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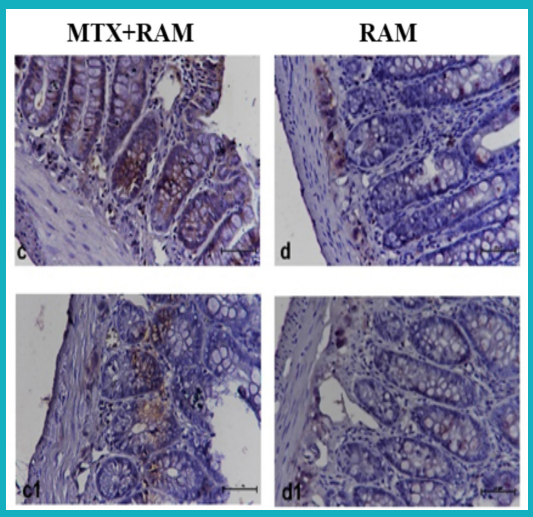

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Original Articles

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EDITORIAL

Dear Readers;

We are with you again in the last issue of 2022. We had a very busy period this year. We had to increase the number of publications to 6 this year in order to allow more studies to be published. In the upcoming period, we will continue our publication life with 4 issues a year. The high number of articles received in our journal caused your valuable articles to be rejected last year. We had to do much more screening for the diversity and originality of the publications for each issue. This difficult situation actually contributed to the increase in the quality of our journal. In the coming period, we will be together with you again with an intense effort and cooperation.

In this issue, I would like to talk about our cover art and selected articles; For this issue, we chose our cover art from the study of Çataklı et al. titled "Ramelteon Protects Intestinal Tissue Against Injury Caused by Methotrexate Via Showing Anti-apoptotic, Anti-inflammatory and Antioxidant Properties". I hope that the subject will draw your attention as well as the cover art, as it is an experimental study and it touches on the subject of reducing the side effects of the drugs we have to use today.

Among the selected articles Büyükkılıç Altınbaşak et al. "Anticholinesterase and Anti-inflammatory Activities of Essential Oils of Naturally Grown Daucus L. Species in Turkey", Alkendi et al. "The Evaluation of Multidrug Resistance-Related Protein 1 as a Prognostic Factor in the Pediatric B-cell Acute Lymphoblastic Leukemia: A Pilot Study", Fazlıoğlu et al. "The Color Change of a Novel Single-shade Composite Immersed in Different Beverages", Semiz and Sağlam Aksüt. "Validity and Reliability of the Symptom-Management Self Efficacy Scale for Breast Cancer Related to Chemotherapy", were our featured articles.

As the year 2023 approaches, I would like to state that we are waiting for your new articles, especially those containing clinical studies and experimental studies.

As it is known, each issue of our journal requires a lot of effort. I would like to thank my assistant editors for their hard work. I wish you all the best to meet new goals and successes in the new year with our referees, publisher and you, our esteemed readers.

Kind regards,
Prof. Dr. Adem AKÇAKAYA
Editor in Chief

Original Article

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Bezmialem Science 2022;10(6):674-82

Sleep Habits and Quality of Life of Intellectually Disabled Children with and without Regular Physical Activity

Düzenli Fiziksel Aktivite Yapan ve Yapmayan Zihinsel Engelli Çocukların Uyku Alışkanlıkları ve Yaşam Kaliteleri

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ABSTRACT

Objective: This study was conducted to determine sleep habits and life qualities of intellectually disabled children who performed or did not perform regular physical activity.

Methods: This descriptive study was done with 126 children between February and June 2017. The data were collected by using the Children's Sleep Habits Questionnaire-Short Form and KIDSCREEN-27. The data were analyzed by using the SPSS package software. The descriptive properties of demographic characteristics are given as percentiles and means. Chi-squared test was used for the comparison of two groups. Independent paired-sample t-test was used to compare intergroup measures. The confidence interval was 95%; $p<0.05$ was considered to be statistically significant.

Results: The children with regular physical activity obtained lower score from Children's Sleep Habits Questionnaire-Short Form. It was found that the children who did not engage regular physical activity, obtained a lower score from KIDSCREEN-27 and its subscales than the children who engaged regular physical activity. The differences between the two groups in terms of general mood and mean scores of your child's feeling, friends, school and learning subscales were also found to be statistically significant ($p<0.05$).

Conclusion: A significant difference in sleep patterns and sleep habits was not found between groups. It was found that intellectually disabled children who engaged regular physical activity had better quality of life.

Keywords: Intellectually disabled child, quality of life, regular physical activity, sleep

ÖZ

Amaç: Bu çalışma, düzenli fiziksel aktivite yapan ve yapmayan hafif düzeydeki zihinsel engelli çocukların uyku alışkanlıklarını ve yaşam kalitelerini belirlemek amacıyla yapılmıştır.

Yöntemler: Bu tanımlayıcı çalışma, 126 çocuk ile Şubat-Haziran 2017 tarihleri arasında gerçekleştirilmiştir. Veriler, Çocuk Uyku Alışkanlıkları Anketi-Kısa Formu ve KIDSCREEN-27 kullanılarak toplanmıştır. Veriler, SPSS paket yazılımı kullanılarak analiz edilmiştir. Demografik özelliklerin tanımlayıcı özellikleri yüzdelik ve ortalama olarak verilmiştir. İki grubun karşılaştırılmasında ki-kare testi kullanıldı. Gruplar arası ölçümleri karşılaştırmak için bağımsız eşleştirilmiş örneklem t testi kullanıldı. Güven aralığı %95 idi; $p<0,05$ istatistiksel olarak anlamlı kabul edildi.

