heart rate recovery 1 minute, HRR2: Heart rate recovery 2 minutes, HT: Hypertensiton, LDL-C: Low density lipoprotein cholesterol, RPP: Rate pressure product, SBP: Systolic blood pressure, TC: Total cholesterol, TG: Triglyceride

In our study, although exercise time was longer and maximal HR was higher in control group, with age included as a covariate, the differences became statistically insignificant. This also explained the similar values of CI in two groups. Hence, differences in exercise time and peak HR between groups were attributed to younger age and better physical performance of the subjects in control group. Although we did not find any differences in HRR indices, further analysis showed that prevalence of abnormal HRR1 values was higher in study group than that of control group. It has been reported that exercise-induced increase in HR is primarily mediated by sympathetic activity whereas post-exercise 1 minute decrease in HR depends on parasympathetic activation (22,23). Our results were in accordance with previous

data, suggesting that parasympathetic system had an important role in the pathogenesis of ED or impotence.

Study Limitations

Limitations of our study were as follows: (1) the sample size was small; (2) it was a single center study; (3) number of patients who had severe-moderate ED was relatively low (1.7 %); and (4) testosterone levels of the subjects were not measured.

Conclusion

Abnormalities of autonomic system activity is more prevalent in patients with ED. Since, exercise treadmill testing is a useful tool for the assessment of autonomic nervous system activity, it can be used to assess autonomic activity in that group of patients.

Ethics

Ethics Committee Approval: The study was approved by local ethical committee and informed consents were obtained from all participants.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: E.O., A.S.E., F.N.T.Ç., İ.F.A., Design: E.O., A.S.E., F.N.T.Ç., İ.F.A., Data Collection or Processing: E.O., C.Y., D.K., Analysis or Interpretation: C.Y., D.K., A.S.E., F.N.T.Ç., Literature Search: E.O., C.Y., İ.F.A., Writing: C.Y.

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Original Article

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Assessment of Calcaneal Morphology and Radiological Parameters in Haglund's Syndrome

Haglund Sendromunda Kalkaneus Morfolojisi ile Radyolojik Ölçümler Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Objective: To compare the radiological parameters of the normal population with the patients with Haglund's syndrome (HS) and to seek a correlation between pain scores and radiological parameters in patients with HS.

Methods: Thirty-one patients surgically treated for HS (Haglund group) and 36 healthy subjects free of hindfoot pathology seen in consultation for any other foot pathologies (control group) were included in the study. Demographic data of the patients were acquired, and radiological measurements such as; Fowler Philip Angle (FPA), lateral talus-first metatarsal angle (LTFMA), and Calcaneal pitch angle (CPA) were recorded. The visual analog scale (VAS) and the American Orthopaedic Foot & Ankle Society (AOFAS) scores of the patients in the Haglund group were assessed.

Results: Mean CPA, FPA, and LTFMA in the Haglund group were 23.88±4.6, 58.16±5.7, and 4.92±1.88, respectively, and the same recordings in the control group were 21.16±4.81, 59.1±4.3, and 4.25±2.57, respectively. CPA differences between Haglund and control groups were statistically significant (p=0.021). The average values of the VAS and AOFAS scores of the patients in the Haglund group were 8.45±1.06 and 47.4±7.58, respectively. There was no correlation between the scores and the radiological measurements of

ÖZ

Amaç: Bu araştırmadaki amacımız normal popülasyonun radyolojik ölçümlerini, Haglund sendromlu (HS) hastalarla karşılaştırmak ve HS hastalarda ölçümsel değişimlerin ağrı ve fonksiyonel skorlar üzerindeki etkisini incelemektir.

Yöntemler: HS nedeniyle cerrahi tedavi uygulanan 31 hasta (Haglund grubu) ve diğer ayak patolojileri için konsültasyonda görülen arka ayak patolojisi olmayan 36 sağlıklı birey (kontrol grubu) çalışmaya dahil edildi. Hastaların demografik verileri sorgulandı ve radyolojik ölçümler olarak; Fowler Philip açısı (FPA), lateral talus-birinci metatarsal açı (LTFMA) ve kalkaneal eğim açısı (CPA) ölçümleri yapıldı. Haglund grubundaki hastaların ayrıca görsel analog ölçeği (GAÖ) ve Amerikan Ortopedik Ayak ve Ayak Bileği Derneği (AOFAS) skorları değerlendirildi.

Bulgular: Haglund grubunda ortalama CPA, FPA ve LTFMA sırasıyla 23,88±4,6, 58,16±5,7 ve 4,92±1,88, kontrol grubunda ise sırasıyla 21,16±4,81, 59,1±4,3 ve 4,25±2,57 olarak bulundu. Haglund ve kontrol grupları arasındaki CPA farklılıkları istatistiksel olarak anlamlıydı (p=0,021). Haglund grubundaki hastaların GAÖ ve AOFAS skorlarının ortalama değerleri sırasıyla 8,45±1,06 ve 47,4±7,58 idi. Haglund grubundaki hastaların skorları ve radyolojik ölçümleri arasında anlamlı korelasyon bulunamadı.

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patients in the Haglund group.

Conclusion: Radiological assessments are widely debated. Many studies support our results that measurements defined for the morphology of calcaneus are not associated with the Haglund's disease severity. Increase in the CPA is found to be related to HS. This may be explained by the extension of the calcaneus to irritate the Achilles tendon and cause heel pain.

Keywords: Calcaneal pitch angle, Fowler Philip Angle, Haglund's syndrome, lateral talus-first metatarsal angle

Sonuç: Radyolojik değerlendirmelerin Haglund sendromu üzerindeki etkisi halen tartışılmaktadır. Pek çok çalışma, kalkaneus morfolojisi için tanımlanan ölçümlerin Haglund hastalığı ile ilişkisi olmadığı yönündeki sonuçlarımızı destekler niteliktedir. CPA'daki artışın HS ile ilişkili olduğu bulunmuştur. Bu, Kalkaneus'un Aşil tendonunu direkt yaralayacak şekilde ekstansiyona gelmesi ve topuk ağrısına neden olmasıyla açıklanabilir.

Anahtar Sözcüker: Kalkaneal eğim açısı, Fowler Philip açısı, Haglund sendromu, lateral talus-birinci metatarsal açı

Introduction

Heel pain is a clinical condition that is quite common in almost every decade of life. Due to various differential diagnosis, correct treatment is hard to decide. Haglund's syndrome (HS) is the most common reason of heel pain. It was first described in 1927 as retrocalcaneal bursitis caused by posterior calcaneus's superior bony prominence (1). Over time, painful heel conditions such as; retrocalcaneal bursitis, supracalcaneal bursitis, and Achilles tendinitis were also defined as HS. Although the cause is unknown; etiological factors, including wearing tight shoes and repetitive trauma, are blamed. To understand and predict HS, several different measurement techniques have been reported in the literature, unfortunately none of these methods have been consistent or reliable to be associated with Haglund's deformities (2).

This retrospective study aims to compare the radiological parameters of the normal population with the patients diagnosed as having HS to measure the reliability of the radiological parameters in the diagnosis of Haglund's disease. We hypothesized that increased abnormality of the radiological parameters would associate with more pain and decreased functionality in patients with HS.

Methods

Patients

After acquiring ethical committee approval, 31 patients treated with the diagnosis of HS over a 5-year period between January 2014 and September 2019, and 36 healthy subjects without hindfoot pathologies who were seen at the consultation for other foot pathologies, were included in the study. Informed consent was signed by all of the included patients. The Haglund group consisted of 17 female and 14 male patients, with a mean age of 47.61 years (range, 31-68 years), with 16 right and 15 left feet. Inclusion criteria comprised calcaneal insertion pain on palpation of the posterior tuberosity and distal calcaneal tendon insertion, calcaneal hump, irritation of adjacent skin by shoe wear back-strip, painful pre or retro-calcaneal bursitis, and radiologic enthesophytes. The control group consisted of 24 female and 12 male patients, with a mean age of 44.7 years (range, 18-68 years), with 17 right and 19 left feet. These patients had no posterior heel pain and hindfoot pathology or radiographic enthesophytes in the calcaneal insertion region. The exclusion criteria were as follows; history of foot surgery, foot implant, amputation of part of the foot, fractures, injury requiring cast or surgery, calcaneal tendon body tendinopathy, posterior impingement, osteochondral defect, neuromuscular pathology, diagnosed as having muscle or skeletal abnormalities, inflammatory rheumatism, osteoarthritis, congenital foot deformity, paralysis or current pregnancy. The exclusion criteria were the same in both groups. Visual analog scale (VAS) and AOFAS scores were recorded only for patients in the Haglund group.

The study was approved by the local ethics committee (date 16/07/2020 and decision number 2020-10) and was conducted in line with the principles of the Declaration of Helsinki.

Imaging

A standard lateral radiograph of the weight-bearing foot, also including ankle, was taken. All radiographs were available digitally in our database. The software available in our system was radiographically able to measure angles with a precision of two decimal points. The following angles were recorded from the lateral radiographs: Fowler Philip angle (FPA), lateral talus-first metatarsal angle (LTFMA, Meary's angle), and calcaneal pitch angle (CPA).

The FPA is subtended by a tangent to the posterior edge of the greater tuberosity of the calcaneus and a line between the lowest weight-bearing point of the posteromedial tuberosity and the end of the calcaneocuboid joint line (Figure 1). Normal values range between 44° and 69°, and the values >75° are considered pathological (3). CPA is the angle determined by the intersection of the baseline tangent to the anterior tubercle and the medial tuberosity with the horizontal surface (Figure 2). The normal

Figure 1. The fowler philip angle

range is 17-32 (4). LTFMA is the angle created between the bisection of the first metatarsal and a line perpendicular to a line connecting the anterodorsal and anterior-plantar extremes of the talar head (Figure 3). The positive value indicates the talus is plantarflexed according to the first metatarsal. The negative value indicates pes planus (5).

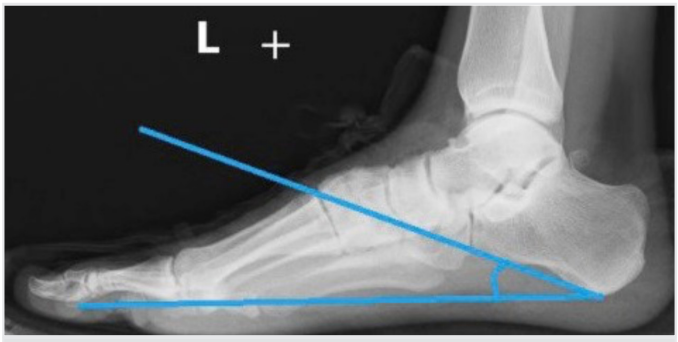

Figure 2. The calcaneal pitch angle

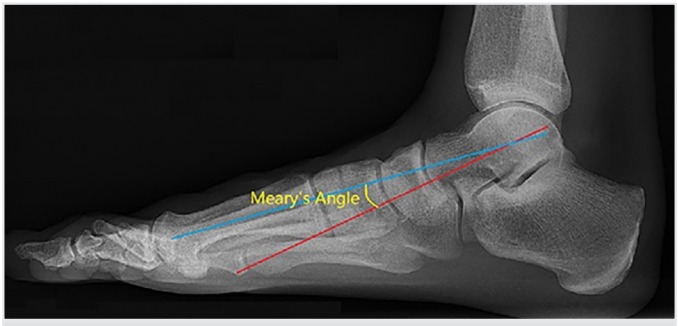

Figure 3. The lateral talus-first metatarsal angle

To determine interobserver reliability, radiologic measurements were made by 2 independent observers (orthopedic surgeons). To assess intra-observer reliability, measurements were made twice by each observer at a minimum 14-days interval. Measurements were made independently by the two observers, blind to the patient's group, known only to the principal investigator.

Statistical Analysis

The data obtained in this study were analyzed with IBM Statistical Package for Social Sciences (SPSS) Statistics 26 software (SPSS Inc., Chicago, IL, USA). The normality of the distribution for the variables was tested with the Kolmogrov-Smirnov test. The comparison of the means' values from two variables with normal distribution was performed with Independent Samples t-test. To evaluate intra-and inter-observer reliability, Pearson correlation analysis was used according to the normality test results. In the evaluation of the correlation coefficient, r:=0-0.24 was considered as poor, r=0.25-0.49 as moderate, r=0.50-0.74 as strong, and r=0.75-1.0 as very strong. Quantitative data were expressed as mean, standard deviation, minimum and maximum values. The confidence interval was 95%, a p-value less than 0.05 was considered statistically significant.

Results

Mean CPA, FPA, and LTFMA in the Haglund group were 23.88±4.6, 58.16±5.7, and 4.92±1.88, respectively, and the same recordings in the control group were 21.16±4.81, 59.1±4.3, and 4.25±2.57 respectively. There was no significant difference between the Haglund and control groups in terms of FPA and LTFMA (p=0.47, p=0.22). However, CPA was found significantly higher in the Haglund group (p=0.021). All the results are summarized in Table 1.

Table 1. Range, average, median, SD, and p value of control and Haglund groups

Measurements	Haglund group				Control group				p-value
	Min	Max	Mean	SD	Min	Max	Mean	SD	
FPA	49.7	70.3	58.16	5.7	50.7	67.1	59.1	4.3	0.47
CPA	15.3	32.1	23.88	4.6	11.7	32.4	21.16	4.81	0.021
LTFMA	1.3	9.1	4.92	1.9	-3.5	9.9	4.25	2.56	0.22

FPA: Fowler Philip angle, LTFMA: Lateral talus-first metatarsal angle, CPA: Calcaneal pitch angle, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2. FPA, CPA, and LTFMA measurements stratified by gender and group

Measurements		Haglund group		Control group	
FPA		Male	Female	Male	Female
	Means	57.7±6.27	58.6±5.34	59±4.74	59.13±4.16
	p-value	0.525		0.987	
CPA		Male	Female	Male	Female
	Means	25.41±4.2	22.62±4.7	21.62±5.4	21±4.6
	p-value	0.074		0.854	
LTFMA		Male	Female	Male	Female
	Means	5.37±1.33	4.56±2.21	3.68±3.43	4.53±2.03
	p-value	0.218		0.906	

FPA: Fowler Philip angle, LTFMA: Lateral talus-first metatarsal angle, CPA: Calcaneal Pitch angle

Table 3. Haglund group's means of VAS and AOFAS scores

VAS Score	Male	Female	Total
Means (std)	8.36±0.84	8.53±1.23	8.45±1.06
Min-max	7-10	6-10	6-10
AOFAS Score	-	-	-
Means (std)	48.21±5.32	46.71±9.15	47.4±7.58
Min-max	37-54	32-63	32-63

VAS: Visual analog scale, AOFAS: American Orthopaedic Foot & Ankle Society, std: Standard, Min: Minimum, max: Maximum

Gender and age showed no correlation with the radiological parameters (CPA, FPA, and LTFMA) in both groups (Table 2).

The average values of the VAS and AOFAS scores of the patients in the Haglund group were 8.45±1.06 and 47.4±7.58, respectively. Statistical evaluation of the patients' VAS and AOFAS scores in Haglund group showed no correlation with CPA, FPA, and LTFMA measurements. Also, there was no significant difference in terms of the AOFAS and VAS scores of the patients in the Haglund group between genders (p=0.393 and p=0.509, respectively) (Table 3).

Discussion

HS is one of the many causes of chronic posterior heel pain and is usually characterized by a posterosuperior bony prominence of the calcaneus, which is also defined as Haglund's deformity (6). There are various radiological parameters used to measure the bursal projection of calcaneus in the literature, such as; Fowler Philip angle, Angle of Steffensen and Evensen, and the parallel pitch lines (7). However, the relationship between any of these measurement techniques and symptomatic posterior heel pain is unclear. FPA angle (> 75 degrees), with a "prow-like" projection of the posterosuperior prominence of the calcaneus and painful swelling of the soft tissues surrounding the Achilles tendon insertion, were found to be related (3). However, it was also reported that the CPA or vertical inclination of the calcaneus might be more theoretically relevant than FPA in the HS (8).

Lu et al. (9) and Fiamengo et al. (10) found that the radiographic measurement techniques associated with heel pain were inconsistent and not reliable enough to assist in the decision making for surgery. Heneghan and Pavlov (11) also observed 100% false-negative results using FPA. Ruch (12) and, Fugslang, and Torup (13) observed 86 to 88% false-negative results while using FPA. Also, Fugslang and Torup (13) observed wide range of FPA variations in the non-Haglund group. Similarly, in other studies, FPA measurement below 75 degrees was detected at rates ranging from 86% to 100%, and high false-negative ratios were reported (9,11-13).

Our study analyzed and compared the radiographic measurements of LTFMA, CPA, and FPA of patients with HS with the normal population. FPA, LTFMA, and CPA are considered to be indicators of foot shape (14). We hypothesized that the presence of high arch or cavus foot will irritate Achilles tendon due to the positioning of the calcaneus and exacerbate HS. According to our hypothesis, CPA and FPA will be used to detect calcaneal positioning and LTFMA will be used for detecting cavus foot. An increase in CPA and FPA would cause the calcaneus' posterosuperior tubercle to become more prominent and thus, the Achilles tendon and retro-calcaneal bursa would be more irritated. According to our current knowledge, no previous study had reported comparison of both CPA, FPA and LTFMA in the normal population with the patients with HS .

In our study, there was no significant difference between the Haglund group (58.16±5.7) and the control group (59.1±4.3) in terms of FPA (p=0.47). In the Haglund group, there was no patient with FPA more than 75 degrees, and 100% false-negative results were observed using FPA in our study so that our study was compatible with the findings of the studies of Fiamengo et al. (10) and Pavlov et al. (8) in this respect.

Singh et al. (15) concluded that CPA was increased in 57% of the control group and 63% of patients with Haglund's deformity. Bulsara et al. (4) reported that CPA was significantly (p=0.021) higher in the Haglund group (22.14±4.74) than in the control group (20.28±5.12). Similarly, in our study, CPA was higher in the Haglund group (23.69±4.64) than in the control group (21.16±4.81). Although this value was within the normal range for CPA, it was higher than the normal control group, and this difference was statistically significant (p=0.021). Our study is compatible with the findings of Bulstra et al. (4) and Singh et al. (15) in this respect.

The LTFMA > + 4° is an indication of the cavus foot (6). In our study, the average LTFMA of the Haglund group was higher than the patients in the normal population (4.92±1.88 and 4.25±2.57, respectively). Although there is no statistically significant difference (p=0.22), this situation, together with increased CPA, shows that the arch height of the patients' feet in the Haglund group is higher than in the control group, which can be attributed as a predisposing factor for the HS. The LTFMA in the normal population was reported as 4±5.5 in the study of Thomas et al. (16) and 5.5±3.9 in the study of Lamm et al. (17). Ahn et al. (6) reported that the lateral LTFMA and CPA were higher in patients with symptomatic HS. Researchers measured LTFMA as 5.9±5.0 preoperatively, and 6.3±4.7 postoperatively in patients with HS, and they reported that postoperative LTFMA was increased, but this increase was not statistically significant. However, only patients with symptomatic HS were included in this study, and patients in the normal population were not evaluated. Unlike the other studies, radiological parameters were measured in normal and Haglund groups and were compared in our study. Our findings have shown that LTFMA is not suitable for the assessment of the HS because of the difficulty in the measurement of LTFMA as it is in a narrow range, but increased CPA may be an indicator of HS.

Study Limitations

Our study's reliability among observers was 0.902 for FPA, 0.874 for CPA, and 0.662 for LTFMA. The observers' reliability was higher between the observers for FPA and CPA, and this was similar to the previous studies. (4,9,18) Although reliability

within the observer increases with observer experience, we believe that the selected measurements should be easy to teach to acquire higher reliability (18).

Conclusion

Our results showed that LTFMA and CPA were higher in patients with symptomatic HS than in the normal population. This was statistically significant in CPA but not in LTFMA, but there was no correlation between the increment of CPA in regard of pain and functional status (VAS and AOFAS) of the Haglund group. We think that the measurement of CPA is more important in patients with heel pain. Bursal projection in a cavus foot is more prominent due to a more vertical calcaneus, even with a normal FPA. Increasing the vertical angle of the calcaneus increases the dorsal prominence of the bone and creates an important pathogenic factor. This situation can lead to an inflammatory process caused by relatively increased traction in the tendon. In other words, a dorsiflexed calcaneus may cause heel pain and HS.

The study's retrospective nature, the relatively small number of patients involved and unavailability of MRI were limitations of the study. Studies with a larger number of patients using more adequate radiographic tools such as 3D CT scans may give more insight into the increase in the vertical angle of the calcaneus, the relationship between HS and cavus foot deformity, and the pathological formation in the calcaneus. Also, MRI could be useful to exclude other pathologies that might cause heel pain to homogenize the population.

In conclusion, in everyday practice of an orthopedic foot and ankle surgeon, Haglund's deformity is an important part of heel pain complaints; although the posterolateral calcaneus's swelling is seen on physical examination, it is not possible to distinguish the orientation of the calcaneus. In our study, HS was found to be associated with a higher CPA, but the pain intensity and functionality were not correlated with the increment of CPA. Although the clinical symptoms are the main indicators of the HS, radiological measurements especially showing the cavus deformity should be taken into account as an auxiliary tool to start the medication or decide on surgery.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (decision number: 2020-10 and date:16/07/2020) and was conducted in line with the principles of the Declaration of Helsinki.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: K.B., B.K., G.A., M.U.M., Concept: K.B., L.A., B.K., G.A., M.U.M., Design: B.K., O.Y., G.A., M.U.M., Data Collection or Processing: K.B., L.A., B.K., O.Y., G.A., Analysis or Interpretation: L.A., O.Y., Literature Search: K.B., L.A., B.K., Writing: K.B., L.A., B.K., M.U.M.

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Original Article

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Turkish Adaptation of the Scale of the Attitudes to Patient Safety: Nursing Student Sample

Hasta Güvenliğine Yönelik Tutum Ölçeğinin Türkçe Uyarlaması: Hemşirelik Öğrencisi Örneği

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ABSTRACT

Objective: Medical errors are very important problems in all health institutions around the world. Creating a patient safety culture is very important in reducing medical errors. Nurses have a special importance in terms of maintaining patient safety. Knowledge, skills and attitudes that can help improve patient safety and reduce medical errors can be gained through nursing education. There is no valid and reliable tool to measure nursing students' attitudes towards patient safety culture. The aim of the research was to investigate whether the Turkish version of the patient safety attitude scale was a valid and reliable tool for nursing students.

Methods: The study, which was designed methodologically, was carried out between February and April 2019. It was conducted with third- and fourth-year students (n=226) in the nursing department of a private university in Istanbul after obtaining ethical approval and institutional permissions.

Results: According to the results of the analysis, it was found that the original scale did not generally comply with the factor structure. The scale was composed of 22 items and 4 factors. The total variance of the scale was 65.14%. As a result of the reliability analysis, Cronbach's alpha coefficient of the scale was calculated as 0.91.

Conclusion: The scale that was developed by Carruthers et al. in 2009, was found to be a valid and reliable measurement tool that could be used to measure the attitude of nursing students towards patient safety in Turkey.

Keywords: Patient safety, nursing, student, validity and reliability

ÖZ

Amaç: Tıbbi hatalar, dünyadaki tüm sağlık kuruluşlarında çok önemli bir sorundur. Hasta güvenliği kültürü oluşturmak tıbbi hataların azaltılmasında çok önemlidir. Hemşireler hasta güvenliğini sağlamada özel bir öneme sahiptir. Hemşirelik eğitimi ile hasta güvenliğini artırmaya ve tıbbi hataları azaltmaya yardımcı olabilecek bilgi, beceri ve tutumlar kazanılabilir. Hemşirelik öğrencilerinin hasta güvenliği kültürüne yönelik tutumunu ölçmek için geçerli ve güvenilir bir araç yoktur. Araştırmanın amacı, hemşirelik öğrencileri için hasta güvenliği tutum ölçeğinin Türkçe versiyonunun geçerli ve güvenilir bir araç olup olmadığını araştırmaktır.

Yöntemler: Metodolojik olarak tasarlanan çalışma, Şubat-Nisan 2019 tarihleri arasında gerçekleştirilmiştir. Araştırma, İstanbul'da bir özel üniversitenin hemşirelik bölümünde okuyan üçüncü ve dördüncü sınıf öğrencileri (n=226) ile etik onay ve kurum izni alınarak yürütülmüştür.

Bulgular: Analiz sonuçlarına göre orijinal ölçeğin genel olarak faktör yapısına uymadığı görülmüştür. Ölçek, 22 madde ve 4 faktörden oluşmaktadır. Ölçeğin toplam varyansı %65,14'tür. Güvenilirlik analizi sonucunda ölçeğin Cronbach alfa katsayısı 0,91 olarak hesaplanmıştır.

Sonuç: İki bin dokuz yılında Carruthers ve ark. tarafından geliştirilen ölçeğin, Türkiye'deki hemşirelik öğrencilerinin hasta güvenliğine yönelik tutumlarını ölçmek için kullanılabilecek geçerli ve güvenilir bir ölçme aracı olduğu bulunmuştur.

Anahtar Sözcükler: Hasta güvenliği, hemşirelik, öğrenci, geçerlilik ve güvenilirlik

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Introduction

Medical errors are a very important problem in all health institutions around the world. For this reason, studies to ensure patient safety and to improve the culture of safety in institutions are among the issues that are primarily handled by the managers (1,2). The fact that healthcare professionals have information about professional education and patient safety is a major factor in creating a safety culture (3,4). Nurses are the largest group in healthcare system. As the largest health care workforce, nurses interact more with patients and their relatives because they spend longer working hours in institutions than other healthcare professionals. Therefore, they play a critical and essential role in ensuring patient safety (5). The World Health Organization (WHO) published the "WHO patient safety curriculum guide for medical schools" in 2009. In this guide, it was emphasized that patient safety and quality improvement should be in the curriculum during undergraduate medical education (2-6). In 2011, it was expanded to include nurses, midwives and other health professionals (2).

Patient safety education is required not only for healthcare professionals working in institutions, but also for undergraduate students in healthcare (7). Health institutions strongly emphasize educations on how to avoid medical errors for healthcare professionals who will graduate in order to develop a patient safety culture and what to pay attention to minimize these errors (8-10). Prevention of adverse events and increasing patient safety in health care services should be among the key goals of nursing education. Knowledge, skills and attitudes that can help improve patient safety and reduce medical errors can be gained through education (11,12). Awareness of patient safety to be gained in nursing education contribute to the development of the quality of nursing care and patient safety (13). The knowledge and competencies of nurses on patient safety can be increased and improved with in-service training programs in health institutions. However, the study by Steven et al. (14) emphasized that patient safety education should be included in the curriculum in order to increase the competence of nurses in patient safety. The training to be given in undergraduate nursing programs is the first step towards improving patient safety and service quality in clinical trainig (15). Nursing students should be properly educated about patient safety with theoretical lectures, clinical practice experiences, laboratory and simulation applications, and their competencies should be improved (16,17). In addition, it is important to evaluate and develop trainings to standardize students' competencies in patient safety (15-18). Therefore, the result of the training given should be evaluated with a valid and reliable tool. Evidence is limited in healthcare professional curricula about how patient safety is addressed, and how safe practitioners develop in schools (19).

In our country, there is no standard and compulsory course on patient safety in nursing undergraduate education curriculum. In addition, nursing students do not have a valid and reliable scale to measure the attitude towards the patient safety culture. For this reason, this study was conducted to adapt the internationally accepted scale (20), which measured the attitude of medical students to the patient safety, to Turkish in nursing students.

Methods

Type of the Study

The aim of this methodological study was to test the validity and reliability of the Turkish version of the Attitudes to Patient Safety scale with nursing students.

Sample and Setting

The study was carried out between February and April 2019, in a private nursing school in Istanbul, which offered four years of undergraduate education. The school accepts students from all parts of the country, and its graduates work in public or private hospitals, responsible for health care. The data were collected by reaching the students between lectures after obtaining permission from the school administration. After the students were informed about the subject and purpose of the research, the data collection tool was given in the envelope and it was taken back after it was filled. The subject of patient safety is included in various sections of the curriculum such as medical-surgical nursing. However, the education curriculum does not include a standard and compulsory course on patient safety.

The number of third- and fourth-year students was 350. In order to determine the size of the sample, we used the criteria that accepted the number of participants ranging from 5 to 20 times the number of items in the scale when testing psychometric properties (21). The scale consisted of 26 items, the required sample size was at least 130 participants. Therefore, the questionnaires were distributed to 300 students (50 students were absent or did not agree to participate). The sample consisted of 226 students who agreed to participate in the research, filled the forms correctly and provided the appropriate data. The response rate was 75.3%.

Instrument

In the study, a data collection tool consisting of two parts was used. In the first part, there was the "student personal information form" (age, class, gender, high school graduated) and in the second part, "The Attitudes to Patient Safety questionnaire" (APSQ) which was developed by Carruthers et al. (20) was used. This scale was translated into Turkish and validity and reliability were tested in this study.

The scale included 9 factors consisting of 26 items designed to measure medical students' attitudes towards patient safety culture.

The scale is a 7-point Likert-type scale including options from "1: absolutely disagree" to "7: absolutely agree". Since 6 items (11,14,15,16,17,25) were negative, these items were re-coded in the opposite direction. The maximum score that can be obtained from the scale is 154 and the minimum score is 26. High scores from the scale show a positive attitude to patient safety. It was stated that the internal consistency coefficient of the original scale was 0.73, and cronbach alpha values of the subscales were

as follows; 0.82 (3 items) for "patient safety training received", 0.77 (3 items) for "error reporting confidence", 0.71 (3 items) for "working hours as error cause", 0.63 (3 items) for "error inevitability", 0.68 (4 items) for "professional incompetence as error cause", 0.68 (3 items) for "disclosure responsibility", 0.69 (2 items) for "team functioning", 0.68 (2 items) for "patient's role in error", and 0.66 (3 items) for "importance of patient safety in the curriculum" (20). In Turkey, in the validity and reliability study done with medical students, the Cronbach alpha value was found as 0.79 for the total scale, but its subscales were between 0.66-0.85 (22). In this study, Cronbach's alpha value for the total scale was 0.91, the Cronbach's alpha values of the sub-scales were found to be between 0.76 and 0.91.

Statistical Analysis

Statistical analyzes were made in IBM SPSS Statistics 21.0 program. Descriptive statistics (frequency, percentage and average) were used to define students' sociodemographic characteristics. In the validity of the study, exploratory factor analysis (EFA) and confirmatory factor analyses (CFA) were used. In the reliability study of the scale, Cronbach's alpha coefficient for determining internal consistency was calculated.

Procedure

Adaptation Process

During the adaptation of the scale to Turkish, the guidelines of the International Testing Commission and the WHO for cross-cultural adaptation studies and the consensus-based standards for the selection of health status measurement instruments (COSMIN) were followed (23-25).

There were four steps in the adaptation process: "translation, back translation, expert opinion and pilot study" (23,25,26) (Figure 1).

Firstly, the scale was translated from English to Turkish independently by three instructors whose native language was Turkish, and who were well educated in English. Later, after the three translations were combined and evaluated by the researchers, two translators independent of the other three translators translated the Turkish version into English. In the third stage, the original scale and translated version were evaluated by a committee (8 academic and clinical nurses) specializing in content validity. Davis technique was used to calculate the content validity index (CVI) (27). According to this technique, the opinions of the experts were evaluated in four categories from 1 to 4, from "absolutely acceptable" to "incompatible". Then the number of experts who chose (1) and (2) was divided by the total number of experts for each item, as Davis suggested, and the tool's CVI value was calculated as 0.98. Finally, The draft scale which was revised according to expert opinion was applied to 30 nursing students outside the sample group in pilot study. No problems were experienced with students' understanding of scale.

Validation Process

In the confirmation process, CFA and EFA were used (28). The second step involved the internal consistency of the APSQ and its sub-scales (26) (Figure 1).

Ethical Considerations

Before collecting data, permission was taken from Carruthers by e-mail, who developed the scale, to adapt APSQ to Turkish. This study was ethically approved by Gelisim University Ethical Review Committee (no.01/2019). Informed consent forms were obtained from each student who agreed to participate in the study by informing the students about the aim and process of the study. No questionnaire was distributed to students who did not agree to participate. Informed consents were obtained before applying the questionnaires.

Limitations of the Research

While performing reliability analyzes, no test repetition showing the reliability of the scale over time was performed. The fact that the research was conducted in a center and the answers were based on student opinions were the limitations of the study.

Results

The Distribution of Participants' Characteristics

The mean age of students was 21.57±2.16, 77.9% were women and 38.1% were third-year students. It was found that most of

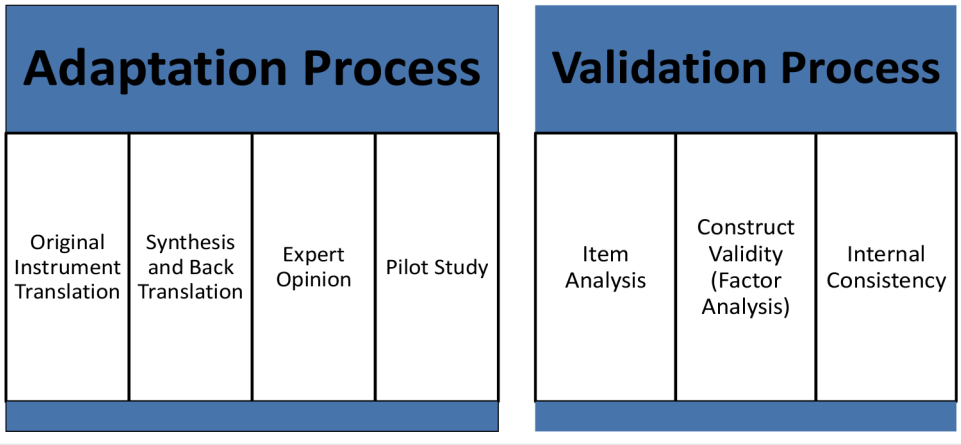

Figure 1. Study process

the students (50.9%) graduated from Anatolian/Science High School.

Validity Analysis of APSQ

Before starting the construct validity analysis of the scale, all items were analyzed and item total correlations were examined. In the scale, the correlation coefficients of 3 items (11,17,18) were between r=0.00 and 0.15, while the item-total correlation coefficients of other items were between r=0.35 and 0.68. After this analysis, it was decided not to include 3 items below 0.30 in the scale, and the number of items was reduced to 23.

To determine the sampling adequacy, KMO test was performed and the result was found to be 0.90. Bartlett's test result was 3,283.78 [degree of freedom (df):231, p=0.00] (Table 1).

Anti-image r-values of the scale were between 0.65-0.96 and eigen value was accepted as 1. When the analysis was made with the Varimax rotation technique, factor loading of the items was more than 0.30 but 14th item was migrated to its alternative factor and thus was removed. When the scale analysis was repeated, it was found that 22 items were collected in 4 sub-scales. These 4 sub-scales explained 65% of the total variance of the scale.

In the scree plot graph of the factors, it was seen that the break point of the curve was in the fourth factor and then the curve progressed at the same level (Figure 2).

When the factor distribution of the items were taken as eigen-value 1, the scale was divided into 4 subscales, and the factor loading was above 0.30 (Table 2).

As a result, the final model consisted of a total of 22 surviving items that were loaded on the four factors, namely, "awareness of medical error causes", "medical error reduction precursors", "importance of patient safety education", and "professional incompetence in medical error".

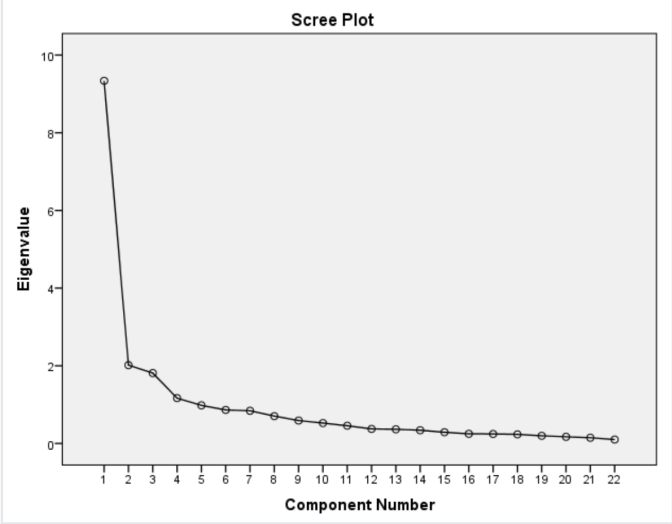

Figure 2. Scree plot graph

Confirmatory Factor Analysis

CFA was performed in order to test how extent the factor structure fit with the data obtained. Factor loading of items was found to be above 0.30 (Figure 3). As a result of this analysis of the model in which 22 items were explained with four factors, chi-square (χ^2) was 205.07; df 120 and root mean square error of approximation (RMSEA) 0.071 (Table 3).

Reliability Study of APSQ

The internal consistency of the scale was examined within the extent of reliability and Cronbach's alpha value was found to be 0.91. When the sub-scales of the scale were analyzed, the Cronbach's alpha values of the sub-scales were found to be between 0.76 and 0.91 (Table 4).

Discussion

Before starting the research, patient safety attitude scales developed for the students studying in nursing, medical and health sciences were examined by scanning in national and

Table 1. KMO and Bartlett''s test values

Kaiser-Meyer-Olkin Sampling Adequacy 0.90		
Bartlett's test of sphericity	Chi-square value	36283.78
	df	231
	p	0.00

df: Degree of freedom

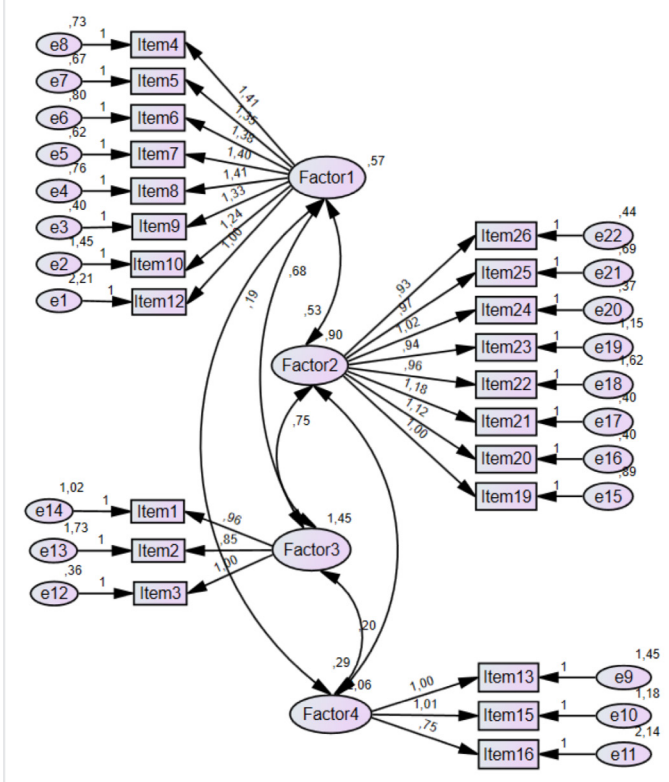

Figure 3. Confirmatory factor analysis results for the adapted version of the Attitudes to Patient Safety scale

Table 2. Factor solution for APSQ

Items	1.	2.	3.	4.
Item 4	0.65			
Item 5	0.74			
Item 6	0.71			
Item 7	0.74			
Item 8	0.72			
Item 9	0.78			
Item 10	0.68			
Item 12	0.49			
Item 19		0.60		
Item 20		0.66		
Item 21		0.69		
Item 22		0.68		
Item 23		0.74		
Item 24		0.79		
Item 25		0.76		
Item 26		0.73		
Item 1			0.79	
Item 2			0.72	
Item 3			0.69	
Item 13				0.83
Item 15				0.85
Item 16				0.73

APSQ: Attitudes to patient safety questionnaire

international literature. As a result, the instrument that was used by nursing students to measure the attitude of patient safety in Turkey was determined not to be valid and reliable instrument. APSQ developed by Carruthers et al. (20) was considered for nursing students in Turkey as appropriate scale in terms of content and fit. The language validity of the scale in the study was done by using the method that WHO recommended in adapting the measurement tools developed in different languages (25). With content validity analysis, the extent to which the measurement tool covered the subjects, and target behaviors or features that were wanted to be measured were tested so content validity

Table 3. CFA of APSQ

Fit index	Obtained value
RMSEA	0.071
NFI	0.81
RMSR	0.076
GFI	0,88
AGFI	0.81
⅄2	205.07
Df	120
⅄2/df	1,709

RMSEA: Root mean square error of approximation, NFI: Normed fit index, RMSR: Root mean square residual, GFI: Goodness of fit index , AGFI: Adjusted goodness of fit index, df: Degree of freedom

analysis was done (29-31). In order to evaluate the Turkish form prepared to test the content validity of APSQ, the opinions of 8 experts who studied on patient safety were received. As a result of the analysis made according to the Davis technique, the content validity criterion of this scale was determined as 0.98. In the literature, it is recommended to make a pilot study in a small group that has similar characteristics with the sample of the scale after language validity (32). In this study, 30 students who were not included in the sample were included in pilot study, so the comprehensibility of the scale items were evaluated and the content validity process was completed by making arrangements in line with the suggestions as a result of the application.

Before the construct validity analysis, the items in the scale were analyzed and the total correlation coefficient values of 26 items were examined. According to Tavşancıl (21), item-total score correlations must be 0.30 and higher in order to measure the features in the best way. Therefore, as a result of the analysis, it was decided to exclude 3 items below r=0.30 from the scale and factor analysis was carried out with 23 items.

In scale adaptation studies, Kaiser Meyer-Olkin (KMO) was performed to evaluate whether the sampling was adequate. KMO results range from 0 to 1. KMO's value must be over 0.50; values above 0.80 couldbe considered as perfect sampling adequacy (21). Bartlett's test is a test that measures whether the correlation

Table 4. Factor analysis and reliability results of APSQ

Factor number	Items	n	Total variance explained	Factor loading	Corrected item total correlation	Cronbach alpha
1	4,5,6,7,8,9,10,12 (4,5,6,7,8,9,10,11)	8	22,936	0.49-0.78	0.38-0.67	0.89
2	19,20,21,22,23,24,25,26 (15,16,17,18,19,20,21,22)	8	21,643	0.60-0.79	0.52-0.70	0.91
3	1,2,3 (1,2,3)	3	11,031	0.69-0.79	0.46-0.62	0.79
4	13,15,16 (12,13,14)	3	9,537	0.73-0.83	0.32-0.35	0.76
Total		22	65,147	0.49-0.83	0.32-0.70	0.91

APSQ: Attitudes to Patient Safety questionnaire
*The last version of the scale is given in parentheses

matrix between the variables in the scale is sufficient for factor analysis. This test is expected to be significant in the analysis (21). In this study, the KMO being 0.90 showed that the sample was sufficient for factor analysis, and the result of Bartlett's test showed that the correlation matrix of variables was suitable for analysis. In the EFA, principal components analysis was preferred, which was one of the most frequently used techniques. During the factor analysis, Varimax rotation technique, one of the most frequently used vertical rotation techniques, was used to provide clarity in independence and interpretation (33). In the first EFA made with 23 items, the factor loading of the items was more than 0.30 but the 14th item was found to be in more than one sub-scale and the values in the factors that were included were less than 0.10. Therefore, 14th item was removed and the analyzes were continued with 22 items. No exact limit has been specified in the literature for factor load values that explain the relationship of items with the factor. However, Büyüköztürk (29) states that factor loading should not be lower than 0.30 and factor loading of 0.45 and above are ideal for selection. When the analysis was repeated with 22 items, it was found that the scale was divided into four factors, explaining 65% of the total variance. If there is a single factor in the scales, it is expected to explain at least 30% of the total variance, while it is expected that this ratio will be higher in structures with multiple factors (33). According to the findings obtained, it can be said that the factor structure is strong since the four factors explain the majority of the total variance.

CFA is one of the two most common methods used in scale development studies to analyze structure validity (34,35). There are different goodness of fit indices used in the evaluation of model suitability, and there are statistical functions of these indices. According to Jöreskog and Sörbom (36), the most used indices were; goodness of fit index (GFI), adjusted goodness of fit index (AGFI), normed fit index (NFI), root mean square residual (RMSR) and RMSEA indices (36). In CFA, it was stated that GFI should be at the desired level. In chi-square fit statistics, the result obtained indicates model-data fit. The chi-square value is divided by the degree of freedom, and if the resulting value is 2 or below, the model is a good model. The value of 5 or less indicates that the model has an acceptable goodness of fit (37,38). In this study, it can be said that the model is a good model with x2/df=1,709. The closer the fit goodness indexes to 1, the model-data fit is good, and the goodness of fit indexes are acceptable to be 0.90-0.95 and values higher than 0.95 indicate a high fit (39). Also, if RMSR is less than 0.10 and RMSEA is equal to or less than 0.08, it shows good fit (37,38). In this study, it was found to be RMSEA=0.071; NFI=0.81; GFI=0.88; AGFI=0.81; SRMR=0.076 and this results showed acceptable fit. Accordingly, the good fit and acceptable fit of the fit measurement, as well as good fit of the corrective chi-square value show that the data have good fit and the 4 factor and 22-item model is statistically significant and valid. Within the content of the reliability study, it is necessary to test the internal consistency of the items in the scale (21,40). The most widely used analysis method in Likert-type scales is the Cronbach alpha coefficient. In the literature is stated about coefficient value, 0.80-1.00 as high reliable, 0.60-0.79 as very reliable, 0.40-0.59 as low reliability, and 0.00-0.39 as unreliable (21,32,41). The Cronbach alpha coefficient of APSQ was found to be 0.91 for the total of the scale. This result shows that the scale items have high internal consistency and high reliability.