Bulgular: Düzenli fiziksel aktivite yapan çocuklar Çocuk Uyku Alışkanlıkları Anketi-Kısa Formundan daha düşük puan aldı. Düzenli fiziksel aktivite yapmayan çocukların KIDSCREEN-27 ve alt ölçeklerinden düzenli fiziksel aktivite yapanlara göre daha düşük puan aldıkları saptandı. Genel duygulanım ve çocuğunuzun duyguları, arkadaşlar, okul ve öğrenme alt ölçek puan ortalamaları açısından iki grup arasındaki fark istatistiksel olarak anlamlı bulundu ($p<0,05$).

Sonuç: Gruplar arasında uyku düzeni ve uyku alışkanlıkları açısından anlamlı bir fark bulunmadı. Düzenli fiziksel aktivite yapan zihinsel engelli çocukların daha yüksek yaşam kalitesine sahip oldukları saptandı.

Anahtar Sözcükler: Zihinsel engelli çocuk, yaşam kalitesi, düzenli fiziksel aktivite, uyku

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Introduction

Intellectual disability (ID) is defined as a limitation in the child's adaptive behaviors and mental functions that begins during the developmental period. Severity levels (mild, moderate, severe, and profound), as defined in the Diagnostic and Statistical Manual on Mental Disorders-V (DSM-V), are based on adaptive functioning in the conceptual, social, and practical domains (1). The definition in DSM-IV utilizes four degrees of severity that reflect the level of intellectual impairment: IQ levels between 50-55 to approximately 70 characterize mild ID, 35-40 to 50-55 characterize moderate ID, 20-25 to 35-40 characterize severe ID, and IQ levels below 20-25 characterize profound ID (2).

Many diseases such as cardiovascular diseases, respiratory system diseases, and gastrointestinal system disorders are more common in people with ID (3). Literature about young people with IDs suggests that adolescents have a greater risk of having chronic diseases compared to adolescents without IDs (4). This systematic literature review shows that the prevalence rates of chronic health conditions in children with IDs are higher than the prevalence rates reported in studies on children without IDs (5). Therefore, people with ID may have higher morbidity and mortality rates and lower quality of life (QoL) (3). The results of the study of Başgül et al. (6) showed that the scores of children with IDs were lower on QoL and also that children with IDs should be supported in all QoL dimensions (physical, social, emotional, and school functioning). Durukan et al. (7) identified that children with IDs had low QoL score in subcategories of emotional well-being, self-esteem, social relationship and school problems. Biggs and Carter (8) observed that people who were aged 13-21 years and suffered from autism or ID had lower scores in subcategories of physical well-being, psychological well-being, social support, and friend than those of children with normal ongoing growth.

Sleep is an important variable of health affecting QoL and well-being. Sundell and Angelhoff (9) reported a significant negative relationship between children's sleep initiation and maintenance problems and health-related QoL in their study on children aged 3-10. Thus, it is important to conduct adequate interventions to eliminate sleep problems. Sleep disorders among children with IDs are very common (10,11). Of intellectually disabled children 34-84% have sleep disorders. Of children with IDs 39% are sleeping together with their mother (12). The children experience problems of being afraid of sleeping in the dark environment, having restlessness in sleep, waking up in the night, having nightmares, speaking, and teeth grinding (bruxism) during sleep (12). Breslin et al. (13) reported in their study that children with Down syndrome were more likely to have a nighttime sleepiness, sleep anxiety, night wake, parasomnia, sleep breathing disorders, and daytime sleep problems.

Based on a comprehensive literature search, the 2018 Physical Activity Guidelines Advisory Committee concluded that physical activity had benefits on brain health including improved cognitive function, reduced anxiety and depression risk, and improved sleep and QoL (14). However, studies on this subject have shown that physical activity rate among children with mental disabilities is low (8,15-17). In the study conducted by Boddy et al. (16) among mentally retarted and 5-15 age group children, it was reported that a very small rate of children (23%) were active enough to benefit their physical health, and they preferred to be alone instead of participating in large groups. Stanish et al. (17) reported that youth with IDs did less moderate and vigorous physical activity than youth with normal development. In the review study investigating physical activity of intellectually disabled individuals in Turkey, Yılmaz et al. (18) reported a total of 55 studies, 42 of which were experimental. They also stated that none of these studies was conducted by nurses. Moreover, these studies investigated the effects of physical activity on motor development, social development and children's activities of daily living and emphasized that there was not enough number of studies on QoL and sleep, and the number of related studies should be increased (18). In conclusion, more research is needed to understand the effect of regular physical activity on sleep and QoL in children with IDs.

Methods

Study Design

This descriptive study was conducted between February and June 2017 in Special Education School and private sports clubs, offering service for children with IDs which were located in the city centers of three neighboring provinces in west Turkey.

Special education school is consisted of both primary and secondary schools. It is an education instution that gives compulsory basic education to children having intelligence scores between 50-55 and 70. All children with mild IDs study in different classes between 1-8.

Private Sport Clubs are organisations which aim to reinforce the social adaptation behaviors of young people with special needs and relations between them and scociety. These Private Sport Clubs conduct volleyball, basketball, football, athletics, table tennis, bowling, gymnastics, and swimmings activities. Children who are members of the sports clubs practice 3 times a week (in different days) for 2 hours. Children with parents come to sports clubs with their own resources. Children do sports with coaches. The studies start with a warm-up period about 5-10 minutes (only running during this time), then continue with sports related movements and finish with a 5-10 minute cooling cycle (stretching and stretching exercises) (19).

Setting and Samples

The population of the study consisted of 94 disabled children enrolled in special education primary/secondary school in the spring semester of 2016-2017 as well as 87 disabled children who engaged regular physical activity program in special sports clubs. In this study, the purpose was to reach the entire universe without using sampling method.

The criteria of inclusion in the study (Group 1):

- Eight to 18 years of age,
- Having mild intellectual disability,

- Having no medical problems, physical disability and/or drug use preventing regular physical activity,
- Not doing regular physical activity.