Conclusion

In present study, where the validity and reliability of the Turkish version of APSQ in the student nurse sample were examined, it was determined that the 4 sub-scales and the 22-item scale met the validity and reliability criteria at an acceptable level so it was found to be a valid and reliable measurement tool that could be used to measure the attitude of nursing students in our country. It is also suggested that the scale should be tested for validity and reliability before applying it to different cultures, considering that it is affected by intercultural differences.

Ethics

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gelişim University Ethical Review Committees (no.01/2019).

Informed Consent: Informed consent forms were obtained from each student who agreed to participate in the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: F.K., H.A., Design: F.K., H.A., Data Collection or Processing: F.K., Analysis or Interpretation: F.K., H.A., Literature Search: F.K., H.A., Writing: F.K., H.A.,

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

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Original Article

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Antidiabetic and Antioxidant Effect of the Aerial Parts of *Lysimachia verticillaris* and its Isolated Phenolic Compounds on Streptozotocin-induced Diabetic Rats

Lysimachia verticillaris'in Toprak Üstü Kısmının ve İzole Edilmiş Bileşiklerinin Streptozotosin ile İndüklenen Diyabetik Sıçanlarda Antidiyabetik ve Antioksidan Etkisi

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ABSTRACT

Objective: *Lysimachia* genus has been recorded to be used in diabetes, traditionally and to have antidiabetic effect and antioxidant effects. The aim of this study was to evaluate the antidiabetic and antioxidant effects of aqueous extract of *Lysimachia verticillaris* (LV) and its isolated compounds, the percentage of apoptosis and histological changes in pancreatic β-cells in rats with streptozotocin (STZ)-induced type 1 diabetes mellitus.

Methods: Male Spraque Dawley rats were divided into 6 groups. STZ (40 mg/kg) induced diabetic rats were treated orally with aqueous extract (400 mg/kg) and isolated compounds (20 mg/kg). To interpret antidiabetic effect, serum glucose and insulin levels were measured and morphological changes of pancreas were examined. Serum samples were analysed for catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) levels to investigate antioxidant effect. The terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-biotin nick end-labeling (TUNEL) assays were performed to reveal apoptosis of pancreatic β-cells.

ÖZ

Amaç: *Lysimachia* cinsinin geleneksel olarak diyabet için kullanıldığı ve antidiyabetik ve antioksidan etkilere sahip olduğu kaydedilmiştir. Bu çalışmanın amacı, *Lysimachia verticillaris*'in (LV) su ekstresinin ve izole edilmiş bileşiklerinin streptozotosin (STZ) ile tip I diyabet oluşturulan sıçanlardaki antidiyabetik ve antioksidan etkilerini, pankreas β-hücrelerindeki apoptoz yüzdesini ve histolojik değişiklikleri değerlendirmektir.

Yöntemler: Erkek Spraque Dawley cinsi sıçanlar 6 gruba ayrılmıştır. STZ (40 mg/kg) ile indüklenen diyabetik sıçanlara, su ekstresi (400 mg/kg) ve izole bileşikler (20 mg/kg) oral yoldan uygulanmıştır. Deney sonunda antidiyabetik etkiyi yorumlamak için kan glukozu ve serum insülin düzeyleri tespit edilmiştir. Pankreastaki morfolojik değişiklikler incelenmiştir. Serum biyokimyasal parametreleri, katalaz (CAT), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPX) açısından da antioksidan etkiyi araştırmak için analiz edilmiştir. Ayrıca pankreas β-hücrelerinin apoptozunun tespiti için terminal deoksinükleotidil transferaz aracılı dUTP-biyotin çentik uç etiketleme (TUNEL) deneyleri gerçekleştirilmiştir.

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Results: Oral administration of the extract and isolated compounds reduced high blood glucose levels (p<0.005). Morever, the treatments were resulted in increased serum insulin (p≤0.05), CAT (p≤0.05), SOD (p≤0.05) and GPX (p≤0.05). All applications with compounds, especially with quercetin 3-O-β-glucopyranoside reduced the morphological impairment of pancreas. The percentage of TUNEL positive cells was higher (p≤0.05) in the pancreatic islets of untreated diabetic group than others groups.

Conclusion: According to results, the extract and isolated compounds of LV displayed antidiabetic effect, while quercetin 3-O-β-glucopyranoside showed the highest antidiabetic effect. The antidiabetic effects of LV and phenolic compounds may be due to their antioxidant effects. Thus, LV and isolated compounds can be a potential source of herbal medicine for DM. However, it requires further investigations such as toxicological analysis studies and clinical trials.

Keywords: Antidiabetic, antioxidant, apoptosis, *Lysimachia verticillaris*, TUNEL

Bulgular: Ekstre ve izole bileşiklerin oral uygulamasının yüksek kan glikoz seviyelerini düşürdüğü saptanmıştır (p<0,005). Oral uygulamalar, serum insülin (p≤0,05), CAT (p≤0,05), SOD (p≤0,05) ve GPX (p≤0,05) düzeylerinin artışıyla sonuçlanmıştır. Ayrıca, kersetin 3-O-β-glikopiranozit için belirgin olmakla birlikte, uygulamaların pankreasın morfolojik bozukluğunu azalttığı saptanmıştır. TUNEL pozitif hücrelerin yüzdesi, tedavi edilmeyen diyabetik grubun pankreas adacıklarında diğer gruplara göre daha yüksek gözlenmiştir (p≤0,05).

Sonuç: Sonuçlara göre, LV'nin ekstresi ve izole edilmiş bileşikleri antidiyabetik etki gösterirken, kersetin 3-O-β-glikopiranozit en yüksek antidiyabetik etkiyi sergilemiştir. LV ve fenolik bileşiklerin antidiyabetik etkileri, DM'den kaynaklanan hasarlar üzerindeki antioksidan etkilerinden kaynaklanıyor olabilir. Bu nedenle, LV ve izole bileşikleri, diyabet ve komplikasyonları için potansiyel bir bitkisel ilaç kaynağı olabilir. Bununla birlikte, yeni bitkisel ilaçlar oluşturmak, toksikolojik analiz çalışmaları ve klinik araştırmalar gibi daha ileri araştırmalar gerektirir.

Anahtar Sözcükler: Antidiyabetik, antioksidan, apoptoz, *Lysimachia verticillaris*, TUNEL

Introduction

Diabetes mellitus (DM) which is a chronic and metabolic disease characterized by hyperglycemia gives rise to risk of cardiovascular, peripheral vascular and cerebrovascular disorders (1,2). Estimates indicated that 463 million people were living in the world with DM in 2019. The number is expected to increase to 700 million by 2045. DM is a global threat for the future due to health problems and economic burden it causes (3,4).

Oxidative stress in DM has been evidenced to occur due to increase of generation of reactive oxygen species and decrease of antioxidant defenses. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) are biomarkers to detect oxidative stress in DM (5). Deficient of insulin is directly related with the death of insulin-producing β-cells by apoptosis. The terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-biotin nick end-labeling (TUNEL) tecnique is mostly used to detect apoptosis of pancreatic β-cells in DM (6,7).

Successful treatment of DM is not yet uncovered via synthetic drugs, therefore natural products have become more popular for treatment of DM. More than 400 medicinal plants have been proven to have hypoglycemic activity. Investigations of new antidiabetic natural herbal drugs are still remarkable owing to phytochemicals exhibiting potent and safe effects on DM (8).

The genus *Lysimachia* (Primulaceae) consists of 140-200 species worldwide (9) and is represented by 8 taxa in Turkish flora (10). *Lysimachia* species locally known as "karga otu, adi karga otu, altın kamış" in Turkey have been recorded to be used for expectorant, antipyretic, and wound healing purposes as well as against cough and bronchitis in Anatolian folk medicine (11). Also, *Lysimachia* genus has been used for the treatment of diabetes, hepatitis, urinary tract disorders, high blood pressure in the world, traditionally (12-15). *Lysimachia* genus has been reported to contain assorted secondary metabolites including flavonoids, triterpene saponins, steroidal saponins, benzodilactones, and quinones (14-17). Besides, several *Lysimachia* species have been demonstrated to exhibit many desirable biological activities such as anti-inflammatory, hepatoprotective, and vasorelaxant properties, etc. (18-20).

Previous studies showed that *Lysimachia* genus possessed therapeutic potential for the treatment of DM with traditional uses, antidiabetic and antioxidant effects. The methanolic extract of *Lysimachia candida* has proven to reduce insulin resistance in rats fed with high-fat high-fructose diet (21). It was reported that *Lysimachia foenum-graecum* and its isolated compound foenumoside B improved insulin sensitivity and metabolic profiles in ob/ob mice by PPARγ antagonism (22). *Lysimachia paridiformis stenophylla* has been evidenced to ameliorate lipid and carbohydrate metabolism in alloxan-induced diabetic mice due to its antioxidant and a-glucosidase inhibitory properties (23). It was demonstrated that the ethyl acetate fraction of *Lysimachia christinae* showed hypoglycemic effect based on its aldose reductase inhibitor effect (24). Previos study showed that *Lysimachia christinae* exhibited decreasing effect on lipid peroxidation levels and increasing effect on glutathione-*S* transferase (GST), GPX, SOD and CAT levels in alcohol-induced mice (25). *Lysimachia paridiformis* has been revealed to possess hepatoprotective properties in mice with CCl4-induced liver injury owing to its decreasing effect of glutamic-oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and increasing effect of SOD (26). Also, our previous study demonstrated that LV had high antioxidant capacity related with its phenolic compounds isolated (27).

Present study aims to investigate the antidiabetic effects of the aqueous extract of the aerial parts of LV and its phenolic compounds gallic acid, myrcetin 3-O-α-rhamnopyranoside and quercetin 3-O-β-glucopyranoside isolated in our previous

study (27), through detection of the biochemical parameters, the percentage of apoptosis and histological changes in pancreatic β-cells in rats with streptozotocin (STZ)-induced type 1 diabetes mellitus. To the best of our knowledge, it is the first study about antidiabetic properties of LV.

Methods

Drug and Chemicals

Streptozotocin was purchased from Sigma-Aldrich. Kits of insulin (Rat INS (Insulin) ELISA Kit: Catalog No: E-EL-R2466), CAT (Rat CAT (Catalase) ELISA Kit: Catalog No: E-EL-R2456), SOD (Rat SOD1 (Superoxide Dismutase 1, soluble) ELISA Kit: Catalog No: E-EL-R1424), GPX (Rat GPX1 (Glutathione Peroxidase) ELISA Kit: Catalog No: E-EL-R2491) obtanied from Elabscience. Other chemicals used were of analytical quality.

Plant Material and Extraction

The aerial parts of LV were collected from Kafkasör vicinity at altitude of 1.300 m (Artvin province, Turkey). The plant sample was identified by one of us (PhD. Ufuk Özgen). Voucher specimen (AEF 26311) is deposited at the Herbarium of Faculty of Pharmacy, Ankara University (Ankara, Turkey).

Air-dried and finely powdered aerial parts of the plant (100 g) were extracted with water (H_2O, 400 mL x8 h) at room temperature and the same process was repeated three times. The combined extracts were lyophilized to obtain approximately 15 g of the crude residue.

Experimental Animals

Fifty four male Sprague Dawley rats, weighing about 200-270 g obtained from Karadeniz Technical University, Surgical Researching Center. They were allowed to acclimatize for 14 days under standard house conditions of temperature (22±3 °C), humidity (40-65%) and 12- hour light and 12- hour dark cycle. The animals were fed with a standard pellet diet and administered water ad libitum. All experiments were executed in accordance with the ethical norms. The study was approval by the Institutional Ethical Committee of Karadeniz Technical University, Trabzon, Turkey.

Induction of Experimental Diabetes

Diabetes was induced by injecting streptozotocin (STZ) (40 mg/kg body weight) intraperitoneally. STZ was dissolved in freshly prepared 0.1 M sterile sodium citrate buffer (pH 4.5). Control rats were only injected with sodium citrate buffer. Two days after STZ administration, blood glucose level of each animal was measured by using automated glucose sensor machine (AccuCheck Active glucometer). Animals with blood glucose levels more than 200 mg/dL were perceived for the experiment (28).

Experimental Design

In the experiment, the rats were divided into 6 groups each consisting of 9 animals (Table 1).

Aqueus extract of LV and isolated compounds (gallic acid, myrcetin 3-O-α-rhamnopyranosid, quercetin 3-O-β-glucopyranoside) were dissolved in equal volume of saline and administered orally using an intragastric tube daily for 14 days.

At the end of the experiment, blood was collected and serum was separated for the estimation of levels of insulin and other biochemical parameters and pancreas was dissected out after euthanasia of rats by decapitation after anesthesia with ketamine.

Body Weight Analysis

The body weights of animals were measured in first and last days of the experiment.

Biochemical Analysis

The level of blood glucose concentration in mg/dL was evaluated by automated glucose sensor machine (AccuCheck Active glucometer) by collecting the blood from rat tail vein. For the estimation of insulin and other biochemical parameters, the blood was collected and serum was separated by centrifugation. Commercial diagnostic kits were utilized to determine of insulin, CAT, SOD, and GPX levels.

Histopathological Analysis

The pancreatic tissues obtained from all groups were fixed in formaldehyde 10% for 3 days and then dehydrated in 70%, 90%, 96%, 100% alcohol series. All the tissues applied xylene were embedded in paraffin and blocked. The paraffin-blocks were sectioned at thickness of 5 μm using microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany). The tissue sections were deparafinized with xylene and rehydrated in graded series of alcohol. Afterwards, all sections were stained with hematoxylin eosin (H & E). All histopathological analyzes were performed using a light microscope (Olympus BX-51; Olympus Co., Tokyo, Japan) equipped with an integrated camera (Olympus DP 71 Olympus Co., Japan). Injury in the pancreas was scored in terms of vacuole formation, pyknosis of nucleus and irregular pancreatic islet borders. Each criterion was rated from 0 to 3 (0, no damage, 1, mild damage, 2, moderate damage, 3, severe damage). Maximum score was evaluated as 9 (29).

Table 1. Control and diabetic animal groups

Groupings	Treatment
Group I	Normal rats received saline (0.5 mL/kg., p.o.)
Group II	STZ-induced diabetic rats received saline (0.5 mL/kg., p.o.)
Group III	STZ-induced diabetic rats received aqueus extract of LV (400 mg/kg., p.o.)
Group IV	STZ-induced diabetic rats received gallic acid (20 mg/kg., p.o.)
Group V	STZ-induced diabetic rats received myrcetin 3-O-α–rhamnopyranoside (40 mg/kg., p.o.)
Group VI	STZ-induced diabetic rats received quercetin 3-O-β-glucopyranoside (40 mg/kg., p.o.)

Apoptosis Analysis

Apoptosis was evaluated using the TUNEL technique. The technique was performed using In Situ Cell Death Detection Kit POD (catalog no: 11 684 817 910, Roche, Mannheim, Germany) and evaluated by light microscopy. Apoptosis was appraised for 300 islet cells in the four regions selected randomly under a magnification of X400 and values of apoptotis were expressed as percent (30).

Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Compatibility with normal distribution was determined using the Kolmogorov-Smirnov test. Kruskal-Wallis and Mann-Whitney U tests were used to compare differences among the groups. Statistical significance level was considered as $p<0.05$.

Results

Results of Body Weight Analysis

At the end of the experiment, untreated diabetic group (II) showed significiant reduction of the body weight compared to control group (I) and the other groups had similar body weights compared to their initial weights although weight loss was observed (Figure 1).

Results of Biochemical Analysis

Oral administration of the extract and isolated compounds reduced high blood glucose levels ($p<0.005$) (Figure 2). Morever, the treatments resulted in increased serum insülin level compared to untreated diabetic groups ($p\leq0.05$) (Table 2). The highest hypoglycemic effect and healing effect were observed in quercetin 3-O-β-glucopyranoside treated group.

In comparison to the normal control, the untreated diabetic group displayed with considerable reduction of the levels of GPX ($p\leq0.05$), SOD ($p\leq0.05$) and CAT ($p\leq0.05$) (Table 2). The others groupsshowed increase in levels of antioxidant effective enyzmes, GPX ($p\leq0.05$), SOD ($p\leq0.05$) and CAT ($p\leq0.05$) (Table 2). The most significiant increase in the levels of CAT and SOD enyzmes was observed with quercetin 3-O-β-glucopyranoside, but highest level of GPX was detected with myrcetin 3-O-α-rhamnopyranoside.

Results of Histopathological Analysis

Microscopically, it was observed that control group (Group I) had regular langerhans islet margin consisting of normal appearing cells. In untreated diabetic rats (Group II), formation of cytoplasmic vacuoles and pyknosis of nucleus were observed, as well as irregular islets borders. Group III, IV and V were observed to have formation of vacuoles and pyknotic nucleus. Group III, IV, V and VIexhibited irregular langerhans islet margins more less than Group II. Group III, IV, V and VI had improved cell appearance of the islets. The histopathological appearance of most notable group (Group VI) was similar to the control group (Group I). The histological analysis of rat pancreas and scoring data are shown in Figure 3 and Table 3.

Results of Apoptosis Analysis

In the present study, TUNEL technique was used to evaluate apoptosis. The percentage of TUNEL-positive cells reduced in the pancreatic islets of groups compared with the untreated diabetic group (Figures 4, 5).

Discussion

Herbal medicines and their active ingredients have been proven to have antidiabetic effect owing to lots of mechanisms. So, natural products are getting more and more attractive for DM treatment day by day (31). Natural phenolic compounds prevent and treat DM through insulin-dependent or insulin-independent ways. Insulin-dependent effects of phenolic compounds on DM are expressed as activation of insulin signaling, stimulation of secretion of insulin, protection of pancreatic β-cell, reduction of β-cell apoptosis, supporting of β-cell proliferation, and decrease of oxidative stress. Insulin-independent effects of phenolic compounds for DM include inhibition of glucose absorption

Table 2. The values of biochemical parameters of control and diabetic groups

	Insulin (ng/mL)	CAT (pg/mL)	SOD (ng/mL)	GPX (pg/mL)
Group I	13.97±1.60$^{*\#}$	685.33±4.50$^{*\#}$	5.84±0.11$^{*\#}$	498.33 ±13.570$^{*\#}$
Group II	8.93±0.07$^{\$\#}$	428.01±10.39$^{\$\#}$	3.13±0.08$^{\$\#}$	187.33±8.08$^{\$\#}$
Group III	10.66±0.38$^{*\$}$	493.33±6.35$^{*\$}$	3.60±0.21$^{*\$}$	336.66±17.95$^{*\$}$
Group IV	10.86±0.12$^{*\$}$	537.00±9.53$^{*\$}$	5.15±0.14$^{*\$}$	444.01±36.59$^{*\$}$
Group V	11.20±1.02$^{*\$}$	586.01±18.08$^{*\$}$	5.74±0.22$^{*\$}$	531.00±15.52$^{*\$}$
Group VI	13.61±0.73$^{*\$}$	651.23±13.00$^{*\$}$	6.44±0.06$^{*\$}$	528.21±17.32$^{*\$}$

CAT: Catalase, SOD: Superoxide dismutase, GPX: Glutathione peroxidase

Table 3. The values of biochemical parameters of the control and diabetic groups

	Group I	Group II	Group III	Group IV	Group V	Group VI
Injury in pancreas	0.00±0.00	7.20±0.42*	4.31±0.31*	3.09±0.21*	3.30±0.28*	0.62±0.17*

(values are mean ± SD, n=9), *P≤0.05 (compared to diabetic group)

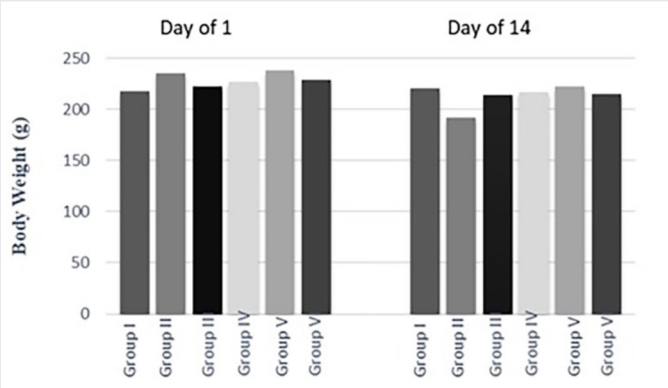

Figure 1. Column graph represents the body weight of the control and diabetic groups

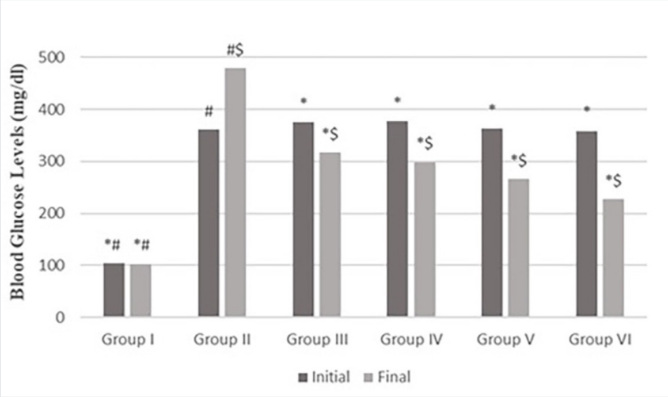

Figure 2. Column graph represents the blood glucose levels of the control and diabetic groups. *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

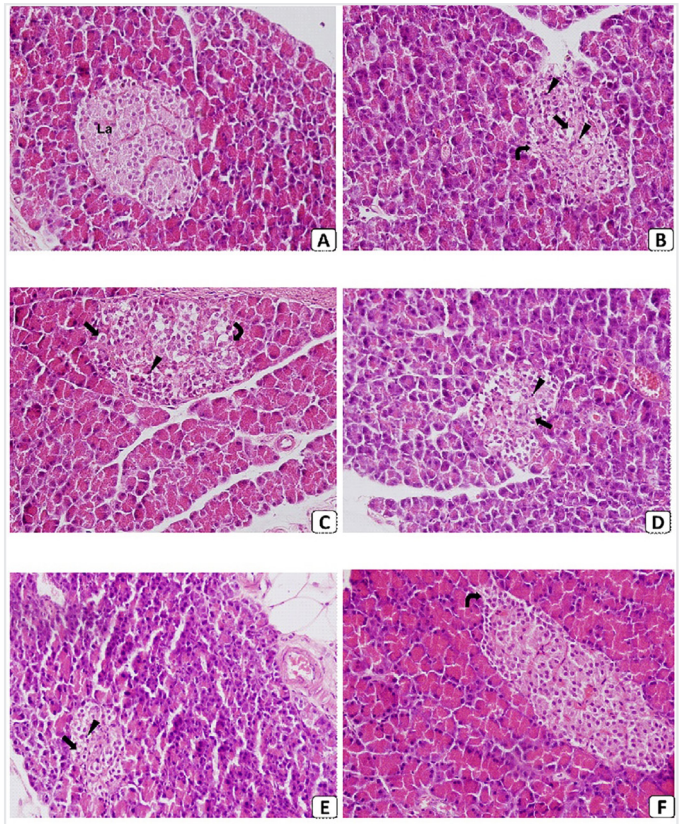

Figure 3. Group I (A), Group II (B), Group III (C), Group IV (D), Group V (E), Group VI (F) Langerhans islet (La), vacuole (arrow), pyknosis of nucleus (arrow head), irregular islet borders (curved arrow) (H & E x400). H & E: Hematoxylin/Eosin

or digestive enzymes, adjustment of intestinal microbiota, and replacement of inflammation response (32).

Gallic acid has been uncovered to have ameliorating effect on hyperglycemia in diabetic rats with STZ-induced pancreatic dysfunction (33). An *in vivo* study of STZ-induced type I diabetes in rats reported that gallic acid exhibited healing effects leading immuno- and thrombo-regulatory responses in DM through the modulation of purinergic signaling pathways (34). Gallic acid, one of the natural phenolic compounds, was used for positive control for the study due to previous literature.

It was reported that administration of myricetin gave rise to reduction of hyperglycemia in STZ-induced diabetic rats. Myricetin has been proven to alleviate high glucose levels and remedy insulin resistance by modulation of β-endorphin generation in fructose-induced insulin-resistant rats (34). Myricetin has been evidenced to protect from tert-butyl hydroperoxide-induced oxidative stress in erythrocytes of patients with type 2 DM. Myricetin has been revealed to have inhibitory effect on aldose reductase and α-amylase (35).

The antidiabetic effect of quercetin arise from the stimulation of glucose uptake related with MAPK insulin-dependent mechanism (36). Quercetin has been found to ameliorate oxidative stress in STZ-induced diaberic rats (37). A further study demonstrated that quercetin displayed beneficial effect in STZ-induced diabetic nephropathy via antioxidative mechanism (38). It was reported that quercetin-3-O-glucoside cured postprandial glycemic control in rats and decreased glucose uptake in Caco-2 cells owing to reduce the expression of glucose transporters (39).

The STZ, a naturally occurring compound, is used to create diabetes in experimental animals. STZ possesses selective cytotoxicity to pancreatic β-cells which adjust blood glucose levels via insulin hormone (40). STZ transported to pancreatic cells by glucose transporter 2 brings about activation of poly adenosine diphosphate ribosylation and nitric oxide release. The STZ causes devastation of pancreatic cells by necrosis and ultimately creates insulin-dependent diabetes (41).

The DM characterized by rising levels of glucose in blood arises from inadequate production and action of insulin (42). STZ induces significant reduction of plasma insulin levels in rats (43). The present study showed that oral administration of the extract and isolated compounds exhibited significantly decreasing effect on blood glucose levels and healing effects of insulin levels. As mentioned above, myrcetin and quercetin have been proven to

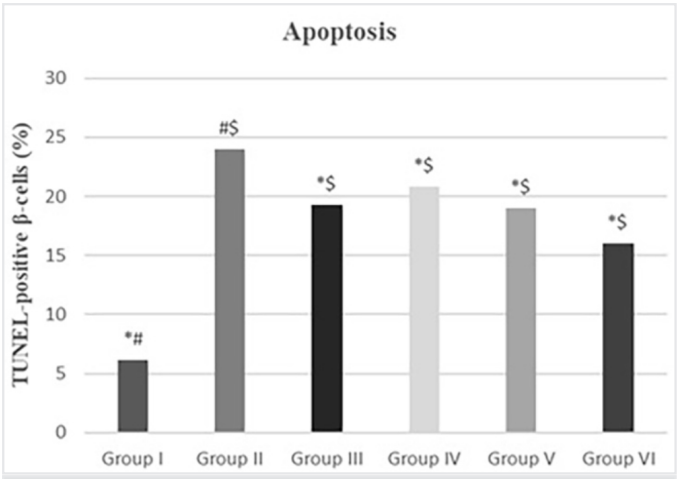

Figure 4. Column graph represents the percentage of TUNEL-positive β-cells in the control and diabetic groups. *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

possess antidiabetic effect via many mechanisms such as MAPK insulin-dependent mechanism, modulation of β-endorphin generation, antioxidant effect and enyzme inhibitions (35-39). The antidiabetic effect of the extract on blood glucose levels may be related to myricetin and quercetin derivative compounds in its content.

The STZ-induced diabetes gives rise to loss of body weight. The significative loss of body weight may be related with increment of muscle wasting and reduction of tissue proteins (44). In present study, untreated diabetic group (II) showed significiant reduction of the body weight compared to control group (I). Significant reduction of body weight may be related to the effects of STZ such as muscle wasting and reduction of tissue proteins. However, the others groups owned body weight similar to initial weight although they lost some weight. This situation indicated that the extract and its isolated compounds improved some toxicological effects of STZ.

In diabetes, oxidative stress is increased by diverse mitochondrial, enyzmatic and non-enyzmatic sources (45,46). The potential reason of oxidative stress includes increase of free radicals and impairment of antioxidant defense mechanism due to reduction in the levels of GPX, SOD and CAT (47,48). GPX metabolizes peroxide to water and oxgen. Alterations of the enyzme in DM induce the cells to prone for oxidative stress and cell injury (49,50). SOD, a primary defender against superoxide on cell injury, is responsible for catalyzing superoxide anion into hydrogen peroxide and molecular oxygen (51). CAT catalyses H_2O_2 into water and oxygen. Deficieny of CAT causes β-cell dysfunction and finally diabetes (42). While the levels of antioxidant effective enzymes (GPX, SOD, CAT) were decreased in untreated diabetic group, the levels of antioxidant effective enzymes (GPX, SOD, CAT) were increased in the other treatment groups in the present study. The antioxidant effects of myrcetin and quercetin were proven (35-39). The antidiabetic effect of myrcetin 3-O-α-

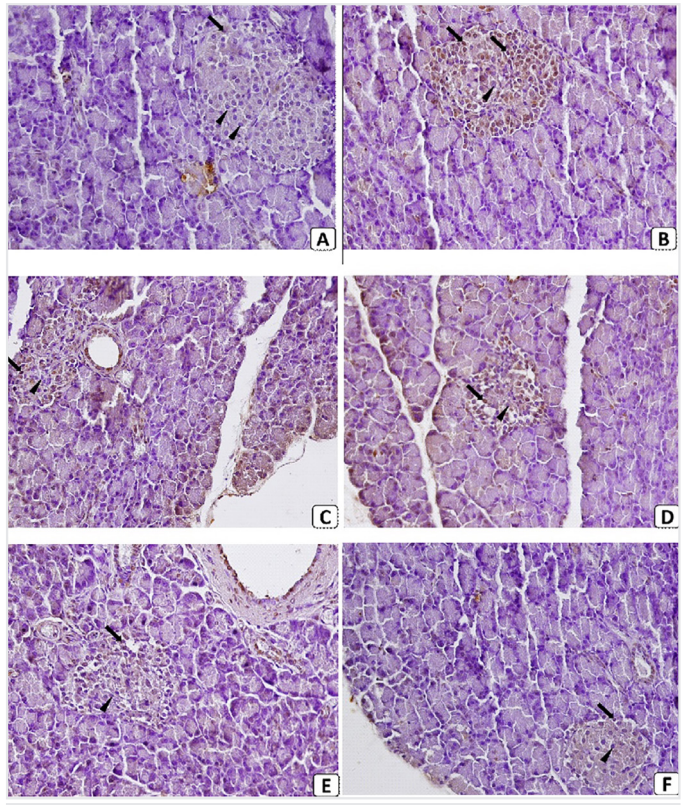

Figure 5. Group I (A), Group II (B), Group III (C), Group IV (D), Group V (E), Group VI (F) Apoptotic cells (arrow), normal cells (arrow head) (TUNEL, x400)

rhamnopyranoside and quercetin 3-O-β-glucopyranoside groups are probably directly related to the antioxidant effect of these phenolic compounds. Similarly, these phenolic compounds in the extract contributed to the antidiabetic and antioxidant effects of the extract.

Values are determined from day 14 after STZ administration, (values are mean ± SD, n=9), *P≤0.05 (compared to control group), $P≤0.05 (compared to diabetic group), #P≤0.05 (compared to control group with diabetic group)

Pancreatic β cell damage by apoptosis is evaluated as a most important factor for improving of hyperglycemia and diabetes (52). The exract and all isolated compounds were proven to have healing effect on STZ-induced pancreatic β cell damage with this study.

Previous studies reported microscopic analyses that Lymphocyte filteration, nucleus pyknosis, cytoplasmic vacuolization and irregular margins were observed in the islet beta cells of untreated diabetic rats (53). Present study revealed that Group III, IV, V and VI showed ameliorating effect when compared to negative control group, histopathologically. Group IV, V and Group VI displayed healing effects on langerhans islet margins. Most notable group, Group VI, decreased pathological effects related with diabetes considerably and exhibited improving effect on langerhans islet margins and cell appearance in most of the islets, similar to the control group (Group I). Thus, histopathological

and apoptosis analyzes supported the antidiabetic effect of LV and its isolated compounds.

Consequently, LV and its active ingredients, gallic acid, myrcetin 3-O-α-rhamnopyranoside and quercetin 3-O-β-glucopyranoside, possess therapeutic potential in DM in accordance with previous literature about importance of phenolic compounds and therapeutic potential of *Lysimachia* genus.

Conclusion

Successful treatment of DM is not yet found and the prevalence of DM is increasing day by day, thus DM is still a global problem. Accordance with traditional uses, the extract and isolated compounds of LV display antidiabetic effect through antioxidant properties and ameliorating effect on STZ-induced histopatholigical changes and pancreatic β cell damage. Thus, LV and isolated compounds especially quercetin 3-O-β-glucopyranoside can be a potential source of herbal medicine for the global problem and related complications through antioxidative mechanism or diminution of apoptosis. Further investigations and human trials are required for therapeutic effect of LV and isolated compounds on the global problem.

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Ethics

Ethics Committee Approval: All experiments were executed in accordance with the ethical norms. The study was approval by the Institutional Ethical Committee of Karadeniz Technical University, Trabzon, Turkey.

Informed Consent: Externally peer reviewed.

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Surgical and Medical Practices: S.Ö.Ş., M.B., U.Ö., R.A., G.K., E.Y., Concept: S.Ö.Ş., Design: S.Ö.Ş., M.B., N.K., Ş.K., Data Collection or Processing: S.Ö.Ş., M.B., U.Ö., N.K., Ş.K., R.A., Analysis or Interpretation: S.Ö.Ş., M.B., N.K., Ş.K., R.A., G.K., E.Y., Literature Search: S.Ö.Ş., U.Ö., E.S.K., Writing: S.Ö.Ş., E.S.K.

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Original Article

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Clinical Practice and Knowledge of Pediatric Surgeons about Eosinophilic Esophagitis in Children with Esophageal Atresia

Çocuk Cerrahlarının Özofagus Atrezili Çocuklarda Eozinofilik Özofajit Konusunda Klinik Uygulamaları ve Bilgi Düzeyleri

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ABSTRACT

Objective: The study examined the clinical practice and knowledge of pediatric surgeons regarding diagnosis and treatment of eosinophilic esophagitis (EoE) in children with esophageal atresia (EA).

Methods: Pediatric surgeons who were involved in diagnosis and treatment of EA from Turkey were included. An online survey, which included 26 questions related to clinical practice and knowledge of pediatric surgeons was administered via Google forms. The survey included questions regarding descriptive information of participants, diagnosis, treatment, and knowledge about EoE in children with EA.

Results: Fifty seven pediatric surgeons responded to the study. Reflux symptoms resistant to treatment and recurrent anastomotic strictures were most commonly reported as symptoms of EoE. Endoscopy with biopsies was the most commonly selected diagnostic method for EoE with nearly half of the surgeons obtaining appropriate biopsies. Diet elimination, proton pump inhibitors and systemic steroids were most commonly reported to be used in the treatment of EoE. Of pediatric surgeons 17.5% (n=10) had low-level knowledge, 45.6% (n=26) had moderate level knowledge, and 36.8% (n=21) had high-level knowledge.

Conclusion: The EoE can be seen in association with EA. This association may cause dysphagia, food impaction, vomiting and decreased quality of life. The study results suggest that pediatric surgeons have some strengths and weaknesses in terms of clinical

ÖZ

Amaç: Çalışmada, çocuk cerrahlarının özofagus atrezili (ÖA) çocuklarda eozinofilik özofajitin (EÖ) tanı ve tedavisi ile ilgili klinik uygulamaları ve bilgileri incelenmiştir.

Yöntemler: Türkiye'de ÖA'nın tanı ve tedavisinde görev alan çocuk cerrahları dahil edildi. Google formları aracılığıyla çocuk cerrahlarının klinik uygulamaları ve bilgileriyle ilgili 26 sorudan oluşan çevrimiçi bir anket uygulandı. Anket; katılımcıların tanımlayıcı bilgileri ve ÖA'lı çocuklarda EÖ'nün tanısı, tedavisi ve bunlarla ilgili bilgilerini içeren sorular içeriyordu.

Bulgular: Çalışmaya 57 çocuk cerrahı yanıt verdi. Tedaviye dirençli reflü semptomları ve tekrarlayan anastomoz darlıkları en yaygın olan EÖ semptomları olarak bildirildi. Biyopsi ile endoskopi, EÖ için en yaygın olarak seçilen tanı yöntemiydi ve cerrahların yaklaşık yarısı uygun biyopsiler alıyordu. Diyet eliminasyonu, proton pompası inhibitörleri ve sistemik steroidler en yaygın EÖ tedavi yöntemleri olarak bildirildi. Çocuk cerrahlarının %17,5'i (n=10) düşük düzeyde bilgiye sahipti, %45,6'sı (n=26) orta düzeyde bilgiye sahipti ve %36,8'i (n=21) yüksek düzeyde bilgiye sahipti.

Sonuç: EÖ, ÖA ile birlikte görülebilmektedir. Bu ilişki disfaji, gıda sıkışması, kusma ve yaşam kalitesinin düşmesine neden olabilmektedir. Çalışma sonuçları, ÖA'lı çocuklarda EÖ tanısı ve tedavisi ile ilgili klinik uygulama ve bilgi açısından çocuk cerrahlarının bazı güçlü ve zayıf yönlerinin olduklarını göstermektedir. Bu nedenle bu çocukların bakımında iyileşme, çocuk cerrahlarının farkındalığını ve bilgisini artırarak, uygulamaların heterojen

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practice and knowledge regarding diagnosis and treatment of EoE in children with EA. Therefore, improvement in the care of these children could be achieved by increasing awareness and knowledge of pediatric surgeons, decreasing heterogeneous nature of practice patterns and creating a consensus in clinical settings.

Keywords: Esophageal atresia, eosinophilic esophagitis, pediatric surgeon, knowledge

doğasını azaltarak ve klinik ortamlarda bir fikir birliği oluşturarak sağlanabilir.

Anahtar Sözcükler: Özofageal atrezi, eozinofilik özofajit, çocuk cerrahı, bilgi

Introduction

Esophageal atresia (EA) is the most common birth defect of the esophagus and affects 1 in 2,500 live births (1). Children with EA experience a high rate of morbidity, which lasts lifelong. Patients have varying degrees of morbidities like esophageal dysmotility, anastomotic strictures, gastroesophageal reflux disease (GERD), dysphagia and feeding difficulties (2).

Eosinophilic esophagitis (EoE) is a chronic allergic disorder, which is characterized by esophageal eosinophilia and esophageal dysfunction. It causes vomiting, dysphagia, feeding difficulties, food allergy, and decreased quality of life (3). Children with EA are shown to have a significant prevalence of EoE ranging between 3-17% (4-7). There are several contributing and underlying factors including food impaction, treatment-resistant strictures and increased allergen exposure due to motility problems underlying EoE development in children with EA. However, it is difficult to diagnose EoE in patients with EA due to similarities of symptoms with GERD, dysphagia and esophageal dysmotility (3). For instance, the study with the largest number of patients with EA and EoE suggested that in addition to fluoroscopic swallowing studies, endoscopic evaluation with multiple biopsies was important in children who suffered from increasing dysphagia (5). Vomiting, dysphagia or feeding problems were more common in patients with EA and EoE resulting in a higher likelihood of undergoing fundoplication and gastrostomy surgeries in comparison to those who did not have associated EoE (5). Pediatric surgeons not only have an important role in the management of children with EA but they also design their long-term follow-ups (1,2). Therefore, awareness of pediatric surgeons about the symptoms suggestive of EoE, and necessity to perform biopsies and referral of the patients to appropriate disciplines if needed are important. Pediatric surgeons are to be involved in developing standardized consensus and providing improvement in care.

In a recent study, dysphagia as one of the most common complication in these children from the perspective of pediatric surgeons was investigated, and it was suggested that there was a need for a standardized protocol in dysphagia management and their knowledge was found to be high (8). The importance of the awareness and knowledge of pediatric surgeons is also known to handle EoE in children with EA. However, there is no study to evaluate their current clinical practice and knowledge in terms of EoE in children with EA in literature. Determination of the current practice and knowledge may provide better understanding of the pitfalls of management delivered by pediatric surgeons, reveal good practices, and contribute to create consensus in national and international areas. Therefore, the current study aimed to define clinical practice and knowledge of pediatric surgeons from Turkey regarding diagnosis and treatment of EoE in children with EA.

Methods

The online survey study was carried out at Hacettepe University. The Hacettepe University Non-invasive Clinical Research Ethics Committee approved the study protocol (approval number = GO20/528).

Participants

Pediatric surgeons who currently worked in Turkey and were involved in diagnosis and treatment of EA were included in the study. The invitation was done by using a national e-mail platform already in use for online communication among pediatric surgeons in Turkey. The online survey was administered via Google forms. All volunteering participants were required to provide informed consent prior to reach survey questions by clicking the start button of the survey. All data were collected anonymously.

Procedure

Two pediatric surgeons with more than 20 years of clinical and research experience in the treatment of children with EA developed the draft questions. A pilot study was performed to test clarity of the questions before survey distribution. Two pediatric surgeons and one physical therapist who were university-affiliated experts with a minimum of 10-year work experience and routinely encountered patients with EA in practice discussed each question, and minor changes were made through their feedback. The survey included 26 questions, which was estimated to take 15 minutes to complete.

The survey included (i) 4 questions related to descriptive information of participants, (ii) 8 questions related to diagnosis, (iii) 4 questions related to treatment, and (iv) 10 questions related to knowledge. Descriptive section questioned the type of hospital, occupational experience, the number of children with EA in annual follow-up and the number of children with EA who had EoE. In the diagnosis section, there were questions related to consultations in the long-term follow-up, the endoscopy applications and the diagnosis of EoE. There were two questions related to the treatment of GERD and two questions regarding the treatment of EoE. In the knowledge section, participants were asked to state "yes" or "no" for the statements. Only one

option was the correct answer, which received one point, and the incorrect answer received zero point. Therefore, the total knowledge score ranged between 0 to 10 points. In addition, the total score was assessed based on Bloom's cut off point (9). According to Bloom's cut off point, the level of knowledge was classified into three as "low-level", "moderate-level", and "high-level" knowledge. The total score less than 60% (0-5 scores) refered to low-level knowledge, 60-79% (6-7 scores) indicated moderate-level knowledge, and 80-100% (8-10 scores) refered to high-level knowledge. The statements were related to time of occurrence of esophagitis (Q1), frequency by gender (Q2), risk related to type of atresia (Q3), condition in prematurity (Q4), genetic relationship (Q5), relationship between esophagitis and anti-acid therapy (Q6), relationship between esophagitis and motility impairment (Q7), relationship between esophagitis and atopic diseases and food allergies (Q8), basal analysis (Q9), and cautions before anti-reflux treatment (Q10). Appendix 1 indicates the survey questions in detail.

The survey was started in July 2020. A reminding e-mail message was sent after each four weeks, and the survey was closed twelve weeks after initial posting, and responses were analyzed.

Statistical Analysis

The statistical analysis was performed by using IBM-SPSS for Windows version 20. Descriptive statistics were expressed as number/percent for qualitative data, and mean, standard deviation, minimum and maximum values for quantitative data.

Results

A total of 57 pediatric surgeons were participated in the current study. The descriptive characteristics of participants are shown in Table 1.

Diagnosis: In the long-term follow-up of children who underwent EA operation, 73.7% (n=42) of pediatric surgeons got routine consultation from pediatric gastroenterology, 36.8% (n=21) from pediatric pulmonology and 26.3% (n=15) from pediatric allergy/immunology units. The rate of pediatric surgeons who did not get any support from other disciplines was 22.8% (n=13). The responses related to the endoscopy applications and the diagnoses of EoE are presented in Table 2 and Table 3, respectively.

Treatment: In treatment section, the questions were related to GERD and EoE. The answers are given in Table 4.

Knowledge: The percentage of correct answers for each questions was as follows: Q1: 66.7% (n=38), Q2: 71.9% (n=41), Q3: 70.2% (n=40), Q4: 64.9% (n=37), Q5: 47.4% (n=27), Q6: 56.1% (n=32), Q7: 89.5% (n=51), Q8: 96.5% (n=55), Q9: 21.1% (n=12), Q10: 93% (n=53). The mean knowledge score was 6.64±1.92 (min=1, max=9). The rate of pediatric surgeons with low-level knowledge was 17.5% (n=10), moderate-level knowledge was 45.6% (n=26) and high-level knowledge was 36.8% (n=21).

Discussion

The care of children with EA, which requires multidisciplinary follow-up should be handled systematically from childhood to adulthood (10,11). Therefore, standard management guidelines in terms of gastrointestinal and nutritional complications in

Table 1. The descriptive characteristics of pediatric surgeons and their clinical practice

	N	%
Hospital		
University hospital	39	69.6
Training hospital	14	25.0
Non-training state hospital	1	1.8
Private hospital	2	3.6
Occupational experience		
0-5 years	8	14.0
6-10 years	15	26.3
11-15 years	12	21.1
>16 years	22	38.6
Number of children with EA treated per year		
< 5	18	31.6
5-10	22	38.6
>10	17	29.8
Number of patients who were diagnosed as having EoE while being under follow-up for EA		
None	27	48.2
<2	19	33.9
2-5	9	16.1
>5	1	1.8

EA: Esophageal atresia, EoE: Eosinophilic esophagitis

Table 2. The responses related to the endoscopy applications in children with EA

	N	%
The percentages of indications for endoscopy		
Esophageal dilatation	50	87.7
Suspected GERD	29	50.9
Reflux esophagitis follow-up	42	73.7
After stopping proton pump inhibitors	1	1.8
Suspected EoE	35	61.4
In asymptomatic adolescents	3	5.3
Routine surveillance for esophagitis	3	5.3
Which type of endoscopy		
Does not perform endoscopy	2	3.5
Rigid endoscopy	8	14
Flexible endoscopy	33	57.9
Both rigid and flexible endoscopy	14	24.6

EA: Esophageal atresia, EoE: Eosinophilic esophagitis

children with EA are important to improve the quality of care to these patients and guide clinicians (12). In addition to clinical studies' results and what is suggested in the guidelines, defining the clinical practice and knowledge of primary responsible clinicians regarding children with EA is also important to reveal what is exactly performed. Therefore, we aimed to determine the current practice and knowledge of pediatric surgeons in terms of EoE in children with EA.

Table 3. The responses related to the diagnosis of EoE in children with EA

	N	%
Signs and symptoms of EoE in children with EA		
Food impaction	25	43.9
GERD symptoms resistant to treatment	50	87.7
Recurrent anastomotic strictures	44	77.2
Recurrence of GERD findings	23	40.4
Airway aspiration	17	29.8
Diagnostic methods for EoE		
Contrast upper GI studies	3	5.3
pH meter/impedance	2	3.5
Endoscopy and biopsy	51	89.5
Esophageal manometry	0	0
Videofluoroscopic swallowing assessment	1	1.8
Endoscopy findings for EoE		
Red streaks and white exudates	42	73.7
Trachealization or ringing	26	45.6
Crepe paper mucosa, fragile mucosa	32	56.1
Varicose veins in the lower end of the esophagus	4	7
Mucosal bleeding centers	30	52.6
Location of endoscopic biopsies and the number of samples in a patient with suspected EoE		
No need for a biopsy	3	5.3
Two from each quadrant from the lower esophagus	12	21.1
Two from each quadrant from the upper esophagus	3	5.3
Two each from the lower, middle and upper esophagus	24	42.1
Only the areas seen as suspicious	15	26.3
Number of eosinophils/high power field required to diagnose EoE in endoscopic biopsy specimens		
Less than 5	2	3.5
Between 5-14	11	19.3
Less than 15	4	7
Equal to and more than 15	27	47.4
No idea	13	22.8

EA: Esophageal atresia, EoE: Eosinophilic esophagitis

In the long-term follow-up of children who were operated on for EA, pediatric surgeons reported that they mostly got help from pediatric gastroenterology unit. However, approximately one quarter of them could not get any collaboration from other disciplines. The percentage of clinicians who did not have any help from other discipline was remarkable. A multidisciplinary approach is necessary to have a favorable long-term outcome and better quality of life (12-14). Therefore, we believe that the awareness of other team members including pediatric gastroenterologists, pediatric pulmonologists, etc. should be increased as well as pediatric surgeons to improve clinical care of these children.

A substantial number of pediatric surgeons (42.1%) favor routine medical therapy for GERD in the first year of life in all patients with EA as recommended in ESPGHAN-NASPGHAN

Table 4. The responses related to the treatment of GERD and EoE in children with EA

Medical treatment approach for GERD in children with EA	N	%
Only to symptomatic patients	18	31.6
All cases with anastomotic strictures	8	14
First 1 year in all EA cases	24	42.1
Treatment after diagnostic testing	7	12.3
First choice for GERD treatment in children with EA		
H_2 receptor blockers	9	15.8
Proton pump inhibitors (PPI)	20	35.1
Alginate and/or prokinetics and H_2 receptor blockers together	19	33.3
Alginate and/or prokinetics, PPI	9	15.8
Other disciplines which are involved in the consultation of patients with suspected EoE		
Pediatric gastroenterology	54	94.7
Pediatric hematology	1	1.8
Pediatric allergy	45	78.9
Pediatric immunology	19	33.3
Treatment of EoE		
Diet elimination	42	73.7
Systemic steroids	25	43.9
Proton pump inhibitors	38	66.7
Esophageal dilatation	12	21.1
Intralesional applications (mitomicin -C, etc.)	6	10.5

EA: Esophageal atresia, EoE: Eosinophilic esophagitis

guidelines (12). One third of responders prescribe anti-reflux medication only in symptomatic patients and 12% of them prescribe after testing for reflux. Proton pump inhibitors (PPI) (35.1%) and alginate and/or prokinetics and H_2 receptor blockers together (33.3%) were the first line of choice for GERD treatment by pediatric surgeons.

The most common indications for endoscopy for pediatric surgeons are esophageal dilatation, follow-up of reflux esophagitis, and suspicion of EoE or GERD in accordance with the literature (12). Only one pediatric surgeon reported that he/she planned endoscopy after stopping proton pump inhibitors. More than half of the pediatric surgeons preferred flexible endoscopy for children with EA, and approximately 25% preferred both rigid and flexible endoscopy. The results show that pediatric surgeons in Turkey follow international consensus regarding endoscopy applications (12).

The responding pediatric surgeons reported that they suspected EoE in patients with GERD symptoms resistant to treatment (87.7%), recurrent anastomotic strictures (77.2%) and food impaction (43.9%). In addition, they also reported that recurrent gastroesophageal reflux and airway aspiration were also signs of EoE in children with EA. These findings suggest that most pediatric surgeons are aware of the considerable overlapping between GERD and EoE symptoms in children with EA. It has been reported that patients with long gap EA and EoE have 1.9 times higher relative risk to develop strictures and surgical treatment of strictures are needed if they become resistant to dilatation treatment (3). The results of survey confirm that majority of pediatric surgeons suspect EoE in case of recurrent strictures.

Endoscopic evaluation with biopsies is crucial for diagnosis of EoE (12,15). EoE can be definitely diagnosed by esophageal biopsy revealing more than 15 eosinophils/HPF (12,15). In our survey, 89.5% of pediatric surgeons reported that they performed endoscopy and took biopsies whenever they suspected from EoE. The finding of "red streaks and white exudates" was the most frequently associated endoscopy finding with EoE (73.7%) by pediatric surgeons followed by crepe paper and fragile mucosa, mucosal bleeding centers and trachealization or ringing, in descending order. Interestingly, half of them consider mucosal bleeding as an endoscopic sign for EoE and 7% of them find varicose veins in the lower end of esophagus suggestive for diagnosis.

Since EoE is described as a patchy disease, it is important to take biopsies form widespread locations (3). In our study, 42.1% of surgeons preferred two biopsies each from the lower, middle and upper esophagus for suspicion of EoE, and 47.4% of them considered more than 15 eosinophils/HPF diagnostic for EoE. These results suggest that nearly half of the surgeons obtain appropriate biopsies and consider the results correctly according to the guidelines. However, 26% of responders obtain biopsies only from the suspected areas and 22.8% of them had no idea about the number of eosinophils in the biopsy specimens for EoE diagnosis. These percentages suggest that there is a need to increase awareness, knowledge and clinical applications of pediatric surgeons in terms of endoscopic evaluations and interpretations for EoE in children with EA. Pediatric gastroenterology (94.7%), pediatric allergy (78.9%) and pediatric immunology (33.3%) disciplines are the most commonly co-involved disciplines in patients with EA with suspected EoE. This may simply be dependent on the availability of the discipline in the institution at which the pediatric surgeon works or a personal preference depending on the enthusiasm of the other disciplines to collaborate in the care of the patients with EA in a given institution.

Although, pediatric surgeons do not directly dictate the treatment of EoE, they report diet elimination (73.7%), proton pump inhibitors (66.7%) and systemic steroids (43.9%) as most frequent treatment options in EoE. This is compatible with the recommended treatment protocols for children with EA (16). In a study performed in 2016, the authors reported that dilatations could be needed for strictures in patients with coexistent EoE with EA although seldom (17). The role of pediatric surgeons in the treatment of EoE is mainly limited to esophageal dilatations for strictures. About one fifth of pediatric surgeons in our study mentioned that they carried out dilatations for these patients. Interestingly, 10% of pediatric surgeons reported intralesional applications for the treatment for EoE. The use of any intralesional applications has not been reported in EoE before and possibly has no beneficial effect on EoE related esophageal strictures.

Study Limitations

Our study had a limitation because only volunteering pediatric surgeons answered the questions and they might be the ones with a special interest in EA and/or EoE. Bearing this fact in mind, the knowledge of pediatric surgeons was found to be above average in this study. Most participants had moderate (45.6%) to high (36.8%) level of knowledge. Wrong answers were mostly related to basal impedance analysis and genetic relationship. The most correct answers were related to relationship between esophagitis and atopic diseases and food allergies, and cautions before anti-reflux treatment. These results are also very important because determining the lack of knowledge as to the degree or aspect may provide clinicians to organize and participate in educational programs, improve their clinical applications, and increase the success of care regarding EoE in children with EA.

Conclusion

The EoE is a chronic inflammatory disease of the esophagus and can be seen in association with EA. Children with EA and also suffering from EoE have higher incidence of dysphagia, food impaction, vomiting and feeding difficulties, which result in decreased quality of life of both patients and their families. Therefore, the clinical practice and knowledge regarding its diagnosis and treatment are important in addition to clinical studies and management guidelines. The current study results suggest that pediatric surgeons have some strengths and weaknesses in terms of clinical practice and knowledge about this

association. Therefore, their awareness, knowledge and clinical practice should be increased to improve the care of these children.

Ethics

Ethics Committee Approval: The online survey study was carried out at Hacettepe University. The Hacettepe University Non-invasive Clinical Research Ethics Committee approved the study protocol (approval number=GO20/528).

Informed Consent: provided informed consent prior to reach survey questions by clicking the start button of the survey.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: S.S.A., Ç.U.D., T.S., Design: S.S.A., Ç.U.D., T.S., Data Collection or Processing: S.S.A., T.S., Analysis or Interpretation: S.S.A., Ç.U.D., T.S., Literature Search: S.S.A., Ç.U.D., T.S., Writing: S.S.A.

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Original Article

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Evaluation of Parathyroid allo-transplantation with the Presence of Auto-CASR Antibody

Paratiroit Allo-Nakli ile Oto-CaSR Antikor Varlığının Değerlendirilmesi

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ABSTRACT

Objective: Parathyroid tissue is responsible for the regulation of calcium and vitamin D metabolism. It regulates parathyroid hormone release by the calcium-sensing receptor (CaSR) which is found on the parathyroid cell surface. Auto-antibody formation against this receptor has been reported in the literature. In this study; the probability of parathyroid transplantation triggering an auto-immune response against this receptor was evaluated. Individuals were screened for the presence of auto-CaSR antibodies after transplantation.

Methods: The nine individuals who underwent parathyroid transplantation were evaluated for the survival rate and were screened for the possible presence of auto-CaSR antibodies. Data were determined by ELISA from peripheral blood samples. A peripheral blood sample from one healthy volunteer was included as a negative control.

Results: Survival rate assessment of nine recipients was as follows; less than one month in three individuals and more than one year in six individuals. Any trace of possible auto-CaSR antibodies was not detected in any individual.

Conclusion: Auto-CaSR antibody formation was not observed after parathyroid transplantation. Thus, it has been shown for the first time in the literature that the recipients did not show an autoimmune-related response after parathyroid transplantation even with having heterogeneous survival rates. Moreover, it has

ÖZ

Amaç: Paratiroit dokusu kalsiyum ve D vitamini metabolizmasının düzenlenmesinde hayati öneme sahiptir. Parathormon salınımını taşıdığı kalsiyum-algılayıcı reseptör (CaSR) ile düzenler. Bu reseptöre karşı vücutta oto-antikor oluşumu literatürde bildirilmiştir. Bu çalışma da; paratiroit nakli uygulanmış bireylerde oto-CaSR antikor varlığı taranarak, paratiroit naklinin oto-immün bir yanıtı tetikleme ihtimali değerlendirilmiştir.

Yöntemler: Paratiroit nakli uygulanmış dokuz birey, nakil sağkalım verileri ile beraber periferik kan örneklerinde oto-CaSR antikor varlığı ELISA ile belirlenmiştir. Negatif kontrol grubu olarak bir sağlıklı gönüllüden alınan periferik kan örneği değerlendirmeye dahil edilmiştir.

Bulgular: Değerlendirmeye alınan dokuz bireyde sağkalım oranları; üç bireyde bir aydan daha az, altı bireyde ise bir yıldan fazladır. Oto-CaSR antikor varlığı ise tüm bireylerde negatif olduğu belirlenmiştir.

Sonuç: Paratiroit nakli sonrasında otoimmün ilişkili olarak oto-CaSR antikor oluşumu gözlenmemiştir. Böylelikle heterojen sağkalım farklılıklarına sahip alıcıların, paratiroit nakli sonrasında otoimmün bir yanıtı göstermemesi literatürde ilk defa bildirilmiştir. Ayrıca, paratiroit naklinin başarısını öngörmede alıcıların semptomatik olarak gösterdikleri iyileşmelerin sağkalım oranını belirlemede önem arz ettiği gösterilmiştir. Paratiroit transplantasyonundan sonra oto-CaSR hakkında güçlü sonuçlar çıkarılmadan önce daha geniş kohortlarla çalışmalara ihtiyaç vardır.

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been shown that the symptomatic improvements of the recipients in predicting the success of parathyroid transplantation are important in determining survival rate. Larger cohort studies are required before strong conclusions can be drawn about the auto-CaSR after parathyroid transplantation.

Keywords: Parathyroid transplantation, parathyroid hormone, survival rate, auto-CaSR antibodies

Anahtar Sözcükler: Paratiroit nakli, parathormon, sağkalım, oto-CaSR antikorları

Introduction

The causes of hypoparathyroidism are autoimmune, congenital, and most often iatrogenic. After thyroid surgery and neck dissection, excision of the parathyroid glands or disruption of their vitality causes a decrease in parathormone (PTH) secretion secondary to hypocalcemia (1, 2). When we look at the literature, the incidence of hypoparathyroidism after total thyroidectomy varies between 7% and 37% (3). This hypoparathyroidism is mostly temporary and resolves within a few weeks to a month (4). During this period, patients receive symptomatic treatment in the form of calcium and vitamin D replacement. However, post-operative hypoparathyroidism exceeding six months is considered permanent hypoparathyroidism (PH) (3,5).

Although calcium and vitamin D replacement and, whenever possible, PTH injections are used in PH, the only curative treatment is parathyroid transplantation (10). Bezmialem Vakıf University Parathyroid Transplantation Unit is the first unit in Turkey to receive a parathyroid transplant permission from the Ministry of Health. Parathyroid transplantation in patients with PH shows survival of 1 to 5 years in some patients, but a survival of 1 to 6 months is observed in some patients (6-8).

Parathyroid tissue has an important role in the regulation of calcium and vitamin D metabolism. Parathyroid tissue provides cell functions with its surface receptor, the calcium-sensing receptor (CaSR) (9,10). CaSR senses the level of calcium in the blood, regulates PTH secretion, and plays an important role in calcium metabolism through urinary calcium excretion. The gene encoding the CaSR protein is located on the 3rd chromosome in humans (11). The parathyroid glands are a rare target for autoimmunity. It has been shown that antibodies to CaSR found on the surface of the parathyroid cells are present in the serum of patients with autoimmune hypoparathyroidism (12). In some individuals, auto-CaSR antibodies are thought to play a direct pathogenic role in hypocalcemia (12,13).

In this context, the relationship of parathyroid cell/tissue transplantation with the possibility of triggering the development of antibodies against CaSR (auto-CaSR antibody) during survival was investigated. In this sense, the presence/absence of auto-CaSR antibodies in blood serum samples of transplanted recipients with a transplant survival of less than 1 month and more than 1 year was evaluated in this study.

Methods

Informed consent forms were obtained from the recipients and one healthy individual for their peripheral blood samples to be used in this study, for which Clinical Researches Local Ethics Committee Approval was obtained in accordance with the World Medical Association Declaration of Helsinki (Document date and number of the approval of the ethics committee: 24/05/2018-8283).

Recipients

The ABO compatible parathyroid allotransplant was performed for the first time in 9 individuals diagnosed as having PH between 2013 and 2018, and individuals who had only one parathyroid transplant were included. There was no autoimmune disease in these individuals and no immunosuppressive drug use was reported during the collection of peripheral blood samples to be used in this study. Of the 9 individuals who voluntarily agreed to participate in the study, the survival of three was less than 1 month and the survival of six of them was more than 1 year. All transplant operations were carried out with a single application. Tissue transplantation (Recipients 1, 3, 4) of the three volunteers in the study was provided by applying the Cleaveland protocol (14), and the other five recipients (Recipients 2, 5, 6, 7, 8, 9) were transplanted parathyroid tissue with cell isolation (15) applied by mechanical method.

Peripheral blood samples were subjected to 10 min centrifugation at 4000 rpm. Serum samples were stored at -80°C until ELISA experiments were performed. As the negative control group, a peripheral blood sample from a healthy volunteer was included in the evaluation.

ELISA

A commercially available ELISA kit for Auto-CaSR antibodies was used (EDITM Human Anti-CaSR Autoantibody ELISA Kit, Epitope Diagnostics, Inc., San Diego, CA, USA). All samples were studied in pairs, in accordance with the kit's protocol. The absorbance values at 450 nm were evaluated relative to the positive and negative control.

Results

In Table 1, the type of transplantation, the region of administration, intact parathormone (iPTH) levels, serum calcium levels and current clinical status of the 9 parathyroid allotransplant recipients participating in the study are presented. Current clinical status of recipients were as follows: K0: no change was observed, P1: The doses of the drugs for the PH treatment were not decreased, but clinical improvement was observed, K2: the use of drugs due to PH treatment was reduced, and K3: drug use was discontinued (Table 1). In Figure 1, the

Table 1. Recipients of parathyroid allo transplant (n=9); type of transplant. place of administration. pre- and post-op intact PTH and serum calcium values. Post-op follow-up data are given for each recipient based on current survival times. clinical situation; It was scored according to the response (change) during the survival and the transplant follow-up status at the end of 2020 (K0: no change was observed. K1: The drugs in the CH treatment were not reduced. but clinical improvement was observed. P2: The drug use due to CH treatment was reduced. and K3: The drug use was discontinued)). PTH: Parathormone. CH: Permanent Hypoparathyroidism. iPTH: pg/mL. Serum calcium (Ca): mg/dL.

	Transport type	Place of ıssue	Pre-op		Post-op		Overal survival ratio (month)	Clinical status (change/current status)
			iPTH	Ca	iPTH	Ca		
A1	Tissue	Deltoid muscle	0	8.8	0.3	8.5	<1	K0/K0
A2	Tissue	Deltoid muscle	9.9	8.9	27.3	7.5	≤20	K2/K1
A3	Tissue	Deltoid muscle	6	8.5	n/a	7.9	≤36	K2/K0
A4	Tissue	Deltoid muscle	3.1	8.5	2.9	9.2	<1	K0/K0
A5	Mechanical cell isolation	Deltoid muscle	0.1	12.5	15.5	10.4	60>	K2/K3
A6	Mechanical cell isolation	Deltoid muscle	10.4	7.3	10.6	7.8	<1	K0/K0
A7	Mechanical cell isolation	Omentum	10.7	8.4	18.6	6.6	<1	K1/K0
A8	Mechanical cell isolation	Omentum	2.2	8.2	8.4	3.5	<12	K1/K1
A9	Mechanical cell isolation	Omentum	9.7	8.4	12.7	8.8	24>	K2/K3

absorbance values at 450 nm obtained as a result of the ELISA experiment for auto-CaSR antibody levels, recipient samples (n=9), values of one healthy individual, and positive and negative controls are given.

Recipient #1 (A1); A 44-year-old woman underwent a parathyroid tissue transplant into the deltoid muscle three years after the diagnosis of PH and her survival time was <1 month. A1 continues on pretransplant medication regimen for PH symptoms. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 4th year after transplantation (Figure 1).

Recipient#2 (A2); A 28-year-old woman underwent parathyroid cell transplantation on the omentum with a laparoscopic approach, approximately 2 years after she was diagnosed as having PH. It was observed that the patient did not use medication until the 20th month after the transplant. Since the patient became pregnant 20 months after transplantation, it was reported that the drug doses used for the diagnosis of PH were reinitiated by half. It was observed that the transplant survival period lasted up to 20 months and her clinical status was still good in terms of drug doses. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 3rd year after transplantation (Figure 1).

Recipient #3 (A3); A 45-year-old woman underwent a parathyroid tissue transplant to the deltoid muscle 8 years after she was diagnosed as having PH and her survival time was 3 years. The recipient returned to the drug doses used for the symptoms of PH at 35 months after transplantation. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 2nd year after transplantation (Figure 1).

Recipient #4 (A4); A 45-year-old man underwent a parathyroid tissue transplant to the deltoid muscle ten years after the diagnosis of PH and his survival time was <1 month. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 2nd year after transplantation (Figure 1).

Recipient #5 (A5); A 56-year-old woman underwent a parathyroid cell transplant to the deltoid muscle two years after she was diagnosed as having PH. Her survival time is more than 5 years and she is still being followed up. The recipient stated that she did not show symptoms of PH and did not use any medication. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 5th year after transplantation (Figure 1).

Recipient #6 (A6); A 51-year-old woman underwent a parathyroid cell transplant into the deltoid muscle six years after being diagnosed as having PH. Survival time was less than 1 month. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 1st year after transplantation (Figure 1).

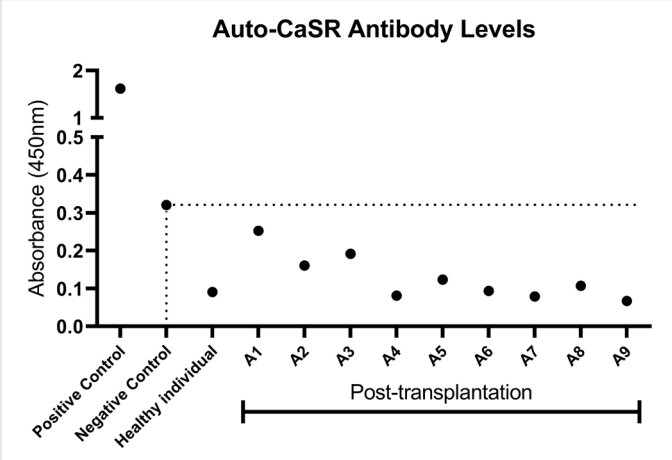

Figure 1. Comparison of auto-CaSR antibody levels by absorbance values at 450 nm

Recipient #7 (A7); A 39-year-old woman underwent parathyroid cell transplantation on the omentum using a laparoscopic approach two years after she was diagnosed as having PH. The transplant survival time was approximately 29 months. It was reported that the drug doses used for the treatment of PH symptoms were reduced to 1/3 of the initial doses, and then the doses were returned back to the initial drug doses. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 1st year after transplantation (Figure 1).

Recipient #8 (A8); A 49-year-old woman underwent a laparoscopic approach to parathyroid cell transplantation on the omentum 7 years after she was diagnosed as having PH, and the survival time of the transplantation was approximately one year. The drug doses used by the recipient for the treatment of PH symptoms were halved and she reported that she continued her medication as it was. The presence of auto-CaSR antibodies was found to be negative in this study performed at the 9th month after transplantation (Figure 1).

Recipient #9 (A9); A 51-year-old woman underwent parathyroid tissue cell transplantation into the deltoid muscle two years after she was diagnosed as having PH. Transplant survival is >2 years and she is still being followed-up. The recipient stated that she did not show symptoms of PH and did not use any medication. In this study performed at the 10th month after transplantation, the presence of auto-CaSR antibodies was found to be negative (Figure 1).

Discussion

The patients with PH need lifelong, regular oral or parenteral calcium and vitamin D replacement, but in some patients, hypocalcemia and symptoms do not improve despite regular supplements. In addition to the morbidity due to hypocalcemia and hypoparathyroidism, the quality of life of the patients is seriously impaired and the cost of these lifelong preparations is high (16). Serum calcium level is primarily affected by PTH level in these patients, and also affected by the levels and functions of vitamin D, phosphate, renal system, calcium transporter/binding protein and CaSR (17-19).

The CaSR auto-antibodies were first found in patients with familial hypocalciuric hypercalcemia (FHH) (20,21). However, Kifor et al. stated that these auto-antibodies were not only found in patients with FHH, leading to a search for a basis for autoimmune symptoms (22). Autoimmune hypoparathyroidism may present as a clinical abnormality. Auto-CaSR antibodies can be seen in autoimmune polyendocrinopathy syndrome-1 (APS-1) or in APS-2 (23,24). APS-1 most commonly includes mucocutaneous, candidiasis, hypoparathyroidism, and Addison's disease. APS-2 includes two or more of the following: Addison's disease, Graves' disease, autoimmune thyroiditis, type 1 diabetes mellitus, primary hypogonadism, myasthenia gravis, or celiac extension (23,25,26). Studies have shown that auto-CaSR antibodies are present in approximately one third of these diseases (23,27). On the other hand, it has been reported that some patients with primary hypoparathyroidism may harbor auto-antibodies against human CaSR (28). Therefore, it is known that detecting this auto-antibody has clinical value to assess the autoimmune origin of the disease.

Study Limitations

In this study, according to the results of parathyroid allo-transplantation, tissue transplantation was applied to four individuals and parathyroid cell transplantation was applied to five individuals. In terms of administration site, six transplants were performed by injection into the deltoid muscle. The other three transplants were performed with the laparoscopic approach to the omental tissue. Survival rates were less than one month in four individuals and less than one year in one individual. Survival of up to two to three years was observed in two individuals. In the other two individuals, survival still continues. The presence of auto-CaSR antibody, on the other hand, was evaluated as negative despite differences in administration site, transplant type and survival times. It was observed that parathyroid allo-transplants did not pose any risk factor for auto-CaSR antibody formation. In addition, the criteria for clinical improvement were determined depending on the survival status in the examination made among the clinical conditions of the recipients. Based on our experience with parathyroid allo-transplants since 2013, it is thought that changes in PTH ratio alone are not sufficient to evaluate the outcome of transplantation treatment of PH. Contribution to clinical improvement should also be considered in cases such as the reduction of drug doses used for PH symptoms, long-term symptomatic relief at low doses, and/or complete disappearance of the need for intravenous calcium.

Conclusion

As it can be understood from the findings obtained as a result, genetic differences between individuals can lead to different course of the same disease with similar symptoms. This reveals the importance of personalized and translational medicine approaches. By expanding the patient cohort in future studies, it will be possible to minimize the individual differences resulting from genetic heterogeneity and to reconsider the transplantation processes taking into account the mentioned differences.

Ethics

Ethics Committee Approval: Clinical Researches Local Ethics Committee Approval was obtained in accordance with the World Medical Association Declaration of Helsinki (ethics committee consent document date and number 24/05/2018-8283).

Informed Consent: Informed consent forms were obtained from the recipients and a healthy individual for the peripheral blood samples to be used in this study.

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Authorship Contributions

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Original Article

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mir210 and BCL2 Expressions in Patients with Acute Myeloid Leukemia

Akut Myeloid Lösemi Hastalarında mikroRNA-210 ve *BCL-2* Ekspresyon Seviyeleri

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ABSTRACT

Objective: Acute myeloid leukemia (AML) is a heterogeneous, malignant disease, characterized by disruption of differentiation of hematopoietic stem cells. The effect of microRNAs (miRNA)-210 on the prognosis of AML is a subject of research. *BCL-2* is a protooncogene encoding a protein that inhibits apoptosis. Overexpression of *BCL-2* is seen in AML cells. The presence or absence of cytogenetic abnormalities is an important prognostic marker in AML. In our study, we aimed to compare the effect of miRNA-210 and *BCL-2* expressions on the prognosis of AML and the relationship with the cytogenetic findings.

Methods: Cytogenetic analyzes were performed in bone marrow and/or peripheral blood samples taken from patients with AML and healthy individuals. miR-210 and *BCL-2* mRNA levels were determined by quantitative real-time polymerase chain reaction method.

Results: No abnormality was found in healthy individuals. Clonal structural and numerical anomalies were detected in some patients with AML. *BCL-2* mRNA expression levels of leukocytes and bone marrow samples of patients with AML were higher than that of the leukocytes of healthy individuals. miR-210 levels did not differ between patients and healthy individuals. The miR-210 level of leukocytes of patients with AML was significantly higher than that of the bone marrow samples of the patients with AML. A positive correlation was found between *BCL-2* and miR-210 in bone marrow samples from patients with AML.

ÖZ

Amaç: Akut myeloid lösemi (AML), hematopoietik kök hücrelerin farklılaşmasının bozulması ile karakterize, heterojen, malign bir hastalıktır. microRNA'lar (miRNA), AML'nin oluşumunda ve ilerlemesinde etkili olan faktörlerden biridir. mikroRNA-210'un AML'nin prognozuna etkisi ve AML gelişimdeki etki ettiği yolaklar araştırılan bir konudur. *BCL-2* bir protoonkogendir ve apoptozu inhibe eden proteini kodlar. AML hücrelerinde *BCL-2*'nin aşırı ekspresyonu görülmektedir. Sitogenetik anormalliklerin varlığı veya yokluğu, AML'de önemli bir prognostik belirteçtir. Bu çalışmada, miRNA-210 ve *BCL-2* ekspresyonlarının AML prognozu üzerindeki etkisi ve sitogenetik bulgularla ilişkisinin karşılaştırılması amaçlanmıştır.

Yöntemler: AML'li hastalardan ve sağlıklı bireylerden alınan kemik iliği ve/veya periferal kan örneklerinde sitogenetik analizler gerçekleştirilmiş, RNA izolasyonunu takiben miR-210 ve *BCL-2* mRNA seviyeleri kantitatif gerçek zamanlı polimeraz zincir reaksiyonu yöntemi (qRT-PCR) ile saptanmıştır.

Bulgular: Sitogenetik analiz sonucu sağlıklı bireylerde herhangi bir anomaliye rastlanmazken, bazı AML'li hastalarda klonal yapısal ve sayısal anomaliler tespit edilmiştir. AML'li hastaların lökositlerindeki ve kemik iliği örneklerindeki *BCL-2* mRNA ekspresyon seviyelerinin sağlıklı bireylerin lökositlerindeki ekspresyon seviyelerine göre arttığı saptanırken, miR-210 seviyeleri açısından hasta ve sağlıklı bireyler arasında bir fark bulunmamıştır. Ancak AML'li hastalara ait lökositlerin miR-210 seviyesi yine

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Conclusion: The increase in *BCL-2* mRNA and miR-210 levels may have negative effects on the prognosis of the disease by causing disruption in the apoptosis mechanisms.

Keywords: AML, miRNA-210, *BCL-2*, cytogenetics, prognosis

hastaların kemik iliği örneklerindeki miR-210 seviyesinden anlamlı derecede yüksek olarak saptanmıştır. Ek olarak, AML'li hastaların kemik iliği örneklerinde *BCL-2* ve miR-210 arasında pozitif bir korelasyon bulunmuştur.

Sonuç: *BCL-2* mRNA ve miR-210 seviyelerinde görülen artış apoptoz mekanizmasında bozulmaya sebep olarak hastalığın prognozu üzerinde olumsuz etkiler yaratabilir.

Anahtar Sözcükler: AML, miRNA-210, *BCL-2*, sitogenetik, prognoz

Introduction

Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder, and this malignant change occurs in hematopoietic cells, resulting in loss of function in the cells (1). The disease progresses rapidly when it is untreated and results in death according to its clinical progression (2). AML was first classified in 1976 by French-American-British classification (FAB) as M0 to M7 according to its morphological features (3,4).

According to the classification made by the World Health Organization based on cytogenetic and molecular properties, AMLs are divided into 4 groups; AML with certain genetic abnormalities, AML with myelodysplasia-related changes, AML related to previous chemotherapy or radiation, AML not otherwise specified (3,5). AML is more common in patients over 65 years of age, but it can occur at any age (6). Its incidence is approximately 3.7/100,000 per year, and the incidence of AML has increased significantly over the past decade (7).

The AML is characterized by the accumulation of over 20% myeloid blast cells in the bone marrow (8). However, in the presence of t(15;17), t(8;21), inv(16) or t(16;16) cytogenetic anomalies, AML can be diagnosed even if the number of blasts is below 20% (1). Cytogenetic findings are important parameters in the classification and prognosis of AML and chromosome anomalies are found in approximately 55% of newly diagnosed patients (9,10)

MicroRNAs (miRNA) are non-coding RNA molecules that have 19-25 nucleotides which are binding to target mRNA's 3' UTR regions and it regulates gene expression (11). Disorders of miRNA regulation affect cancer development and progression. These dysregulations cause different miRNA profiles to appear between normal and cancer tissues. The different profiles have shown that there is a relationship between cancer diagnosis and prognosis and miRNAs (12). In recent years, expressions of some specific miRNAs have been noticed to be associated with AML. miRNA-210 is also one of the miRNAs that affect prognosis of AML. Overexpression of miRNA-210 indicates poor prognosis in patients with AML (13).

The *BCL-2* protein is an inhibitor of apoptosis and is located in the outer membrane of the mitochondria, endoplasmic reticulum, and nucleus membrane (14). *BCL-2* oncogene is located on chromosome 18. This *BCL-2* locus is translocated with the immunoglobulin H locus on chromosome 14 and this translocation results in overexpression of *BCL-2* (15). Over-expression of *BCL-2* has been associated with a low complete remission rate and shorter survival after chemotherapy (16). Over-expression of *BCL-2* is associated with low treatment response in some hematological cancers such as follicular lymphoma, chronic lymphocytic leukemia, and AML (17).

In this study, it was aimed to detect chromosomal anomalies in patients with AML and to associate them with miR-210 and *BCL-2* expression levels. In this way, it would be evaluated whether miRNA-210 could be used as a biomarker with differences in treatment and prognosis.

Methods

Study Population

In this study, bone marrow materials were obtained from 7 patients who were newly diagnosed as having AML in the Cerrahpaşa Medical Faculty Hematology department and peripheral blood samples were also taken from 5 patients. The mean age of the patients was 49.4±22.4 years. Peripheral blood samples of 7 healthy volunteers (mean age was 36.8±10.6) as a control group were studied and the results were compared with the patient group. This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (07.09.2016-327301). Written consent form was obtained from all patients and volunteers before participating in the study.

Conventional Cytogenetics Analysis

Chromosomal analysis was performed on bone marrow samples by 24 and 48 hours cultures using standard cytogenetic technique. Seventy-two hour culture procedure was performed for peripheral blood samples (18). All samples were stained with the gas-to-liquids-banding method (19). Chromosome analysis was performed on each of the patient and control groups. The analyzes were evaluated according to The International System for Human Cytogenetic Nomenclature 2016 (20).

miRNA and mRNA Expression Analysis

The RNA was isolated from bone marrow samples of the patients by using MIRCURY Tissue RNA Isolation Kit (Exiqon, 300110). While RNA isolation from peripheral blood samples of both patients and healthy subjects was done with Qiagen RNA Isolation Kit (Qiagen, 52304). Both RNA isolation experiments were performed according to the manufacturers' protocols.

A fixed amount of RNA was fixed to 20 ng/µL for each sample for cDNA synthesis reaction and a miRCURY LNA™ microRNA PCR, Polyadenylation, and cDNA synthesis kit II (8-64 rxns) (product no. 203301, Exiqon, Denmark) were used for cDNA synthesis. miRNA expression level of miR-210 was detected by qRT-PCR using miRCURY LNA™ microRNA PCR, ExiLENT SYBR® Green master mix (Product no: 203421, Exiqon, Denmark) and LightCycler 480 instrument (Roche). UniSp6 RNA Spike-in control was used as an endogenous reference gene for normalization.

For mRNA expression analysis, cDNA synthesis was done with iScript cDNA synthesis kit (Biorad, 170-8891). From blood samples, 50 ng/µL of RNA was isolated and 150 ng/µL of RNA was isolated from bone marrow samples was used for cDNA synthesis. mRNA expression level of BCL-2 was analyzed by quantitative real-time

polymerase chain (qRT-PCR) using Universal Probe Library probe (Prob No. 75, Roche, 04688988001), Lightcycler 480 Probe Master Mix kit (Roche, 04707494001) and LightCycler 480 instrument. Actin beta (ACTB Gene Assay, Roche, 05532957001) was used as endogenous reference gene for normalization.

Statistical Analysis

The computed tomographic (CT) values obtained from qRT-PCR experiments were put into the formula ΔCT=2(reference CT - target gene CT) to determine the expression levels of both miR-210 and *BCL-2*. Microsoft Office Excel was utilized for calculations and graphics.

Raw data of each group were statistically analyzed on GraphPad InStat DTCG 3.06 software by performing Kruskal-Wallis test followed by Dunn's multiple comparisons test. $p<0.05$ was considered statistically significant. Data are presented as mean ± standard deviation.

Results

Cytogenetic Findings

The karyotype results of 7 patients with AML constituting the patient group as a result of conventional cytogenetic methods performed in bone marrow material are shown in Table 1. Clonal structural and numerical abnormalities were observed in some of the patients (Figure 1).

Peripheral blood of the control group consisting of 7 volunteers was also studied by using the conventional cytogenetic method and karyotype analysis was performed. Normal karyotype findings were seen in all volunteers.

miRNA-210 and *BCL-2* Expression Levels

The *BCL-2* mRNA expression levels in both leukocytes and bone marrrow of patients with AML were found to be increased compared to the expression levels of leukocytes of the healthy individuals ($p<0.05$ and $p<0.05$, respectively) (Figure 2).

The miR-210 expression level of leukocytes of patients with AML was higher than the level of bone marrow of the patients with AML ($p<0.05$). Yet, there was no difference between patients and healthy individuals ($p>0.05$) (Figure 3). It was important to note that one patient was not included in statistical analysis given the low levels of miR-210 expression (cycle threshold was higher than 35 in qRT-PCR).

A correlation analysis was also performed between *BCL-2* and miR-210 expression levels. A positive correlation between *BCL-2* and miR-210 was found in bone marrow samples of patients with AML ($r2=0.89$; 95% confidence interval: 0.77-0.99; $p<0.0001$) (Figure 4).

Discussion

Dysregulation of miRNA regulation leads to disruption in the hematopoietic system and can cause leukemia (21). It has been supported by studies that miRNA-210 plays a role as a tumor suppressor or oncomir according to cancer type (22). Most studies have shown that high miR-210 levels in cancerous tissues are associated with poor prognosis (23-27). However, there are also studies showing opposite results (23,24,28-30).

In the literature, a single study has been found showing the expression of miR-210 in patients with AML. In this study, the level of miR-210 expression in the peripheral blood and bone marrow material of patients with AML was investigated. In the findings obtained, overexpression of miR-210 in patients with AML in both serum and bone marrow was compared with the healthy control group. There was no relationship between serum miR-210 level and age, sex, white blood cell amount and complete remission. It was found to be related to cytogenetic findings and FAB classification. In addition, it was found that the overall survival rate was worse and the survival rate without relapse was worse in patients with high miR-210 levels. The findings showed that miR-210 might be a prognostic marker for AML (13).

In our study, the level of miR-210 expression detected from peripheral blood of patients with AML was found to be significantly higher than the expression level detected from bone marrow, yet there was no difference between patients and healthy individuals. This result is not fully compatible with the literature. A reason for this may be due to the low number of patients.

The *BCL-2* is classified as an oncogene (31). Ongoing studies show that *BCL-2* damage causes cell death. It is also known that *BCL-2* plays a role in developing resistance to chemotherapeutic agents (32).

In AML, over-expression of *BCL-2*, resistance to chemotherapy and low overall survival rates were first investigated in a study by Tóthová et al. (16). Data from another study conducted in later years shed some points light on *BCL-2*'s expression and its role in disease. In most of the patients studied, *BCL-2* expression was significantly higher and was associated with poor clinical prognosis and poor response after intense chemotherapy. High *BCL-2* expression level supports the hypothesis that the apoptotic

Table 1. Patients age, gender, FAB classification, and results of conventional cytogenetic analysis

Patient	Age	Sex	Diagnosis	Karyotyping
1	24	M	AML M2	46,XY[4] *NCA[2]
2	21	M	AML M4	41~44,XY,-18[3],-21[3],-22[3][cp6]/46,XY[5]
3	62	M	AML M4	40~47,XY,+4[2],-5[3],-9[3],inv(9)(p11q13)[8],add(21)(p11)[3],-22[3],+mar1[3],+mar2[2] [cp9]/46,XY,inv(9)(p11q13)[10]**
4	52	F	AML M5	***-
5	42	F	AML	46,XX[2] *NCA[3]
6	61	M	AML	25~27,X,+2[3],+5[2],+9[2],+12[2],+20[2][cp3]/35~44,XY,-6[4],-8[4],-9[3],-21[3] [cp6]/46,XY[7]
7	84	M	AML	27~34,X,+2[2],+6[2],+13[2],+18[2],+21[2],+22[2][cp2]/39~45,XY,-16[4],-21[3] [cp7]/46,XY[16]

*non-clonal abnormalities, **inv(9)(p11q13) finding is polymorphic, ***No quality metaphase to evaluate,
FAB: French-American-British classification, AML: Acute myeloid leukemia

mechanism contributes to tumorigenesis. In addition, anomaly in *BCL-2* protein has been shown to cause longer survival of cancerous cells, thereby negatively affecting drug resistance and chemotherapy response with increased white blood cells. And also, data obtained as a result of experiments suggested that the functional role of *BCL-2* was to block apoptosis without affecting cell proliferation (33-35). In our study, we found that mRNA expression level of *BCL-2* in bone marrow samples of newly diagnosed patients with AML was found to be significantly higher than the levels in peripheral blood samples of healthy subjects. In addition, the level of *BCL-2* expression in peripheral blood from patients was significantly increased compared to the healthy individuals. The data we obtained are compatible with the data in the literature. Compared to the level of *BCL-2* expression in bone marrow and peripheral blood, *BCL-2* expression in the bone marrow was found to be higher. This may be due to the high expression of *BCL-2* in tissues with more apoptosis, such as bone marrow (36). In our patients, higher *BCL-2* expression in the bone marrow is compatible with the literature in this regard.

Among the target genes of miR-210, when the databases showing miRNA target genes were examined, *BCL-2* could not be found. In our study, it was investigated whether miR-210 and *BCL-2* levels showed correlation in AML. When the studies on this subject in the literature were examined, a study supporting the increase theory was found. Data obtained in the study of neurons in cell culture and PC12 cell line in mice showed that over-expression of miR-210 suppressed apoptosis in a correlation with increased *BCL-2* level (37). The data we obtained in our study showed that *BCL-2* expression and miR-210 expression in the bone marrow of patients with AML were positively correlated. The increase of miR-210 and hence *BCL-2* confirms the theory that leukemia cells increase by suppressing apoptosis in AML, and therefore causing disease deterioration. This findings may indicate that *BCL-2* is not a target of miR-210 and miR-210 may regulate apoptosis through other targets, such as HIF-1-a (38). More comprehensive studies are needed to clarify this mechanism more clearly.