The criteria for inclusion of the child in the study (Group 2):

- Eight to 18 years of age,
- Having mild intellectual disability,
- Having no medical problems, physical disability and/or drug use preventing regular physical activity,
- Being engaged in any sport activity regularly for at least 6 months in a sports club.

The inclusion criteria of the caregiver:

- Agreeing to participate in study,
- The caregiver should not have psychological, mental issues or any health problem that prevent communication,
- Being a Turkish speaker.

The criteria for exclusion of the child and caregiver in the study: A medical problem that would prevent the child from doing regular physical activity, being physically disabled and/or using medication, the caregiver's mental disability, psychological problems or any health problems that prevent communication, and having caregiver not speaking Turkish. The trainers in private sports clubs were contacted to collect data on frequencies of the children's participation in the sporting activities. Children who were not considered to engage regular physical activity based on the observation by the researchers were not included in the study. Children who did not egage regularly and did not participate in physical activities done 3 times in a week were excluded from the study.

Group 1 consisted of 64 of 94 children who were enrolled in special education primary and secondary school and not engaging in regular physical activity. Goup 2 consisted of 62 of the 87 children who regularly engaged in physical activity in private sports clubs in three neighboring provinces. Children in group 2 were determined according to the World Health Organization (WHO) criteria related to the regular physical activity. According to WHO, children aged between 5-17 years are expected to do medium-intensity physical activity for 60 minutes per day (20).

Data Collection Tools

Children's Sleep Habits Questionnaire (CSHQ)-Short Form and Child Screening Index Short Family Form were used in the study.

Children's Sleep Habits Questionnaire (CSHQ)-Short Form:

This form developed by Owens et al. (21) was used to investigate children's sleep habits and sleep-related problems. The CSHQ includes 33 items. Perdahlı Fiş et al. (22) conducted the Turkish validity and reliability study of the questionnaire. The questionnaire has eleven subscales as morning wake up problems, parasomnias related to sleep interruption, sleep anxiety, sleep disordered breathing, other parasomnias, morning wake up style, sleep duration, transition to sleeping, needing someone to sleep with, daytime sleepiness, and night-wetting (22). Parasomnias, as described in the the International Classification of Sleep Disorders, are "undesirable physical events or experiences" occurring during sleep transition, during arousal from sleep, or within the sleep period (23).

The items in the questionnaire are rated based on the three-point likert scale. The total score of 41 is accepted as the cut-off point and the values above the cut-off point are considered as clinically meaningful. The questionnaire also includes four open-ended questions about the child's sleep habits (bedtime, sleeping duration during the day, stay-awake duration on wake-up at night, morning wake-up time). In their study, Perdahlı Fiş et al. (22) determined the Cronbach alpha coefficient of the questionnaire as 0.78. In this study, the Cronbach alpha coefficient of the scale was 0.82.

Child Screening Index Short Family Form (KIDSCREEN-27):

The validity and reliability of the index in Turkish children/adolescents was confirmed by Baydur et al. (24). KIDSCREEN-27 parent form has a total of 27 items and 5 subscales; physical activity and health (5 items), general mood and your child's feeling (7 items), family and your child's free time (7 items), friends (4 items), and school and learning (4 items). The index is a Likert-type scale. Meral and Fidan (25) examined the psychometric properties of the parent form in Turkish version and Cronbach's alpha value was found as 0.88. In this study, the Cronbach alpha coefficient of KIDSCREEN-27 parents form was 0.86.

Data Collection

In the first stage of data collection, the caregivers of the children, who met the inclusion criteria of the study conducted by the researcher and the related school/sports club managers, received an envelope containing the documents confirming the fact that the school/club management was aware of the study and granted permission. A form explaining the purpose of the study and the permission form indicated whether the caregivers accepted to participate in the study were put into the envelopes. School and sports club managers were asked to send these envolopes to children's families. Envelopes were sent to families by children. Within a week, families were asked to sign the permisison form and send it back to their teachers in school or trainers in sports clubs. The telephone numbers of the families who gave written permission by this way were taken from the managers of the schools and sports clubs. The caregivers were contacted by telephone to check their available time slot. Later, the researcher obtained the contact addresses of caregivers of the children enrolled in the school/sports club. Home visits were made in the specified time slot. The CSHQ and KIDSCREEN-27 were completed during the face-to-face interview made with the families who gave written consent. The forms were filled out by primary caregivers such as parents, fathers, and grandparents.

Data Analysis

The data were analyzed by using the Statistical Package for Social Sciences (SPSS), version 15.0 (SPSS Inc., 2007). Independent paired-sample t-test was used to compare intergroup measures. The confidence interval was 95% and p<0.05 was accepted as statistically significant.

Results

It was determined that 53.13% of the children who did not engage regular physical activity were males, 73.44% were secondary school students, 37.50% were second children and the mean age was 12.90±2.72 years. In the children who engaged regular physical activity, 54.84% were males, 64.52% were secondary school students, 40.32% were the first-born children of the family, and the mean age was 13.70±2.97 years. The two groups were found to be similar (p>0.05).

It was determined that 24.19% of the children who engaged regular physical activity were interested in the athletics branch, half were performing sports activities for 1-2 years, and 64.52% engaged in sports 2 days a week.

It was determined that the CSHQ total mean scores of the children in the group 1 were higher than those in the group 2 and the difference between them was statistically significant (p<0.05). It was found that children in the group 1 obtained higher scores from all subscales of the CSHQ compared to those in the group 2 but the difference between them was not statistically significant (p>0.05) (Table 1).

Nineteen (29.69%) of the children in the group 1 had a score of 41 or less, did not experience any sleep problems at the clinical level, and had a total mean score of 36.57±3.27. Forty-five children (70.31%) had a score of 42 or higher, experienced sleep problems at the clinical level, and had a total mean score of 52.66±10.06. Twenty-seven (43.55%) of the children in the group 2 had a score of 41 or less in the sleep habits questionnaire, did not experience any sleep problems at the clinical level, and had a total mean score of 36.18±2.48. Thirty-five children (56.45%) had a score of 42 or higher, experienced sleep problems at the clinical level, and had a total mean score of 49.28±7.24 (Table 2).

It was found that children in both groups who had sleep problems at the clinical level, had higher scores from CSHQ and the difference between them was statistically significant (p<0.05). The number of children, who had sleep problems at the clinical level with respect to the cut-off point in CSHQ, was higher in both groups and they had higher mean scores from CSHQ. However, it was determined that the difference between the total mean scores was not statistically significant (p>0.05; Table 2).

In this study, the Cronbach's alpha coefficient of the CSHQ was 0.82. The Cronbach's alpha coefficient of the subscales were 0.83 in morning wake-up difficulty subscale, 0.67 in the parasomnias related to sleep interruptions subscale, 0.71 in the sleep anxiety subscale, 0.79 in the sleep disordered breathing subscale, 0.54 in the other parasomnias subscale, 0.34 in morning wake-up style subscale, 0.66 in sleeping duration subscale, 0.40 in transition to sleep subscale, 0.58 in needing someone to sleep with, 0.50 in day-time sleepiness, and -0.035 in the nighttime wetting subscale. Cronbach's alpha coefficients of wake-up style, transition to sleep, daytime sleepiness and night-wetting

Table 1. Children's CSHQ total and subscale mean scores in terms of their regular physical activity status

	Regular physical activity		
	Group 1 Mean ± SD	Group 2 Mean ± SD	Test and significance
Morning wake up problems	6.78±2.91	6.22±2.49	t=1.147 p=0.254
Parasomnias related to sleep interruption	6.14±1.64	6.00±1.68	t=0.474 p=0.636
Sleep anxiety	4.85±2.23	4.85±2.25	t=0.011 p=0.991
Sleep disordered breathing	4.14±1.58	4.03±1.67	t=0.373 p=0.710
Other parasomnias	3.98±1.21	4.22±1.58	t=-0.958 p=0.340
Sleeping duration	3.82±1.38	3.69±1.24	t=0.572 p=0.568
Needing someone to sleep with	2.85±1.25	2.74±1.10	t=0.557 p=0.579
CSHQ total score	47.75±11.28	43.58±8.64	t=2.323 **p<0.05**

t: Independent paired-sample t-test, SD: Standard deviation, CSHQ: Children's Sleep Habits Questionnaire

Table 2. Children's sleep problem at clinical level with respect to the cut-off points of their CSHQ mean scores

Sleep problem at clinical level	Regular physical activity				
	Group 1		Group 2		Test and significance
	n (%)	Mean ± SD	n (%)	Mean ± SD	
Did not have	19 (29.69%)	36.57±3.27	27 (43.55%)	36.18±2.48	x^2=2.610
Had	45 (70.31%)	52.66±10.06	35 (56.45%)	49.28±7.24	p=0.106
Test and significance		t=-9.970 **p=0.000**		t=-9.469 **p=0.000**	

n: Number, x^2: Chi-squared tests, t: Independent paired-sample t-test, SD: Standard deviation, CSHQ: Children's Sleep Habits Questionnaire

subscales were found to be between 0.30 and 0.50, hence, they were not used in the study.

It was found that the hourly mean bedtime of the children in the group 1 was 21.17±4.74, the mean daytime sleep duration was 9.21±1.04, staying awake time at night was 4.37±7.41, and the mean wake-up time was 7.39±0.83. The mean sleep duration of the children in the group 2 was 22.28±0.74, mean daytime sleep duration was 99.05±1.33, mean stay awake time at night was 4.24±6.19 minutes, and the mean wake-up time was 7.34±1.57 hours (Table 3).

In this study, the Cronbach's alpha coefficient of KIDSCREEN-27 parent form was 0.86. The Cronbach's alpha coefficients of the subscales were 0.74 in the physical activity and health subscale, 0.78 in the general mood and your child's feeling subscale, 0.73 in the family and your child's free time subscale, 0.80 in the friends subscale, and 0.73 in the school and learning subscale.

The mean scores of the children in the group 1 for KIDSCREEN-27 and its subscales were 16.45±3.73 in physical activity and health, 24.78±4.38 in general mood and your child's feeling, 26.84±4.04 in family and your child's free time, 14.20±3.65 in friends, and 15.03±3.25 in school and learning. Total mean score of KIDSCREEN-27 was 97.31±12.24. The mean scores of KIDSCREEN-27 in the children in the group 2 for and its subscales were 17.85±3.58 in physical activity and health, 26.98±3.54 in general mood and your child's feeling, 28.75±4.24 in family and your child's free time, 14.85±3.17 in friends, and 16.79±2.25 in school and learning and KIDSCREEN-27 and total mean score was 105.24±11.37 (Table 4).

Comparison of KIDSCREEN-27 and its subscales based on children's the regular physical activity status indicated that the total and subscale mean scores of the children in the group 2, except for the subscale of the friends were higher than the children in the group 1 and the difference between the mean scores was statistically significant (p<0.05, Table 4).