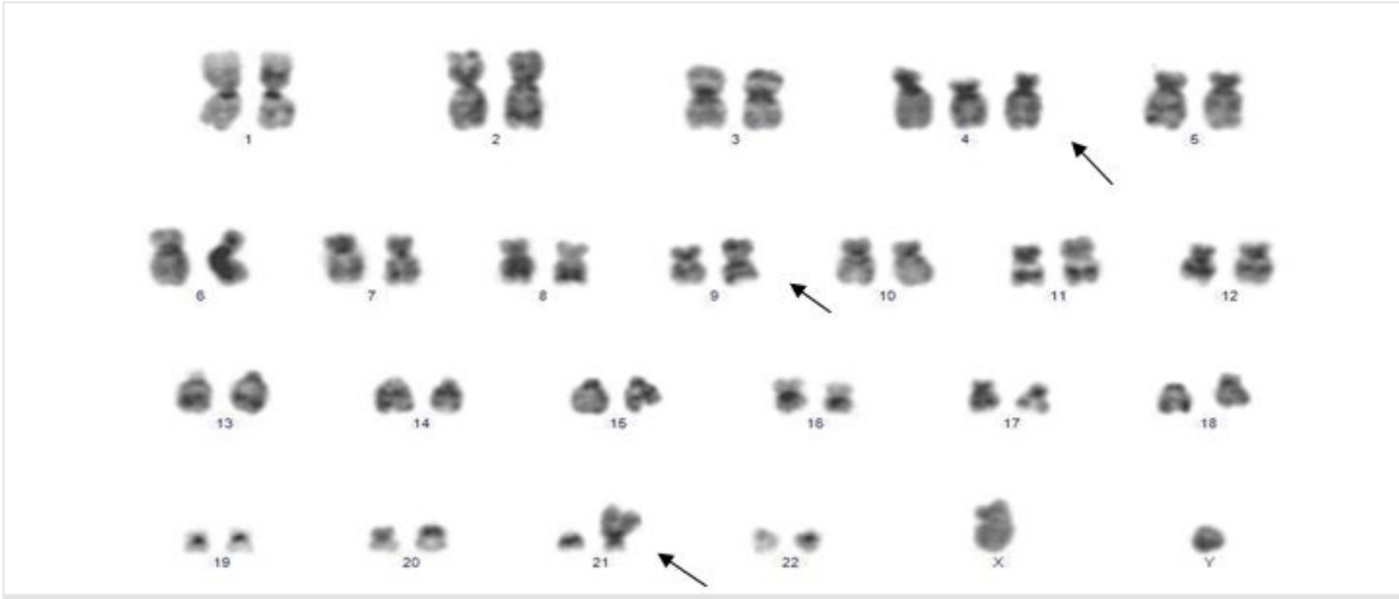

Figure 1. Karyotype analysis of patient 3 including numerical and structural chromosomal abnormalities.
Karyotype: *47,XY,+4,inv(9)(p11q13),add(21)(p11)*

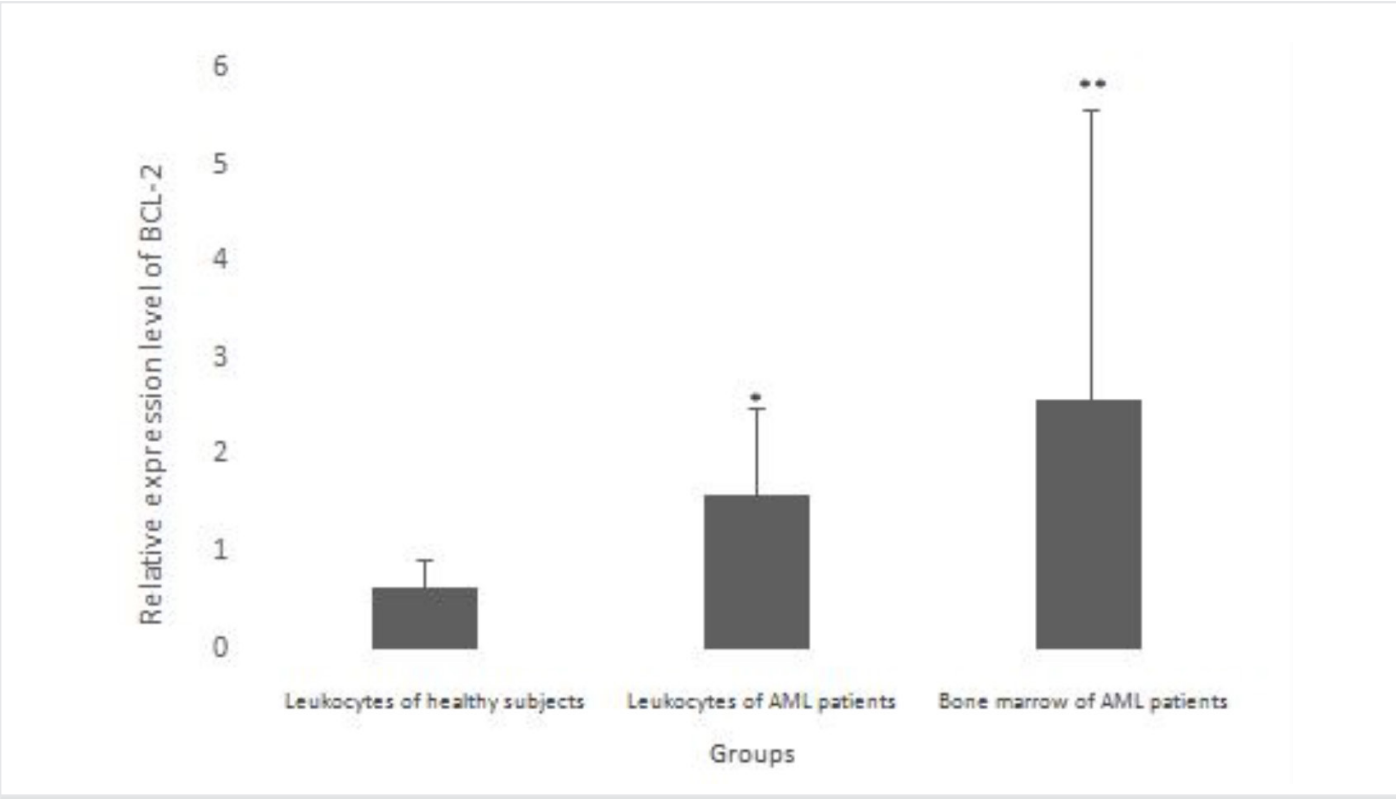

Figure 2. BCL-2 mRNA expression levels of patients with AML and healthy individuals
AML: Acute myeloid leukemia

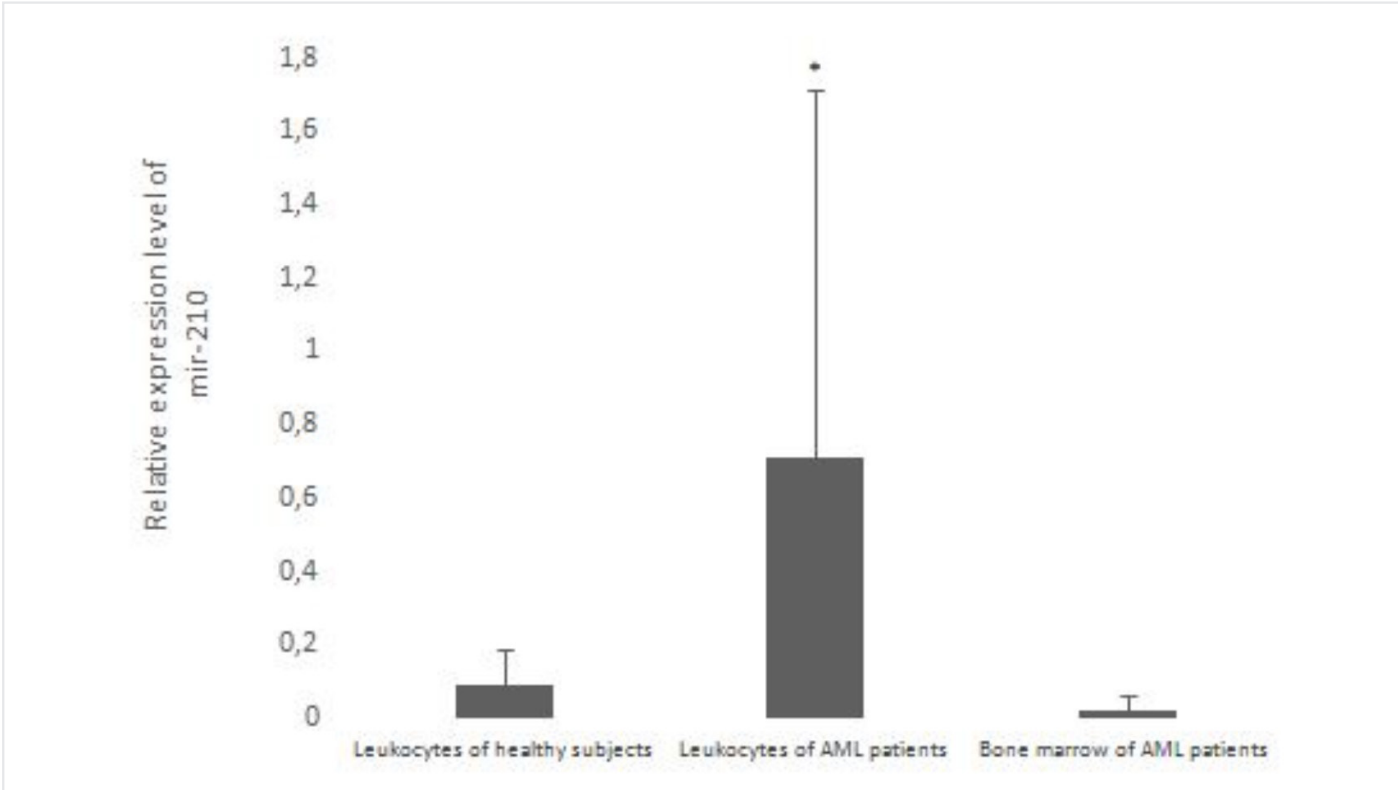

Figure 3. mir-210 expression level of patients with AML and healthy individuals
AML: Acute myeloid leukemia

Cytogenetic findings play an important role in the prognosis of AML. In a study of patients with AML, the presence of only trisomy 21 (+21) or the presence of +21 with additional anomalies was found to be associated with poor prognosis. A poor prognosis is predicted especially in patients with AML over 60 years old with only +21 (39). In our study, clonal +21 findings were found in one patient. Also, other numerical chromosomal anomalies were seen in this patient. Although our findings were consistent with the literature, the age of our patient was 84, which supported the prediction of poor prognosis in patients over 60 years of age.

In the bone marrow material of 3 patients examined in our study, monosomy 21 (-21) was found clonally. However, the finding of -21 was accompanied by several numerical chromosomal anomalies. In the literature, there were patients in whom only -21 was seen in AML and other hematological diseases (such as multiple myeloma and myelodysplastic syndrome (MDS) (40). In an article, it was predicted that the loss or increase of chromosome 21 in MDS would cause a moderate prognosis (41). MDS is a hematological disease that has the potential to transform into AML. Therefore, the -21 anomaly seen in the patients examined in our study may be significant in terms of prognosis.

According to the data obtained from the literature, patients with monosomal karyotype and complex karyotype are associated with poor prognosis. Patients who have a poor prognosis or complex karyotype have a worse prognosis than patients who have a monosomy karyotype. According to the data obtained from previous studies, -5, -7, -8, -17, -21, -22 are the most commonly seen monosomies (42). In our study, monosomies of chromosomes -5, -8, -21 and -22 were observed in some patients. In these patients, both the monosomal karyotype and complex karyotype were detected. Especially in one patient, the disease progressed very shortly after diagnosis, resulting in hospitalization and death. In patient 6, the monosomal karyotype (monosomy 8 was also present) and complex karyotype findings, which were indicative of poor prognosis, were seen together. At the same time, because our patient was over 60 years old, it was compatible with the thesis that poor prognosis in patients with AML over 60 years old.

Study Limitations

We encountered some limitations during our study. One of them was to find a patient with *de novo* AML who had not yet received treatment. For this reason, we could not reach the target number of patients. Another limitation was the lack of sufficient financial resources to perform apoptotic tests. Therefore, additional studies are needed to support our apoptosis theory. The last limitation was that healthy bone marrow samples could not be part of our study due to the ethical reasons. For this reason, *BCL-2* and miR-210 expression levels in bone marrow samples of patients with AML were compared with the leukocytes of healthy volunteers instead of bone marrow samples.

Conclusion

As a result, in our study, increased expression levels of miR-210 and *BCL-2* were observed in the bone marrow material in AML, and findings indicating that this increase occurred in a correlation were obtained. It was concluded that this increase might cause disruption in the apoptosis mechanism and might have negative effects on prognosis. In addition, as a result of the data we obtained, we believe that the expression of miR-210 and *BCL-2* can be useful as a biomarker for AML disease. In addition, we think that the cytogenetic findings obtained will be beneficial in terms of prognosis evaluation. We believe that the effects of miR-210 and *BCL-2* on the apoptosis pathway, especially in AML, should be investigated and a larger number of samples and comprehensive tests are needed to clarify this mechanism and obtain precise results.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine (07.09.2016-327301).

Informed Consent: Written consent form was obtained from all patients and volunteers before participating in the study.

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Authorship Contributions

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Original Article

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Effects of Sulfur Containing Glycine Imine Derivatives Compounds on Multidrug Resistance Proteins (MRPs) and Apoptosis Mechanism in MCF-7 and DLD-1 Cell Lines

Kükürt İçeren Gilisin İmin Türevi Bileşiklerin MCF-7 ve DLD-1 Hücre Hatlarında Çoklu İlaç Direnci Proteinleri (MRP'ler) ve Apoptoz Mekanizması Üzerine Etkileri

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ABSTRACT

Objective: Glutathione (GSH) is a tripeptide consisting of glycine, glutamic acid and cysteine. If the sulfur containing amino acids like methionine and cysteine increase in the cells, the level of GSH increases. GSH is one of the most important and powerful antioxidants in the body and it reduces oxidative stress. Reduction of GSH affects apoptosis activity and multidrug resistance proteins (MRPs). In the present study, we investigated the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanism in the MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines.

Methods: In MCF-7 and DLD-1 cell lines; mRNA levels of MRPs (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2), apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), heat shock proteins (HSPs) and endoplasmic reticulum chaperone proteins (GRPs) were determined by qRT-PCR method.

Results: Compounds decreased gene expression of multiple drug resistance (MDR) genes and increased gene expression of pro-apoptosis mechanism genes (BAX, P53, CASP3). HSPs and BCL-2 and PARP gene expressions decreased. There was no significant decrease in gene expression of GRPs. The compounds were shown

ÖZ

Amaç: Glutatyon (GSH), glisin, glutamik asit ve sisteinden oluşan bir tripeptiddir. Metiyonin ve sistein gibi kükürt içeren amino asitler hücrelerde artarsa GSH seviyesi yükselmektedir. GSH vücuttaki en önemli ve güçlü antioksidanlardan biridir ve oksidatif stresi azaltmaktadır. GSH'nin azaltılması, apoptoz aktivitesini ve çoklu ilaç direnci proteinlerini (MRP'ler) etkilemektedir. Bu çalışmada, kükürt içeren glisin imin türevlerinin, MCF-7 (meme kanseri) ve DLD-1 (kolon kanseri) hücre hatlarında çoklu ilaç direnci proteinlerine (MRP'ler) ve apoptoz mekanizmasına etkisini araştırmayı amaçladık.

Yöntemler: MCF-7 ve DLD-1 hücre hatlarında; çoklu ilaç direnci proteinleri (ABCB1, ABCC3, ABCC10, ABCC11 ve ABCG2), apoptoz mekanizması proteinleri (BAX, BACL-2, P53, PARP, CASP3), ısı şoku proteinleri (HSP'ler) ve endoplazmik retikulum (ER) GRP'ler mRNA seviyeleri qRT-PCR yöntemi ile belirlendi.

Bulgular: Bileşikler çoklu ilaç direncinde (MDR) gen ekspresyonunu azalttı ve pro-apoptoz mekanizması genlerinde (BAX, P53, CASP3) ekspresyonu artırdı. HSP'ler, BCL-2 ve PARP gen ekspresyonları azaldı. GRP gen ekspresyonunda önemli bir azalma olmadı. Bileşiklerin, ABCC3 dışındaki çoklu ilaç direnci genleri (ABCB1,

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to have remarkable effects on MDR genes (ABCB1, ABCC10, ABCC11 and ABCG2) other than ABCC3. Compounds were found to have significant effects on apoptosis mechanism genes and HSPs.

Conclusion: Our results indicate that the sulfur-containing glycine imine derivatives can be a potent option as a cancer drug, especially in breast and colon cancers.

Keywords: MCF-7, DLD-1, sulfur, glycineimine, MRPs, apoptosis

ABCC10, ABCC11 ve ABCG2) üzerinde önemli etkilere sahip olduğu gösterilmiştir. Ayrıca bileşiklerin apoptoz mekanizması genleri ve HSP'ler üzerinde önemli etkilere sahip olduğu tespit edilmiştir.

Sonuç: Sonuçlarımız, sülfür içeren glisin imin türevlerinin, özellikle meme ve kolon kanserinde anti-kanser ajanı olabilecek önemli ve güçlü bir seçenek olabileceğini göstermektedir.

Anahtar Sözcükler: MCF-7, DLD-1, kükürt, glisinimin, MRP'ler, apoptoz

Introduction

Glycine (NH_2CH_2COOH), which is unique because it has no isomeric forms and is a structural unit for many proteins, has been the subject of numerous studies (1). Sulfur-containing amino acids play major roles in synthesis, structure, and function of proteins (2). Sulfur is a critical element and it is found in the structure of cysteine and methionine, and it is necessary for the biosynthesis of sulfolipids, antioxidants, cofactors, secondary metabolites and amino acids (3,4). Glutathione [g-glutamyl-L-cysteinylglycine; (GSH)] containing glycine and cysteine amino acids is the strongest antioxidant in the body. It is found in almost all tissues (at concentrations of 1-10 mM) (4-6). GSH is a necessary endogenous tripeptide for the prevention of neoplasms, liver disorders, corneal disorders, eczema, heavy metal poisoning, and to prevent the negative effects of radiation therapy (7,8). Supplementing of component amino acids (cysteine, glycine and glutamate) increases GSH synthesis in tissue. Studies have reported that high GSH concentration is effective against cellular damage, tissue degeneration and disease progression (8,9). GSH is a protein that fights oxidative stress and regulates cell proliferation, apoptosis, signal transduction, gene expression, insulin resistance, immune function and fibrogenesis (4,10,11). It has been reported that negative changes in GSH homeostasis cause obesity, cancer, AIDS, diabetes mellitus and heart disease (8,12) and GSH regulates cell death. GSH level affects the expression and activity of caspases and signal molecules that are important in cell death (12). GSH levels decrease during apoptosis. Many studies suggest that GSH output through the cell during apoptosis is associated with multiple drug resistance proteins (MRP) (4,13-15).

Several members of the MRP family, ABCC1 (MRP1), ABCC2 (MRP2), ABCC4 (MRP4), ABCC5 (MRP5) and ABCC7 (CFTR) have been shown to mediate GSH transportation (14). MRPs can transport structurally and mechanically different drugs, including natural anticancer drugs, nucleoside analogues, antimetabolites and tyrosine kinase inhibitors (16). MRPs are included in the ABCC subfamily of the ABC transporter family (17,18). Expression of ABCC proteins is seen in most human tissues (18). P-glycoprotein (P-gp; ABCB1), the first discovered member of the ABC (ATP-binding cassette) transporter family, is a membrane protein that works as an efflux pump (19-21). Proteins specific to breast tissue; ABCB1 (MDR1-P-gp), ABCC11 (MRP8), ABCC12 (MRP9), ABCG2 (BCRP) and colon tissue-specific proteins; ABCB1 (MDR1-P-gp), ABCC3 (MRP3), ABCC10 (MRP7), ABCC11 (MRP8), ABCC13 (Pseudogene), ABCG2 (BCRP) belong to the ABC family (18,22).

The p53 protein is a tumor suppressor gene consisting of 393 amino acids weighing 53 kDa. The p53 gene makes a choice between life and death (apoptosis) (23-25). Generally, p53 is upregulated or mutated in MRPs of cancer cells (26,27). Intracellular BCL-2/BAX ratio is extremely important in determining whether the cell will go to apoptosis. If BAX is too much, the cell will go to apoptosis, or if BCL-2 is too much, apoptosis will be inhibited (28-30). Activation of effector caspases such as Caspase-3 causes downstream of PARP and similar substrates. Thus, it initiates apoptotic cell death (31). PARP down regulation suppresses cell proliferation and causes apoptosis via the p53 signal pathway (32). HSPs play a role as molecular chaperones that ensure the correct folding of the newly synthesized proteins or stress-dependent misfolded proteins and also prevent aggregation of the proteins (33-35). Expression of HSPs plays a role in regulating apoptosis, immune response to tumors, and multiple drug resistance. Increased HSPs levels make cells more resistant to apoptosis (36,37). Glucose regulated proteins (GRPs) are molecular chaperones in cancer tissues that are upregulated by cellular response to various stressful conditions such as glucose deprivation, oxidative stress and hypoxia (38-40). At the present time, the elimination of drug resistance (41,42) and increasing the ability to direct resistant cells to apoptosis (43,44) are the main targets for research and development of anti-cancer agents.

In this study, it was aimed to investigate the effects of sulfur containing glycine imine derivatives compounds on MRPs and apoptosis mechanism in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines.

In our previous study, three different compounds were synthesized from glycine imine containing sulfur. Antioxidant and cytotoxic activity studies of the compounds were carried out using five different methods. The compounds were shown to exhibit antioxidant and cytotoxic activities in MCF-7 and DLD-1 cells (45). As a continuation of the study, the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanisms in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines. In our study, we showed that sulfur glycine imine-derived compounds had the ability to direct drug-resistant cells to apoptosis and exhibited significant activity in MCF-7 and DLD-1 cells.

Methods

In MCF-7 and DLD-1 cell lines; mRNA levels of MRPs (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2), apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), heat shock proteins (HSPs) and endoplasmic reticulum (ER) chaperone proteins (GRPs) were determined by qRT-PCR method.

Cell Culture

In this study, *in vitro* studies were used for MCF-7 (ATCC® HTB22™) (breast adenocarcinoma) DLD-1 (colon cancer) (ATCC® CCL221™) human cell lines. MCF-7 and DLD-1 cells were cultured using 25 g/100 mL sodium bicarbonate, 10% fetal bovine serum (FBS) RPMI-1640 (Roswell Park Memorial Institute) mediums containing 1 % penicillin/streptomycin in 25 cm^2 or 75 cm^2 flask, 5% CO_2 and it was produced by incubation for 24 hoursat 37 °C. Stock solutions were made in dimethyl sulfoxide (DMSO) (Sigma, Steinheim, Germany) and were made with culture medium. Controls were cultured in RPMI 1,640 medium with a final concentration of 0.1% of DMSO but without compounds (46).

qRT-PCR Assay

Total RNA Isolation

The MCF-7 (breast cancer) and DLD-1 (colon cancer) cells were incubated at 37 ºC for 24 hours, they were added to the 6 plates using a number of $2x10^6$ cells. Cells with compound dose determined as 50 µM according to MTT results were washed with 1X PBS at the end of 24 hours and 1X trypsin was removed with EDTA. In order to evaluate expression at the gene level, mRNA extraction was studied in the control and dose groups in accordance with the method recommended in the kit (Thermo Fisher; RNA isolation kit [K0731]). The concentration and purity of the isolated RNA were carried out with the aid of the nanodrop device. In the process of measuring the RNA samples with nanodrop, the RNA samples were diluted at appropriate concentrations (ng/µL) and they were measured at 260 and 280 nm (µg/mL) = [optical density 260x dilution rate x 40 (µg/mL)] (A260/A280≅2.0). These RNA samples were included in the study.

cDNA Synthesis

The complementary chains of isolated total RNAs were converted into cDNA by synthesizing them with "Thermo Fisher; cDNA synthesis kit (K1622)" using oligo d (T) primer and reverse transcriptase enzyme (RT). In the 1st stage of cDNA synthesis; it was kept at 65 °C for 5 minutes. In the 2nd stage; it was kept for 1 hour at 42 °C and 5 minutes at 70 °C to inhibit the enzyme after incubation. Synthesized cDNAs were kept at -20 °C for qPCR.

qReal Time PCR (qRT-PCR)

The sequences of genes were determined by NCBI database. In line with the accessory numbers listed in the Table 1, the primers required for real-time PCR were designed with the help of the NCBI primary blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast). (Thermo Fisher; K0221); Syber Green Master Mix (2x) 5 µL Forward primer (10 mM) 1 µL, Reverse primer (10 mM) 1 µL, cDNA 2 µL and Nuclease free water 1 µL, using Piko Real 96 (Thermo Scientific) device and by applying the amplification program, mRNA expression levels were examined. All samples were studied in duplicate. After real time PCR, the melt curve analysis was performed and the specificity of the primers was tested.

Results

Chemistry

We performed this study to understand MRPs and apoptosis mechanism of ethyl 2-((bis(propylthio)methylene)amino)acetate (1), ethyl 2((bis(ethylthio)methylene)amino)acetate (2), and ethyl 2-((bis(methylthio)methylene)amino) acetate (3) on the two different cancer cell lines (Figure 1).

Glycineimine Derivatives Compounds Regulate mRNA Expression of MRPs

In MCF-7 cells; *ABCB1* gene expression was reduced by 0.6-fold, ABCC10 gene expression by 0.6-fold, *ABCC11* gene expression by 0.5-fold, and *ABCG2* gene expression by 0.3-fold with compound **I** compared with the untreated control. *ABCB1* gene expression was reduced by 0.6-fold, *ABCC10* gene expression by 0.6-fold and *ABCC11* gene expression by 0.4-fold with compound **II** compared with the untreated control. *ABCB1* gene expression was reduced by 0.7-fold, *ABCC10* gene expression by 0.4-fold, *ABCC11* gene expression by 0.5-fold, and *ABCG2* gene expression by 0.2-fold with compound **III** compared with the untreated control.

In DLD-1 cells; *ABCB1* gene expression was reduced by 0.4-fold, ABCC10 gene expression by 0.6-fold and *ABCG2* gene expression by 0.8-fold with compound **I** compared with the untreated control. ABCB1 gene expression was reduced by 0.2-fold and ABCG2 gene expression by 0.7-fold with compound **II** compared with the untreated control. ABCB1 gene expression was reduced by 0.7-fold, *ABCC10* gene expression by 0.4-fold and *ABCG2* gene expression by 0.2-fold with compound **III** compared with the untreated control.

Compound **I** reduced the expression of all other *MDR* genes except ABCC3 compared to negative control in the MCF-7 cells. It was found that expression was decreased in other genes except ABCC3 and ABCC11 in DLD-1 cells . Compound **II** reduced expression in only *ABCB1* and *ABCC11* genes by half in MCF-7 cells. In DLD-1 cells, expression was decreased only in *ABCB1* and *ABCG2* genes. Compound **III** reduced expression in ABCB1, ABCC11 and *ABCG2* genes by half in MCF-7 cells. Expression in *ABCB1*, *ABCC10* and *ABCG2* genes in the DLD-1 cells decreased significantly (Figure 2).

Glycineimine Derivatives Compounds Regulate mRNA Expression of HSPs and Apoptotic Genes

Compared to untreated control; gene expression of BAX was increased by 2-fold and p53 by 4.3-fold, and gene expression

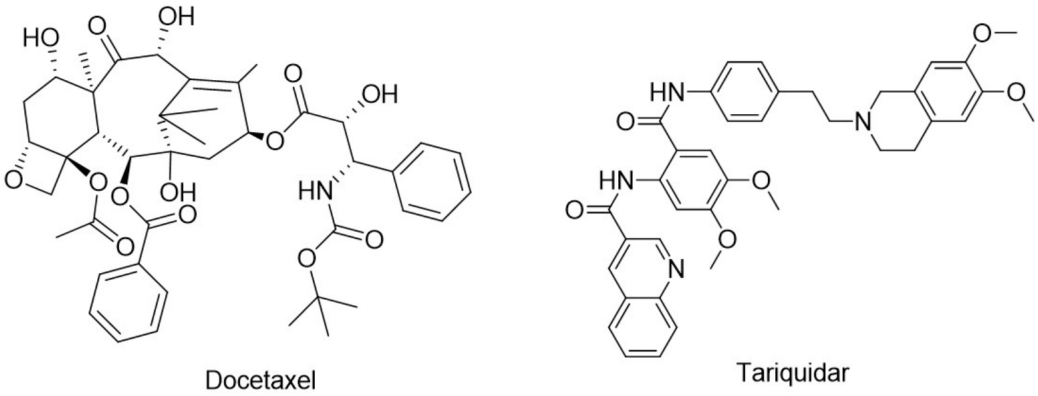
Table 1. Primer sequences, references, and product lengths

Genes	Primer sequences (5'-3')	References	Product length	Melting temperature
β-Actin F	TGACGTGGACATCCGCAAAG	NM_001101.5	205	51
β-Actin R	CTGGAAGGTGGACAGCGAGG			
ABCB1 F	GTTCAGGTGGCTCTGGATAAG	NM_001348946.1	93	55
ABCB1 R	AGCGATGACGTCAGCATTAC			
ABCC3 F	TACTCCAAGACAGAGACAGAGG	NM_003786.4	111	53
ABCC3 R	CCGGTAGCGCACAGAATAAT			
ABCC10 F	TCACCCTGTCTCCACTGTAT	NM_001198934.1	133	49
ABCC10 R	AACTGGCACCTCTGGTTTAG			
ABCC11 F	GTGGTGCTGATCGTCTTCTT	XM_017023801.2	106	53
ABCC11 R	CCATGGTTCCATTGCTCTCT			
ABCG2 F	TCGTACTGGGACTGGTTATAGG	XM_017008852.2	101	53
ABCG2 R	GTTGGTCGTCAGGAAGAAGAG			
BAX F	TCATGGGCTGGACATTGGAC	NM_001291428.1	114	59
BAX R	GAGACAGGGACATCAGTCGC			
BCL2 F	ATCTGGGCCACAAGTGAAGTC	NM_000633.2	209	59
BCL2 R	TTCGACGTTTTGCCTGAAGAC			
PARP F	GAATGCCAGCGTTACAAGCC	NM_001618.4	212	59
PARP R	TCTCCCTGAGACGTATGGCA			
P53 F	GTTTTCCCCTCCCATGTGCTC	NM_001126112.2	170	53
P53 R	CAGTCTGGCCAATCCAGGGAAG			
CASP3 F	GCGGTTGTAGAAGAGTTTCGT	NM_001354777.1	146	59
CASP3 R	TTATTAACGAAAACCAGAGCGCC			
HSP27 F	GAGGAGCATAAAAGCGCAGC	NM_001540.5	347	60
HSP27 R	CTAACCACTGCGACCACTCC			
HSP40 F	GAGGGGTTGTGAATGCAGGAG	NM_001300914.1	246	59
HSP40 R	CTCAGCAAACATGGCATGAGG			
HSP60 F	GACGACCTGTCTCGCCG	NM_002156.5	258	60
HSP60 R	GGACTTCCCCAACTCTGCTC			
HSP70 F	AAGGAGACAGCCGAAAGTGT	L12723.2	212	57
HSP70 R	CTTGGTTTCTCTTCTAAGCGAGG			
HSP90 F	GCGCTAGCAGGAGATGGTTA	NM_005348.3	103	59
HSP90 R	TTTCTGTGCCTACGTGTGCT			
GRP78 F	GAACGTCTGATTGGCGATGC	NM005347.5	143	59
GRP78 R	ACCACCTTGAACGGCAAGAA			
GRP94 F	GCCAGTTTGGTGTCGGTTTC	NM_003299.3	168	59
GRP94 R	GGGTAATTGTCGTTCCCCGT			

of BCL-2 was decreased by 0.2-fold, PARP by 0.5-fold, HSP60 by 0.5-fold, HSP70 by 0.8-fold, and HSP90 by 0.7-fold with compound **I** in MCF-7 cells. Gene expression of p53 was increased by 3.2-fold and gene expression of HSP27 was decreased by 0.5-fold, HSP40 by 0.9-fold, and HSP90 by 0.7-fold withcompound **I** in DLD-1 cells.

Compared to untreated control; gene expression of BAX was increased by 3-fold, Caspase-3 by 3-fold, p53 by 14.4-fold, and gene expression of BCL-2 was decreased by 0.8-fold, PARP by 0.2-fold, HSP60 by 0.3-fold, and HSP70 by 0.7-fold with compound **II** in MCF-7 cells. Gene expression of p53 was increased by 3.8-fold and gene expression of HSP40 was decreased by 0.8-fold, and HSP90 by 0.4-fold with compound **II** in DLD-1 cells.

Compared to untreated control; gene expression of Caspase-3 was increased by 2.7-fold, and p53 by 9.5-fold, and gene expression of BCL-2 was decreased by 0.9-fold, PARP by 0.6-fold, HSP40 by 0.5-fold, HSP60 by 0.4-fold, HSP70 by 0.8-fold, and HSP90



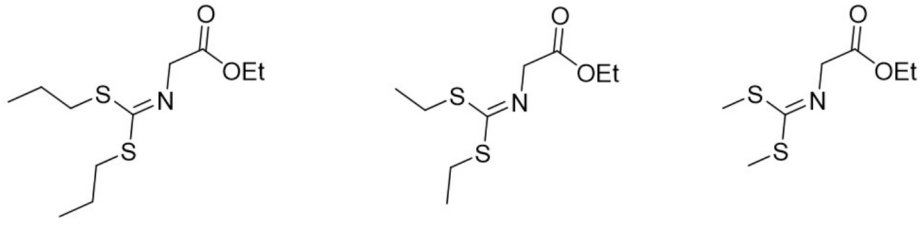


Figure 1. Docetaxel, Tariquidar chemotherapeutic drug (include aminoaside ester units) and alkyl substituted sulfur-containing glycine imine (**I**, **II**, and **III**)

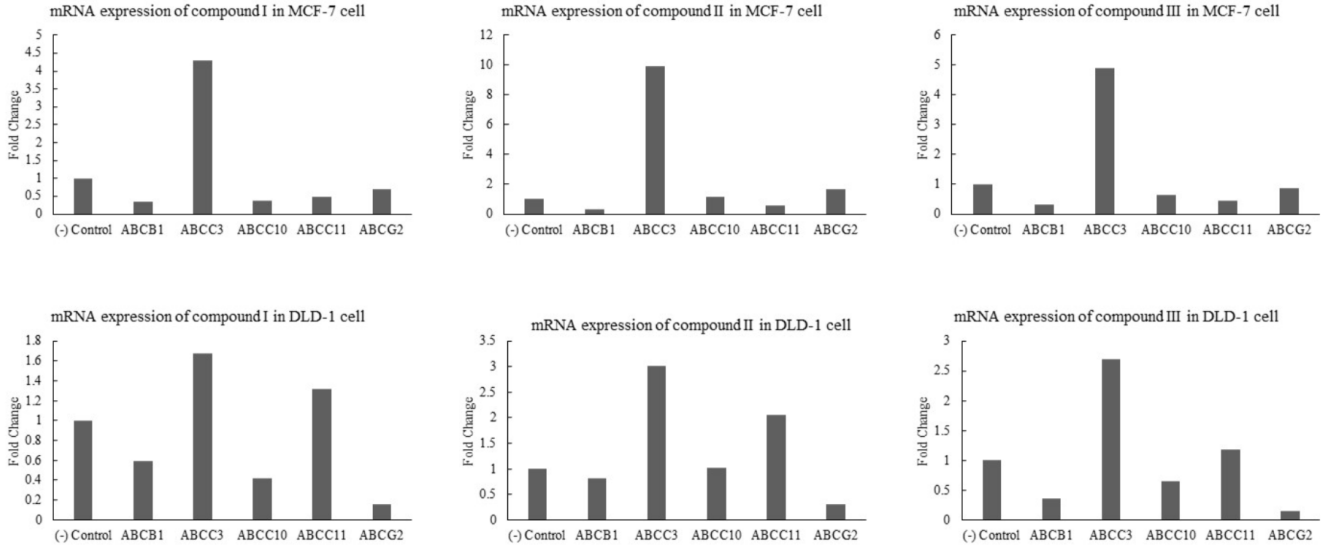


Figure 2. Glycine imine derivatives compounds regulate multidrug resistance in MCF-7 and DLD-1 cell lines. mRNA expressions of ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2 in cells are measured by qRT-PCR, normalized to β-actin

by 0.5-fold with compound **III** in MCF-7 cells. Gene expression of p53 was increased by 1.8-fold, and gene expression of PARP was decreased by 0.3-fold, HSP70 by 0.5-fold, and HSP90 by 0.3-fold with compound **III** in DLD-1 cells.

Compound **I** increased the expression of p53, BAX, CASP3 and GRPs in the MCF-7 cells. BCL-2 reduced the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cells and the expression of HSPs was decreased except HSP60. Compound **II** increased the expression of p53, BAX and CASP3 in the MCF-7 cells. It only reduced HSP60 and HSP70 expressions. Expression of p53 was increased in DLD-1 cells. HSP40, HSP70 and HSP90 expressions were decreased. Compound **III** increased the expression of p53 and CASP3 in the MCF-7 cells. BCL-2 reduced the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cells. PARP, HSP27 and HSP90 expressions were decreased. Compounds (**I**, **II** and **III**) did not have a significant effect on *GPRs* genes' (GRP78 and GRP94) expression (Figure 3).

Discussion

In our previous study, it was demonstrated that sulfur-containing glycine imine derived compounds had antioxidant and cytotoxic activities in MCF-7 and DLD-1 cell lines (45). It was stated that the compounds gave parallel results with other studies in both antioxidant and cytotoxic activities. As a continuation of the study, the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanisms were determined in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines. It was observed that biological activity studies were limited in the literature reviews of glycine imine derived compounds containing sulfur. Biological activity studies with these compounds were carried out for the first time by our group.

GSH, (2S) -2-amino-5 - [[(2R) -1- (carboxymethylamino) -1-oxo-3-sulfanylpropan-2-yl] amino] -5-oxopentanoic acid, and tripeptide are found in all tissues. GSH consists of L-cysteine, L-glutamic acid and glycine, forming L-gamma-glutamyl-Lcysteinylglycine amino acids (47). GSH in cancer cells is important in the regulation of mutagenic mechanisms, DNA synthesis, growth, and multidrug and radiation resistance (48). GSH showed inhibition of apoptosis in HeLa (49), in HSC-2 cancer cell lines (50), and in Calu-6 lung cancer cells (51). GSH levels decrease rapidly during apoptosis, and this is known as a biochemical feature of oxidant-induced programmed cell death (52). GSH inhibits the induction of apoptosis in human lung cancer cells via down-regulation of survivin (inhibitor of the apoptosis protein family). In drug-resistant cells, GSH protects healthy cells from oxidative stress, but prevents induction of apoptosis in tumor cells (53). In addition, GSH describes a new pathway of apoptosis dependent on PKC-delta activation and independent of p53, Bcl-2 and Bax levels (54). Fico et al. (13), 2008 reported that 2-deoxy-D-ribose (dRib) induced apoptosis by activating an oxidative stress by consuming GSH. In our study, the effects of sulfur containing glycine imine derived compounds on apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), HSPs and ER GRPs in MCF-7 and DLD-1 cell lines. The compounds decreased expression in anti-apoptotic (*HSPs*, *BCL-2* and *PARP*) genes, while increased expression in pro-apoptotic (BAX, P53, PARP) genes. Compound **I** increased the expression of p53, BAX, CASP3 and GRPs in MCF-7 cells. BCL-2 decreased the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cell and expression of HSPs was decreased. Compound **II** increased p53, BAX and CASP3 expressions in MCF-7 cells, as well as decreased HSP60 and HSP70 expressions. In DLD-1 cells, p53 expression was increased, moreover HSP40, HSP70 and HSP90 expressions were decreased. Compound **III** increased p53 and CASP3 expressions in MCF-7 cells. BCL-2 effectively reduced the expression of PARP and HSPs. In DLD-1 cells, p53 expression was increased, furthermore PARP, HSP27 and HSP90 expressions were decreased. Decreasing anti-apoptotic gene expressions and increasing pro-apoptotic gene expressions drive cancer cells to apoptosis. The data of the study determined that the compounds decreased HSPs, BCL-2 and PARP gene expressions and increased BAX, P53, CASP3, PARP gene expressions, leading MCF-7, and DLD-1 cancer cell lines to apoptosis.

In studies, Hammond et al. (14), 2007 showed evidence that GSH was released through MRP1 during both internally and externally induced apoptosis. In our study, the effects of sulfur containing glycine imine derived compounds multidrug resistance proteins (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2) in MCF-7 and DLD-1 cell lines were investigated. The compounds decreased expression in multidrug resistance (ABCB1, ABCC10, ABCC11 and ABCG2) genes. Compound **I** effectively reduced the expression of MDR genes in the MCF-7

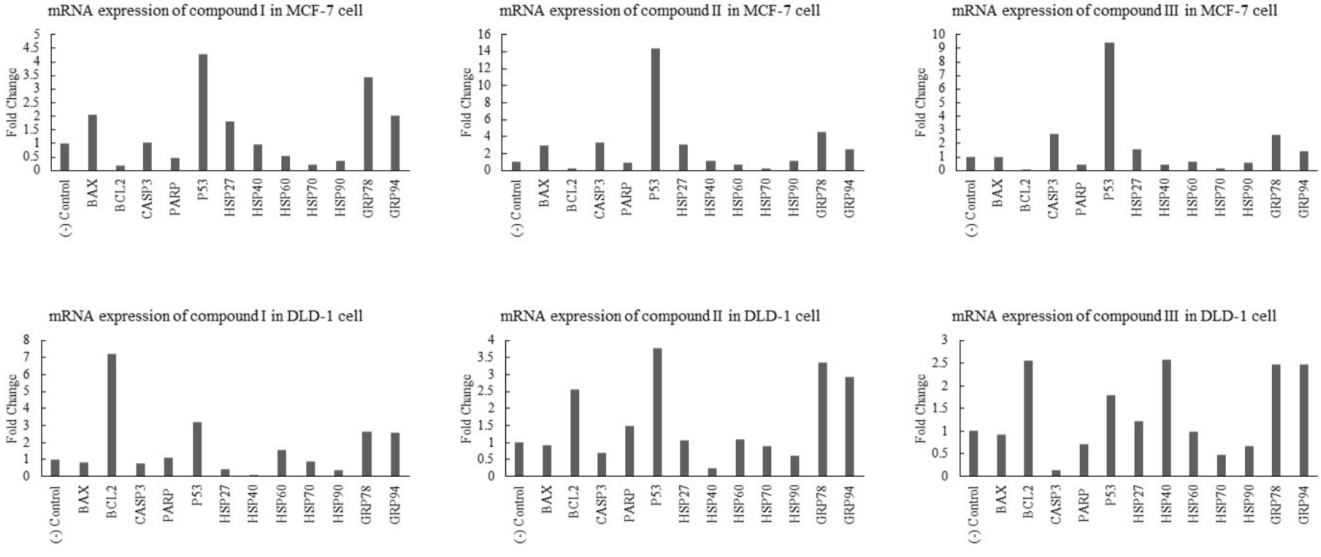


Figure 3. Glycine imine derivatives compounds regulate apoptosis mechanism, HSPs and GRPs in MCF-7 and DLD-1 cell lines. mRNA expressions of apoptosis mechanism, HSPs and GRPs in cells are measured by qRT-PCR, normalized to β-actin

cell compared to the negative control. Compound **I** reduced the expression of *ABCB1*, *ABCCC10* and *ABCG2* genes in DLD-1 cells compared to the negative control. Compound **II** reduced expression of *ABCB1* and *ABCC11* genes by half in MCF-7 cells . Expression of ABCB1 and ABCG2 genes was decreased in DLD-1 cells . Compound **III** halved the expression in the ABCB1, ABCC11 and ABCG2 genes in the MCF-7 cells. Expression in the ABCB1, ABCC10 and ABCG2 genes significantly decreased in DLD-1 cells. The expression of MRPs in the cell membrane prevents cancer cells from drifting into apoptosis. With the compounds used in the study, the expression of proteins that caused drug resistance was suppressed and it was proven that cancer cells could drift into apoptosis.

Conclusion

In brief, in our study, compounds increased gene expression in MDR and apoptosis mechanism genes (BAX, P53, PARP). HSPs and BCL-2 and PARP gene expressions decreased. There was no significant decrease in gene expression of GRPs. The compounds (**I**-**II**-**III**) were shown to have remarkable effects on ABCB1, ABCC10, ABCC11 and ABCG2 genes. Compounds **I**, **II**, and **III** were found to have no effect on the ABCC3 gene in MCF-7 and DLD-1 cells. No significant effect of the compounds (**I**, **II** and **III**) on GPRs genes (GRP78 and GRP94) was detected. Compounds (**I**-**II**-**III**) were found to have significant effects on apoptosis mechanism genes and heat shock proteins (HSPs). Compared to DLD-1 cells, the effects of the compounds on MCF-7 cells were more marvelous.

According to the results of the used compounds on MDR in cells, they had the ability to direct cancer cells to apoptosis. The compounds used in the study were useful for anticancer studies. Derivatives of the compounds were thought to contribute to further studies. Our results showed that sulfur glycine imine derivatives had a potent ability to direct resistant cells to apoptosis, although MCF-7 cells exhibited remarkable activity.

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Ethics

Ethics Committee Approval: The article is a cell culture study. It does not require an ethics committee.

Informed Consent: No patients were used in the study. Informed consent is not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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

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Original Article

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Effect of Pilates Exercises on the Body Composition of Fasting Females

Oruç Tutan Kadınlarda Pilates Egzersizinin Vücut Kompozisyonu Üzerine Etkisi

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ABSTRACT

Objective: This study aimed to evaluate the changes in the body composition of individuals who fasted and did Pilates at least twice a week during Ramadan versus those who fasted but did no Pilates.

Methods: This was an experimental study conducted on 60 healthy women who were admitted to the Noi Pilates in İstanbul Turkey, which was a private Pilates studio. The study was carried out on 60 grown-up, healthy women between 18 and 65 years of age who fasted in Ramadan 2019, some of whom did Pilates and some who did not. The women involved in the study had been doing Pilates for 6 months prior to Ramadan and did Pilates at least 3 times a week during Ramadan.

Results: Significant increases were observed in body mass index (BMI), triceps skin-fold thickness (SFT), muscle mass and fat percentage in the fasting and non-pilates group ($p<0.05$). Our study found the change in the percentage of triceps SFT and fat in women who did Pilates to be more than those who did not. BMI, muscle mass, waist circumference and hip circumference of women who did Pilates and who did not do Pilates during Ramadan did not differ compared to the first measurements, and neither of the two methods dominated in terms of these variables.

Conclusion: While a long period of fasting in Ramadan leads people to inactivity, personalized exercise and nutrition programs planned by experts may result in weight loss, especially for obese and slightly overweight people.

Keywords: Ramadan, pilates, diet, healthy nutrition, exercise, weight management

ÖZ

Amaç: Bu çalışmanın amacı, ramazan ayında oruç tutup haftada en az 2 kez pilates yapan ve sadece oruç tutup pilates yapmayan bireylerin vücut kompozisyonundaki değişimlerin değerlendirilmesidir.

Yöntemler: Bu çalışma, İstanbul, Türkiye'de özel bir pilates stüdyosu olan Noi Pilates'e başvuran 60 sağlıklı kadın üzerinde yapılan deneysel bir çalışmadır. 2019 Ramazan ayında oruç tutan, pilates yapan ve yapmayan 18-65 yaş arası 60 yetişkin sağlıklı kadın çalışmaya dahil edilmiştir. Çalışmaya ramazan ayı öncesinde 6 aydır pilates yapan ve ramazan ayında da haftada en az 3 kez pilates yapan kadınlar dahil edilmiştir.

Bulgular: Oruç tutup pilates yapmayan grupta beden kitle indeksi (BKİ), triseps deri kıvrım kalınlığı (DKK), kas kütlesi ve yağ yüzdesinde anlamlı artışlar gözlenmiştir ($p<0,05$). Bizim çalışmamızda pilates yapan kadınların triseps DKK ve yağ yüzdesindeki değişim sadece oruç tutan kadınlara kıyasla üstün bulunmuştur. Pilates yapan ve yapmayan kadınların ramazan ayında BKI, kas kütlesi, bel çevresi ve kalça çevresi son ölçümlerinin ilk ölçümlere göre farklılaşmadığı ve bu değerler açısından iki yöntemin birbirine üstün olmadığı görülmüştür.

Sonuç: Ramazan ayında uzun süren açlık süresi kişileri inaktiviteye yönlendirirken, kişilere özel uzman eşliğinde planlanan egzersiz ve kişiye özgü beslenme programları ile Ramazan ayı özellikle obez ve hafif kilolu kişiler için ağırlık kaybı ile sonuçlanabilir.

Anahtar Sözcükler: Ramazan, pilates, diyet, sağlıklı beslenme, egzersiz, ağırlık yönetimi

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Introduction

One to 1.5 billion of the world's population consists of Muslims. Healthy and grown-up members of this religion make up approximately 18 to 25% of the total population who fast during a holy month, Ramadan (1,2). Fasting is not mandatory for everyone. Pregnant women, women going through menstruation, women in lactation, mentally challenged individuals, children who have not reached puberty, individuals with chronic illnesses who are unable to fast, and senile people are exempt from this religious duty (3).

The timing of Ramadan is determined according to the Muslim calendar, and it takes place at different times over a 33-year cycle (3). Fasting for around 14 hours a day during this month, Muslims do not eat or drink anything from the dawn called "sahur" until the sunset called "iftar". Smoking, drinking water, and taking medicine are also prohibited during fasting. Nonetheless, there are no restrictions from iftar to sahur and fasting people are allowed to eat and drink as they wish during this period of time (4,5).

Previous studies have demonstrated that during the period of fasting, eating habits change significantly, and the type and amount of food consumed are very different from the rest of the year. It is known that in some countries special dishes with more sugar are preferred, unlike regular eating habits (3). Also, during this month, the decrease in the frequency of meals of people who eat and drink at night after iftar leads to an increase in free fatty acid concentrations in the body (6). The long time gap between main meals when fasting affects appetite, hormonal responses to food, energy, and glucose metabolism. Studies have revealed that particularly the lack of breakfast is associated with low exercise-induced thermogenesis and causes impaired insulin sensitivity in people with diabetes (7).

During this worship considered sacred by Muslims, alterations in weight are observed depending on the levels of energy taken and consumed. A meta-analysis obtained from 35 studies has put forward that fasting causes a statistically significant weight loss. Other than its impact on weight, the way it affects body composition cannot be interpreted clearly (8). Biochemical studies have confirmed that blood glucose decreases significantly with the decrease in total energy intake during Ramadan. At the same time, compared to the period before Ramadan, significant decreases in the total serum cholesterol by 7.9% and triglycerides by 30% have been observed. While there has been a significant increase in high-density lipoprotein (HDL) cholesterol, a decrease has been observed in low-density lipoprotein (LDL) cholesterol (9). Another study conducted on active and inactive men examined the effects of fasting and physical activity on biochemical parameters and found a significant increase in HDL cholesterol in both groups. Additionally, hematocrit level, red blood cell count, LDL, very LDL, and total cholesterol greatly decreased after fasting (10).

Although it was confirmed that fasting affected physical performance and activity, in a study conducted on inactive individuals, the heart rate was found to be lower in moderate aerobic exercises while fasting. In these individuals, a significant decrease in performance was also observed. Likewise, a study conducted on combat pilots demonstrated that fasting during Ramadan had negative effects on muscle performance and lowered physical performance scores (11). Measurements done during the Ramadan periodrevealed a decrease in the total body mass of sportspersons regularly performing general aerobic training, footballers, elite athletes, referees, and rugby players, alongside dehydration and negative energy balance (12).

Several studies have emphasized that due to the consumption of food and liquids late at night, the changes in sleeping duration and habits, as well as less fluid intake deteriorate performance in sports during Ramadan (12). That is why sportspersons are advised to track their dehydration levels regularly. In order to avoid dehydration or over-hydration, athletes must consume 1.5 times the amount of fluid they have lost through exercise. In the case of excessive consumption, sleep will be interrupted by the need to urinate and sleep quality will be negatively impacted (13).

In a study investigating the effects of fasting on aerobic performance, maximum oxygen intake was directly measured using a treadmill. No difference was observed in the highest oxygen-carrying capacity in the period before and during Ramadan. Although there was a decrease in maximum oxygen intake in the first week of Ramadan, the values in the fourth week returned to normal (14).

Most of the studies on aerobic and anaerobic performances during Ramadan have focused on individual sports, and those examining team sports have mostly studied football. A study conducted on basketball players concluded that fasting did not make a significant difference in aerobic and anaerobic performance based on the results of small games and repeat sprint ability (15). For this study, Pilates was picked on the grounds that it favored lymphatic and blood drainage, ameliorated posture, increased flexibility, and expanded motion range and muscular strength, improved cardiometabolic parameters, and augmented blood glucose (16,17).

While team sports or individual sports are generally examined to evaluate performance during Ramadan, there is no study on Pilates in the literature. This study aimed to evaluate the changes in the body composition of individuals who fasted and did Pilates at least twice a week during Ramadan versus those who fasted but did no Pilates.

Methods

This was an experimental study conducted on 60 healthy women who were admitted to the Noi Pilates in İstanbul Turkey, which was a private Pilates studio. The study was carried out on 60 grown-up women who fasted during Ramadan 2019, some of whom did pilates and some of whom did not. The participants in the study were 18-65 years old and were healthy adults who had been doing Pilates for 6 months prior to Ramadan and did Pilates at least 3 times a week during Ramadan. Individuals

aged <18 or >65, not fasting or fasting less than twice a week in Ramadan did not take part in the study.

The women were divided into 2 groups by random sampling using simple randomization. Participants were randomly allocated to the Pilates exercise group (PL) or control group (No PL), with 30 participants in each (simple allocation using www.random.org). The ethical review committee of İstanbul Kültür University approved the study (number: 2019.04). All participants provided written consent in accordance with the declaration of Helsinki and informed consent was obtained from each participant before participation.

The Evaluation of Nutritional Status

To identify the dietary habits of the patients, a food consumption frequency form was applied. To determine the dietary status of the patients, a three-day food consumption form was used at the beginning of the study.

The researcher provided the participants who got trained about portions with consumption forms and they were instructed to write down on the form the food and drinks they consumed for three days, including a weekend and two weekdays, in the morning, afternoon, and evening, with the details of time and place. The portion education was given to the participants through the food and nutrition catalog (18). The average daily intake of participants' macro and micronutrients were analyzed by the National Food Composition Database (Türkomp).

Assessment of Anthropometric Measurements

The height measurement was done with a Seca-206 height meter (Hamburg, Germany). While the height measurement was done, the standing position and head at the Frankfurt plane were noted. The body analysis of the patients was done using a regularly calibrated Tanita SC-330 analyzer (Tokyo, Japan). BMI can be determined with the weight/height^2 equation (19). While evaluating the results of BMI, the BMI classification of WHO was used (20). The smallest waist circumference between bottom costa and processus spina iliaca anterior superior was measured and saved with a measure parallel to the ground from navel by the researcher. Two measurements, at the beginning and end of the study, were done.

Subcutaneous fat thickness was measured in 3 body parts, including the triceps, thigh, and above the pelvis; the subcutaneous fat was measured by caliper (Saehan skinfold caliper, SH5020, Korea). Triceps skin-fold thickness (SFT) was measured on the right arm bent 90° from the elbow by locating and marking the bump between the acromion (shoulder) and olecranon (elbow). The arm was positioned loosely and the skin fold was held 1 cm above the mark between the index finger and thumb of the left hand. The measurement was done on the marked spot by the right hand with a caliper. The participant was standing upright while the measurement was done (21,22). All measurements were done on the right side of the body. All body parts (to the nearest 0.1 mm) were measured with three rotational measurements. The caliper measurements were read 4 seconds later than releasing the folding handle. To obtain reliable and accurate measurements, they were carried out at a certain time of the day, preferably in the afternoon. The SFT of all participants was measured by a specialist. Jackson-Pollock nomogram was used in measurements, and the final step was to measure the ratio of the waist-to-hip circumference.

Training Program

As diaphragmatic breathing was an effective method for improving glycemic parameters in participants, a trained physiotherapist taught patients diaphragmatic breathing before leading the effects of pilates based mat exercise (PBME) program (23). During Ramadan 2019, Pilates participants performed PBME at 11 a.m. every Monday, Wednesday, and Saturday. All of the sessions took place in a private Pilates studio in Istanbul. The sessions were initially 50 minutes long. There were three stages of the Pilates exercise: 1) warm-up and stretching; 2) Moderate Pilates to train arms, legs, and body; 3) cool-down and relaxing. The participants were monitored in case of any possible complications, such as fatigue, dizziness, headache, feeling light-headed, shortness of breath, decreased reflexes, numbness in hands or feet, any sort of pain, and cold sweating.

Statistical Analyses

The Turkomp program was used to calculate the data related to nutrients. Then, the nutrient data and other anthropometric data were coded into the statistics program. Mean and standard deviation analyses were used to describe the anthropometric measurements before and after Ramadan for the PL and non-PL groups. Minimum and maximum values, mean, and standard deviations were calculated for the number of meals and nutrient variables. Mann-Whitney U Test and Wilcoxon Signed Rank Test were used to compare the pre- and post-Ramadan anthropometric measurements of the PL and non-PL groups. Analyses were made with the SPSS 20 program. A p-value less than 0.05 was accepted statistically significant.

Results

The means of anthropometric variables and standard deviation values of the PL and non-PL groups are given in Table 1. Accordingly, anthropometric values of the PL group before and after Ramadan were 28.77±3.14 and 27.59±3.22 kg/m^2 for BMI, 26.31±1.87 and 25.13±1.93 for triceps SFT, 35.21±4.80 and 32.23±4.16 for fat percentage, 24.07±1.27 and 23.03±1.77 kg for muscle mass, 79.52±5.80 and 78.88±14.37 cm for waist circumference, and 111.69±7.20 and 110.88±8.30 cm for the hip circumference, respectively. In the non-PL group, anthropometric values before and after Ramadan were 28.60±2.94 and 29.13±3.10 kg/m^2 for BMI, 26.23±1.19 and 26.77±1.25 mm for triceps SFT, 34.68±3.26 and 36.03±3.54 for fat percentage, 23.47±2.11 and 23.87±2.22 kg for muscle mass, 77.15±6.30 and 78.07±6.33 cm for waist circumference, and 108.30±9.49 and 107.37±9.62 cm for hip circumference, respectively.

Table 2 shows the number of meals and nutrient values of all participants. The daily average of meals was 2.33. Also, 1.51 liters

Table 1. The means and standard deviations of the anthropometric variables of the groups

Variable	PL group		No PL group	
	Before Ramadan	After Ramadan	Before Ramadan	After Ramadan
BMI (kg/m²)	28.77±3.14	27.59±3.22	28.60±2.94	29.13±3.10
Triceps skin-fold thickness (mm)	26.31±1.87	25.13±1.93	26.23±1.19	26.77±1.25
% fat	35.21±4.80	32.23±4.16	34.68±3.26	36.03±3.54
Muscle mass	24.07±1.27	23.03±1.77	23.47±2.11	23.87±2.22
Waist circumference (cm)	79.52±5.80	78.88±14.37	77.15±6.30	78.07±6.33
Hip circumference (cm)	111.69±7.20	110.88±8.30	108.30±9.49	107.37±9.62

PL: Pilates exercise group

Table 2. The mean of meal number and dietary nutrient values of all participants

Variable	Min	Max	X ± SD
Number of meals	1.00	3.00	2.33±0.71
Water consumption (lt)	1.00	2.50	1.51±0.42
Energy	544.50	2,102,00	1,274.98±457.56
% protein	13.30	26.69	18.52±3.09
% carbohydrates	26.00	60.40	42.68±7.53
% fat	22.40	53.00	38.89±6.40
Fiber (g)	3.20	13.50	6.27±2.33
Cholesterol (mg)	100.60	416.00	223.39±81.71

Min: Minimum, Max: Maximum, SD: Standard deviation

of water, 1,274.98 kcal of energy, 6.27 g of fiber, and 223.39 g of cholesterol were consumed. The nutrient distribution was as follows: 42.68% carbohydrates, 18.52%, and 38.89% fat.

The comparison of the preliminary measurements of the PL and non-PL groups are shown in Table 3. The groups had to be equal at the beginning (in terms of preliminary measurements). In experimental models, the groups were organized equivalently to determine the role of application in the difference. In the preliminary measurements, no significant difference was found between anthropometric variables of fasting women who did pilates and did not do Pilates ($p>0.05$).

Table 4 shows the comparison of the final measurements of the test and control groups. This demonstrated that there was a significant difference between the fat percentage and triceps SFT of fasting women who did pilates and who did not do pilates ($p<0.05$). According to the final measurements, the two groups did not differ in terms of BMI, muscle mass, waist circumference, and hip circumference ($p>0.05$).

Table 5 shows the comparison of the preliminary and final measurements of the PL group. Based on these results, fasting when combined with Pilates led to a significant decrease in the fat percentage and triceps SFT ($p<0.05$). In terms of other variables, there was no significant difference between the preliminary and final measurements ($p>0.05$).

Table 6 shows the comparison of the preliminary and final measurements of the non-PL group. According to this table, in those who fasted without doing Pilates, BMI, triceps SFT, muscle mass, and fat percentage increased significantly ($p<0.05$). There was no significant difference between the preliminary and final measurements in terms of waist and hip circumference ($p>0.05$).

Table 3. The comparison of the preliminary measurements of the test and control groups

Variable	Group	Mean rank	Sum of rank	U	p
BMI (kg/m²)	PL	31.06	1,025.0	427.0	0.783
	No PL	29.81	805.0		
Triceps skin-fold thickness (mm)	PL	30.06	992.0	431.0	0.829
	No PL	31.04	838.0		
% fat	PL	30.59	1,009.5	442.5	0.964
	No PL	30.39	820.5		
Muscle mass (kg)	PL	34.17	1,127.5	324.5	0.071
	No PL	26.02	702.5		
Waist circumference (cm)	PL	32.92	1,086.5	365.5	0.232
	No PL	27.54	743.5		
Hip circumference (cm)	PL	32.74	1,080.5	371.5	0.270
	No PL	27.76	749.5		

Table 4. The comparison of the final measurements of the test and control groups

Variable	Group	Mean rank	Sum of ranks	U	p
BMI (kg/m²)	PL	26.71	881.5	320.5	0.063
	No PL	35.13	948.5		
Triceps skin-fold thickness (mm)	PL	23.47	774.5	213.5	**0.001**
	No PL	39.09	1,055.5		
% fat	PL	23.86	787.5	226.5	**0.001**
	No PL	38.61	1,042.5		
Muscle mass (kg)	PL	30.14	994.5	433.5	0.858
	No PL	30.94	835.5		
Waist circumference (cm)	PL	31.17	1,027.5	424.5	0.754
	No PL	29.72	802.5		
Hip circumference (cm)	PL	34.29	1,031.5	320.5	0.063
	No PL	25.87	689.5		

Discussion

This study aimed to evaluate the changes in the body composition of individuals who fasted and did Pilates at least twice a week during Ramadan versus those who fasted but did no Pilates. According to the American Diabetes Association (ADA), the daily energy intake of a healthy adult should consist of 50-60% carbohydrates, 10-20% protein, and <30% fat, whereas Turkey Dietary Guidelines (TÜBER-2015) advises 55-60% of daily energy should be provided from carbohydrates, 10-15% from protein, and <30% from fat (23-25). Three-day food consumption records provided by the women participating in this study showed they consumed an average of 2 meals during Ramadan and took in 1,270 kcal of energy. When their diet pattern was examined, 18.5% of the energy was provided from protein, 42.6% from carbohydrates, and 38.8% from fat. It was presumed that the increase in the consumed protein rate during Ramadan caused an increase in dietary fat. In the study conducted by Hsouna et al. (26), they monitored 20 healthy and exercising men throughout Ramadan. They found that the men participating in the study consumed more fat as a result of the increasing carbohydrate amount in their diet, and the dietary protein ratios were lower compared to their regular levels until the end of Ramadan. In another study, the components of individuals' diets were examined during Ramadan (the first 10 and the last 10 days) and it was found that people

Table 6. The comparison of the preliminary and final measurements of the group who did not do pilates

Variable	Rank		Mean rank	Sum of rank	z	p
BMI (kg/m²)	Negative	5	9.20	46.00	-3.29	**0.001**
	Positive	21	14.52	305.00		
	Same	1				
Triceps skin-fold thickness (mm)	Negative	2	4.75	9.50	-4.32	**0.000**
	Positive	25	14.74	386.50		
	Same	0				
% Fat	Negative	0	0.00	0.00	-4.53	**0.000**
	Positive	27	14.00	378.00		
	Same	0				
Muscle mass (kg)	Negative	7	12.43	87.00	-2.25	**0.024**
	Positive	19	13.89	264.00		
	Same	1				
Waist circumference (cm)	Negative	11	12.27	135.00	-1.64	0.102
	Positive	8	6.88	55.00		
	Same	8				
Hip circumference (cm)	Negative	12	17.13	205.50	-1.60	0.111
	Positive	12	7.88	94.50		
	Same	3				

Table 5. The comparison of the preliminary and final measurements of the group who did Pilates

Variable	Ranks	2	Mean rank	Sum of rank	z	p
BMI (kg/m²)	Negative	33	17.00	561.00	-5.01	**0.000**
	Positive	0	0.00	0.00		
	Same	0				
Triceps skin-fold thickness (mm)	Negative	31	17.13	531.00	-4.48	**0.000**
	Positive	2	15.00	30.00		
	Same	0				
% fat	Negative	31	17.52	543.00	-4.69	**0.000**
	Positive	2	9.00	18.00		
	Same	0				
Muscle mass (kg)	Negative	25	14.46	361.50	-1.83	0.068
	Positive	7	23.79	166.50		
	Same	1				
Waist circumference (cm)	Negative	22	13.50	297.00	-0.62	0.535
	Positive	10	23.10	231.00		
	Same	1				
Hip circumference (cm)	Negative	13	15.27	198.50	-0.41	0.680
	Positive	16	14.78	236.50		
	Same	4				

consumed more carbohydrates at the beginning of Ramadan, and the amount decreased significantly during the last 10 days (27). In this study, contrary to the literature, an increase was found in the rate of dietary fat consumption. This may have occurred because the people gave priority to protein foods in order to stay full for a long time during Ramadan. A study by Lessan et al. (28) evaluated basal metabolic rates and activity pace of 29 individuals who were healthy, non-obese, and fasting during Ramadan. Basal metabolic rate was found to be 2,200 kcal and 2,100 kcal during and after Ramadan, respectively. While there were no significant fluctuations observed in basal metabolic rate during Ramadan, the results showed that it was associated with fasting, decreased activity levels, and sleep duration. Another study evaluated the performance and eating habits of adolescent football players during Ramadan. A performance decrease was seen in the athletes during Ramadan; however, this decrease could not be associated with low calorie intake or change in sleep times (29).

Changes in eating habits may influence liquid consumption adversely. Inadequate fluid intake causes dehydration, especially in athletes during Ramadan. That is why tracking hydration levels is important during Ramadan (12,13). This study showed that people consumed 1.5 liters of water on average.

In one of the studies on sportpersons' performance in Ramadan, there was no deterioration observed in the aerobic exercise performance and body composition of the football players who had been regularly training before Ramadan (30). This study compared the body compositions of women who did Pilates and who did not do Pilates during Ramadan. BMI, triceps SFT measurements, and fat percentage decreased significantly in women who did Pilates during Ramadan when compared to those who did not. The change in the triceps SFT and fat percentage of women who did Pilates were superior to only that of those who fasted without Pilates. BMI, muscle mass, waist circumference, and hip circumference of women who did Pilates and who did not do Pilates during Ramadan did not differ in the final measurements, and neither of the two methods dominated in terms of these variables. In another study, the body composition of athletes was evaluated during Ramadan. It found that athletes' post-Ramadan BMI and body fat ratio were lower, and muscle and water mass did not change (31). According to a meta-analysis, fasting causes a statistically significant decrease in weight, but its effect on body composition cannot be interpreted clearly (8).

Thirty-four volunteers practicing karate and Taekwondo were divided into 2 groups in a study. One group exercised an hour before iftar and the second group exercised 3 hours later. Their anthropometric measurements were evaluated. The study revealed that training before or after iftar during Ramadan did not affect agility and power performance (32). In this study, the diet and exercise programs of women were planned individually, and no distinction was made duration-wise. A meta-analysis evaluated the physical performance of athletes during Ramadan in the morning and afternoon. The results showed that the performance of the participants was not affected during the day. It is concluded that as long as sleep and diet programs are planned individually by considering iftar and sahur during Ramadan, physical performance will not be adversely affected (33).

Study Limitations

Although the limitation of this study on Ramadan and Pilates was that it was conducted only on women, this study was a first in its field. Evaluating anthropometric measurements such as food consumption, body analysis and SFT together were the strengths of the study. With these features, it is thought that this study will shed light on future studies.

Conclusion

People's diets change during Ramadan. However, a balanced diet pattern to provide sufficient energy should be preserved. While a long period of fasting in Ramadan leads people to inactivity, personalized exercise and nutrition programs planned by experts may result in weight loss, especially for obese and slightly overweight people.

Acknowledgments

The experiments complied with the current laws of the country where they were performed. The authors have no conflict of interest to declare.

Ethics

Ethics Committee Approval: The ethical review committee of İstanbul Kültür University approved the study (number: 2019.04).

Informed Consent: All participants provided written consent in accordance with the declaration of Helsinki.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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The Relationship of Depression and Stress with Tryptophan Consumption among University Youth

Üniversite Öğrencilerinde Triptofan Tüketim Düzeyleri ile Depresyon ve Stres Arasındaki İlişki

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ABSTRACT

Objective: The most common psychological health problems are depression and stress. Depressive symptoms and stress are increasing day by day in university youth. Since depression and stress negatively affect the health of the young population, it is important that it contribute to the increase of the healthy population. The objective of the study was to evaluate the relationship of depression and stress with tryptophan consumption among university students.

Methods: A questionnaire which included descriptive questions, amounts of tryptophan consumption, "Beck depression" inventory and "perceived stress (PS) scale" were conducted voluntarily to 900 undergraduate students studying at İstanbul Aydın University in this correlation study.

Results: There was no statistically significant difference was found between students' depression levels and tryptophan consumption levels ($p>0.05$). A statistically significant difference was found between the perceived coping level and the mean tryptophan consumption level ($p<0.05$). As tryptophan consumption increases, stress decreases and coping with PS increases.

Conclusion: There was no relationship between tryptophan consumption and depression, but there was a relationship between tryptophan consumption and stress.

Keywords: Tryptophan, depression, students, nutritional status, anxiety

ÖZ

Amaç: En yaygın psikolojik sağlık sorunları depresyon ve strestir. Her geçen gün üniversite gençliğinde depresif belirtiler ve stres artmaktadır. Depresyon ve stres genç nüfusu sağlık bakımından olumsuz etkilediği için, sağlıklı popülasyonun artışına katkı sağlaması açısından önemlidir. Çalışmanın amacı, üniversite öğrencilerinde depresyon ve stres ile triptofan tüketimi arasındaki ilişkiyi değerlendirmektir.

Yöntemler: Bu korelasyon çalışmasında İstanbul Aydın Üniversitesi'nde okuyan 900 lisans öğrencisine gönüllü olarak tanımlayıcı sorular, triptofan tüketimi miktarları, "Beck depresyon" envanteri ve "algılanan stres ölçeği" içeren bir anket uygulandı.

Bulgular: Öğrencilerin depresyon düzeyleri ile triptofan tüketim düzeyleri arasında istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0,05$). Algılanan başa çıkma düzeyi ile ortalama triptofan tüketim düzeyi arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0,05$). Triptofan tüketimi arttıkça, stres azalmakta ve algılanan stresle başa çıkma artmaktadır.

Sonuç: Triptofan tüketimi ile depresyon arasında bir ilişki yoktur, ancak triptofan tüketimi ile stres arasında bir ilişki vardır.

Anahtar Sözcükler: Triptofan, depresyon, öğrenciler, beslenme durumu, anksiyete

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Introduction

The nutritional status of university students; many factors such as body perceptions, genetic factors, lifestyle changes, difficulties in adapting to the school, environment and dormitory environment, and unconscious nutrition and fast-food feeding tendencies on top of economic inadequacies constitute the nutritional status of university students and the psychological health problems they bring (1-3). Stress is quite common among university students due to intense processes such as insomnia, exams, homework, presentations and situations in which they can not cope sufficiently with psychological-social pressures. The most common psychological health problem is depression (4,5).

There are many definitions of depression; according to Beck's (6) model, depression occurs with emotions, thoughts, motivation, and symptoms of a physiological nature and depression starts with thought distortions first, emotional depression comes as a secondary condition. For this reason, Beck states that the depressed person perceives reality by distorting it, and depression arises as a result of the thought system of "negative thought structure towards oneself, negative perception of the world and a negative future perception", which he describes as "cognitive triad". It is possible to group the symptoms and clinical appearance of depression as behavioural, emotional, cognitive, somatic and motivational symptoms (7). A study has pointed out that the depressive symptoms seen in university youth are increasing day by day and young people face many different situations, events and expectations that require reintegration after starting university. In other words, life changes are serious sources of stress (8,9). Studies show that perceived stress (PS) situations in university students differ (10-12). Stress, which is an important part of daily life, occurs as a result of a person's reactions to events arising from her environment or herself (13). Scientific studies on stress show that stress has a strong relationship with mental health (14). Scientific data indicate a strong causal relationship between exposure to stressful events and major depression (15).

Tryptophan has also been associated with depression, as it has a calming effect. The content of the diet affects the amount of tryptophan in the blood and consequently ensures the secretion of serotonin. Serotonin plays a role in regulating anxiety and mood, and low serotonin levels can cause an increase in anxiety and depression (16,17). Tryptophan is taken into the body through diet and is essential for humans. Tryptophan taken into the body affects protein synthesis in the brain and increases serotonin release. In a study conducted from the data of NHANES (2001-2012), it was observed that as the amount of tryptophan intake increased, the severity of depression decreased (16). Another study reported that tryptophan supplementation is as effective as tricyclic antidepressant drugs in the treatment of depression (18). In a similar study, individuals on a diet rich in tryptophan and low in tryptophan were administered for 4 days, and after a 2-week break, it was observed that anxiety and depression symptoms decreased according to the other kinds of diet The anxiety scale in those who consumed a diet rich in tryptophan (17) determines the relationship of tryptophan consumption of university students with depression and stress.

There are many studies showing that tryptophan consumption has an effect on depression and stress. Studies have shown that low consumption of tryptophan has an increasing effect on depression and stress (13,19,20) (Figure 1).

Methods

This research utilizes the relational (correlation) scanning model, which is a type of scanning model. The term "relational scanning model" refers to a method for determining the existence of co-variance between two or more variables. Whether the variables change concurrently or not, the relational survey model attempts to determine how the change occurred (21).

Universe and Sample

This study's universe is comprised of students enrolled at the University of İstanbul Aydın. The universe of this study consists of 25,0000 students studying at İstanbul Aydın University. For the purpose of the study, stratified sampling method was used. 384 students would be sufficient in the calculation of the sample volume within the range of 0.05 sample error and 95% confidence. Study was concluded to 900 students. Written informed consent was obtained from the participants.

Tools for Data Collection

Procedure

A questionnaire including sociodemographic questions, amounts of tryptophan consumption (for determining the frequency of tryptophan consumption, one week food frequency record was taken), the Beck depression inventory (BDI) and PS scale were applied to the students.

Inclusion and Exclusion Criteria

Criteria for inclusion in the study; having undergraduate education at İstanbul Aydın University, is not using psychological drugs and nutritional supplements.

Criteria for Exclusion

Those with not having other comorbid mental disorders (e.g., attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, and seizure disorder), pregnant women.

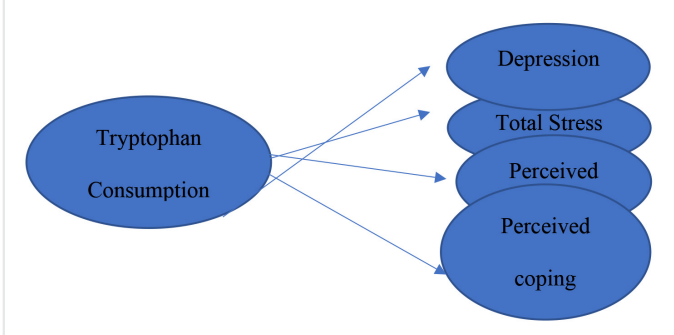

Figure 1. Conceptual model of the effects of tryptophan consumption on depression total stress, perceived stress, and perceived coping

Measures

For determining the frequency of tryptophan consumption, foods with high, normal and low tryptophan content were listed according to the average amount of tryptophan in 100 g of foods using the Turkish Food Composition Database (22). According to the data from EFSA, 250-500 mg (average 5 mg/kg) tryptophan intake from the foods consumed daily by healthy individuals has been recommended. In this report, it is also recommended that the individual should take at least 3 mg/kg tryptophan daily in order to protect her mental health. It has been stated that there is no harm in consuming tryptophan up to 750 mg as the upper limit. Based on this data, students' tryptophan consumption is classified as low tryptophan consumption of 0-250 mg, normal tryptophan consumption of 251-750 mg, and high tryptophan consumption of >751 mg (23).

Beck Depression Inventory

BDI was developed by Beck in 1961. BDI is used to determine the risk of depression and to measure the level and severity of depressive symptoms (24). The validity and reliability study in Turkey was carried out by Hisli (25) in 1989. Each item of BDI determines a depression-specific behavioral pattern in the past week and includes 21 self-assessing sentences with four options, going from low to high (0-3). The total score that can be obtained from the scale varies between 0-63. Evaluation; 1-10: normal, 11-16: mild mood changes, 17-20: clinical depression at the border, 21-30: moderate depression, 31-40: severe depression, >40: very severe depression. It takes about 15 minutes to complete the test (25).

Perceived Stress Scale (PSS-10)

Developed by Cohe et al. (26) in 1983, the Cronbach's alpha value was found at 0.86 in the reliability study. In 2007, the scale adapted into Turkish by Bilge et al. (27) was used, and the Cronbach's alpha value was found 0.81 in the reliability study. The PSS-10 has 10 items on a 5-point Likert scale (0= never, 1= almost never, 2= sometimes, 3= fairly often, 4= very often) indicating how often they have felt or thought a certain way within the past month. Four positively stated items (item 4,5,7, and 8) are reversely scored (0= very often, 1= fairy often, 2= sometimes, 3= almost never, 4= never). It has two subscales: PS (items 1,2,3,7,8) and perceived coping (PC) (items 4,5,6). The scale is evaluated on both total score and subscale scores (26,27).

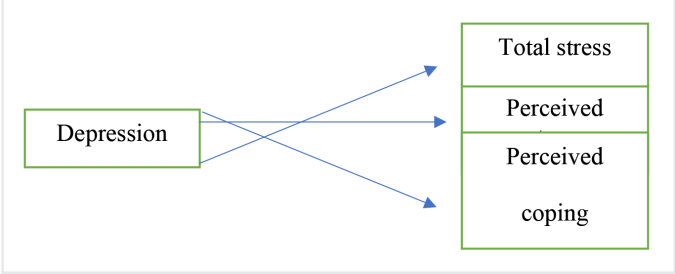

Figure 2: Conceptual model of the effects of tryptophan consumption on depression, total stress, perceived stress, and perceived coping.

Acquiring Data

This study is not sponsored by any institution and is not governed by any authority.

The study was completed with 900 students; 391 male, 509 female who agreed to participate in the study between February and March 2020 in İstanbul Aydın University.

Statistical Analysis

SPSS 22.0 program was used for data analysis. Number, percentage, average, standard deviation, minimum, maximum values are shown in a Table 1. The data showed normal distribution according to the normality test results. Independent sample t-test, Annova (one way variance analysis), Pearson's correlation test analyzes were performed in the analysis of the data providing parametric test assumptions (Figure 3). The hypothesis tests that reveal the relationship between variables were analyzed in this study, which is based on the relational screening model.

According to the CFA performed, the PS scale is divided into two factors: PS and PC. PS; it shows distribution according to 1, 2, 3, 7 items and PC; it shows distribution according to 4, 5, 6 items. Item loads of PC and PS factors are shown in Figure 1. The factor loads of all the items are high, the scale questions are sufficient to measure the desired sub-factors. As a result, it was established through fit indices of CFA that the Turkish version PTS comprised 8 items and two factors and this model was found to be appropriate theoretically and statistically.

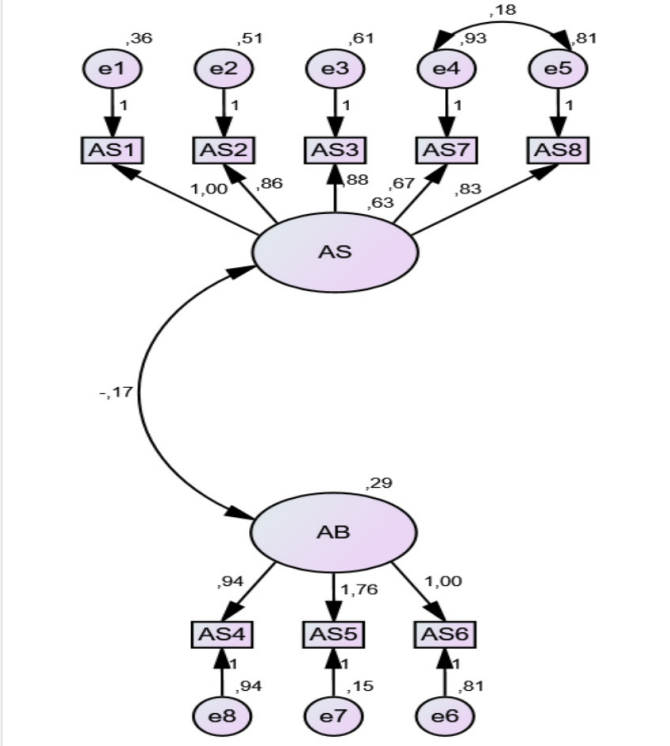

Figure 3: Standardized factor loadings of items in confirmatory factor analysis.

Results

Nine hundred students participated in the study. 78% of the students are female and 76.5% are male; 97.0% of them are single and 2.4% are married. 77.3% of the students are between 19-22 years old, 22.1% are between 23-25 years old and 0.6% are between 26 years old and above. 34.8% of the students smoke, 56.5% do not smoke and 8.7% quit smoking. 41.6% of the students use alcohol, 54.1% do not use alcohol, and 4.3% stopped using alcohol (Table 1).

The average daily tryptophan consumption of male students is 1,395.6±750.01, while the average consumption of females is 1611.5±967.35. A statistically significant difference was found between the levels of tryptophan consumption of female and male students ($p<0.05$), and female students consume higher levels of tryptophan than males. No statistically significant difference was found between the age distribution of the students and the tryptophan consumption rates ($p>0.05$). The highest tryptophan consumption is between the ages of 19-22 with 78% (Table 2).

The average depression of male students is 11.17±10.4, while the average of females is 13.10±11.08. A statistically significant difference was found between the depression levels of the students according to gender ($p<0.05$). Female students have a higher level of depression than male students. No statistically significant difference was found between the age distributions of the students and their depression levels ($p>0.05$). The extreme depression rate is in the group between the ages of 19-22 with 94.4% (Table 3).

There is no statistically significant difference was found between students' depression levels and tryptophan consumption levels ($p>0.05$). 53% of those with normal depression consume high amounts of tryptophan, 22.0% of those with mild mood depression consume high amounts of tryptophan, 7.3% of those at the border depression consume high amounts of tryptophan, 9.6% of those with moderate depression. 5.3% of those with severe depression consume high amounts of tryptophan, 2.2% of those with very severe depression consume high amounts of tryptophan (Table 4).

There was no statistically significant difference between the genders of the students and total stress, PS ($p>0.05$), and the average PS was higher in females than in males. A statistically significant difference was found between gender and PC levels ($p<0.05$) The average of male students in the sub-dimension of coping with PS is higher than that of women. There is no statistically significant difference between students' age and total stress, PS and PC levels ($p>0.05$) (Table 5).

Table 1. Demographic information of students

Variables	Male		Female		Total (n=900)	
Age (years)	n	%	n	%	n	%
19-22	299	76.5	397	78.0	696	77.3
23-25	91	23.3	108	21.2	199	22.1
26≤	1	0.2	4	0.8	5	0.6
Total	391	100.0	509	100.0	900	100.0
Mean ± SD/min. max	Mean: 21.18 SD: 1.79				Min:19 Max:39	
Marital status						
Married	14	3.6	8	1.6	22	2.4
Single	377	96.4	501	98.4	878	97.0
Total	391	100.0	509	100.0	900	100.0
Chronic disease status						
Yes	18	4.6	38	7.5	56	6.2
No	373	95.4	471	92.5	844	93.8
Total	391	100.0	509	100.0	900	100.0
Smoking status						
Yes	175	44.8	138	27.1	313	34.8
No	163	41.7	346	68.0	509	56.5
Quitting smoking	53	13.6	25	4.9	78	8.7
Total	391	100.0	509	100.0	900	100.0
Drinking alcohol						
Yes	177	45.3	197	38.7	374	41.6
No	188	48.1	299	58.7	487	54.1
Quitting drinking alcohol	26	6.6	13	2.6	39	4.3
Total	391	100.0	509	100.0	900	100.0

SD: Standard deviation

Table 2. Tryptophan consumption amount-levels of students according gender and age

Gender		Tryptophan consumption amount (mg/day)				
		n	Min.	Max.	Mean	SD
Male		509	243.40	4,629.10	1,395.6	750.01
Female		391	122.25	6,084.50	1,611.5	867.35
t-test: -3.996, p-value: ,000						
Gender		**Tryptophan consumption levels**				
		Low (<250 mg)		**Normal (251-750 mg)**		**High (>751 mg)**
Male	n	4		55		332
	%	66.7		35.7		44.9
Female	n	2		99		408
	%	33.3		64.3		55.1
Total	n	6		154		740
	%	100.0		100.0		100.0
Chi-square: 5.670, p value: .05						
Age (years)		**Tryptophan consumption levels**				
		Low (<250 mg)		**Normal (251-750 mg)**		**High (>751 mg)**
19-22	n	6		113		577
	%	100.0		73.4		78.0
23-25	n	0		40		159
	%	0.0		26.0		21.5
26 ≤	n	0		1		4
	%	0.0		0.6		0.5
Total	n	6		154		740
	%	100.0		100.0		100.0
Chi-square: 3,307, p value: .508						
SD: Standard deviation, Max: Maximum, Min: Minimum						

The averages of total stress, perceived stress, PC and depression according to the tryptophan consumption levels of the students; a statistically significant difference was found between the PC level and the mean tryptophan consumption level ($p<0.05$). According to the post hoc Tukey test result, it was determined that this significant difference ($p<0.05$) was due to the difference between the averages of those with normal and high tryptophan consumption levels (Table 6).

A negative and insignificant relationship was found between tryptophan consumption level and total stress. (r=-0, 019, p=0.575 <0.05); a negative and insignificant relationship was found between tryptophan consumption level and PS. (r=-0. 021, p=0.522 <0.05); a positive and insignificant relationship was found between tryptophan consumption level and PC. (r=-0. 008, p=0.815 <0.05); a negative and insignificant relationship was found between tryptophan consumption level and depression. (r=-0.005, p=0.892 <0.05); a positive and significant relationship was found between total stress and perceived stress. (r=0. 791, p=0.001 <0.01); a negative and significant relationship was found with perceived coping. (r=-0.348, p=0.001 <0.01); a positive and significant relationship was found with depression. (r=0, 243, p=0.001 <0.01); there is a positive and significant relationship between depression and perceived stress. (r=0.473, p=0.001 <0.01); there is a negative and significant relationship between depression and PC (r=-0.333, p=0.001 <0.01) (Table 7). That is, as the level of depression increases in students and the level of stress increases, depression and stress nourish and increase each other. Between the PC and depression and stress in a negative way, that is, as the PC increases, the level of depression and stress decreases. In this case, hypotheses 4, 5, 6, 7, 8 and 9 have been accepted.

In addition, according to the results of the regression analysis, the relationship between tryptophan consumption level between depression, total stress, PS and PC. The effect of tryptophan consumption level between depression, total stress, PS and PC was not significant. In other words, a significant effect of the increase in tryptophan consumption levels of students in this study on depression, total stress, PS and PC was not determined (Table 8).

Table 3. Depression scores and levels of students by gender and age

Gender					Depression scores			
	n		**Min.**		**Max.**	**Mean**		**SD**
Male	391		1		63.00	11.17		10.24
Female	509		1		63.00	13.10		11.08
t-test : 2,681					P value: .007			
Depression levels								
Gender	**Normal**		**Slight**	**At the border**	**Moderate**	**Severe**	**Extreme**	**Total**
Male	n	225	87	21	31	24	3	391
	%	47.3	43.5	33.9	33.0	48.0	16.7	43.4
Female	n	251	113	41	63	26	15	509
	%	52.7	56.5	66.1	67.0	52.0	83.3	56.6
Total	n	476	200	62	94	50	18	900
	%	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Chi-square: 15,012					p value: .010			
Age (years)	**Normal**		**Slight**	**At the border**	**Moderate**	**Severe**	**Extreme**	**Total**
19-22	n	369	158	43	74	35	17	696
	%	77.5	79	69.4	78.7	70	94.4	77.3
23-25	n	103	41	19	20	15	1	199
	%	21.6	20.5	30.6	21.3	30	5.6	22.1
26≤	n	4	1	0	0	0	0	5
	%	0.8	0.5	0	0	0	0	0.6
Total	n	476	200	62	94	50	18	900
	%	100	100	100	100	100	100	100
Chi-square: 9,581					p value: .478			
SD: Standard deviation, Max: Maximum, Min: Minimum								

Table 4. Distribution of tryptophan consumption level by depression level

Depression level		Tryptophan consumption level			
		Low (<250 mg)	Normal (251-750 mg)	High (>751 mg)	Total
Normal	n	4	80	392	476
	%	66.7	51.9	53.0	52.9
Slight	n	2	30	168	200
	%	33.3	19.5	22.7	22.2
At the border	n	0	8	54	62
	%	0.0	5.2	7.3	6.9
Moderate	n	0	23	71	94
	%	0.0	14.9	9.6	10.4
Severe	n	0	23	39	94
	%	0.0	14.9	5.3	10.4
Extreme	n	0	11	16	50
	%	0.0	7.1	2.2	5.6
Total	n	6	154	740	900
	%	100.0	100.0	100.0	100.0
Chi-square: 8,255, p value: .604					

Table 5. Distribution of perceived stress scale sub-factors by gender and age and the relationship between total stress, perceived stress, perceived coping and total beck according to tryptophan level

Stress scale sub-factors	Gender	N	Mean	SD	t-test	p
Total stress	Women	509	15.58	3.86	-.984	.325
	Male	391	15.85	4.08		
Perceived stress	Women	509	9.06	3.71	.785	.433
	Male	391	8.86	3.87		
Perceived coping	Women	509	6.65	2.31	-2.555	.011
	Male	391	7.05	2.42		
	Age (years)				F	p
Total stress	19-22	696	15.64	3.93	1.774	.170
	23-25	199	15.98	4.00		
	26≤	5	13.00	5.38		
Perceived stress	19-22	696	8.97	3.76	.850	.428
	23-25	199	9.03	3.87		
	26 ≤	5	6.80	3.56		
Perceived coping	19-22	696	6.79	2.42	.448	.639
	23-25	199	6.93	2.15		
	26 ≤	5	6.20	2.94		

SD: Standard deviation

Table 6. The relationship between total stress, perceived stress, perceived coping and total beck according to tryptophan level

Variables	Tryptophan consumption level	n	Mean	SD	F	p
Total stress	Low	6	14.8	4.02	2.140	.118
	Normal	154	15.1	4.31		
	High	740	15.8	3.87		
Perceived stress	Low	6	9.3	3.72	.178	.837
	Normal	154	8.8	3.58		
	High	740	9.0	3.83		
Perceived coping	Low	6	5.5	1.64	4.078	.017
	Normal	154	6.4	2.50		
	High	740	6.9	2.33		
Beck depression	Low	6	7.6	4.17	.568	.567
	Normal	154	12.1	11.22		
	High	740	12.3	10.70		

SD: Standard deviation

Table 7. Descriptive statistics and correlations for study variables

	n	Mean	SD	1	2	3	4	5
1. Tryptophan	900	1,489.4	809.74	-	-	-	-	-
2. Beck depression	900	12.26	10.76	.485*	-	-	-	-
3. Total stress	900	15.70	3.96	.355*	.000**	-	-	-
4. Perceived stres	900	8.98	3.78	.941*	.000**	.000**	-	-
5. Perceived coping	900	6.82	2.37	.045*	.000**	.000**	.000**	-

*p<.05., **p<.01.
SD: Standard deviation

Table 8. A simple regression analysis was conducted to determine the effect of total tryptophan consumption on depression and stress

Depression	B	Standard error	Beta	t	Sig.
Constant*	11,806	.752		15,703	.000
Total tryptophan	.000	.000	.023	.699	.485
Total stress					
Constant*	15,479	.277		55,951	.000
Total tryptophan	.000	.000	.031	.924	.355
Perceived stress					
Constant	8,997	.264		34,019	.000
Total tryptophan	-1,159E-005	.000	-.002	-.074	.941
Perceived coping					
Constant	6,536	.165		39,558	.000
Total tryptophan	.000	.000	.067	2.004	.045

Total depression: *R: .23 R^2: .001. Total stress: *R: .31 R^2: .001. Perceived stress: *R: .002 R^2: .000. Perceived coping: *R: .067 R^2: .004

Each factor was tested for goodness-of-fit and the criterion for goodness. The statistics used to evaluate the confirmatory factor analysis (CFA) models are shown in Figure 3. According to the fit values examined, the first-order multi-factor model, except for chi-square/degrees of freedom, shows good acceptable fit to the data. CFA was not performed for the Beck depression scale. Because, when scoring the scale, it is divided into 6 sub-factors and there is no specific scale item revealing each factor. Each sub-factor of the scale is determined according to the total score. Therefore, factor analysis was not done. Cronbach's alpha value was found to be 0.889.