Table 3. Children's sleeping patterns in terms of their physical activity status

	Regular physical activity		
	Group 1 Mean ± SD	Group 2 Mean ± SD	Test and significance
Bedtime	21.17±4.74	22.28±0.74	t=-1.853 p=0.068
Sleeping duration during the day (hour)	9.21±1.04	9.05±1.33	t=0.755 p=0.452
Stay-awake duration on wake-up at night (minute)	4.37±7.41	4.24±6.19	t=0.109 p=0.913
Morning wake-up time	7.39±0.83	7.34±1.57	t=0.226 p=0.822

t: Independent paired-sample t-test, SD: Standard deviation

Table 4. Children's mean scores of KIDSCREEN-27 and its subscales in terms of their physical activity status

	Regular physical activity		
	Group 1 Mean ± SD	Group 2 Mean ± SD	Test and significance
Physical activity and health	16.45±3.73	17.85±3.58	t=-2.148 **p<0.05**
General mood and your child's feeling	24.78±4.38	26.98±3.54	t=-3.098 **p<0.05**
Family and your child's free time	26.84±4.04	28.75±4.24	t=-2.592 **p<0.05**
Friends	14.20±3.65	14.85±3.17	t=-1.067 p=0.288
School and learning	15.03±3.25	16.79±2.25	t=-3.538 **p<0.001**
KIDSCREEN-27 total score	97.31±12.24	105.24±11.37	t=-3.763 **p<0.001**

t: Independent paired-sample t-test, SD: Standard deviation

Discussion

In this study, it was found that the mean CSHQ's scores (47.75±11.28) of children in the group 1 were higher than the children in the group 2 (43.58±8.64). However, the difference between the groups was not statistically significant in terms of the mean scores of CSHQ's subscales. Total mean scores of the two groups were 42 or higher and they had clinically significant sleep problems. In addition, it was noteworthy that more than half of the children who engaged or did not engage regular physical activity had regular sleeping problems at the clinical level per CSHQ cut-off point, but this rate was quite higher among the children who did not. Hence, it might be concluded that regular physical activity decreased the sleep problems in children with IDs. In their study, Wachob and Lorenzi (26) reported that children with autism spectrum disorder aged between 9-16 years had a mean score of 44.4±11.01 and physically more active children had better sleep quality. Köse et al. (11) reported that the mean score was 51.78 in children with ID and 41.56 in children with normal development and sleep disturbances were more frequent in children with ID than children with normal development. The comparative and interventional studies on the relationship between physical activity and sleep have showed that physical activity has a positive effect on sleep (26-30), but there are also studies that do not support this view (10,31,32). In the study performed by Temel et al. (31) with high school students and in the study by Aktaş et al. (29) there was no significant relationship between regular and adequate physical activity and sleep quality. Similar to the results of the present study, in their study, Ghanizadeh and Faghih (10) found that children with IDs did not have more sleep problems in terms of daytime sleepiness, parasomnia, resistance to bedtime, sleep time and sleep anxiety compared to their sibling and healthy children groups. This result of the study suggests that the sleep quality in mentally retarded children is not only related to regular activity, but many factors may be effective in sleep problems.

When comparing the sleeping patterns of the children in the group doing regular physical activity compared to the other group in this study, it was determined that bed-time was later, total sleeping duration was less, time to fall asleep again after wake-up at night was shorter, and the morning wake-up time was earlier. In the present study, it was found that there was no statistically significant difference between children's regular physical activity and sleep patterns ($p>0.05$). Contrary to the findings of this study, Brand et al. (33) determined that children with autism spectrum disorder did aerobic exercise for 60 minutes per week, and at the end of the third week, children's sleep activities increased, their transition to sleep time shortened, and the sleep interruption decreased. In their study, Dodds et al. (34) applied a 5-week physical activity program to children with neurodevelopmental disorder in the age group of 1-14 years and found that children had an increased sleep duration and decreased sleeping problems. When the studies in the literature are reviewed, it is observed that, among children and adolescents with IDs, increasing physical activity improves sleep quality and decreases sleep problems. It is stated that the intervention of a three-week pedometer and daily-based physical activity in adolescents are effective in improving the subjective sleep quality and reduce the frequency of night wake-ups (35) and reduce the time to fall asleep again if sleep is interrupted at night (27). In the current studies (27,34), the authors conducted their studies on children with regularly controlled physical activities over a certain period of time. But, in this study, children engaging regular physical activities in a club were compared with those who did not. The source of difference between our results and current studies' conclusions is based upon this.

In comparison of KIDSCREEN-27 and its subscales according to children's regular physical activity status in the present study; mean scores of all the subscales, except for the subscale of the friends and total mean scores were higher in children in the group 2 than the children in the group 1 and the difference between the mean scores was statistically significant ($p<0.05$, Table 4). In contrast to the results of the study, İlhan et al. (36) conducted a physical education and sports activities program twice a week for 10 weeks to a group of children with IDs aged 8-12 years. At the end of the study, it was reported that the QoL of the children participating in the program was higher than the control group, but the difference between the groups was not significant, and it was suggested that the length of the 10-week program used might be insufficient (36). In the study by Top and Akıl (37), a 10-week swimming practice was found to significantly increase the scores of self-esteem and the QoL subscales of the children with mild IDs in the age group of 12-16 years. The study was conducted with children in the group 2 who were doing regular physical activity for at least 6 months and children in the group 1 who were not doing regular physical activity, and the studies in the literature were conducted with the exercise program over a period of 8-10 weeks. Hence, it can be asserted that the difference between the results of these studies in the literature and the results of the present study was associated with the type and duration of physical activity applied. According to the results of our study, it can be concluded that regular physical activity has an important contribution to the improvement of the QoL of intellectually disabled children.

Bayazıt et al. (38) found in their study that an 8-week athletics program developed motor skills in 11-14 year-old children with educable IDs. In the study by İlhan et al. (39) it was found that regular athletics program increased the self-care skills of intellectually disabled children. The results of the present study supported the literature and it was concluded that physical activity increased the QoL of children with IDs, developed motor skills, and thus had a positive effect on their health.