Discussion

The main finding of this content is to reveal the levels and amounts of the nutrients that can have an effect on the stress and depression level of university students on depression and stress. In this study, the majority of the students consume high levels of tryptophan. Similarly; in a study conducted by Gracia-Marco et al. (19) on university students in 2017, it was reported that the vast majority of students consumed tryptophan at high levels. This situation can be explained by the fact that foods rich in tryptophan are easily accessible and liked by students and are found in foods that are sources of protein (milk, chicken, meat, legumes, etc.).

In this study, a difference was found in tryptophan consumption according to gender, and female students have more tryptophan consumption than males. Differently, in the study conducted by Gracia-Marco et al. (19) on university students in 2017, it was observed that male students had more tryptophan consumption than female students. This situation can be explained by the fact that this situation may be due to differences in food preferences among students.

When tryptophan consumption and age groups were examined in this study, the group with the highest tryptophan consumption was students in the 19-22 age group. This may be due to the fact that the younger students consume more foods rich in tryptophan or the individual selection differences among the students.

In this study, when the students' level of depression was examined, the depression level of the majority of students was found to be normal. Similarly, the majority of students' depression levels were found to be normal in many studies (28,29,30). This situation can be explained by the fact that university students can easily adapt to the new system they are in, and they can manage negative events and processes well and overcome their psychological effects.

In this study, when the depression status of students according to gender was examined, female students were found to be more depressed than males. Similarly, in a 2007 study by Liu et al. (30) on university students, females were found to be more depressed than males. This situation can be explained by the fact that female students are more emotionally sensitive, act with more emotions and may be more psychologically affected by negative events and situations.

In this study, when the depression status of the students according to age was examined, the students in the younger age group (19-22 years old) were found to be more depressed. Similarly, in a study conducted by Liu et al. (30). In 2007, it was discovered that university students of a younger age group were more depressed.This situation can be explained by the fact that individuals in a younger age group have less life experience, their adaptation to unfavorable events and processes is difficult, and they have psychological difficulties in managing such situations.

In this study, no relationship was found between students' depression level and tryptophan consumption levels. Similarly, Soh and Walter (31) study in 2011 found that no relationship was found between depression level and tryptophan consumption levels. Differently, Grases et al.'s (20) study in 2019 found that those with low depression levels had high tryptophan consumption. Differently, Lindseth et al.'s (17) study found that depression levels were low in those who consumed high amounts of tryptophan. This may be due to individual differences among students. More scientific studies are needed on this subject.

In this study, the PS was found to be higher in females than in males. A statistically significant difference was found between the PC levels and gender ($p<0.05$). Male students' average coping with PS is higher than girls. Similarly, in the study conducted by Cavallo et al. (11) on university students in 2009, the PS situations were found to be higher for female students than for male students. Similarly, in Almojali et al. (9) study on university students, the PS level of female students was found to be higher than male students. This can be explained by the fact that female students are more emotionally sensitive and are more affected by negative events and situations and get stressed more quickly.

In this study, no relationship was found between students' PS and age ($p>0.05$). Similarly, in Birks et al's (10) study on university students, no relationship was found between PS and age. This situation can be explained by the different perception of stress in the face of events rather than age.

In this study, a statistically significant difference was found between the PC level and the mean tryptophan consumption level ($p<0.05$). Those with normal and high tryptophan levels were found to have a high level of coping with perceived stress. This may be due to individual differences among students. More scientific studies are needed on this subject.

Due to the inadequacy of studies on this subject in the literature, this study will shed light on future studies. In addition, by contributing to the formation of a psychologically healthy, happy and peaceful young population, it is important to contribute to the reduction of social burden by reducing health expenditures.

Study Limitations

Though the current study has addressed a gap in the literature, several methodological limitations should be considered when interpreting the findings. One such limitation is that the participants who completed the questionnaires were not fully representative of the school from which they came. Nevertheless, as the population studied came from a very specific demographic group (i.e. 19-26-year-old university students from different nationalities and different cultures from different cities in Turkey and different countries around the world), further research is needed that focuses on more representative samples. Another limitation of the current research was that the chronicity of tryptophan use was not taken into account. For instance, students' tryptophan consumption may differ, especially during exam periods, so the timing of administration of the questionnaire may have been of importance. A further limitation of the current study is that it utilised a cross-sectional design. This means that all effects observed here are correlational, and that causation can not be inferred. Future research should therefore aim to conduct intervention studies in order to investigate the nature of these relationships further.

Conclusion

While the depression status of the students is not affected by the amount of tryptophan consumption, their stress status is affected. The increase in tryptophan consumption increases the students' ability to cope with stress and reduces their stress levels. In parallel with the result, it can be recommended that students increase their consumption of tryptophan source foods by paying more attention to their nutrition during periods of high stress such as exam periods.

Ethics

Ethics Committee Approval: Our study was approved by Üsküdar University Non-Interventional Research Ethics Committee under the number 61351342-/2020-61 dated 29.01.2020.

Informed Consent: Written informed consent was given to participant.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: B.A.U., A.S.G., Design: B.A.U., Data Collection or Processing: B.A.U., Analysis or Interpretation: B.A.U., A.S.G., Literature Search: B.A.U., A.S.G., Writing: B.A.U.

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Original Article

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Determining the Amount of Iodine in Edible Salts Obtained from Markets and District Bazaars in İstanbul

İstanbul Piyasasında Satılan Yemeklik Tuzlarda İyot Miktarı Tespitinin Yapılması

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ABSTRACT

Objective: Iodine is an element that is rarely found in water and soil in nature. Iodine is a component of thyroid gland hormones and is an essential element for human life. Iodine deficiency disease (IDD) is an important public health problem all over the world, which occurs as a result of iodine deficiency. Visible symptoms of iodine deficiency are defined as goiter. On the other hand, excessive intake of iodine into the body may disrupt thyroid hormone secretion and result in iodine deficiency.

Methods: In this study, the iodine amounts of 26 edible salt samples collected from markets and district bazaars in Istanbul were analyzed according to the method "Determination of Iodine in Iodized Salts with Iodized Ion" in TS 933 Edible Salt Standard, and the compliance of salts sold in the market with the Turkish Food Codex Salt Communiqué was investigated.

Results: Thirteen of 26 salt samples were appropriate at the level of 28.0-39.8 mg/kg (50%), high at the level of 44.0-52.9 mg/kg (27%) in 7 samples and low at the level of 2.0-20.0 (23%) in 6 samples have been found.

Conclusion: It has been determined that the amount of iodine in 50% of the salt samples was not appropriate. Iodine deficiency detected in salts in the market increases the risk of IDD, and excess iodine increases the risk of goiter. In this case, it is important that the sustainability of food control and inspection by administrative authorities is effective for the protection of public health.

Keywords: Iodine, salt, food control, public health

ÖZ

Amaç: İyot doğada sularda ve toprakta nadir olarak bulunan bir elementtir. İyot, tiroid bezi hormonlarının bir bileşeni olup insan hayatı için gerekli bir elementtir. İyot yetersizliği hastalığı (İYH), iyot eksikliği sonucu ortaya çıkan ve tüm Dünya'da önemli bir halk sağlığı sorunudur. İyot eksikliğinin gözle görülen belirtileri guatr olarak tanımlanmaktadır. Diğer yandan, iyotun vücuda fazla miktarda alınması tiroit bezi hormon salınımı bozup iyot eksikliği ile sonuçlanabilir.

Yöntemler: Bu çalışmada İstanbul'da marketlerden ve semt pazarlarından toplanan 26 yemeklik tuz örneklerinin iyot miktarları TS 933 Yemeklik Tuz Standardındaki "İyodat İyonu ile İyotlanmış Tuzlarda İyot Tayini" metoduna göre analizi yapılarak piyasada satılan tuzların Türk Gıda Kodeksi Tuz Tebliği'ne uygunluğu araştırılmıştır.

Bulgular: Yirmi altı tuz örneğinin 13'ü 28,0-39,8 mg/kg düzeyinde (%50) uygun, 7 örnekte 44,0-52,9 mg/kg düzeyinde (%27) yüksek ve 6 örnekte ise 2,0-20,0 düzeyinde (%23) düşük miktarlarda bulunmuştur.

Sonuç: Tuz örneklerinin %50'sinde iyot miktarlarının uygun miktarda olmadığı tespit edilmiştir. Piyasadaki tuzlarda tespit edilen iyot eksikliği toplumda İYH, iyot fazlalığı ise guatr riskini artırmaktadır. Bu durumda; idari otoriteler tarafından halk sağlığının korunması için gıda kontrol ve denetimlerinin sürdürülebilirliğinin etkin olması önemlidir.

Anahtar Sözcükler: İyot, tuz, gıda kontrolü, halk sağlığı

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Introduction

"Ioeides" means "purple" in Greek. Iodine is a very rare, black, solid and active element with symbol I, atomic number 53, and atomic weight of about 126.09 g/mol. It was defined by Gay Lussac in 1812 (1). Chemically, iodine is the least reactive of the halogens. Iodine has the least solubility in water, it dissolves in organic solvents. The most important inorganic iodine is hydrogen iodide, which is colorless. Iodine is mainly used in medicine, photography, and paint manufacturing. Many living things need trace amounts of iodine (1,2). Iodine, which is found in rocks, soil, minerals, sea water and underground water resources, is mostly found in dark colored algae (2,3). On earth, iodine is mostly taken from the surface layers of the soil with glacier, rain and snow, carried to the oceans and seas by wind, rivers and floods, and returns to the clouds as vapor and then to the soil as rain. In particular, seafoods are considered the best food sources rich in iodine (3).

Iodine is an essential element for the synthesis of the thyroid hormone. The body does not produce iodine, so it is an important part of the diet (4). If there is not enough iodine in the body, enough thyroid hormone cannot be produced. Therefore, iodine deficiency can cause thyroid enlargement, hypothyroidism and mental retardation in infants and children whose mothers are iodine-deficient during pregnancy. Iodine deficiency is the most common preventable cause of mental retardation (5). Iodine deficiency affects 30% of the world's population, especially in developing countries. In addition, iodine deficiency is common in pregnant women worldwide. Among the devastating consequences of this condition are cretinism and congenital abnormalities (3,6). When sufficient fetal iodine is not taken, it can lead to multiple iodine deficiency disorders such as maternal hypothyroidism, and neuropsychological developmental disorder and cretinism in children (6-8). Iodine deficiency is an important public health problem that concerns all countries (9). Iodine plays a key role in early growth and development of the organs, particularly the brain, between fetal life and post-adolescent life. It is stated that the main source of food in infants is breast milk during breastfeeding (10). Breastfeeding mothers who do not take iodine supplements are a risk group for iodine deficiency. In addition, breastfed newborns of mothers who do not take iodine supplementation have a low urinary iodine concentration and an increased risk of neurological disorders (9). Inadequate fertility, preeclampsia, postpartum hemorrhage, maternal anemia, and increased sensitivity of the thyroid gland to nuclear radiation are the main clinical manifestations in mothers with iodine deficiency. The effects of iodine deficiency on the fetus are early and late abortions, stillbirth, low birth weight, congenital malformations, microcephaly, increased perinatal mortality, cretinism, fetal goiter, and increased sensitivity of the thyroid gland to nuclear radiation. In newborns, neonatal goiter, neonatal hypothyroidism, a 13-fold increase in recall rate in congenital hypothyroidism screening, and an increase in the sensitivity of the thyroid gland to nuclear radiation can be seen. Goiter, hypothyroidism, increase in infant mortality, failure in phagocyte functions and late cellular immune response, physical developmental delay, failure in school, and increased sensitivity of the thyroid gland to nuclear radiation can be observed in infants, and childhood and adolescence periods. In adults, goiter and nodularity, hypothyroidism, mental dysfunction, insufficient physical performance, hyperthyroidism that may occur with iodine loading, and an increase in the risk of autoimmune thyroid diseases can be seen (4). In animals, decrease in reproduction, decrease in the number of live births, decrease in birth weight, increase in the rate of deformed birth, loss of strength, low yield in terms of meat, milk and wool can be seen (3).

The basic approach to iodine deficiency is to increase the daily iodine intake of individuals and the recommendations to ensure this are based on enriching frequently consumed foods with iodine (10). In order to eliminate iodine deficiency, many countries allocate large budgets to medicine for the production of iodine-enriched foods. The amount of iodine in meat, milk, eggs, cereals, and plant-based foods depends on the iodine level of the region, season, and reproductive conditions (2,11). The iodine levels of the soil during the cultivation of fruits and vegetables, irrigation water used and fertilization of the plants affect the iodine content in plant-based foods, while the iodine level of the feeds affects the iodine content of the animal based foods (5).

Patients with goiter mostly vary in relation to features such as geographical region and age of the patient (4,6-9,12). In prospective studies conducted in the USA and Japan, the incidence of goiter is 6% in school-age children living in areas where iodine deficiency is not observed, and different results are obtained in North America, Europe, Asia (Japan) and Oceania when goiter is frequent due to differences in iodine intake in Europe. The prevalence of endemic goiter in economically developed countries (New Zealand and Australia) was found to be 0-5%. According to the latest data, it is reported that goiter is still seen in adults in European countries such as Bulgaria, Czech Republic, Slovakia, Netherlands, Switzerland and Belgium, but it is rare in children. There are regions with high prevalence of goiter in Austria, Hungary, Poland, Yugoslavia and Western Russia (11). It is estimated that 37% of school-age children (285 million children) and approximately two billion people worldwide have iodine deficiency (12,13). It has been reported that iodine supplementation programs should be applied when clinical cases of iodine deficiency are seen in a region and when urinary iodine excretion is found to be low in parallel (2). In many studies, iodine deficiency is determined by combining clinical findings with urine iodine excretion analyzes. Four parameters for evaluation of iodine nutrition in the population are generally recommended to be used: Urinary iodine concentration (UIC), goiter size, serum tiroit stimulating hormone and serum Tg. These parameters are complementary to each other. As over 90% of dietary iodine eventually appears in the urine, UI is an excellent indicator of recent iodine intake (14). A cross-sectional national study was conducted in Switzerland in 2015 to assess the current iodine status in school-aged children, women of reproductive age, and pregnant women. As a result of this study, a longitudinal analysis was performed by the same team on UIC

detected in the same population in 1999, 2004, 2009 and 2015, and the effect of increasing the iodine concentration in salt by 5 mg/kg was evaluated. As a result, the investigators observed a modest improvement in median UIC in children compared to the previous three national studies by increasing the salt iodine content from 20 mg/kg to 25 mg/kg. The investigators showed that iodine intake remained low in reproductive-age adult women and pregnant women, and that increasing iodine in salt by 5 mg/kg did not benefit women with higher dietary iodine requirements. The reason for this is that women working in Switzerland tend to prefer ready-to-eat foods, and since there has not been any study on the amount of iodine in ready-made foods and the salts used in these foods, a definite conclusion has not been reached on this issue. As a result, it was concluded that there was insufficient iodine nutrition in women of reproductive age and pregnant women. It was concluded that in Switzerland, when using continuous iodized salt, an overall increase in iodine intake of about 25 μg/day would likely to be sufficient to provide average iodine nutrition (15).

Seafoods and foods from iodine-rich soils may be suitable sources for iodine uptake (4). However, the contribution of iodine taken from different food sources to human health cannot be measured directly. However, it shows that a significant portion of daily iodine intake may come from sources other than salt, and iodized salt may partially compensate for the deficiency (15). Iodization of salt is currently the most common strategy to control and completely eliminate iodine deficiency disorder. However; for the amelioration of iodine deficiency to be fully effective, salt must be delivered to the entire affected population, particularly to susceptible individuals such as pregnant women and young children. Especially in production, iodized salt should be monitored. This type of monitoring requires close cooperation between public authority and the salt industry. Ideally, monitoring of the iodine content of the salt should be done internally by the salt producer at the iodization site, and also externally by the health authorities. Internal monitoring of the production process should be done routinely and external (public) monitoring should be done intermittently, and where possible, both of these monitoring systems should use the titration method to determine the iodine content of the salt (14).

Although the first legal regulation on the iodization of salt was made in our country in 1968, the most meaningful initiative to prevent iodine deficiency was supported by UNICEF and T.C. The issue was handled by the Ministry of Health AÇSAP General Directorate, and in this context, a real intersectoral cooperation was made to inform the public about the iodization and consumption of all table salt produced. This has been the most comprehensive cooperation in our country under the coordination of the Ministry of Health, with the contribution and participation of many organizations such as salt producers, the Ministry of Agriculture and Forestry, universities and some international organizations (3). During this period, trainings were organized for technical personnel in salt production facilities on the preparation of homogeneous salt in terms of iodine and titrimetric salt analysis. According to the Salt Communique numbered 2004/25 (16), which was out of date in our country, both KI and KI could be added to salts. However, since the titrimetric chemical analysis of KI is complex and difficult compared to KI, according to the current Turkish Food Codex Communique on Salt No. 2013/48 (17), adding potassium iodate at the rate of 25-40 mg/kg to table salt is mandatory. In this study, the compliance of potassium iodate amounts in the edible salt samples sold in the Istanbul market with the Turkish Food Codex Salt Communique No. 2013/48 was investigated.

Methods

Chemicals

Hydrochloric acid (25%), potassium iodide (KI) (15%), sodium thiosulfate and 0.5% (m/v) starch were obtained from Merck.

Determination and Collection of the Sample

Assuming that the inappropriate sample rate varied between 5% and 20%, it was calculated by power analysis that at least 26 samples should be studied, taking into account the 95% confidence level and 80% power coefficients, in order to reveal this frequency approximately. For this purpose, 26 salt samples, 50 grams each, were collected from the Istanbul market. Ethics committee approval of the study was obtained from Bezmialem Vakif University Non-interventional Research Ethics Committee with the decision dated 04.09.2018 and numbered 16/197. For the salt samples included in the study, it was stated on the labels that potassium iodate was added to the salts.

Analysis of Iodine Content in Salt

The amount of iodine analysis was made by the method of "Determination of Iodine in Iodized Salts with Iodate Ion" in TS 933 Edible Salt Standard (18). For this purpose, 0.1 g of 50 g salt sample was weighed on a precision balance (Ohaus Company, USA) and dissolved in 200 mL distilled water. 2.5 mL of 25% hydrochloric acid solution and 10 mL of 15% KI solution were added. The solution was titrated with 0.01 M sodium thiosulfate solution until the color of the solution became light yellow, then 2.5 mL of 0.5% (m/v) starch solution was added and the titration was continued with adding 0.01 M sodium thiosulfate solution without delay until the blue color disappeared. The total volume of sodium thiosulfate solution spent was recorded. The amount of iodine was calculated in terms of potassium iodate.

Statistical Analysis

Power analysis was performed to determine the number of samples. Collected salt samples were analyzed twice and the values were calculated as mean ± standard deviation.

Results

Potassium iodate analysis was carried out in 26 edible salts, which were stated to be iodized on their labels, which were offered for sale in different markets and neighborhood markets in Istanbul (Table 1). Iodine content was found to be 28.0-39.8 mg/kg in 13 of the analyzed samples, 44.0-52.9 mg/kg in 7 samples, and 2.0-20.0 mg/kg in 6 samples. While 50% of the salts were

Table 1. Evaluation of the results of the iodine amount analysis in terms of potassium iodate of 26 edible salt samples collected from the market according to the Turkish Food Codex Communiqué on Salt (2013/48). Results are given as mean ± standard deviation

n	Mean ± standard deviation (mg/kg)	Suitability
1	46.3±2.47	**High**
2	47.0±0.99	**High**
3	47.5±4.38	**High**
4	34.8±2.83	Appropriate
5	3.3±0.00	**Low**
6	39.5±0.71	Appropriate
7	47.5±0.49	**High**
8	38.7±1.41	Appropriate
9	44.0±0.28	**High**
10	44.4±2.69	**High**
11	20.0±1.20	**Low**
12	34.4±0.49	Appropriate
13	34.8±0.28	Appropriate
14	28.0±0.42	Appropriate
15	28.5±0.78	Appropriate
16	37.2±0.14	Appropriate
17	2.0±0.00	**Low**
18	33.7±0.85	Appropriate
19	22.5±0.00	**Low**
20	39.8±0.14	Appropriate
21	30.7±0.28	Appropriate
22	30.1±0.49	Appropriate
23	4.76±0.00	**Low**
24	52.9±0.00	**High**
25	2.05±0.78	**Low**
26	34.1±1.13	Appropriate

suitable according to the Codex, 23% were determined to have low iodine levels. Iodine content was high in 27% of salts.

Iodine deficiency, which is one of the most common micronutrient deficiencies in the world, is the main cause of mental retardation which can potentially be prevented in childhood, and morbidity series called iodine deficiency disorders (4,5). It is suggested that iodization of salt is the best way to prevent and cure many of these disorders (15). In this study, 26 samples of edible salt sold in the Istanbul market were taken and their potassium iodate amounts were evaluated in accordance with the Turkish Food Codex Salt Communiqué No. 2013/48.

As a result of the titrimetric analysis, it was determined that 50% of the iodized salts contained iodine in the appropriate amount according to the Turkish Food Codex Salt Communiqué, while 50% of the samples contained iodine in low or high amounts. On the other hand, iodine was not found in 8 (38%) of 21 salt samples collected and analyzed in Ankara in 2007 (19). All these results show that our country has made progress in terms of 'iodized salt' health policies. However; it was reported that an average of 39.85±5.46 ppm was found, with the lowest iodine content being 6.30 ppm and the highest 82.50 ppm, which was analyzed by a titrimetric and spectrometric methods performed on 15 salt samples from India (20). The large difference between the lowest amount of iodine in salts and the highest amount of iodine indicates that there may be a production error. The researchers urge the Ministry of Health authority in the countries of this study to reassess iodized salt quality as an urgent public health imperative. In the iodine analysis performed on 1803 (n=1,533 iodized salt, n=270 crystal salt) salt samples brought from home by primary school children from 7 different districts in Nepal; the mean iodine content in iodized and crystalline salts was 40.8±12.35 ppm and 18.43±11.49 ppm, respectively (21). There was a significant difference between the use of iodized and crystalline salts and the salt iodine content of iodized and crystalline salts between different districts, and it was revealed that only 169 samples (9.4%) of the total samples had an iodine content of <15 ppm, and that most Nepalese households had access to iodized salt. The iodization of salt is the world's most widely used method for fortifying foods with iodine. On the other hand, it is obvious that iodine deficiency is still an important and widespread public health problem. Although salt iodization programs have been initiated by the International Council for Control of Iodine Deficiency Disorders (ICCIDD) in some countries such as the Philippines, Indonesia, New Zealand, Papua New Guinea, Srilanka and Vietnam, successful results have not been achieved, but it has been reported the targeted results have not yet been achieved (5).

Conclusion

In this study, the amount of iodine in the salts stated to be iodized on the labels, 50% of the samples in terms of potassium iodate were found to be in compliance with the Turkish Food Codex Table Salt Communiqué, and 50% (27% high and 23% low) were not found to be in accordance with the aforementioned codex. In this case, it is seen that the salt iodization is not done as it should be in accordance with the target. Considering that high iodine content can cause hyperthyroidism and low iodine content can cause iodine deficiency disease, it is imperative that the Ministry of Health and the Ministry of Agriculture and Forestry should carry out regular, widespread and target-oriented food checks in this area, and that they should also issue necessary warnings to producers with codex violations. In addition, periodic studies in this area will lead to the solution of public health problems such as hyperthyroidism and iodine deficiency in our country.

Ethics

Ethics Committee Approval: Ethics committee approval of the study was obtained from Bezmialem Vakif University Non-interventional Research Ethics Committee with the decision dated 04.09.2018 and numbered 16/197.

Peer-review: Externally peer reviewed.

Authorship Contributions

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Original Article

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Determining the Compliance of Intern Nursing Students with Isolation Precautions in the COVID-19 Pandemic Period

COVID-19 Pandemisi Döneminde İntörn Hemşirelik Öğrencilerinin İzolasyon Önlemlerine Uyumunun Belirlenmesi

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ABSTRACT

Objective: This study aimed to determine the compliance of intern nurses with isolation precautions in the coronavirus disease-19 (COVID-19) pandemic period.

Methods: This descriptive and cross-sectional study was conducted with 4th-year intern nurses (n=90) receiving education at the vocational school of health of a state university in November 2020. The data were collected by using a Questionnaire Form and the "Isolation Precautions Compliance Scale". In data analysis, percentage, mean, Kruskal-Wallis test and Mann-Whitney U test were used in the SPSS software.

Results: The mean age of the intern nurses who participated in the study was 22±1.43 years, 74.4% were female, 51.1% were graduates of Anatolian high schools. Intern nurses 95.6% had received training on isolation precautions, and 81.1% received this training from a course instructor during their undergraduate studies. In was determined that 71.1% of the participants provided nursing care for patients who were under isolation during clinical practice, and 91.1% had knowledge that isolation precautions need to be implemented in the nursing care of patients diagnosed with COVID-19. The mean Isolation Precautions Compliance Scale total score of the participants was 71.92±5.74. There was no significant difference in the mean Isolation Precautions Compliance Scale total scores of the participants based on their sociodemographic characteristics and isolation precautions.

Conclusion: It was determined that the compliance of the intern nursing students, whose nursing labor could be needed in the

ÖZ

Amaç: Araştırma, koronavirüs hastalığı-19 (COVID-19) pandemisi döneminde intörn hemşirelerin izolasyon önlemlerine uyumlarının belirlenmesi amacıyla yapılmıştır.

Yöntemler: Tanımlayıcı ve kesitsel tipteki bu araştırma, Kasım 2020 tarihinde bir devlet üniversitesinin sağlık yüksekokulunda öğrenim gören 4. sınıf intörn hemşireler (n=90) ile gerçekleştirildi. Veriler, Kişisel Bilgi Formu ve "İzolasyon Önlemlerine Uyum Ölçeği" kullanılarak toplandı. Verilerin değerlendirilmesinde yüzde, ortalama, Kruskal-Wallis testi ve Mann-Whitney U testi kullanıldı.

Bulgular: Araştırmaya katılan intörn hemşirelerin yaş ortalaması 22±1,43 yıl olup, %74,4'ü kadın, %51,1'i anadolu lisesi mezunudur. Öğrencilerin %95,6'sının izolasyon önlemleri hakkında eğitim aldığı ve %81,1'inin bu eğitimi lisans öğreniminde öğretim elemanı/üyesinden aldığı bulundu. Katılımcıların %71,1'inin klinik uygulama esnasında izolasyon uygulanan hastaya hemşirelik bakımı verdiği ve %91,1'inin COVID-19 tanılı hastanın hemşirelik bakımında izolasyon önlemlerinin uygulanması ile ilgili bilgi sahibi olduğu belirlendi. Öğrencilerin izolasyon önlemlerine uyum ölçeği toplam puan ortalaması 71,92±5,74 olarak bulundu. Öğrencilerin sosyodemografik özellikleri ve izolasyon uygulamaları ile izolasyon önlemlerine uyum ölçeği toplam puan ortalamaları arasında istatistiksel olarak anlamlı farklılık olmadığı saptandı.

Sonuç: COVID-19 pandemisi döneminde hemşirelik iş güçlerine ihtiyaç duyulabilecek intörn hemşirelerin izolasyon önlemlerine uyumlarının iyi düzeyde olduğu belirlendi.

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COVID-19 pandemic period, with isolation precautions was on a good level.

Keywords: COVID-19 pandemic, internship, isolation, nurse

Anahtar Sözcükler: COVID-19 pandemisi, intörn, izolasyon, hemşire

Introduction

The World Health Organization (WHO) reported patients with pneumonia of unknown etiology in the city of Wuhan, Hubei province of China, on 31 December 2019. These patients were identified as infected by a new coronavirus (2019-nCoV) which was not detected in humans before on January 7, 2020. Later, the name of the 2019-nCoV disease was determined as coronavirus disease-19 (COVID-19) (1). WHO classified COVID-19 as an international public health emergency on 30 January 2020. The disease was declared as a pandemic by WHO on March 11, 2020, due to the spread of the virus and severity of the disease, and its occurrence in 113 countries outside of China, where COVID-19 first started (1,2). The first case of COVID-19 in our country was detected on March 11, 2020 (1). WHO reported that as of January 24, 2021, COVID-19 had spread to 224 countries and caused 2,098,879 deaths (2).

Despite all the precautions taken in the world and in our country, the COVID-19 pandemic continues and the number of morbidity and mortality is increasing (2-4). Not knowing when the pandemic will end puts responsibilities on universities as well as health services (3). There are countries that give an active role in the field to nursing students in the last years of their undergraduate education in the fight against the pandemic (5,6). This has not been the case in our country, and most universities have postponed the clinical and internship practices of nursing departments due to the COVID-19 pandemic.

Internship system is implemented in many universities in our country (7). In this system, senior nursing students, who have gained a certain professional knowledge and skill, are given an active role as an intern nurse in the field and gain professional awareness and experience before graduation (8). The insufficiency of nurses struggling with the pandemic due to overwork, increased workload or the emergence of the need for nurses as a result of being infected is a situation that may cause intern nurses to be needed in the field. In addition, if the pandemic continues, intern nurses will have to complete their clinical practice in this environment and will take an active role in the pandemic when they graduate (3,6,9).

Adequate and correct application of infection control measures by nurses in the clinical management of COVID-19 is important in breaking the chain of infection (10-12). Nurses are at risk of transmission of COVID-19 during the care and treatment of patients, and they have responsibilities both for themselves and for the prevention of transmission in the healthcare setting (7,9,12). The causative agent of COVID-19 is transmitted from person to person through droplets, contact and, in some cases, aerosols. Therefore, isolation measures should be applied to patients with COVID-19 (11,13). Isolation is the separation of the patient in order to prevent the transmission of microorganisms from the infected patient to health professionals, other patients and visitors. Isolation precautions are divided into two as standard and contagious precautions (14). Isolation measures to be applied to patients diagnosed or suspected as having COVID-19 include contact and droplet isolation for transmission in addition to standard precautions (15).

The knowledge and attitudes of intern nurses who can work in the field during the COVID-19 pandemic period regarding isolation measures are extremely important in terms of safe, quality and sustainable health care (16-19). In this study, it is aimed to determine the compliance of intern nurses with isolation measures during the COVID-19 pandemic period.

Methods

Purpose and Type of Research

This research was carried out in a descriptive and cross-sectional type with the aim of determining the compliance of intern nurses with isolation measures during the COVID-19 pandemic period.

Population and Sample of the Research

The universe of the research consisted of 4th grade intern nurses studying at a public university health school in November 2020 (n=90). Sample calculation was not made in the study, and the entire universe was reached.

Data Collection Tools

Personal Information Form and Isolation Precautions Compliance Scale were used to collect data.

Personal Information Form: The personal information form developed by the researchers in line with the literature consists of 7 questions to determine the sociodemographic characteristics of intern nurses (age, gender, graduated high school) and isolation practices (training about isolation, caring for isolated patients, etc.) (10,20-25).

Isolation Precautions Compliance Scale: The scale, which was developed by Tayran and Ulupınar (16) in 2011 and of which validity and reliability studies were conducted, consisted of 18 items and 4 sub-dimensions. These sub-dimensions are; route of transmission (items 3, 8, 9, 10 and 11), employee and patient safety (items 2, 5, 12, 14, 16 and 17), environmental control (items 1, 13, 15 and 18), and hand washing and the use of gloves (items 4, 6 and 7). It is a five-point Likert type (1= strongly disagree, 2= disagree, 3= no idea, 4= agree, and 5= strongly agree). Items 5, 7, 12 and 17 in the scale are negative statements. The total scores that can be obtained from the scale vary between 18-90 points, and an increase in the score indicates that nurses' compliance with isolation measures increases. The authors of the

scale suggest the use of the scale as one-dimensional. In the study of Tayran and Ulupınar (16), the Cronbach alpha value of the scale was found to be 0.85. In this study, it was found to be 0.79.

Data Collection

Intern nurses were reached online after obtaining the necessary permissions to conduct the research. Students were asked to answer the Personal Information Form and Isolation Precautions Compliance Scale by explaining to the students the purpose of the research, the identity information and the information they provided would be kept confidential by complying with the ethical principle of confidentiality and secrecy.

Ethical Aspect of Research

In order to carry out the research, ethics committee approval was obtained from the Scientific Research Ethics Committee of the Trakya University Faculty of Medicine with the date 12.10.2020 and number 2020/326, and the institutional permission was obtained from the Y School Directorate with the date 09.11.2020 and number 33505391-044-E.472785. In accordance with the Declaration of Helsinki, the purpose of the study was explained to the intern nurses who constituted the sample of the study. It was informed that the participation was voluntary and that the personal information and privacy of the participants would be protected.

Evaluation of Data

The data obtained in the research were analysed by using the SPSS 21.0 package program. The compatibility of the variables with normal distribution was evaluated with the Kolmogrov-Smirnov test and it was found that the data did not show compliance with the normal distribution. While evaluating the study data, non-parametric tests such as Kruskal-Wallis test and Mann-Whitney U test were used for comparisons between groups as well as descriptive statistical methods (number, percentage, mean, standard deviation). Statistical significance level was accepted as $p<0.05$.

Results

The average age of the intern nurses participating in the study was 22±1.43 years. It was determined that 74.4% of the students were women, 51.1% were Anatolian high school graduates, 95.6% received training on isolation precautions and 81.1% received this training from an instructor/member in their undergraduate education. It was found that 71.1% of the participants provided nursing care to the patients who were isolated during clinical practice, and 91.1% had knowledge about the need to apply isolation measures in the nursing care of a patient with a diagnosis of COVID-19 (Table 1).

In our study, the average score of the intern nurses on the Isolation Precautions Compliance Scale was 71.92±5.74, and the sub-dimension mean scores of the scale was as follows; 23.66±2.25 for route of transmission, 18.81±2.56 for employee and patient safety, 18.42±1.86 for environmental control, 11.02±1.28 for hand hygiene and use of gloves (Table 2).

Intern nurses participating in the study had knowledge about gender, graduated high school, getting training about isolation precautions, where and from whom they received training on isolation measures, providing nursing care to the patients who were isolated during clinical practice, and the need for isolation measures to be applied in the nursing care of the patient with COVID-19. It was determined that there was no statistically significant difference between the characteristics of being an individual and the mean scores of the Isolation Precautions Compliance Scale ($p>0.05$) (Table 3).

Discussion

In this study, which aimed to determine the compliance of intern nurses with isolation precautions, the total score average of the students' Isolation Precautions Compliance Scale was 71.92±5.74. Accordingly, it could be said that the students' compliance with the isolation measures was at a good level. It is expected that nursing students who are studying at the undergraduate level and who are entitled to be interns by completing the theoretical courses in this education have a good level of compliance with isolation measures. Because nursing education at the undergraduate level plays an important role in helping students acquire the right knowledge, attitude and skills (10). Most of the students who participated in our study stated that they received the training about compliance with isolation measures during their undergraduate education.

In the literature, there are no other studies evaluating the compliance of nursing students with isolation precautions, both in the world and in our country, but there are studies evaluating nurses (10,20,21). In these studies with nurses, the level of compliance of nurses with isolation measures is higher than the intern nurses participating in our study. It is thought that this situation is due to the fact that the clinical experience of the students is less than that of the nurses. In the literature, it is stated that experience increases nurses' compliance with isolation measures (20,22-25). The fact that the students who participated in our study did not have clinical experience other than clinical practices and that only 71.1% of them gave care to the patients in isolation in these clinical practices supported the literature.

During the pandemic process, nursing department students should have knowledge about COVID-19 (9). The majority of the students (91.1%) who participated in our study had knowledge about the necessity of following isolation precautions in the nursing care of patients with COVID-19. However, in our study, intern nurses who were not informed about the need to comply with isolation precautions in the nursing care of patients with COVID-19 were more likely to comply with isolation measures ($p>0.005$). It is thought that this situation is due to the fact that intern nurses are generally well-adapted to isolation measures, even if they do not have the experience of caring for a patient with a diagnosis of COVID-19, and cannot go to clinical practice during the COVID-19 pandemic. Implementation of isolation measures in the nursing care of patients with a diagnosis of COVID-19 is critical to prevent transmission (11,26). Studies have shown that the knowledge and awareness of nursing

Table 1. Sociodemographic features and isolation practices of intern nurses (n=90)

Year (age)	Min-max 20-31 n	Mean ± SD 22±1.43 %
Gender		
Female	67	74.4
Male	23	25.6
Graduated high school		
Anatolian High School	46	51.1
Health Vocational High School	25	27.8
Other	19	21.1
Get training on isolation precautions		
Yes	86	95.6
No	4	4.4
Where and from whom was the training on the importance of insulation received?		
From the infection control nurse at the hospital	13	14.4
From an instructor/member in undergraduate education	73	81.1
Providing nursing care to a patient who is in isolation during clinical practice		
Yes	64	71.1
No	26	28.9
To have knowledge about the necessity of applying isolation measures in the nursing care of a patient with a diagnosis of COVID-19		
Yes	82	91.1
No	8	8.9

Min: Minimum, Max: Maximum, SD: Standard deviation, COVID-19: Coronavirus disease-19

Table 2. The mean scores of the total and sub-dimensions of the Intern Nurses' Isolation Precautions Compliance Scale

Scale and its sub-dimensions	Min-max	Median	Mean ± SD
Compliance with Isolation Measures Scale total score	55-90	73	71.92±5.74
Transmission route	14-25	25	23.66±2.25
Employee and patient safety	14-30	18	18.81±2.56
Environmental control	12-20	19	18.42±1.86
Hand hygiene, use of gloves	7-15	11	11.02±1.28

SD: Standard deviation, Min: Minimum, Max: Maximum

department students about COVID-19 is good or moderate (9,27,28) and that the majority of students (75%) know the infection control measures to be followed in COVID-19 (9). In a study conducted with intern nurses, it was concluded that it was important for students to have knowledge about infection control measures before starting the profession (29). In a study conducted with nursing students in Korea, it was determined that infection was prevented as students' knowledge and attitudes towards infection control increased (30).

In the study, the level of compliance with the isolation precautions of the intern nurses who received training from the infection control nurse in the hospital about the importance of isolation was higher (p>0.005). Isolation measures are an issue in the nursing education curriculum. It was thought that the information that the students received from the infection control nurse in clinical practices after the training they received about isolation measures at school increased their level of compliance with isolation measures.

In our study, it was determined that the compliance of the intern nurses who did not care for the patient who was isolated in clinical practice was higher (p>0.05). In a study where nurses' compliance with isolation measures was determined, it was determined that nurses who did not apply isolation, similar to our finding, had higher compliance with isolation measures (p>0.05) (25). This finding might indicate that the intern nurses, who did not have experience in caring for the isolated patient, learned the isolation practices well in the education they received at school. Supporting intern nurses' knowledge about isolation precautions with clinical practice rather than being limited to theoretical education can significantly increase their compliance with isolation precautions.

Table 3. Comparison of the sociodemographic characteristics and isolation practices of the intern nurses and the total score averages of the Isolation Precautions Compliance Scale

		Min-max	Median	Mean ± SD	Significance
Gender	Female	60-90	73	72.88±4.57	MWU=573.5
	Male	55-85	69	69.13±7.72	p>0.05
Graduated high school	Anatolian High School	57-86	73	72.06±5.62	KW=1.467 p>0.05
	Health Vocational High School	55-90	71	72.76±6.10	
	Other	57-79	71	70.47±5.56	
Getting education about the importance of insulation	Yes	55-90	73	71.90±5.65	MWU=136.5 p>0.05
	No	60-79	73	72.25±8.53	
Where and from whom was the training on the importance of insulation received?	From the infection control nurse at the hospital	62-82	73	73.00±5.24	MWU=424.0 p>0.05
	From the lecturer in undergraduate education	55-90	73	71.71±5.73	
Providing nursing care to a patient who is in isolation during clinical practice	Yes	57-86	73	71.78±4.95	MWU=757.0 p>0.05
	No	55-90	73.5	72.26±7.43	
To have knowledge about the necessity of applying isolation measures in the nursing care of a patient with a diagnosis of COVID-19	Yes	55-90	73	71.87±5.90	MWU=326.5 p>0.05
	No	67-79	72	72.37±3.92	

MWU: Mann-Whitney U test, KW: Kruskal-Wallis test, SD: Standard deviation, Min: Minimum, Max: Maximum

Nurses are health professionals who are at the forefront and play a key role in the fight against the COVID-19 pandemic (24). In order for the pandemic to be managed perfectly, nurses must be sufficiently ready in terms of quantity and quality. Due to the increased workload of nurses and the risk of contamination during the pandemic, increasing the number of nurses in case the number of nurses is affected is an important initiative to prevent the pandemic (6,9,26). Ensuring adequate nurse workforce management in case of need may be possible by assigning responsibilities to intern nurses (6,31,32). In a study conducted with nursing students involved in the fight against COVID-19, it was reported that students were afraid of COVID-19 and the workload of the clinics, but they were aware of their responsibilities towards the society (33). Within the scope of both global and national struggle against COVID-19, intern nurses who are health professional candidates should be ready, aware and knowledgeable about the pandemic (34). Effective fight against the pandemic will be facilitated by scientific measures to be taken by health professionals with enhanced awareness (18).

Study Limitations

The limitation of the study was that it only covered intern nurses studying at a university.

Conclusion

In this study, it was determined that the level of compliance of intern nurses to isolation measures was good during the COVID-19 pandemic period. Most of the students knew that isolation precautions should be followed in the nursing care of patients diagnosed as having COVID-19. The results showed that intern nurses, whose nursing workforces might be needed during the pandemic period, had knowledge and awareness about isolation measures. It is recommended to keep the knowledge and awareness of nursing students at a high level by including the current developments that may arise during the pandemic process into the nursing education curriculum.

Ethics

Ethics Committee Approval: In order to carry out the research, ethics committee approval was obtained from the Scientific Research Ethics Committee of the Trakya University Faculty of Medicine with the date 12.10.2020 and number 2020/326, and the institutional permission was obtained from the Trakya University Keşan Hakkı Yörük School of Health Directorate with the date 09.11.2020 and number 33505391-044-E.472785.

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Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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The Relationship Between Life Satisfaction and Spouse Support in Women with Multiple Sclerosis

Multiple Skleroz'lu Kadınlarda Yaşam Doyumu ve Eş Desteği İlişkisi

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ABSTRACT

Objective: This research aims to define the correlation between life satisfaction and spouse support of women receiving multiple sclerosis (MS) treatment.

Methods: The sample of the descriptive study consisted of 185 patients who applied to the neurology service and outpatient clinics of a university hospital between November 2018 and May 2019. Data was pooled with definitive data sheets for life satisfaction scale and spouse support scale. Correlation and regression analyses has been used for data analysis and percentile calculations.

Results: Data show that 35.7% of the participants were diagnosed with MS for 1-5 years and 50.3% receive care support and 13.5% use ancillary device. Average life satisfaction scale score is found as 16.40±5.13 and spouse support scale score average as 66.37±15.10. There is high correlation in positive direction between average life satisfaction scale and spouse support scale scores ($p<0.01$).

Conclusion: Women diagnosed with MS need huge support to fulfill their daily tasks. The physical and social support coming from their environment positively affects their treatment process. In this process, the importance of spousal support in coping with the disease should be explained to the spouses of the patients, and the life satisfaction of the patients should be tried to be increased with the trainings to be given.