Vogt et al. (40) reported that 30-min moderate intensity running exercise strengthened emotional state, improved self-esteem, and increased perceived social acceptance in intellectually disabled adolescents. In the study by Çokluk et al. (41) it was observed that the 10-week special physical education program had a positive effect on the self-perception of children with mild IDs. Aslan and Çalışkan (42) stated in their study that participation in exercise and sportive games had a positive effect on anger control in children with IDs. In this study, it was determined

that children with IDs in the group 2 had higher scores in general mood and child's feelings subscales than those in the group 1. The results are compatible with those reported in the literature. According to the results of this study, it can be concluded that participation in sports and exercise is important for the positive psycho-social development of children with IDs.

Grandisson et al. (43) stated that parents were proud of their intellectually disabled children participating in sports and reported an improvement in their family relations. In their study, Blick et al. (3) showed that individuals with IDs who exercised regularly had better participation in society than their peers. Similar to the results in the literature, it was found in the present study that children with IDs who made regular physical activity had higher scores in family and leisure subscale. Therefore, physical activity is thought to be the best quality time for children with IDs to utilize their spare time and spend with their family.

McConkey et al. (44) stated that that sportsmen with/without ID playing together in the same sports team improved their friendship and social participation. Biggs and Carter (8) reported in their study that greater participation by children with IDs in activities outside the school provided a significant increase in the scores of the QoL social support and friends subscales (6). Yarımkaya (45) determined that children with moderate mental retardation between the ages of 11-14 who participated in the peer-mediated adapted physical activity program developed characteristics such as interacting with their peers, acting together with the group, obeying the group rules and sharing, and had positive effects on their socialization levels. Based on this information, in the present study, it was found that the mean score of the children in the group 2 as an out-of-school activity was higher than those in the group 1, but this difference was not statistically significant. It was concluded that this result was due to the fact that children who did not do regular physical activity continued their education on a regular basis and their friendship relations were stronger than those who had no education.

In the study by Saygılı et al. (46), it was found that the students who did regular sports had better personality characteristics and academic achievement than the students who did not do regular sports. In the present study, it was found that the mean scores of the children in the group 2 were higher than those in the group 1 and the difference in the scores of school and learning subscale was statistically significant. The results of the present study are compatible with the literature.

Study Limitations

This descriptive study was conducted comparatively. This was one of its limitations. Another limitation was nonqualitative and nonquantitative examinations of physical activities in which children participated actively. Our results showed that new controlled studies to understand the effects of regular physical activities in special sports club would be necessary.

Conclusion

In this study, the sleep quality and QoL of children who engaged or did not engage regular physical activity were compared. A significant difference in sleep patterns and sleep habits was not found between groups. It was found that intellectually disabled children who engaged regular physical activity had better quality of life. It can be recommended that regular school screening is made to identify sleep habits and sleep problems among children with IDs and counseling and education programs for children and parents/caregivers are provided for the solution of the problem. It can be recommended to evaluate physical activity patterns of intellectually disabled children, to train them and their parents/caregivers on the benefits of physical activity, and to ensure that the children with sleeping disorders participate in regular physical activity programs to improve their quality of life. It can be recommended to establish regular physical activity programs outside of school by providing all opportunities for the participation of children with IDs in the programs.

Ethics

Ethics Committee Approval: Atatürk University Faculty of Health Sciences (date: 15.07.2016/decision no: 2016/07/06).

Informed Consent: Obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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The Need for Information and Support among First-degree Relatives of Patients with Breast Cancer What Do We Know?

Meme Kanseri Olan Hastaların Birinci Dereceden Akrabalarının Bilgi ve Destek Gereksinimi: Ne Biliyoruz?

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ABSTRACT

Objective: Since first-degree female relatives (FDFRs) of women with breast cancer (BC) also have a high risk of developing BC, providing them information and support is important and this should not be overlooked. The purpose of the study is to determine the needs of primary relatives of women with BC in Turkey for information and support.

Methods: This article is a descriptive research. A total of 199 volunteer relatives of patients with BC were reached and interviewed by nurses in a university hospital's oncology clinic. They completed the Information and Support Needs Questionnaire. Parametric, non parametric, and multiple regression tests were used in statistical analysis.

Results: In the study, a statistically significant difference was observed between the information and support needs and demographics of FDFRs ($p<0.05$). The rate of information need was higher among those who had a risk of BC and who practiced protective behaviors regularly. And also the rate of unmet support need was higher among those who had a risk of BC and who did not practice protective behaviors ($p<0.05$). The need for information and support is increasing in the FDFRs of women with BC whose diagnosis time prolonged.

Conclusion: This study showed that FDFRs of patients with BC needed information and psychosocial support. Nurses should have an important role in communicating with relatives of patients with BC.

Keywords: Breast cancer, information and supports, primary relatives, nursing

ÖZ

Amaç: Meme kanseri (MK) olan kadınların birinci derece kadın akrabaları da (BDKA) MK'ye yakalanma konusunda yüksek risk potansiyeli taşıdıkları için onlara bilgi ve destek sağlamak önemlidir ve onlar göz ardı edilmemelidir. Bu çalışmanın amacı, Türkiye'deki MK'li kadınların BDKA'nın bilgi ve destek ihtiyaçlarının belirlenmesidir.

Yöntemler: Bu makale tanımlayıcı bir araştırmadır. Toplamda 199 gönüllü akrabaya hastalar aracılığıyla ulaşılmış ve bu akrabalarla bir üniversite hastanesinin onkoloji kliniğinde çalışan hemşireler görüşme yapmıştır. Katılımcılar Bilgi ve Destek Gereksinimleri Ölçeği-Türkçe Formu'nu doldurdu. İstatistiksel analizde parametrik, non-parametrik ve çoklu regresyon testleri kullanıldı.

Bulgular: Çalışmada BDKA'nın demografik özellikleri ile bilgi ve destek ihtiyaçları arasında istatistiksel olarak anlamlı bir fark saptandı ($p<0,05$). MK riski taşıyan ve düzenli koruyucu davranışlar sergileyenlerde bilgi ihtiyacı oranı daha yüksekti. Ayrıca MK riski taşıyan ve koruyucu davranışlar uygulamayanlarda karşılanmamış destek ihtiyacı oranı daha yüksekti ($p<0,05$). Teşhis süresi uzamış olan MK'li kadınların BDKA'nın bilgi ve destek ihtiyacı artmaktadır.

Sonuç: Bu çalışma, meme kanseri hastalarının BDKA'nın bilgi ve psikososyal destek ihtiyacı olduğunu göstermiştir. Sağlık hizmetinde görev alan hemşireler, MK'li hastaların BDKA ile iletişimde önemli bir role sahip olmalıdır.

Anahtar Sözcükler: Meme kanseri, bilgi ve destek, birinci derece akraba, hemşirelik

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Introduction

Breast cancer (BC) in the world and in Turkey, is the most common cancer in women (1,2) BC affects about 2 million women every year in the world and is the leading cause of cancer-related deaths in women. In the WHO 2018 data, it was reported that approximately 627 thousand women died due to BC, and this rate constitutes 15% of all cancer-related deaths. BC rates in developed countries continue to increase as all over the world (1).

One of the most important risk factors of BC especially in is the history of BC in the first degree female relatives (FDFRs), especially in mothers, sisters or daughters (3). BC due to genetic predisposition is seen in one-ninth and 5-10% of women with BC in FDFRs. Genes that increase BC risk are BRCA1 and BRCA2 (4-6).

Women at risk who have BC in FDFRs may experience mental problems such as psychological distress, anxiety and fear (7,8). Approximately 7% of women who are diagnosed as having BC are younger than 40 years of age (9). Based on the rapidly increasing incidence between 25 to 40 years of age, e.g., when a woman is diagnosed as having BC in her 30s, her FDFR should be aware that they may develop BC nearly 8 to 10 years earlier than her diagnosis age (10). While BC was seen in 4.9% of women aged 15-24 years in our country, it was seen in 33.7% of the age group of 25-49 years. Moreover, 45% of women who were diagnosed as having BC were between the ages of 50-69 years and 40% of them were between the ages of 25-49 years (2). Accordingly, it is important to provide guidance on this issue to primary relatives to bring awareness with respect to early screening tests, a healthy lifestyle, and psychosocial support for women in their 20s.

After a cancer diagnosis, some family members already have a high awareness comparable to that of patients with cancer so that they can make healthy lifestyle changes, while some of them do not (11). FDFRs need to be motivated by the possibility of a cancer diagnosis so that they can make healthy lifestyle changes and participate in screening programs rather than experience the challenges that may occur when these changes are not made (12,13).

This study aimed to determine the FDFRs of women with BC information and support needs in Turkey. We are also looking for answers to the following questions: What is the effect of having a hereditary risk for BC and the development status of protective behaviors on information and support needs? What are the effects of socio-demographic features on information and support needs?

Methods

Design

First-degree female relatives (mother, sisters and daughters) were reached for a cross-sectional, descriptive and non-experimental study through patients (n=199) who were under treatment for BC and participated in a survey. The survey sample was calculated using a sample size calculations formula with 95% confidence intervals.

Participants

Nurses of the university hospital interviewed the volunteering patients in the oncology clinic of Pamukkale University Hospital in Turkey. After approval was obtained from the Ethics Committee, a total of 199 FDFRs agreed to participate and signed the informed consent before completion of the surveys. Women over 18 years of age and not previously diagnosed as having BC were included in the study. Data were collected through a Demographic Questionnaire, the ISNQ and the BRAC tools.

Instruments

Demographic Questionnaire

We administered a structured questionnaire that included questions on the following: education, age, number of live births, kinship relations, marital status, biopsy status, menopausal status, breastfeeding, perceived risk of BC. In addition to the potential cancer risk, the respondents were evaluated through a description of their perceived state: "Yes, I am at risk", "No, I am not at risk" or "I do not know".

The Information and Support Needs Questionnaire (ISNQ)

This questionnaire was developed by Chalmers et al. (14) to understand the information and support needs of women who had a family history of BC. This questionnaire is composed of two scales: 1- The importance scale, which contains 18 informational and 11 support items, is evaluated according to a four-point Likert-type scale (4- very important, 1- not important at all); 2- The needs met scale contains twenty-nine items that address whether needs are met, (1 -not met at al to 4- met fully). The Turkish validity and reliability study of the scale was conducted by Aslan and Ceber (15).

BCRA-Breast Cancer Risk Assessment Tool

This interactive tool was developed by Gail et al. (16) to determine the risk factors for the development of BC in women within the next five-year period of their lives and during their lifetimes. BRCA is used in risk calculation for women aged 35 and over. This tool is useful for the estimation of women with a lifetime risk of BC ≥20% (17). Moreover, an estimated 5-year BC risk ≥1.67% is considered high (18).

Statistical Analysis

Independent Samples t-test, Mann-Whitney U test, Kruskal-Wallis test or one-way ANOVA were used for continuous variables. Multiple logistic regression analysis was used to see the effect of dependent variables on information and support scores. $P<0.05$ was considered significant in all statistical tests.

Results

The 199 women were FDFRs of patients with breast cancer: 125 were mothers (62.8%), 53 were sisters (26.6%), and 21 were daughters (10.6%). The mean age was 34.48±10.14 years (minimum: 18; maximum: 58) (Table 1).