Keywords: Multiple sclerosis, life satisfaction, spouse support

ÖZ

Amaç: Bu araştırma, multiple skleroz (MS) hastalığı nedeniyle tedavi gören kadınların yaşam doyumu ve eş desteğini arasındaki ilişkiyi belirlemek amacıyla gerçekleştirilmiştir.

Yöntem: Tanımlayıcı tipteki çalışmanın örneklemini, Kasım 2018-Mayıs 2019 tarihleri arasında, bir üniversite hastanesinin nöroloji servisi ve polikliniklerine başvuran 185 hasta oluşturmuştur. Veriler, tanımlayıcı veri formu, yaşam doyum ölçeği ve eş desteği ölçeği ile elde edilmiştir. Verilerin değerlendirilmesinde, sayı yüzdelik hesaplamaları, pearson korelasyon ve lineer regresyon analizi kullanılmıştır.

Bulgular: Kadınların %35,7'sinin 1-5 yıldır MS hastası olduğu ve %50,3'ünün bakım desteği aldığı saptanmıştır. Katılımcıların yaşam doyum ölçeği puan ortalaması 16,40±5,13 ve eş desteği ölçeği puan ortalaması 66,37±15,10 olarak bulunmuştur. Katılımcıların yaşam doyum ölçeği ile eş desteği ölçeği puan ortalaması arasında pozitif yönde yüksek düzeyde ilişki saptanmıştır ($p<0,01$).

Sonuç: MS hastası olan kadınların günlük işlerini yerine getirmekte desteğe ihtiyaçları vardır. Çevrelerinden aldıkları fiziksel ve sosyal destek tedavi süreçlerini olumlu yönde etkilemektedir. Bu süreçte, hastaların eşlerine hastalıkla baş etmede eş desteğinin önemi anlatılmalı ve verilecek eğitimler ile hastalarının yaşam doyumu artırılmaya çalışılmalıdır.

Anahtar Sözcükler: Multiple skleroz, yaşam doyumu, eş desteği

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Introduction

Multiple sclerosis (MS) is an immune-derived, chronic, inflammatory and demyelinating disease, mostly seen in young adults, characterized by myelin sheath damage in the central nervous system, which can progress with periods of attack and remission, and can be progressive. MS is usually detected between the ages of 20 and 40, but less than 1% occurs in childhood and about 2% to 10% after the age of 50. This pathological condition affects women more than men (gender ratio 2.5: 1) and although the prevalence of the disease varies according to the geographical region, it is given as 120 per 100,000 people. Although the etiology of MS is not clear, it can be considered as a multifactorial disease and includes a genetic predisposition together with environmental effects (1,2).

MS, which was diagnosed in 2.1 million people in the world in 2008, was estimated to reach 2.3 million in 2013. Although MS was seen in all countries in the world, it was found to be higher with a prevalence of 140-108/100,000 in North-American and European countries, and lower with a rate of 2.1-2.2/100,000 in African and East Asian countries. Although there is no national epidemiology study conducted in Turkey, the prevalence of MS was reported as 30/100,000 and 100/100,000 in studies conducted in Edirne and İstanbul (3). According to these data, Turkey is among the medium risk regions.

Due to demyelination, multiple neurological symptoms such as diplopia, blurred vision, optic neuritis, vertigo, weakness, paresthesias, imbalance and cerebellar findings may occur in MS, resulting in deterioration in the quality of life of the patients and their daily living activities. Of patients with MS, 32-41% have weakness, mostly in the lower extremities, due to motor involvement. It is stated that the frequency of depression in patients is 2-3 times higher. Treating the symptoms that can cause serious problems in the patient will allow the patient to improve in daily life activities, to be less dependent, to feel less need for support, and to continue his/her work and education life (4).

MS affects the quality of life because it starts at a young age, is a chronic disease, and has a high disability potential. How to overcome the limitations that will occur and how to strengthen the existing functions of the person can bring important life changes. Things that are seen as simple and ordinary in daily life create insurmountable obstacles in these patients, and this causes the patient to lose his/her life with depressive mood, seeing himself/herself as inadequate in every field, gradually decreasing his/her quality of life and becoming dependent on others. The change in quality of life also affects satisfaction (5).

Life satisfaction is a psychological and sociological concept that expresses the positive or negative judgments that a person has reached as a result of his/her evaluation of his/her whole life and his/her general view of life. The World Health Organization defined life satisfaction as how an individual perceives his/her own life within the culture and value system in which he/she lives. Accordingly, life satisfaction is related to the goals, hopes, concerns, and standards of the individual and is affected by the individual's physical and psychological health, independence status, social relations, and important features of the environment (6).

Marriage is a legal union in which a man and woman share their lives and contain biological, psychological and social functions. In the marital relationship, spouses gain a different priority and importance compared to other support providers in social support resources. Many adults perceive their spouses as the main source of social support. Especially in times of crisis, the most common and most important source of social support in all societies is marriage and the family institution. In other words, with marriage, spouses become one of the most important sources of support in each other's life. The young adult, who has left family life, starts to care primarily about the quality of the support he/she will receive from his/her wife in line with the step he/she has taken to establish his/her own family (7,8).

In cases where people are inadequate or exhausted, social support meets their needs such as love, respect, compassion and belonging, positively affects physical or mental health (9), contributes to positive thinking (10,11) and increases their quality of life. As a matter of fact, studies conducted with patients with MS revealed a positive effect of perceived social support on quality of life (12-19). Studies show that the patient's self-care and mental processes are affected in MS, and the patient needs family and spouse support to cope with his/her problems (20,21).

Considering that MS is a chronic disease that affects the entire family system, determining the life satisfaction of individuals during the disease process and revealing the support of their spouses in this process will contribute to the solution of the problems that the patients and their spouses will encounter during the disease process. This research was carried out to reveal the relationship between life satisfaction and spousal support of women treated for MS.

In the research, answers to the following questions were sought:

- What is the level of life satisfaction and spousal support of women?
- Is there a relationship between women's spousal support and life satisfaction?
- What are the factors that predict women's life satisfaction?
- What are the factors that predict the spousal support of women?

Methods

Type of Research: The research is descriptive, cross-sectional and relationship-seeking.

Population/Sample of the Research: The population of the research consisted of 600 women with MS who were admitted to the hospital where the study was conducted in the last 6 months, and the sample number was determined according to the sample calculation formula when the population was determined. The

sample included 185 female patients with MS who were 18 years of age or older, married and living with their spouses, could speak and understand Turkish, had a diagnosis of MS for at least 6 months, and agreed to participate in the study.

Data Collection Tools: Data in the study were collected using the descriptive data form, the life satisfaction scale and the spouse support scale.

Life Satisfaction Scale (LSS): It consists of a total of 5 items under a single factor structure developed by Diener et al. (22) and a Turkish validity and reliability study conducted by Dağlı and Baysal (6). The scale is in a 5-point Likert structure and is scored as 1-I totally disagree, 5-I totally agree. A minimum of 5 points and a maximum of 25 points can be obtained from the scale. The Cronbach alpha internal consistency coefficient of the scale was found to be 0.88. Higher scores indicate higher life satisfaction (6). In this study, the cronbach alpha value of the scale was found to be 0.75.

Spouse Support Scale (SSS): It was developed by Yıldırım (23) to determine the perceived spousal support level. There are 27 questions in the Spouse Support Scale and the answer is a Likert-type scale ranging from 1-3. The highest score is 81 and the lowest score is 27 points that can be obtained from the scale. Items 10, 20 and 24 in the scale contain negativity. High scores indicate that the level of spousal support is perceived as high. The cronbach alpha coefficient of the SSS was found to be 0.95 (22). In this study, the cronbach alpha value of the scale was found to be 0.93.

Data Collection: The research data were obtained from married women with MS who were hospitalized in the neurology inpatient service of a university hospital between November 2018 and May 2019 and were admitted to the MS Outpatient Clinic for examination. Questionnaires were given to the patients who accepted to participate in the study and they were asked to answer the questions individually. Data collection took approximately 10 minutes. Data collection forms were applied in a way that would not disrupt the care and treatment processes of the patients and pay attention to their privacy.

Evaluation of Data: The data obtained in the study were analyzed using SPSS (Statistical Package_for_Social Sciences) for Windows 22.0 program. In the descriptive statistics of the data, mean, standard deviation, median minimum, maximum, frequency and ratio values were used. Scale score averages were subjected to correlation and regression analysis. The findings were evaluated at 95% confidence interval and 5% significance level.

Ethical Aspect of the Study: Prior to data collection, approval was obtained from the ethics committee of the university (297 issue/17.10.2018) where the study was conducted. After obtaining the approval of the ethics committee, permission was obtained from the institution where the study would be conducted on 31.10.2018, the women were informed about the research and their written consents were obtained.

Results

The mean age of the participants was 40.34±10.02 [minimum (min): 22, maximum (max): 72], 37.8% were in the 33-43 age group, 47% were primary school graduates and 74.1% were not working. Of the participants 88.6% had children and 50.5% of them had a medium income (Table 1).

It was determined that 35.7% of the participants had MS for 1-5 years, 26.5% stated that their disease did not interfere with their daily work, 50.3% received care support and 13.5% used an assistive device (Table 2).

Table 1. Distribution of demographic characteristics of the participants (n=185)

Variables		n	%
Mean age 40.34±10.02 (min: 22, max: 72)			
Age group	22-32 years	50	27.0
	33-43 years	70	37.8
	44 years or above	65	35.1
Education status	Primary school	82	47.0
	High school	52	28.1
	University or above	46	24.9
Working status	Working	48	25.9
	Not working	137	74.1
Status of having a child	Yes	164	88.6
	No	21	11.4
Income status	Low	21	11.4
	Moderate	149	50.5
	High	15	8.1
	Total	**185**	**100.0**

min: Minimum, max: Maximum

Table 2. Distribution of the characteristics of the participants regarding the disease and treatment processes (n=185)

Variables		n	%
Duration of MS	6 months- 1 year	22	11.9
	1-5 years	66	35.7
	6-10 years	52	28.1
	11 years or above	45	24.3
MS interfering with daily activities	Interfering	44	23.8
	Partially interfering	92	49.7
	Not interfering	49	26.5
Status of receiving care support	Receiving	93	50.3
	Not receiving	92	49.7
Assistive device usage status	Using	25	13.5
	Not using	160	86.5
Total		**185**	**100.0**

MS: Multiple sclerosis

The mean age of the spouses of the participants was 44.25±10.1 (min: 22, max: 73). It was determined that 50.8% of the women were married for 16 years or more, 63.2% of them had good relations with their spouses, and spouses of 58.9% always helped them (Table 3).

The mean score of LSS of women with MS was 16.40±5.13 and the mean score of SSS was 66.37 ± 15.10 (Table 4)

A high level of positive correlation was found between the participants' LSS and SSS mean scores (p<0.01) (Table 5).

By applying linear regression, it was determined to what extent the independent variables of "income status", "how the relationship was with the spouse", and "the status of helping the spouse in daily chores" predicted the LSS score. As a result of this procedure, it was found that "F=15.415, p<0.05, R2= .442", and 44% of the total variance of the LSS was explained by these variables, and these variables were found to be significant predictors of the LSS score (Table 6).

By applying linear regression, it was determined to what extent the independent variables of "income status", "the disability of the disease", "the relationship with the spouse" and "the spouse's help in daily tasks" predicted the SSS score. As a result of this procedure, it was found that "F=47.095, p<0.05, R2=.708", and 70% of the total variance of the SSS was explained by these variables, and these variables were significant predictors of the SSS score (Table 7).

Table 3. Distribution of participants' characteristics regarding spouses and spousal relationships (n=185)

Variables		n	%
Mean age of spouses 44.25±10.41 (min: 22, max: 73)			
Duration of marriage	0-5 years	38	20.5
	6-10 years	29	15.7
	11-15 years	24	13.0
	16 years or above	94	50.8
Relationship with spouse	Good	117	63.2
	Moderate	56	30.3
	Bad	12	6.5
Spouse's assistance in daily activities	Always helps	109	58.9
	Sometimes helps	54	29.2
	Never helps	22	11.9
	Total	**185**	**100.0**

min: Minimum, max: Maximum

Discussion

The findings obtained from the study, which was conducted to reveal the relationship between life satisfaction and spousal support of women treated for MS, were discussed in the form of answers to the research questions.

One of the research questions was "What is the level of life satisfaction and spousal support of women?". As an answer to the question, it was determined that women's perceptions of life satisfaction were positive and their spouse support levels were high. There are studies in the literature that support the study finding. In a study conducted by Aşiret et al. (16) on individuals with MS, patients' life satisfaction was moderate. In the studies of Purutçuoğlu and Aksel (24) in disabled women and Yıldız and Baytemir (25) in married individuals, it was determined that life satisfaction was high. Similarly, there are studies in the literature showing that women's perceived support from their spouses is high (26-29). Ghodusi et al. (30) determined that the social support level of the patients was moderate and stated that the majority of the patients perceived support from their spouses and that the perceived support was satisfactory.

Another question of the research was "Is there a relationship between women's spousal support and life satisfaction?". As an answer to the question; it was determined that as the spouse support levels of the women within the scope of the study increased, their life satisfaction also increased. The support provided by the spouse indicates that the spouse is valued, loved and respected (8). In this context, it can be said that the married individual's feeling of value in her relationship with her spouse, together with the support provided by the spouse, affects life satisfaction. Although there are no studies in the literature examining spousal support and life satisfaction, there are studies that examine the relationship between marital satisfaction and spousal support. It is thought that the satisfaction people get from their marriage may affect the satisfaction they get from life. In this context, in the study conducted by Kabasakal and Soylu (31) on married individuals, it was found that as the support received from the spouse increased, the satisfaction obtained from marriage also increased. Similarly, it is stated in the literature that the psychological and physical well-being of individuals who are satisfied with their marriage by getting support from their spouses are also positively affected, and this situation causes individuals to get more satisfaction from life (13,25,30). In the study of Yuca and Beydağ (29), in women with heart disease, it was found that increased support from spouses increased marital satisfaction. In the studies of Çelik and Tümkaya (33) and Kublay and Oktan (34), it was determined that as marital adjustment increased, life satisfaction also increased. Yıldırım and Işık (35) found that

Table 4. Distribution of the mean scores of the scales

Scales	Mean	Sd	Min	Max	α
Life satisfaction scale (LSS)	16.40	5.13	5	25	0.75
Spouse support scale (SSS)	66.37	15.10	27	81	0.95

Sd: Standard deviation, Min: Minimum, Max: Maximum, α: Cronbach alpha

as social support increased, life satisfaction also increased in unemployed married women.

One of the research questions was "What are the factors that predict women's life satisfaction?". As an answer to the question; it was found that the increase in the income level of women, the positive relationship with their husbands and the fact that their husbands always helped them increased their life satisfaction. In a study conducted by Özen et al. (36), in patients with MS, it was determined that patients who had a positive relationship with their spouses had easier psychosocial adjustment to the disease, and this situation positively affected life satisfaction. In the study of Samios et al. (37), it was determined that the harmony between spouses was an important determinant of life satisfaction. In the study of Herbert et al. (38), it was determined that individuals with MS who received adequate support from their spouses had higher life satisfaction despite the increasing disability due to the disease. In the study of Neate et al. (39), it was stated that patients with MS and their spouses made joint decisions and acted together, making it easier to cope with the difficulties brought by the disease and positively affecting the quality of life of the spouses. This result shows that both financial and social support is important in coping with the financial and moral problems brought about by the chronic disease.

The last question of the research questions was "What are the factors that predict the spousal support of women?". As an answer to the question, it was found that the level of spousal support was higher among women who had a high income level, whose illness prevented them from carrying out their daily work, who had a good relationship with their spouses, and whose spouses helped them in daily chores. Ghafari et al. (40) stated in their study that the spouses of individuals with MS did not always have a supportive approach, that the reactions given to the disease might change due to the addictions of the individuals, that the emotional distress of the spouses might affect the process negatively, and that the relationship between the spouses affected the level of spousal support. In the study of Ebrahimi et al. (41), it was stated that the financial support resources of the family and the level of the relationship between the spouses were important in meeting the emotional and physical care needs. In the study conducted by Yuca and Beydağ (27) in women with heart disease, it was determined that how the relationship between spouses was also effective on the level of spousal support (27). The income level of individuals with chronic diseases can be important in terms of financial issues such as receiving care support during the disease process and providing the necessary equipment support. The high income level of the spouses will eliminate the problems arising from financial difficulties and will cause economically troublesome processes to be experienced between the spouses. In addition, the negative situation experienced by one of the spouses can also affect the other spouse and cause mutual exhaustion between the spouses. For this reason, the relationships of spouses who communicate well with each other and support each other become stronger during the illness and can cope with the psychological burden of the illness more easily.

Table 5. Correlation between scales

Scales		Life Satisfaction scale
Spouse support scale	**r_s**	.683
	p	**.000**

r_s: Sperman's correlation; p<0.05

Conclusion and Recommendations

As a result of the research, the life satisfaction scores of the participants with high spousal support were also found to be high. It was determined that the income status of the women, the level of their relationship with their spouse and the state of helping their spouses were the factors affecting life satisfaction and spousal support.

As a result of the study, it is recommended to conduct experimental studies in which psycho-education or

Table 6. Results of linear regression analysis of factors predicting life satisfaction scale score

Variables	B	t	p	F	Model (p)	R^2 (95% CI)*
Income status	.145	2.241	.026	15.415	.000	.442
Relationship with spouse	-.155	-2.073	.040			
Spouse's assistance in daily activities	-.289	-3.700	.000			

B: Regression coefficient; t: degrees of freedom; p: Significance value; R^2: Coefficient of determination, CI: Confidence interval

Table 7. Linear regression analysis results of factors predicting spouse support scale score

Variables	B	t	p	F	Model (p)	R^2 (95% CI)*
Income status	.097	2.031	.044	47,095	.000	.708
MS interfering with daily activities	.096	2.043	.043			
Relationship with spouse	-.402	-7.463	.000			
Spouse's assistance in daily activities	-.419	-7.409	.000			

B: Regression coefficient, t: Degrees of freedom, p: Significance value, R2: Coefficient of determination, CI: Confidence interval

psychotherapy is provided with the joint participation of the spouses, and qualitative studies in which the contribution of the spouses' support to the disease and treatment process is evaluated more comprehensively.

Study Limitations

The fact that the study was conducted in a single center was one of the limitations of the study. The results obtained were limited to the answers given by the patients who were admitted to the hospital where the study was conducted and at the time of data collection.

Conclusion

In line with the findings; the support that women receive from their husbands during the disease process is extremely important and affects life satisfaction. How the patients' relationship with their spouse is, the spouse's ability to help in daily chores, their own and their spouses' character traits, whether the disease interferes with their daily work, and their thinking that they need psychological support affect spouse support and life satisfaction. As seen in the results, spousal support has been interpreted as one of the reasons for getting satisfaction from life. Considering that nurses working in this field interact with patients at all stages of treatment and care; evaluating married female patients with MS in terms of compatibility between spouses, determining their needs, and planning nursing interventions that provide emotional support will be beneficial in terms of patients' compliance with their treatment.

Ethics

Ethics Committee Approval: Prior to data collection, approval was obtained from the ethics committee of the university (297 issue/17.10.2018) where the study was conducted. After obtaining the approval of the ethics committee, permission was obtained from the institution where the study would be conducted on 31.10.2018.

Informed Consent: The women were informed about the research and their written consents were obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: H.M., K.D.B., Design: K.D.B., Data Collection or Processing: H.M., Analysis or Interpretation: H.M., K.D.B., Literature Search: H.M., Writing: H.M., K.D.B.

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Original Article

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Management of Acute Biliary Pancreatitis in Cholecystectomized Patients

Kolesistektomili Hastalarda Akut Biliyer Pankreatit Yönetimi

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ABSTRACT

Objective: We aimed to evaluate the patients who were hospitalized, followed up and treated in our clinic with the diagnosis of acute biliary pancreatitis (ABP) after cholecystectomy.

Methods: The electronic records of 18 patients with a history of cholecystectomy were reviewed retrospectively. The demographic findings of the patients, time passed after cholecystectomy, methods used in diagnosis, amylase levels, treatment choices, clinical follow-ups, mortality and morbidity rates, and length of hospital stay were evaluated. The Ranson criteria and Apache II score were used to determine the severity of pancreatitis.

Results: Thirteen (72.2%) were female and 5 (27.8%) were male. The mean age was 57.83±12.59 (34-77). The mean time elapsed after cholecystectomy was 72.11±38.12 (5-130) months. The mean diameter of the common bile duct (CBD) was measured as 12.39±2.30 (8-15) mm. The average level of amylase was 986.50±323.29 (350-1530) U/L. Fifteen (83.33%) patients had mild, and 3 (16.67%) patients had moderately severe acute biliary pancreatitis. Endoscopic sphincterotomy (ES) was performed on 16 patients during endoscopic retrograde cholangiopancreatography (ERCP). Two patients were operated due to failure of ERCP. Choledochotomy, transduodenal sphincteroplasty and The T-tube drainage were performed on 1 patient. The other patient underwent choledochotomy and choledochoduodenostomy. The average length of stay in hospital was 7.89±4.91 (5-25) days.

Conclusion: It should be kept in mind that ABP may develop months or even years after cholecystectomy. The standard treatment

ÖZ

Amaç: Bu çalışmada kolesistektomi sonrası akut biliyer pankreatit (ABP) tanısıyla kliniğimizde yatan, takip edilen ve tedavi edilen hastaların değerlendirilmesini amaçladık.

Yöntemler: Kolesistektomi öyküsü olan 18 hastanın kayıtları retrospektif olarak incelendi. Hastaların demografik bulguları, kolesistektomi sonrası geçen süre, tanıda kullanılan yöntemler, amilaz düzeyleri, tedavi seçenekleri, klinik takipler, mortalite ve morbidite oranları ve hastanede kalış süreleri değerlendirildi. Ranson kriterleri ve Apache II skoru, pankreatitin şiddetini belirlemek için kullanıldı.

Bulgular: Hastaların 13'ü (%72,2) kadın, 5'i (%27,8) erkekti. Ortalama yaş 57,83±12,59 (34-77) idi. Kolesistektomi sonrası geçen ortalama süre 72,11±38,12 (5-130) aydı. Ana safra kanalının (ASK) ortalama çapı 12,39±2,30 (8-15) mm olarak ölçüldü. Ortalama amilaz seviyesi 986,50±323,29 (350-1.530) U/L idi. On beş (%83,33) hastada hafif ve 3 (%16,67) hastada orta şiddetli akut biliyer pankreatit vardı. Endoskopik retrograd kolanjiyopankreatografi (ERCP) sırasında 16 hastaya endoskopik sfinkterotomi (ES) yapıldı. ERCP'nin başarısızlığı nedeniyle 2 hasta ameliyat edildi. Bir hastaya koledokotomi, transduodenal sfinkteroplasti ve T-tüp drenajı yapıldı. Diğer hastaya koledokotomi ve koledokoduodenostomi yapıldı. Hastanede ortalama kalış süresi 7,89±4,91 (5-25) gündü.

Sonuç: ABP'nin kolesistektomiden aylar, hatta yıllar sonra gelişebileceği unutulmamalıdır. Kolesistektomili hastalarda ASK

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for acute pancreatitis caused by CBD stones in patients with cholecystectomy are ERCP and ES. In patients with failed ERCP and ES, the CBD exploration should be performed surgically.

Keywords: Acute pancreatitis, cholecystectomized patients, endoscopic retrograde cholangiopancreatography

taşlarının neden olduğu akut pankreatit için standart tedavi ERCP ve ES'dir. ERCP ve ES'nin başarısız olduğu durumlarda, cerrahi olarak AKS koledok eksplorasyonu yapılmalıdır.

Anahtar Sözcükler: Akut pankreatit, kolesistektomili hastalar, endoskopik retrograd kolanjiyopankreatografi

Introduction

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas that is characterized by edema, and when severe, necrosis (1). Alcohol and gallstones are responsible for 80% of the etiology (2). While alcohol consumption is the most common cause of AP in developed western countries, gallstones are the most common cause in eastern society. The rate of incidence of gallbladder and common bile duct (CBD) stones in patients diagnosed as having AP changes between 30% and 70% (3). While men are more prone to the development of AP in the presence of gallbladder stones, it is more common in women (4). In acute biliary pancreatitis (ABP), laparoscopic or open cholecystectomy is performed to prevent pancreatitis recurrence. However, ABP may develop due to gallstones months or even years after surgery in patients who undergo cholecystectomy for non-pancreatitis causes (5-7). Approximately 10% to 18% of patients with cholecystectomy have also CBD stones (8). Endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic sphincterotomy (ES) are standard approaches used for treatment of patients with choledochal stones subsequent to cholecystectomy. However, some authors have reported that these techniques are unsuccessful approximately in 10% of the patients. When ERCP and ES fail, laparoscopic or open surgery and choledochal exploration is the approach of choice (9,10). In the literature, we determined that studies on ABP in cholecystectomized patients were rare. Thus, we aimed to evaluate the patients who were hospitalized, followed up and treated in our clinic with the diagnosis of ABP after cholecystectomy, and to share our clinical experience.

Methods

Patients diagnosed as having ABP in the University of Heath Sciences Turkey Derince Training and Research Hospital between August 2010 and July 2020 were listed. The electronic records of 18 patients with a history of cholecystectomy from a total of 585 patients were reviewed retrospectively. The demographic findings of the patients, time passed after cholecystectomy, methods used in diagnosis, amylase levels, treatment choices, clinical follow-ups, mortality and morbidity rates, and length of hospital stay were evaluated. The Ranson criteria and Apache II score were used to determine the severity of pancreatitis. Patients who had a Ranson score ≥3, an Apache II score ≥8 (48th hour), persistent organ failure (>48 hours) and local complications (pancreatic necrosis, pancreatic abscess, pseudocyst) were considered to have severe pancreatitis. However, patients with transient organ failure (<48 hours) were considered to have moderately severe pancreatitis. Patients with a Ranson score <3, an Apache II score <8 and without permanent organ failure and local complications were considered to have mild pancreatitis. Stones in the CBD were detected by using magnetic resonance cholangiopancreatography (MRCP) in patients diagnosed as having pancreatitis and with signs of cholestasis. All patients underwent ERCP. ES was performed on 16 of the 18 patients during ERCP. Two patients were operated due to failure of ERCP. Patients with no evidence of cholestasis and who had no radiologically detected bile duct stones were excluded from the study. The study was approved by the Local Ethical Committee of University of Health Sciences Turkey Derince Training and Research Hospital, (protocol number: 2021/54-25.03.2021).

Statistical Analysis

The Statistical Package for the Social Sciences (IBM SPSS Statistics 23, software, IL-Chicago- USA) was used for data analyses. The frequency and percentage values of thedemographic variables of the qualitative data in our study, and the mean ± standard deviation of the age variable of the quantitative data were used in the descriptive statistics.

Results

Eighteen patients were examined (Table 1). Thirteen (72.2%) of the 18 patients were female and 5 (27.8%) were male. The mean age was 57.83±12.59 (34-77). The mean time elapsed after cholecystectomy was 72.11±38.12 (5-130) months. The rate of incidence of patients with cholecystectomy in the etiology of ABP was found to be 3.08%. Intravenous contrast-enhanced abdominal tomography (CT) was performed on all patients to evaluate the pancreas. CT was repeated 48 and 96 hours later when patients' clinical conditions had not changed. MRCP was performed on all patients to show the CBD diameter and the presence of stones. The mean diameter of the CBD was measured as 12.39±2.30 (8-15) mm by MRCP. The average level of amylase was 986.50±323.29 (350-1530) U/L. Fifteen (83.33%) patients had mild, and 3 (16.67%) patients had moderately severe ABP according to the Ranson's criteria and Apache II score. None of the patients had severe ABP. Patients with moderately severe ABP responded to medical therapy. None of the patients needed intensive care unit. All patients underwent endoscopic retrograde ERCP. ES was performed on 16 of the 18 patients during ERCP. Two patients were operated due to failure of ERCP. CBD exploration was performed on both patients surgically. Four to five stones were removed from the CBD in one patient. The transition from CBD to duodenum was controlled with dilators. Transduodenal sphincteroplasty was performed because there was stenosis in the Oddi sphincter. A T-tube was inserted into the CBD. T-tube cholangiography was executed on the 14th day. The T-tube was removed since no pathology was found in the

CBD lumen, and free passage was observed into the duodenum. A 10-mm stone that was impacted in the Oddi sphincter was removed from the other patient. Due to Oddi sphincter fibrosis, the duodenum could not be passed with CBD dilators, hence choledocoduodenostomy was performed. In our series, the failure rate of ERCP was found to be 11.11%. CBD stones were removed in all patients when ERCP was successful. There was no mortality. The average length of stay in hospital was 7.89±4.91 (5-25) days. Demographic features for each patient are shown in Table 2.

Table 1. Demographic features (n=18)

Age (mean ± std)	
For all patients	57.83±12.59 (34-77)
Male	67.20±8.43 (55-77)
Female	54.23±12.25 (34-70)
Sex (n)	
Male	5 (27.8%)
Female	13 (72.2%)
Severity of AP	
Mild	15 (83.33%)
Moderately severe	3 (16.67%)
Severe	0 (0%)
The mean diameter of the CBD (mm)	12.39±2.30 (8-15)
The average level of amylase (U/L)	986.50±323.29 (350-1,530)
The mean time elapsed after cholecystectomy (months)	72.11 ± 38.12 (5-130)
The average length of stay in hospital (days)	7.89 ± 4.91 (5-25)
Mortality (n)	0 (0%)
Morbidity (n)	0 (0%)
Treatment method (n)	
ERCP + ES	16 (88.9%)
Exploration of CBD + TDS + T-tube drainage	1 (5.6%)
Exploration of CBD +Choledocoduodenostomy	1 (5.6%)

AP: Acute pancreatitis, CBD: Common bile duct, ERCP: Endoscopic retrograde cholangiopancreatography, ES: Endoscopic sphincterotomy, TDS: Transduodenal sphincteroplasty

Discussion

The most common causes of AP, which is considered inflammation of the pancreas, are chronic alcohol use and gallstones/sludge. They appear as the etiological causes in 80% of all patients with AP (1,2). AP caused by gallstones is called ABP. Approximately 10-20% of patients with stones in the gallbladder have stones in the CBD at the same time (11,12). Gallstones in the CBD can be primary or secondary stones. Primary stones are very rare, therefore more of the stones detected in the CBD are secondary gallstones that are poured from the gallbladder. In order to be called a primary choledochal stone, it must occur at least 2 years after cholecystectomy (13). The rate of gallstones in the CBD is 3-18.5% after cholecystectomy (14-16). CBD stones can remain asymptomatic for a long time. However, they may cause symptomatic ABP in some patients months or even years after cholecystectomy (5). Manuel-Vázquez et al. (17) reported that 6% of patients who were rehospitalized within 90 days after cholecystectomy hospitalized due to AP. There are few publications on the rate of developing AP in patients with cholecystectomy. Gloor et al. (6) reported that the rate of cholecystectomized patients was 10% in patients diagnosed as having ABP in their series of 278 patients. There are also publications on the literature reporting that ABP can occur in patients with cholecystectomy without gallstones. Panara et al. (18) reported that an endoclip migrating from the cystic duct to the bile duct caused AP in a patient who had recurrent AP attacks, 15 and 19 months after cholecystectomy.

Contrast-enhanced abdominal tomography is the gold standard in the diagnosis and treatment plan of ABP. Anatomical condition of the pancreas and local complications such as abscess and necrosis can be easily detected by using tomography (1).

Table 2. Demographic features of each patient

Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Sex	F	F	M	M	F	F	F	F	F	F	M	F	M	M	F	F	F	F
Age	60	45	70	77	65	53	39	45	48	69	71	70	55	63	34	46	68	63
Time after cholecystectomy (months)	5	130	120	96	29	120	60	36	72	84	84	72	60	120	6	48	96	60
Diameter of the CBD (mm)	15	14	15	13	8	12	13	10	14	12	10	15	14	13	9	15	12	9
Level of amylase (U/L)	1145	1055	950	1190	450	985	632	1255	1530	350	735	1464	1215	690	1080	945	856	1230
Treatment method	1*	1*	1*	3***	1*	1*	1*	1*	1*	1*	2**	1*	1*	1*	1*	1*	1*	1*
Length of stay in hospital (days)	6	7	7	15	5	5	7	10	5	5	25	7	8	5	6	7	5	7

CBD: Common bile duct, F: Female, M: Male,
1*: Endoscopic retrograde cholangiopancreatography + endoscopic sphincterotomy
2*: Exploration of common bile duct + transduodenal sphincteroplasty + T-tube drainage
3*: Exploration of common bile duct + choledocoduodenostomy

However, the development of necrosis takes time, so it is not desired on the first day (6). It provides important information to differentiate edematous pancreatitis from necrotizing pancreatitis. In cases with necrosis of more than 50% of the pancreas and in which they do not recover clinically, fine needle aspiration biopsy and culture can be taken by using tomography, and antibiotic treatment can be arranged according to the culture result (19). MRCP is used to detect stones in the CBD. Also, the width of the CBD can be measured with MRCP. The development rate of ABP increases in patients whose width of the CBD is over 10 mm (5-7). The mean CBD diameter in our study was 12.39±2.30, and it was consistent with the literature. Although duodenal diverticulum was an important factor in ABP etiology after cholecystectomy (5-7), no duodenal diverticulum was detected in any patient in our study.

While 80% of patients with AP have mild edematous pancreatitis, 20% of patients have necrotizing pancreatitis accompanied by multiple organ failure. Mortality rate in edematous AP is less than 1%. However, in necrotizing pancreatitis, this rate rises to 20-40% and even over 50% in critical patients (1,18). Supportive therapy such as stopping oral intake and starting fluid replacement in the edematous form is usually sufficient. However, patients with severe pancreatitis and multiorgan failure should be followed up in the intensive care unit (20). In our study, 15 of 18 patients had mild AP and 3 had moderately severe AP according to the Ranson's criteria. All patients, including those with moderately severe pancreatitis, were followed up in the normal clinic room and did not require intensive care.

The ERCP and ES are standard treatments accepted by most centers for treatment of ABP after cholecystectomy (21,22). The failure rates of ERCP and ES are around 10-18% in recent studies (5,7). In our study, this rate was found to be 11.11%. ERCP and ES were successful on 16 of the 18 patients, but they failed on 2 patients. Open or laparoscopic exploration of the CBD should be performed if ERCP and ES fail. In order to increase the reliability of the ERCP procedure, it is necessary to determine the risk factors for ERCP complications very well. In the study of Atamanalp et al. (23) on 3,136 patients, 2,965 (94.5%) of 3,136 patients were successfully cannulated, 465 (14.8%) anterior incisions were made, and no successfull procedure was done in 171 (5.5%). In the study of Ciftci and Anuk (7), gallstones and biliary sand were found in CBD of 36 patients upon ERCP, but not observed in the remaining 8 patients. ES was performed and material was extracted in 32 of 36 patients, but stone extraction was unsuccessful in 4 patients; 3 patients underwent open surgery with CBD exploration and 1 patient died. We did an exploration of CBD with the open surgical method on 2 patients who failed to respond to the ERCP. We added transdudodenal sphincteroplasty and T-Tube placement to the procedure in one patient, and we performed choledocoduodenostomy in the other. Another purpose of doing ERCP in ABP is to remove obstruction by cannulation of the pancreatic duct and to provide drainage of pancreatic secretion (24). This drainage helps to reduce the pressure inside the pancreatic duct. In patients with severe necrotic pancreatitis and developing pancreatic fistula, ERCP is again used for stenting of the pancreatic canal. Although serious complications can be seen with ERCP, performing ERCP in the appropriate indication and early recognition of the complications are the most important steps in preventing morbidity and mortality (25-27).

Conclusion

It should be kept in mind that ABP may develop months or years after cholecystectomy. The standard treatment for AP caused by CBD stones in patients with cholecystectomy are ERCP and ES. In patients with failed ERCP and ES, CBD exploration should be performed surgically, and transduodenal sphincteroplasty plus T-Tube drainage or choledocoduodenostomy/ choledocojejunostomy should be added to the procedure.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethical Committee of University of Health Sciences Turkey Derince Training and Research Hospital, (protocol number: 2021/54-25.03.2021).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer review: Externally peer reviewed.

Author Contributions

Concept: A.Ç., Design: A.Ç., M.A.G., M.T.K., Supervision: M.T.K, Funding: A.Ç., M.A.G., Materials: A.Ç., M.A.G., Data Collection and/or Processing: A.Ç., M.A.G., Analysis and/ or Interpretation: M.A.G., M.T.K., Literature Review: A.Ç., M.T.K., Writer: A.Ç., M.T.K.

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Original Article

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Attitude, Knowledge and Donor Card Volunteering of Nursing Students Regarding Organ Donation

Hemşirelik Öğrencilerinin Organ Bağışı Konusunda Tutumu, Bilgisi ve Donör Kartı Gönüllülüğü

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ABSTRACT

Objective: To shed light on the educational curriculum and determining the organ donation attitude, knowledge and donor card willingness of students who study nursing.

Methods: This study is a descriptive and cross sectional. The study is a descriptive cross-sectional design. The research was carried out on 203 students studying in the nursing department of a private university. The data were collected with Organ Donation Attitude and Knowledge Scales, which were validated and reliable in Turkey. The significance value was accepted as $p<0.05$.

Results: The students' organ donation knowledge (9.95±2.14) and donor card volunteer scores (mean ± standard deviation) (2.94±0.89) were low. Although the students' organ donation positive attitude scores were 106.75±13.21, the rate of supporting being a cadaver donor was 73.8%. 91.6% of the students did not intend to donate their organs after death. The semester they study, place they live and the education level of their parents did not seem to have a significant effect on the point average of organ donation knowledge, attitude and donor card willingness ($p>0.05$).

Conclusions: It can be stated that lack of knowledge had more effect on students' fear on attitude points rather than religion. Planned training on subjects such as brain death and the grieving process in undergraduate education may affect the motivation of students about organ donation.

Keywords: Organ donation, attitude, knowledge, donor card volunteering, nursing student

ÖZ

Amaç: Bu çalışmada, hemşirelik eğitim programında okuyan öğrencilerin organ bağışı konusundaki tutum, bilgi ve donör kartı gönüllülükleri belirlenerek eğitim müfredatına rehberlik edilmesi hedeflendi.

Yöntemler: Çalışma, tanımlayıcı kesitsel bir tasarımdır. Araştırma, özel bir üniversitenin hemşirelik bölümünde okuyan 203 öğrenci ile gerçekleştirilen bu çalışmanın verileri Türkiyede geçerlik ve güvenirliği yapılmış Organ Bağışı Tutum ve Bilgi Ölçekleri ile toplandı. Önemlilik değeri $p<0,05$ olarak kabul edildi.

Bulgular: Öğrencilerin organ bağışı bilgi (9,95±2,14) ve donör kartı gönüllülük puan ortalamaları (ortalama ± standart sapma) (2,94±0,89) düşüktü. Öğrencilerin organ bağışı pozitif tutum puanları 106,75±13,21 olmasına karşın, kadavra donör olmayı destekleme oranı %73,8 idi. Öğrencilerin %91,6'sı ölümden sonra organlarını bağışlamayı düşünmüyordu. Öğrencilerin organ bağışı tutum, bilgi ve donör kartı gönüllülük puan ortalamaları üzerinde okumakta oldukları eğitim dönemi, yaşadıkları yer, ebeveynlerinin eğitim düzeyi anlamlı bir etkiye sahip değildi ($p>0,05$).

Sonuç: Öğrencilerin tutum puanları üzerindeki korkularında dinsel değerlerden çok bilgi eksikliğinin ağırlık kazandığı söylenebilir. Lisans eğitiminde beyin ölümü, yas süreci gibi konularda verilecek planlı eğitimlerin, öğrencilerin organ bağışı konusunda motivasyonlarını etkileyebilir.

Anahtar Sözcükler: Organ nakli, tutum, bilgi, donör kartı gönüllülük, hemşirelik öğrencisi

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Introduction

Organ transplantation is an important option in the treatment of patients with organ failure. In countries such as England, America and Germany, the process of identifying potential donors is managed by nurses and nurses are generally trusted to overcome this problem. For this reason, nurses' organ donation attitudes, knowledge and volunteerism are important (1-3).

In the organ donation system in the UK, nurses have responsibilities that involve many complex processes such as identifying potential donors and preparing the family for the possibility of organ donation, explaining the process and obtaining approval. They are given a good education in order to be successful in this field (2). For this reason, training on subjects such as the donor process and brain death before and after graduation is considered important (4,5). The best examples of this are; the 99% rate of identification and referral of potential donations from existing donors after brain death in the UK, and the 91% rate of dealing with families for donations (6). In a study conducted in Turkey between 2013 and 2017, it was reported that 74.3% of the patients who died could not be used for organ donation due to the rejection of their legally responsible relatives (7).

The Spanish model, which cares about the cooperation of nurses and physicians, is adopted in organ donation counseling (8). In studies conducted in Turkey, it is stated that knowing the attitudes of nurses towards organ donation during the school period for donor counseling may contribute to their education (5,9,10).

In this study, it was aimed to determine the attitudes, knowledge and donor card volunteering status of the students studying in different classes of the nursing education program and to guide the education curriculum in this regard.

Research Questions

Nursing students;

• Does he/she have a positive attitude towards organ donation?

• Does he/she have sufficient information about the donor process?

• Does he/she want to have a donor card?

• Are there any situations that affect organ donation attitudes, information and card volunteering?

Method

Design

The research is a descriptive and cross-sectional design.

Center

This research was conducted at the beginning of the 2019-2020 academic year at the nursing department of the faculty of health sciences of a private foundation university.

Nursing department students take the organ transplantation course as a 2-hour theoretical course in the surgical diseases nursing course in the second semester of their 2nd year. During this lesson, very limited information can be given about brain death and the concerns of patients and their relatives about transplantation. There is no systematic educational infrastructure specific to the donor process in the curriculum content.

Time

The research was carried out between October and November in the fall semester of the 2019-2020 academic year, when new student registrations were completed and classes started actively.

Universe and Sample

The universe and sample of this study consisted of all students studying in the nursing department. A total of 236 nursing students (n=54 in 1st grade, n=49 in 2nd grade, n=44 in 3rd grade, n=89 in 4th grade) were enrolled in the 2019-2020 academic year. The number of students who participated in the study voluntarily was 203 (n=54, 100% of 1st grade; n=42, 85.7% of 2nd grade; n=43, 97.7% of 3rd grade; and n=64, 71.9% of 4th grade) which constituted 86.0% of all students. Students who did not volunteer to participate in the study and who filled in the data collection tools incompletely were excluded from the study (14%).

Inclusion criteria: Being a nursing undergraduate student, participating voluntarily in the study, speaking Turkish.

Exclusion criteria: Foreign exchange students, the studens who filled in the data form incompletely.

Data Collection Tool

The Data were collected by using 2 scales as collection tools. The first scale was the Organ Donation Attitude Scale (ODAS) which was adapted by Kent and Owens (11). Its validity and reliability study in Turkish was performed by Yazıcı Sayın (12). The scale was first developed by Parisi and Katz (13) in 1986 and adapted to the present day by Kent and Owens (11) in 1995. The adapted form of the scale included 46 items (23 positive, 23 negative items) showing attitudes towards organ donation. Each item of the scale was in the format of a 6-point Likert scale, ranging from "strongly agree" to "strongly disagree". The Turkish scale was presented in a questionnaire form. The first part of the scale included socio-demographic data. In the second part, there were 40 items (20 positive, 20 negative items) that determined the organ donation attitude. The "charity and moral values and beliefs" sub-dimension of ODAS consisted of 20 items and indicated positive attitudes towards organ donation (PATOD). The possible score for PATOD was between 20-120. Negative attitudes towards organ donation (NATOD) included 2 sub-dimensions. The first was "medically neglect" (MN) and the second was "fear of bodily injury" (FBI). The sub-dimensions of the scale, MN and FBI, each had 10 questions and their scores varied between 10-60. The total NATOD score was between 20-120. High positive and low negative scores indicated strong voluntary attitudes towards organ donation. In the third part, there were questions about the opinions of the participants about

the organ donation, and a question about organ donor card volunteering (ODCV) with a 5-point Likert. ODCV was scored between 1-5. The questions in this section were not mandatory to take an attitude. However, researchers could add any questions they wanted here. Cronbach's alpha (α) of PATOD was 0.92 and Cronbach's α of NATOD was 0.91, and total Cronbach's α was 0.85 (12). These values showed that the scale was reliable.

The second scale used in the study was the Organ Donation Knowledge Questionnaire (ODKQ). This questionnaire was developed by Emiral et al. (14) by examining the current national and international literature and educational materials. It contained a total of 17 questions (9 correct, 8 incorrect) consisting of correct and incorrect answers. The questions consisted of two subgroups, the first one was about donor characteristics (age, organ donation definition, cadaver and living donor type, brain death and medical death, recipient and donor characteristics), the second one was about ethical, legal and medical conditions (permission from the individual and his/her family about organ donation…etc.). The score range was between 0-17, as the score increased, the level of knowledge was evaluated positively, as it decreased, it was evaluated negatively. The Cronbach's α was 0.88.

In the present study, Cronbach's α of ODAS was 0.770; PATOD and NATOD items were 0.866 and 0.920, respectively. Cronbach's α of ODKQ was 0.674. These values showd that both scales were reliable.

Data Collection

The instructors of relevant courses were interviewed to determine the appropriate time according to the course status of the students included in the study. The data were collected 30 minutes before the lesson, with the permission of the lecturer who usually attended the last lesson in the morning. Before delivering the forms, the researcher explained the background and reason for the study, encouraged participants to participate without any pressure, gave explanation on how to fill out the data form, and gave information about the voluntary participation form on the scale. Each student filled out the form in class and gave it back.

Statistical Analysis

Data analysis was done in computer environment with SPSS 24.0 package program. The distribution of data was checked with the Kolmogrow Simirnow test. The data were analyzed for normal distribution. Accordingly, comparisons were made with one-way analysis of variance (ANOVA) test and independent t test. For continuous variables, data were expressed as mean ± standard deviation (SD), median, and range (minimum-maximum). Ratios were used for categorical variables. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

Ethical Aspects

The ethical permission (Ethics committee permission number: 12/11/2018-17105) from the relevant University Hospital Non-Clinical Research Ethics Committee, institutional permission from the Faculty of Health Sciences Nursing Department, and written informed consent from the participants were obtained for the study.

Results

Of the students 93.6% were female, the average age was 20.88±1.77 years (25.6% in the 17-19 age range, 59.6% in the 20-22 age range, 14.8% in the 22-25 age range), 99.5% were single, and 68.5% of them graduated from Anatolian Science High School. All of their parents had a low education level (59.1% of their fathers had primary education; 60.1% of their mothers were literate). Of the students 70.4% were living in the city and 31.5% were fourth grade students.

Table 1 contains some introductory information of the students. When the introductory information about organ donation was examined, 83.7% of the students did not know the working structure of the organ donation registration system in Turkey, and only 2.5% had a donor card. Of the students 73.8% preferred cadaver donors for organ donation, but 91.6% wanted to be buried with their organs when they died. They showed the first three organs that they could donate the least as skin, external genitalia and eye, respectively. Regarding the type of organ donation, 1.5% were against organ donation from opposite sex and 15.8% were against organ donation from animals. Table 2 shows nursing students' organ donation attitude, knowledge and donor card volunteer scores. The students' donor card volunteer scores were very low (2.94±0.89). Organ donation attitudes, knowledge and donor card volunteering of the students were not related to the place of residence and parental education level ($p>0.05$). Only as the education level of the mother increased, the donor card volunteer score increased ($p<0.001$). In addition, there was no statistically significant difference in terms of organ donation knowledge scores, total positive attitudes and negative attitudes between 1st, 2nd, 3rd and 4th grade classes ($p>0.05$). However, the fear of medical neglect score in negative attitudes of the 4st grade class students (28.89±10.51) was significantly higher than that of 1st grade class (23.37±7.78) and 3rd grade class (23.97±8.28) students ($p=0.007$). Organ donation card volunteering had the lowest score in the first grades (2.55±0.92).

Discussion

In this descriptive cross-sectional study, the organ donation attitude, knowledge and donor card volunteerism of students studying in the nursing department of a private university in Istanbul, Turkey were evaluated.

In the present study, the fact that there was no difference between the organ donation knowledge scores of newly enrolled students and students studying in the last year (4th grade), might suggest that sufficient information was not provided in this area during the education process. The fact that the majority of the students did not have a donor card also supported their ignorance in this area. Despite the positive attitude in the studies of nursing students on organ donation in Turkey, the very low rate of having a donor card draws attention to the need for a change in the curriculum related to the donor process (5,9,10). Contrary to the presented study, Martínez-Alarcón et al. (15) reported that senior students had more knowledge about organ transplantation

Table 1. Descriptive features (n=203)

Features	n	%
The class year		
1st year student	54	26.6
2nd year student	42	20.7
3rd year student	43	21.2
4th year student	64	31.5
Knowing the working structure of the organ donation registration system in Turkey		
Yes	33	16.3
No	170	83.7
The presence of donor card		
Yes	5	2.5
Type of donor they support		
Cadaver	150	73.8
Live	39	26.2
The first 4 organs they can donate least (n=93)		
Face	61	14.0
Eye	53	12.2
External genitalia	51	11.8
Skin	46	10.6
Possible will for their post-mortem bodies		
To be buried	186	91.6
Other (respectively): being donated for research, being frozen, being mummified, being cremated	17	8.3
Reaction to organ type¥		
Being against organ transplants of the opposite sex	3	1.5
Not supporting artificial organ transplant	18	8.9
Being against animal transplant	32	15.8
Total	203	100

¥ More than one answer given

than first year students. In addition, in our study, the fact that students' parental education level and place of residence did not have an effect on their organ donation attitude, knowledge and donor card volunteering scores might suggest that the family and environment were not aware of this issue. Tam et al. (16) reported that the education level of the parents did not correlate with the attitudes and knowledge levels of the students. However, in the presented study, only the education level of the mother was effective on the donor card willingness score. This finding was confirmed by Mikla et al. (17). Although there is a similar study in the literature, this effect of the mother's education level on donor card willingness in the presented study may also be a coincidence.

The fact that students' total positive and negative attitudes did not show a difference in terms of the duration of their education could be attributed to the lack of theoretical information on the donor process in the curriculum. When the negative attitudes of the students were examined, the increase in the "fear of medical neglect" scores in the 4th grade students, contrary to the expectations, might be related to the malpractice events they perceived during clinical applications.

The fact that there was no difference in the "fear of bodily injury" among the 1st, 2nd, 3rd and 4th grade students could be explained by the fact that their knowledge deficiencies were the same. In addition, the fact that they showed organs such as the skin, genital organs, eyes and face as the organs that would be donated the least could be associated with the fact that body image was more important at a young age.There was not enough data to say that religious values and judgments played a role in these thoughts. However, the fact that they were not against a donor organ from living or non-living person or from an animal suggested that they could make an evaluation independent of religious beliefs and values. These findings indicated that, contrary to previous research findings in Asia (10,17-21) and Europe (22-24), on the basis of students' organ donation attitudes, ignorance about the donor process and donor counseling might be at the forefront rather than the influence of religious beliefs and values. In this study, students' ignorance of the donor process, transplantation, and brain death might be the reason why they rejected the possibility of being a cadaver donor after death. Although the positive attitude scores were high in the study, the conflict of these scores with the other findings suggested the presence of ignorance and that socially expected responses might be given.

Enriching the education curriculum of students, especially on brain death, the grieving process, the operation of the organ donation registry system in the country, and the obstacles to cadaver donation, can increase positive attitudes and encourage them to become a donor candidate for donor counseling (5,9,10). Some researchers stated in their studies that this young generation studying in the health department at university was confused about brain death and that they were "not sure" about accepting it while encouraging organ transplantation (18,19). However, according to the literature, nurses should encourage potential donors due to the vital role they play in the organ donation process. For this reason, it is reported that they should receive adequate training to be able to explain the organ donation process, get approval, and enable donors and their families to understand the participation process (2). Organ donor organizations provide a standardized approach training to both medical and nursing students in the USA in order to facilitate the process and to use donor resources effectively (25). In fact, it is tried to provide a better education to students with the module in which there are a standardized patient, an actor depicting a living donor candidate and educational materials (film, panel discussion, reading list) used as a supplement (26). In studies conducted in Italy (24) and Spain (27), it was reported that ignorance negatively affected the organ donation attitudes of nursing students. Whinesan et al. (28) showed that the education given to nursing students by experienced clinical nurses made a significant change in their attitudes. However, in some studies, it was reported that there was no relationship between students' level of organ donation knowledge and their attitudes towards

Table 2. Attitude, willingness and knowledge levels of students according to the grade level they are studying

Feature	Negative attitude (NT)	Positive attitude/ helpfulness	NT/ medical neglect	NT/bodily injury	Organ donation card willingness	Knowledge score
	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)
The place of residence						
District	48.03±16.15 (18-85)	106.2±13.19 (63-126)	24.06±9.65 (10-48)	28.76±10.47 (10-51)	10.01±1.08 (7-17)	10.01±1.82 (7-17)
Province	47.59±16.00 (18-93)	106±13.21 (27-124)	26.38±9.35 (10-54)	27.06±10.03 (10-54)	9.92±2.27 (5-27)	9.92±2.27 (5-27)
Test: t; p value	0.178;0.860	-0.355;0.723	-1.575;0.118	0.070;0.278	-1.524;0.130	0.309;0.758
Father's education						
Literate	47.27±16.67 (18-93)	108.01±13.33 (63-126)	25.28±10.39 (10-54)	27.68±9.55 (10-50)	9.79±2.11 (5-17)	2.85±0.91 (1-5)
Primary school and above	48.03±15.59 (18-82)	105.89±13.11 (27-124)	25.98±8.82 (10.48)	27.48±10.61 (10-54)	10.05±2.17 (6-27)	3.00±0.87 (1-5)
t; p value	-0.326;0.745	1.122;0.264	-0.497;0.620	0.142;0.887	1.194;0.234	-0.863;0.389
Mother's education						
Literate and Illiterate	47.13±14.32 (23-93)	107.45±12.05 (63-126)	24.66±8.64 (12-54)	27.86±9.64 (10-54)	9.72±1.79 (5-17)	2.77±0.88 (1-5)
Primary school and above	48.61±18.32 (18-88)	105.70±14.80 ((27-123)	27.25±10.47 (10-49)	27.12±10.96 (10-48)	10.28±2.56 (7-27)	3.19±0.84 (1-5)
t; p value	-0.616;0.539	0.927;0.375	1.850;0.066	0.492;0.624	-1.692;0.093	-3.396;**0.001**
The class year						
1st year student	47.44±13.02 (76-24)	107.77±12.38 (123-63)	23.37±7.78 (48-10)	29.16±8.65 (54-11)	2.55±0.92 (4-1)	9.50±1.42 (13-5)
2nd year student	48.40±18.65 (93-18)	104.23±17.75 (126-27)	25.59±10.52 (54-10)	27.95±11.28 (50-10)	2.97±0.92 (5-1)	10.47±2.28 (17-7)
3rd year student	44.90±13.70 (75-26)	107.65±11.73 (121-77)	23.97±8.28 (40-13)	26.30±8.72 (49-10)	3.02±0.63 (4-2)	9.72±1.35 (12-6)
4th year student	49.40±17.84 (85-18)	10.95±13.31 (124-71)	28.89±10.51 (48-10)	26.81±11.45 (51-10)	3.20±0.89 (5-1)	10.14±2.82 (27-6)
F; p	0.707:0.549	0.684;0.563	4.167;**0.007***	0.802;0.494	5.732;**0.001****	1.995;0.116
Total	47.72±16.01 (93-18)	106.75±13.21 (126-27)	25.69±9.47 (54-10)	27.56±10.17 (54-10)	2.94±0.89 (5-1)	9.95±2.14 (27-5)

F: ANOVA, t: Independent t-test, *There is a significant difference between Classes 1 and 4, and Classes 3 and 4, **The difference between classes 1, 3 and 4 is significant

organ donation (15,22). Studies in Europe and America have reported that increasing knowledge in educational settings that reflect social status increases interest in organ donation and encourages more people to carry donation cards (19,29).

Being informed about the donor process during undergraduate education may encourage them to seek counseling in this field after graduation. Thus, they can motivate themselves for the power that can strengthen their ability to identify and direct potential donors (25). Although lectures and presentations, which are traditional educational initiatives in today's Turkey, seem to meet the information needs of nursing students, they have little effect on their attitudes towards organ donation (30). It is important to include trainings that develop behavior and awareness in improving students' attitudes towards organ donation and encouraging them to donate.

Study Limitations

Considering the possibility that students had to fill in the data forms collectively in the classroom and interact with each other, the effect of these on the results was unknown.

Conclusion

Organ donation attitudes of nursing students were independent of religious beliefs and values. The lack of information about the donor process drew attention to the importance of education on brain death and understanding the patient and his/her family in this process. The low level of donor card volunteering suggested that there were important barriers in their behavior development and awareness in this area.

Although the findings of the study were limited to the students in the study, it was important in terms of showing the importance

of inclusion of trainings that addressed the lack of knowledge among students and concerns about negative attitudes in the curriculum.

Ethics

Ethics Committee Approval: Ethical permission (Ethics committee permission number: 12/11/2018-17105) from the relevant University Hospital Non-Clinical Research Ethics Committee, institutional permission from the Faculty of Health Sciences Nursing Department.

Informed Consent: Written informed consent from the participants were obtained for the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Design: Y.Y.S, M.D., Data Collection or Processing: M.D., Analysis or Interpretation: Y.Y.S, Literature Search: Y.Y.S, Writing: Y.Y.S

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Review

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The Effect of Reiki Therapy on Cancer Pain Management in Palliative Care Patients: A Systematic Review

Palyatif Bakım Hastalarında Reiki Uygulamasının Kanser Ağrısı Yönetimine Etkisi: Bir Sistematik Derleme

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ABSTRACT

This systematic review was conducted to demonstrate the effectiveness of the use of reiki therapy in cancer pain management and to draw attention to the availability of this application in palliative care units. The studies on cancer pain management, both in English and Turkish, of which full text versions were accessible, were systematically analyzed, and the studies without full text and ongoing studies were not included. The study was conducted by reviewing Cumulative Index to Nursing and Allied Health Literature, EBSCOhost, MEDLINE, ScienceDirect, Ovid, ProQuest, Web of Science, and ULAKBİM National DataBases. There were no year restrictions in screening. Keywords such as "cancer pain", "cancer pain management", "reiki" and "palliative care" were used to access the studies. Nineteen research papers were accessed and 5 of them were found to be compatible with the inclusion criteria of our study. Cohrane was established as the guideline to determine levels of evidence. Of the included studies, one was a randomized controlled study, three were experimental studies, the other one was a systematic review. In our day, randomized controlled studies examining the effect of reiki therapy on pain in cancer patients who receive palliative care are limited. In particular, studies have shown that reiki is even more effective in reducing pain when used in addition to pharmacological treatment and conventional nursing care. It is observed that the studies are carried out with a small sample number and generally with a single group. Pain assessments are usually performed with visual scale and numeric scale, reiki sessions are performed in the range of 10-90 minutes and 2-6 sessions per week.

ÖZ

Palyatif bakımda tedavi gören hastalarda reiki uygulamasının kanser ağrısı yönetimine etkisini değerlendiren çalışmaların incelenmesidir. Kanser ağrısı yönetimine ilişkin Türkçe ve İngilizce tam metnine ulaşılabilen araştırmalar sistematik inceleme kapsamına alınmış, tam metin olmayan ve devam eden çalışmalar kapsam dışı bırakılmıştır. Çalışma Hemşirelik ve Yardımcı Sağlık Literatürü Kümülatif İndeksi, EBSCOhost MEDLINE, ScienceDirecf, Ovid, ProQuest, Web of Science ULAKBİM Ulusal Veri Tabanları taranarak yürütülmüştür. Taramada yıl kısıtlaması yapılmamıştır. Çalışmalara ulaşmak için "kanser ağrısı", "kanser ağrısı yönetimi", "reiki", "palyatif bakım" anahtar kelimeleri kullanılmıştır. On dokuz araştırma makalesine ulaşılmış, bu çalışmaların 5 tanesinin araştırmaya dahil edilme kriterlerine uygun olduğu belirlenmiştir. Bu çalışmalar araştırmacılar tarafından ayrı ayrı incelenmiş ve kanıt düzeyleri belirlenmiştir. Kanıt düzeylerini belirlemede Cohrane rehberi temel alınmıştır. Dahil edilen çalışmalardan bir randomize kontrollü çalışma, üçü deneysel çalışma, biri ise sistematik derleme idi. Palyatif bakım alan kanser hastalarında reiki terapinin ağrıya etkisini inceleyen randomize kontrollü çalışmalar günümüzde sınırlı sayıda olmasına karşın yapılan çalışmalar, ağrı şiddetini azaltmada olumlu etkisinin olduğunu göstermektedir. Çalışmaların küçük örneklem sayısıyla ve genellikle tek grupla yapıldığı gözlemlenmektedir. Ağrı değerlendirmelerinin genellikle görsel skala ve numerik skala ile yapıldığı, reiki seanslarının 24-90 dakika aralığında yapıldığı, haftada 2-6 seans şeklinde yapıldığı görülmektedir. Palyatif bakımda tedavi gören kanser hastalarında

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When we examine the effect of reiki on physical and psychological recovery in cancer patients treated in palliative care, it is reported that reiki has a positive effect on reducing pain. However, there are gaps in matters such as which hand position to use in reiki therapy, the duration and frequency of the reiki session, and the application period. For this reason, it is recommended to conduct randomized controlled studies with a large sample group.

Keywords: Cancer pain, cancer pain management, reiki, palliative care

fiziksel ve psikolojik iyileşmede reikinin etkisi incelendiğinde, reikinin ağrıyı azaltmada olumlu etkisi olduğu bildirilmektedir. Ancak reiki terapide hangi el pozisyonunun kullanılacağı, reiki oturumunun süresi ve hangi sıklıkla yapılacağı, uygulama süresi gibi konularda boşluklar bulunmaktadır. Bu nedenle büyük örneklem grubuyla yapılacak randomize kontrollü çalışmaların yapılması önerilir.

Anahtar Sözcükler: Kanser ağrısı, kanser ağrısı yönetimi, reiki, palyatif bakım

Introduction

Many causes, such as the increase in cancer incidence and life expectancy of individuals with cancer, and aggressive treatments administered at the end of patient's life have increased the need for palliative care. Because it is important to manage cancer-related symptoms such as pain in palliative care. Pain often seen in palliative care patients is a condition that patients with cancer fear the most and has been described as "scarier than death itself" (1). There are many causes of cancer-related pain¬. It may be associated with the duration of disease or may develop due to the treatment and procedures administered. Pain due to the duration of disease may develop due to nerve pressure, bone metastasis, local inflammation¬ and thrombophlebitis, usually caused by the primary tumor. Pain associated with the diagnosis and treatment process may be due to biopsy, surgical interventions,-inadequate opioid use, extravasation¬, peripheral neuropathy, or grade 3-4 mucosity (2,3). The incidence of pain varies depending on the type- and cause of cancer. The -incidence of pain in patients treated with a diagnosis of head and neck cancer is reported to be 70%, in gynecologic cancers it is 60% -and in gastrointestinal cancers it is 59% (4). In addition, some studies have shown that one-third of patients with cancer who receive active treatment- and 60-90% of patients with advanced cancer experience moderate or severe pain, and 75% of patients experience pain 2 or 3 times a day with a severity of 5 points or more (5). Medical treatment and nonpharmacological methods are used in the management of cancer pain. Nowadays, one of the methods developed to deal with cancer pain is reiki. Reiki therapy is an energy therapy method performed by the therapist with or without light touch, and it can reduce pain. When held in certain positions, energy is transferred via hands according to the person's needs. Reiki energy provides strength, harmony and balance. It protects physical, emotional, mental and spiritual health. Reiki therapy, which is used in many countries, is usually a reliable, noninvasive and cost effective method with no adverse effect on current treatment and known serious side effects. It can be applied anytime and anywhere, as it does not require special supplies and materials. It is suitable for hospital environment (6,7). Studies indicate that reiki touch therapy is an effective intervention to relieve pain (8,9). A study conducted with women diagnosed as having breast cancer concluded that reiki reduced the experience of physical symptoms such as pain (10). In a study comparing the effectiveness of reiki, massage and yoga, reiki was more effective in reducing cancer pain (11) but it was stressed that there was not sufficient level of evidence regarding the effect of reiki in symptom management of patients with cancer (12). This systematic review was conducted to demonstrate the effectiveness of the use of reiki therapy in cancer pain management in the light of the literature, and to draw attention to the availability of this application in palliative care units.

Methods

This study was conducted as a systematic review. The following steps were followed during the study process:

1. The problem was identified, then the objective was identified.
2. A comprehensive screening of all the studies published by the researchers was carried out.
3. Elimination was carried out according to the inclusion criteria.
4. It was determined which studies would be included in the review.
5. The findings in the studies included were synthesized in the review.

Inclusion and Exclusion Criteria

Studies in Turkish and English that evaluated the effect of reiki on cancer pain in palliative care patients were included. The literature review was conducted independently to assess eligibility criteria by the authors. Discrepancies and disagreements regarding eligibility were resolved by discussion. Articles about palliative care in patients with cancer, of which full texts were not accessible, and ongoing studies were excluded from the review.

Data Collection and Analysis

The study was conducted by reviewing Cumulative Index to Nursing and Allied Health Literature, EBSCOhost MEDLINE, ScienceDirect, Ovid, ProQuest, Web of Science, and ULAKBİM National Data Bases. There were no year restrictions in screening and studies of which publication dates were up until February 2020, were examined. The MESH keywords such as "cancer pain", "cancer pain management", "reiki", "palliative care" were used to access the studies. Also, the reference list of all randomized clinical trials and review papers were checked to find suitable studies that were not identified by electronic search. After the abstracts of the papers were scanned, 19 papers were reached. It was determined that 5 of these studies met the criteria of inclusion in the research. These studies were analyzed separately

by academicians and their levels of evidence were determined. Cohrane was established as the guideline to determine the levels of evidence.

Assessment of Levels of Evidence

Levels of evidence of the studies were evaluated by researchers according to Melnyk and Overholt's guideline. The researchers evaluated each study individually and then combined results. Levels of evidence are evaluated as follows;

Level I evidence:	Evidence that is generated from systematic reviews or meta-analyses of all relevant randomized controlled trials.
Level II evidence:	Evidence that is generated from at least one well-designed randomized controlled trial.
Level III evidence:	Evidence that is generated from well-designed controlled trials without randomization.
Level IV evidence:	Evidence from well-designed case-control and cohort studies.
Level V evidence:	Evidence from systematic reviews of descriptive and qualitative studies.
Level VI evidence:	Evidence from a single descriptive and qualitative study.
Level VII evidence:	Evidence from the opinion of authories and/or reports of expert committees (13).

Results

The overall process of article selection is illustrated with the Preferred Reporting Items for Systematic Reviews flow diagram. In the initial screening, 826 studies were identified. This systematic review included studies examining the effect of reiki on cancer pain in palliative care patients. Following the initial screening of unrelated titles and elimination of duplications, 324 potentially eligible papers were remaining. After the abstracts of the papers were screened, 19 articles of which full texts were accessible were remaining. After the elimination of duplications and other reasons, 5 eligible articles were scrutinized for inclusion in this review (Figure 1).

One of the research included in the systematic review is a randomized controlled study, three is an experimental study, and one is a systematic review (Table 1).

It is noteworthy that all the studies were carried out with patients with mixed cancer diagnoses. All study results report that reiki is effective in reducing cancer pain in palliative care patients (14-18). When the studies are examined, it is observed that reiki application is performed 1-2 times a week with 10-90 minute sessions (7,15-18). Patients' pain scores were evaluated mostly according to the Visual Anologue Scale (VAS) (7,16) and Numerical Scale (18). Studies have shown that reiki therapy practice is more effective in reducing pain when used in conjunction with opioid therapy and nursing care, especially it increases its effectiveness in the 1st and 4th days of therapy (16). The systematic review included in the study not only examined the effect of reiki therapy on cancer pain, but also evaluated the effect of reiki therapy on other chronic pain types (17).

Discussion

This study focuses on the relationship between reiki therapy and cancer pain in palliative care patients. All of the studies has shown that reiki therapy reduces cancer pain and has a positive effect on palliative care patients.

Randomized controlled studies examining the effect of reiki therapy on pain in cancer patients receiving palliative care are today limited. However, existing studies have shown that it has a positive effect on reducing pain intensity. Studies are observed to be conducted with a small sample group and usually with a single group (15-17).

The studies included in the systematic review were analyzed. The pilot study which was conducted to determine the effect of reiki therapy on symptom management in children in palliative care units, only one group undergoing pre-post testing was included. Pediatric patients (n=16) had 24 minutes long, 2 reiki sessions in their houses. At each reiki session, the children put on comfortable clothes, practised 12 hand positions during 2 minutes for each position. Prior to each session, the pre-post test assessed pain with VAS and a significant reduction in pain intensity was found (15) (Level of evidence III). In the study which was conducted with 25 patients receiving palliative care, the patients were divided into 2 groups. One group underwent opioid therapy (analgesia administration of 2-5 doses) for pain treatment, while the other group underwent opioid therapy and 1.5 hours of reiki practice as well. Patients and their VAS scores were recorded and followed up for 7 days, and pain intensity was recorded to reduce drastically in the reiki group in 1st and 4th days (16) (Level of evidence II). In order to evaluate the results of the reiki therapy program in patients with cancer, patients received 10-30 minutes long reiki sessions for at least 3 hours per week, by reiki specialists. During the sessions, specialists applied 3-5 basic hand positions, usually on the head, torso, arms, legs and feet of the patients individually. Pain was assessed with Numerical Rating Scale before and after each session. As a result, the pain score decreased from 2.5 points to 1.2 points (18) (Level of evidence III). The study was conducted with one group to determine the effect of reiki therapy on pain and anxiety in oncology patients in ambulatory and infusion units. Every session was conducted while patients sitting on a chair or lying in bed for 30 minutes. During sessions, therapists hovered their hands from head to toe on the patient's body where the pain or disease was located or gently touched patients' body, focusing on the patient's energy centers. A total of 4 sessions were performed, pain scores decreased by 50% after each session (7) (Level of evidence III). In the systematic review which was conducted to determine whether reiki was beneficial in pain management or not, 2 randomized controlled trial studies were analyzed, and statistically significant decrease in pain was reported when reiki was administered in addition to opioid therapy, resting or conventional nursing care (17) (Level of evidence V).

Table 1. Summary of the research that was included in the study

Study	Design	Sample	Intervention that was administered	Conclusion	Level of evidence
Thrane et al. (15)	Experimental study mixed phase and mixed cancer diagnosis.	E n=16	Pediatric patients (n=200) had 24 minutes long, 2 reiki sessions in their houses. At each reiki session, the children put on comfortable clothes, practised 12 hand positions during 2 minutes for each position. Before each session, pain was assessed with pre-post testing and VAS.	Significant reduction in pain intensity was recorded. However, the fact that there was only a small sample group reduced the statistical significance of the results.	III
Olson et al. (16)	RCT advanced mixed cancer diagnosis.	E n=13	Opioid therapy for pain treatment in a group (administration of analgesia between 2-5 doses)	Pain intensity was evaluated with VAS, 30 mins after the sessions. A significant decrease in pain in the music group was found in the post testcompared to the control group.	II
		C n=11	Opioid therapy and additional 1.5 hours of reiki practice		
Fleisher et al. (18)	Experimental study mixed cancer diagnosis. phase not specified.	Single group n=213	10-30 minutes long reiki sessions were carried out by specialists in total 3 hours per week. Specialists applied 3-5 basic hand positions, usually on the head, torso, arms, legs and feet of the patients individually.	Pain was assessed before and after sessions with NRS. The pain score decreased from 2.5 points to 1.2 points in patients.	III
Birocco et al. (7)	Semi-experimental mixed cancer diagnosis. phase not specified.	Single group n=118	Each session was administered for 30 minutes while patients were sitting in chairs or lying in bed. Therapists hovered over the patient's painful or discomfort areas from head to toe or gently touching their body, focusing on the patient's energy centers. A total of 4 sessions were held.	Pain was assessed before and after sessions according to VAS. Pain scores decreased by 50%.	III
Lee et al. (17)	Systematic review mixed phase and mixed cancer diagnosis.	-	-	When 2 randomized controlled trials were analyzed, it was seen that when reiki was administered during 30 minutes for 4 sessions, in addition to opioid therapy, resting or conventional nursing care, statistically significant reduction in pain was recorded.	V

RCT: Randomized controlled trial, E: Experimental group, C: Control group, VAS: Visual analogue scale

Study Limitations

There were several limitations to this study. The restriction of this review to English language may have resulted in language bias with potentially relevant studies published in other languages being missed. This review also did not include unpublished abstracts from relevant cancer, pain, reiki or complementary therapies conferences. In addition, since some of the investigated studies were conducted with a small sample group, they had methodological limitations.

Conclusion

In conclusion, reiki has a positive effect on reducing pain. The administration of this method to all patients experiencing pain, including patients in palliative care units, can contribute positively to patients' pain management. However, in reiki therapy, randomized controlled trials with a large sample group are needed. The questions such as; which hand position to use for different diseases, the duration of the reiki session and how often it will be practiced, the duration of the practice, and the training of the reiki practitioner, need to be answered (7,17).

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: D.Y., M.E., Design: D.Y., M.E., Data Collection or Processing: M.E., D.Y. Analysis: D.Y., M.E., Literature Search: M.E., D.Y., Writing: D.Y., M.E.

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Review

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Integrated Treatment Methods Applied for Fatigue in Hemodialysis Patients

Hemodiyaliz Hastalarında Yorgunluğa Yönelik Uygulanan Bütünleşik Tedavi Yöntemleri

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ABSTRACT

Chronic kidney disease (CKD), which is an important public health problem both in the world and in our country, is characterized by the irreversible loss of kidney functions, and many organs and systems are affected. Hemodialysis (HD), in addition to being a treatment that should be applied until the end of life and prolonging life unless successful kidney transplantation is performed, also causes the emergence of biological, psychological, social and economic problems arising from the treatment.. Fatigue, which is one of the frequently encountered problems related to its biological dimension, affects the daily life activities of the individual and reduces the quality of life. Fatigue is defined as an irresistible feeling of exhaustion and occurs in situations such as accumulation of waste materials, muscle weakness, inflammatory process, fluid-electrolyte imbalance, anemia. It is important to combine pharmacological and integrated treatment methods with a qualified and comprehensive nursing care in the control of fatigue caused by CKD or HD. While drugs for anemia and depression are often used in pharmacological treatment for relieving fatigue; in integrated treatment, programming of daily activities, helping the individual, exercise, relaxation, yoga, acupressure, hypnosis, reflexology, aromatherapy and massage are used. In this study, it is aimed to provide information about effective, integrated treatment methods on fatigue experienced by patients undergoing HD.

Keywords: Nursing, hemodialysis, fatigue, integrated treatments

ÖZ

Dünyada hem de ülkemizde önemli bir toplum sağlığı problemi olan kronik böbrek yetmezliği (KBY), böbrek işlevlerinin geriye dönüşsüz kaybı sonucunda ortaya çıkan ve beraberinde pek çok organ ve sistemin etkilendiği bir tablodur. Hemodiyaliz (HD) tedavisi, başarılı böbrek transplantasyonu yapılmadıkça yaşamın sonuna kadar uygulanması gereken ve yaşam süresinin uzamasını sağlayan bir tedavi olmasının yanı sıra tedaviden kaynaklı biyolojik, psikolojik, sosyal ve ekonomik sorunların da ortaya çıkmasına sebep olmaktadır. Biyolojik boyutuyla ilgili sıklıkla yaşanan sorunlardan yorgunluk, bireyin günlük yaşam aktivitelerini olumsuz etkileyerek yaşam kalitesini düşürmektedir. Yorgunluk, karşı konulamayan bir tükenme duygusu olarak tanımlanmakta ve daha çok atık maddelerin birikmesi, kas güçsüzlüğü, inflamatuar süreç, sıvı-elektrolit dengesizliği, anemi gibi durumlarda ortaya çıkmaktadır. KBY ya da HD tedavisine bağlı olarak ortaya çıkan yorgunluğun kontrolünün sağlanmasında farmakolojik ve bütünleşik tedavi yöntemlerinin nitelikli ve kapsamlı bir hemşirelik bakımı ile birleştirilmesi önemlidir. Yorgunluğun giderilmesi ya da hafifletilmesine yönelik yapılan farmakolojik tedavide sıklıkla anemiye ve depresyona yönelik ilaçlar verilirken, bütünleşik tedavide ise günlük aktiviteleri programlama, bireye yardımcı olma, egzersiz, relaksasyon, yoga, akupresör, hipnoz, refleksoloji, aromaterapi ve masajdan yararlanılmaktadır. Bu araştırmada, HD alan hastaların deneyimlediği yorgunluk üzerine etkili, bütünleşik tedavi yöntemleri hakkında bilgi verilmesi amaçlanmıştır.

Anahtar Sözcükler: Hemşirelik, hemodiyaliz, yorgunluk, bütünleşik tedaviler

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Introduction

Chronic kidney disease (CKD), regardless of the etiology of the underlying kidney disease, is a progressive and irreversible condition characterized by a decrease in the glomerular filtration rate (GFR) below 60 mL/min/1.73 m², in which many organs and systems are affected (1). In this disorder, there is a decrease in GFR, which indicates the rate of removal of waste materials from the body, along with irreversible losses in the functions of regulating the fluid-electrolyte balance of the kidney and fulfilling metabolic-endocrine functions (2).

The results of epidemiological studies conducted in different countries on CKD show similarity (3,4) and according to these results, the rate of CKD in the world varies between 10-16% (5). Although most of them are in developed countries, more than 2 million people all over the world continue to live with dialysis and kidney transplant treatments (6) and this number is expected to double in the next 10 years (5). In Turkey, the rate of CKD in the adult population is 15.7%. According to the Chronic Renal Disease In Turkey-CREDIT study, the incidence of the disease in men is 12.8% and in women 18.4%. In addition, it has been determined that the incidence of the disease increases with age, and the risk of CKD is much higher in those residing in the Marmara and Southeastern Anatolia regions and rural areas (7).

According to the classification made by the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative/NKF-KDOQI in 2002, CKD consists of 5 stages (5,7). The first stage is the stage in which GFR (≥90 mL/min/1.73 m²) is not affected, but the patient has symptoms of proteinuria/albuminuria or changes in kidney imaging. In the second stage, GFR (60-89 mL/min/1.73 m²) regresses with kidney damage, in the third stage, GFR (30-59 mL/min/1.73 m²) regresses moderately and functional losses become biochemically evident. In the fourth stage, there is a severe regression in GFR (15-29 mL/min/1.73 m²) and the uremic picture becomes evident. In the fifth stage, called end stage renal disease (ESRD), GFR falls below 15 mL/min/1.73 m² and renal replacement therapies (RRT) become mandatory (5,7).

Renal replacement therapies (RRT): Hemodialysis (HD) includes peritoneal dialysis and kidney transplantation. In our country, according to the Joint Report of the Ministry of Health and the Turkish Society of Nephrology, it was stated that the number of patients undergoing RRT increased from year to year, and as of the end of 2019, a total of 83,783 patients, including pediatric patients, were administered RRT, and the most common form of RRT was HD with a rate of 73.21% (8).

Hemodialysis

Hemodialysis is the process of purifying the blood taken from the patient from excess fluid and electrolytes (9), along with uremic toxic substances, through a semi-permeable membrane and HD machine (10). In order to perform the HD procedure, the patient needs an arteriovenous fistula/graft or a central catheter that can provide adequate blood flow (approximately 300-600 mL/min in an adult). By providing anticoagulation in one of these ways, the blood taken to the extracorporeal (outside the body) area is purified from excess fluid, electrolyte and metabolic wastes as a result of filtering towards the dialysis solution moving in the opposite direction and on the other side of the dialyzer while passing through the semipermeable membrane (9,10).

Hemodialysis is a form of treatment that should be applied until the end of life unless successful kidney transplantation is performed (11). The patient is included in the dialysis program 2 or 3 times a week, for an average of 4 or 6 hours, considering creatinine clearance, residual renal function, and clinical status (12).

Problems Experienced by Patients Due to Hemodialysis

In addition to being a form of treatment that prolongs life expectancy, HD also causes biological, psychological, social and economic problems arising from treatment (13). It is stated that patients entering HD are more likely to face malnutrition, inflammation, repeated hospitalization and death compared to other individuals, since it is a primary disease that basically causes kidney failure, and this situation negatively affects general well-being and quality of life (14). At the same time, patients face with negativities such as being dependent on a machine and institution, dietary restriction, social isolation, deterioration in body image, sexual problems, differentiation in family roles, economic problems, difficulties in daily life and social activities, lack of communication with healthcare professionals, and inability to do their job. It is emphasized that the tendency to depression increases in individuals (15). In addition, patients may face acute and chronic complications arising from the HD procedure. While hypotension, cramps, nausea-vomiting, head-chest-back pain, itching and fever-chills are counted among the acute complications (16); dialysis dementia, acceleration in cardiovascular diseases, pericarditis, pleural effusion, uremic bone disease, hypertension, aluminum intoxication, thrombosis, anemia, and hepatitis B and C are among the chronic complications (17).

In the literature, it is stated that patients receiving HD mostly experience fatigue problems (18,19). In a study conducted by Usta and Demir (18) on patients receiving HD, they determined that 52.2% of the patients had fatigue problems in the pre-HD period, 43.3% during HD and 84.4% after HD. Murtagh and Weisbord (19) reported in a systematic review that patients receiving dialysis treatment experienced fatigue problems between 12% and 97%.

Fatigue and Nursing Care in Individuals Receiving Hemodialysis

Fatigue, which is frequently experienced by patients, is defined as an irresistible feeling of exhaustion and occurs mostly in conditions such as accumulation of waste materials, muscle weakness, inflammatory process, fluid-electrolyte imbalance, and anemia (17,20).

Fatigue is a problem that needs to be intervened early, since it negatively affects the patient's quality of life as well as being

life-threatening (21). Therefore, in nursing care, nurses should carefully define the fatigue experienced by the patients, and identify the problems that develop due to fatigue through observation. In addition, by making a detailed evaluation after the determination of the problems; they should determine the effects of the problem on the daily living activities of the patients and make applications to reduce the effects on the patients. In nursing practices; planning activities and determining priorities (22), supporting daily activities at the point where they cannot perform, having active passive exercises, ensuring regular and correct use of prescribed drugs in case of anemia and depression, and enabling patients to share their experiences about fatigue with group meetings (23). In addition, exercise, relaxation, yoga, acupressure, hypnosis, reflexology, aromatherapy and massage are among the interventions for fatigue (24-57). These initiatives are listed below.

Exercise

In patients with CKD, there is a decrease in muscle strength and cardiovascular capacity together with the decrease in kidney functions. With decreasing cardiovascular capacity, aerobic exercise capacity also decreases. This is an important factor affecting mortality in patients with CKD. It is stated that aerobic exercise has positive effects on this factor and reduces fatigue (24-27). Salehi et al. (28) aimed to determine the effect of mini-bike exercise for 20 minutes twice a week for 3 months on fatigue in patients receiving HD in their randomized controlled research; they found a significant decrease in the fatigue level of the intervention group compared to the control group at the third month and 1 month after the intervention. Other studies have shown that exercise may be beneficial in relieving fatigue in patients receiving dialysis (29-31).

Relaxation

Relaxation is a method in which relaxation is achieved by performing stretching and relaxation exercises on the muscles. Amini et al. (32) investigated the effects of progressive muscle relaxation (PMR) and aerobic exercise (AE) on fatigue, sleep quality, and anxiety in patients receiving HD. PMR and AE were applied to the groups other than the control group for 60 days, and especially when PMR was compared with AE, it was found that PMR was more effective in fatigue, sleep quality and anxiety (32).

Yoga

It is the fusion of the body, mind and spirit to form a single whole. It is a philosophy that aims to bring happiness, success and enlightenment through physical, mental and spiritual discipline by increasing the flexibility of the body (33). Yurtkuran et al. (34) performed their randomized controlled study in patients receiving HD. They made the experimental group do yoga-based exercise for 30 minutes/day twice a week for 3 months, and they evaluated the fatigue, pain management, sleep disturbance, grip strength and some biochemical values (urea, creatinine, alkaline phosphatase, cholesterol, erythrocyte and hematocrit), and stated that yoga was effective on them.

Acupressure (Shiatsu)

It is a massage technique applied to the acupuncture points on the fingers and palms and the energy-carrying meridians in the body, based on traditional Chinese Medicine, consisting of the words shi = finger and atsu = pressure. It is also called needle-free acupuncture (35). Cho and Tsay (36) examined the effect of acupressure on fatigue and depression in patients with ESRD in Taiwan. They applied acupressure to both lower extremities of the experimental group for 12 minutes, 3 times a week for 4 weeks, and they stated that depression and fatigue levels decreased in the experimental group compared to the control group (36). Sabouhi et al. (37) applied acupressure treatment to both hands and legs and waist for 20 minutes, 3 days a week for four weeks in their study examining the effect of acupressure on fatigue in patients receiving HD, and they stated that acupressure was an integrated treatment that reduced fatigue. Eğlence et al. (38) applied acupressure treatment to the leg of the patient for 25 minutes, 3 times a week for 4 weeks in their study investigating the effect of acupressure treatment on fatigue in patients receiving HD, and they found that the experimental group had a lower fatigue rate than the control group.

Hypnosis

It is a state between sleep and wakefulness, in which changes in perception, memory, emotion and thought can occur, which creates a tendency for suggestion through gaze or other means (39). Untas et al. (40) found that anxiety, depression, fatigue, and sleepiness decreased after hypnosis in their study examining the effects of hypnosis on anxiety, depression, fatigue, and sleepiness in patients receiving HD.

Reflexology

Reflexology is the stimulation of nerves and blood circulation by applying deep pressure to the reflex points corresponding to all parts of the body, and it activates the nerve energy blocks in the body and ensures a balanced distribution of energy throughout the body. It is preferred because the points where the organs are reflected on the hands and especially on the feet are wider (41). Özdemir et al. (42) applied three sessions of reflexology treatment to the experimental group after HD, each session lasting 30 minutes, for a total of one week in their study to evaluate the effect of foot reflexology on fatigue, pain, and cramps in patients receiving HD. They found that reflexology reduced cramp levels. Unal and Akpinar (43) applied reflexology and back massage for 30 minutes before HD twice a week for 4 weeks to patients in the reflexology and back massage groups in their study examining the effectiveness of reflexology and back massage in increasing the sleep quality of patients undergoing HD and relieving fatigue, and they found that reflexology and back massage improved sleep quality and reduced fatigue. Roshanravan et al. (44) examined the effect of foot reflexology on fatigue in patients receiving HD by forming intervention, placebo and control groups, and they found that the fatigue level of the intervention group decreased significantly after the reflexology application, there was no significant difference in the placebo group, and there was a significant increase in the fatigue in the control

group. Bazzi et al. (45) applied reflexology to the experimental group for 30 minutes, twice a week for 5 weeks in their study examining the effect of foot reflexology on the severity of fatigue in patients receiving HD, and they found a significant difference between the control group and the intervention group, and they found that reflexology reduced fatigue. Ahmadidarrehsima et al. (46) applied foot reflexology and back massage 2 days a week for a total of 3 weeks in their semi-experimental research examining the effect of foot reflexology and back massage on the severity of fatigue in patients receiving HD, and at the end of the application, it was stated that the fatigue level was lower in the group that received foot reflexology than the group that received back massage. In a study conducted by Sharifi et al. (47), in which they examined the effect of foot reflexology on the severity of fatigue in patients receiving HD, they stated that reflexology significantly reduced the level of fatigue in the experimental group compared to the control group.

Aromatherapy

It is performed as a result of taking essential oils obtained from plants, usually by skin route or inhaler (48). Kang and Kim (49) examined the effects of hand massage with aromatic oils on the pruritus, fatigue and stress of patients undergoing HD. They applied aroma hand massage to the hand of the experimental group without arteriovenous fistula 12 times for 5 minutes and stated that aroma hand massage was an effective nursing intervention to reduce pruritus, fatigue and stress in patients undergoing HD (49). Muz and Taşçı (50) applied two-minute aromatherapy (lavender oil and sweet orange) by inhalation before going to bed for a month to the patients in the intervention group receiving HD, and at the end of the fourth week, they found a significant decrease in the fatigue level of the patients in the intervention group compared to the patients in the control group.

Massage

By stimulating the sensitive receptors in the skin and subcutaneous tissue, massage provides relaxation of the muscles, accelerates the blood and lymph circulation, ensures the removal of metabolic wastes such as BUN, creatinine, uric acid, lactic acid, and has a relaxing and energy-increasing effect by stimulating the parasympathetic nervous system (51-53). With its psychosedative effect, it also reduces the feeling of fatigue (53). Hasankhani et al. (54) found that back massage applied for 10 minutes, 3 days a week for 4 weeks to patients receiving HD significantly reduced the level of fatigue. Shahdadi et al. (55) applied back massage to the experimental group for 10 minutes, twice a week for 3 weeks in their study examining the effect of back massage on fatigue in patients receiving HD, and they reported that fatigue in the experimental group decreased significantly. Lee (56) applied hand massage to the experimental group for 5 minutes, 3 times a week for 4 weeks in his study examining the effect of hand massage on fatigue, stress and depression in patients receiving HD, and he found that hand massage reduced the level of fatigue and stress. Habibzadeh et al. (57) applied foot massage for 20 minutes, 3 times a week for 8 weeks to patients who received HD and they stated that the mean fatigue score of the group that received classical foot massage decreased significantly, and that there was a significant decrease in the level of fatigue in the experimental group compared to the control group.

Conclusion

As can be seen from the statistical values announced by the Turkish Society of Nephrology, the number of patients receiving RRT is increasing in parallel with the increase in the number of patients with CKD every year. This situation also increases the possibility of reflection of the biological, psychological, social and economic problems that occur in the patient due to both the disease and dialysis to the society. In this respect, the nurse has a key position in solving the existing or potential problems of the patients. The nurse, who undertakes the holistic, comprehensive and qualified care of the patients, can use integrated treatment methods as well as pharmacological methods to solve the biological problems seen in the patient. The nurse can also solve the patient's problem by utilizing integrated treatments, especially in reducing the level of fatigue.

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