When the information and support needs of the study group were evaluated, the five most significant items were determined to be; information about ways that could help decrease the suffering of relatives with BC (3.60±0.82), information about the treatments of BC (e.g., radiation, chemotherapy, side effects) (3.59±0.75), information about the emotional reactions of women who were newly diagnosed as having BC (3.58±0.78), information about how to support relatives during their experience with BC (3.57±0.79) and information on and a demonstration of a BC examination (3.54±0.81). The five most important items within the "needs met" scale were; information about the treatments of BC (e.g., radiation, chemotherapy, side effects) (2.96±1.06), information about the causes of BC (2.86±1.03), information on and a demonstration of BC examination (2.83±1.05), information about how to support relatives during their experience with BC (2.82±1.00), and information about the emotional reactions of women who were newly diagnosed as having BC (2.81±1.02) (Table 1).

When the importance of information and support needs of FDFRs was evaluated based on their socio-demographic characteristics, it was found that FDFRs who were aged 40 years or older had higher information needs than the other two groups (p=0.032). Those who were widowed and divorced had a greater need for information than single FDFRs (0.042), and those who were graduates of elementary school had a greater need for information than those who were graduates of middle school (p=0.022). Unemployed FDFRs had a greater need than employed FDFRs (p=0.002), and menopausal women had a greater need than women who were not in menopause (p=0.000). The differences between groups were found to be statistically significant (Table 1).

When needs met were evaluated based on the socio-demographic characteristics of FDFRs, it was found that support needs were not met among the 31-40 year-old group compared with the other age groups (p=0.000) and among the relatives with secondary education (p=0.028) and higher education (p=0.018) groups compared with the relatives with the primary education group. The differences between these groups were statistically significant, but no statistically significant differences were observed between the other variables and the importance and needs met (p>0.05) (Table 1).

When the status of FDFRs was evaluated with respect to their risk for BC and the adoption of preventive behavior, it was determined that the need for information was higher among those whose mothers had cancer than in those whose sisters had cancer (p=0.000). The need was also higher in those who were diagnosed after 40 years of age compared with those who were diagnosed before 40 years of age (p=0.042) and in those whose duration of diagnosis was more than one year compared with those whose duration was less than one year (p=0.033). The need for information was also higher in those who had education/information about BC than in those who did not (p=0.003) and in those who performed breast self-examinations than in those who did not (p=0.005). Finally, the need was higher in those who underwent mammography than in those who did not (p=0.038), in those who underwent a breast examination by a healthcare professional (e.g., doctor, nurse, midwife) than in those who did not (p=0.002) and in those who stated that "presence of BC among my relatives creates a risk for me" than in those who stated "I do not know" (p=0.003). These differences were found to be statistically significant (Table 2).

When the status of FDFRs was evaluated with respect to their risk of BC and the adoption of preventive behavior, and when their effects on needs met were examined, it was found that support needs were not met among the women whose duration of diagnosis was less than one year compared with those whose duration of diagnosis was more than one year (p=0.001). Support needs were also not met in those who performed breast self-examinations compared with those who did not (p=0.001)

Table 1. Socio-demographic characteristics of the study group and their information and support needs

	Importance **n=199**	Needs met **n=199**	P value
Age group			
18-30 (69)	3.26 (0.82)	2.78 (0.77)	IN:0.005
31-40 (67)	3.17 (0.87)	2.14 (0.74)	SN:0.000
40 or over (63)	3.59 (0.51)	2.77 (0.99)	
Marital status			
Single (64)	3.17 (0.80)	2.62 (0.82)	IN:0.042
Widowed/divorced (135)	3.41 (0.74)	2.54 (0.92)	SN:0.517
Education level			
Primary (10)	3.85 (0.23)	3.32 (1.08)	IN:0.002
Secondary (93)	3.17 (0.88)	2.56 (0.76)	SN:0.025
University (96)	3.43 (0.66)	2.52 (0.95)	
Work status			
Housewife (88)	3.53 (0.49)	2.51 (0.88)	IN:0.002
Outside work (111)	3.18 (0.91)	2.62 (0.89)	SN:0.391
Income status			
Low (40)	3.20 (0.78)	2.36 (0.83)	IN:0.094
Middle (123)	3.33 (0.82)	2.70 (0.86)	SN:0.141
High (36)	3.52 (0.53)	2.36 (0.98)	
Status of live birth			
Yes (127)	3.36 (0.76)	2.60 (0.92)	IN:0.502
No (72)	3.29 (0.79)	2.50 (0.83)	SN:0.419
Biopsy history			
Yes (13)	3.56 (0.28)	3.05 (0.44)	IN:0.948
No (186)	3.32 (0.79)	2.53 (0.90)	SN:0.078
Menopause			
Yes (29)	3.74 (0.36)	2.87 (1.12)	IN:0.000
No (170)	3.27 (0.80)	2.51 (0.84)	SN:0.065
Breastfeeding			
Yes (124)	3.35 (0.77)	2.64 (0.91)	IN:0.766
No (75)	3.31 (0.78)	2.45 (0.85)	SN:0.149

IN: Information needs, SN: Support needs

Table 2. Protective behavior development in first degree female relatives of patients with breast cancer and their information and support needs

	Importance n=199	**Needs met** n=196	P value
Family relationship			
Mother (125)	3.43 (0.82)	2.63 (0.91)	IN:0.000
Sister (53)	3.14 (0.67)	2.35 (0.86)	SN:0.168
Daughter (21)	3.25 (0.61)	2.67 (0.79)	
Age at diagnosis in relatives			
30-40 age (64)	3.17 (0.80)	2.62 (0.82)	IN:0.042
41 age and (135)	3.41 (0.74)	2.54 (0.92)	SN:0.517
Time of diagnosis (month)			
12 and (112)	3.24 (0.93)	2.38 (0.89)	IN:0.033
13 and (87)	3.46 (0.48)	2.79 (0.84)	SN:0.001
Education/information retrieval related on breast cancer and breast self-examination (BSE)			
Received (132)	3.45 (0.60)	2.65 (0.84)	IN:0.003
Not received (67)	3.10 (0.99)	2.39 (0.97)	SN:0.064
Information/instruction request on early diagnosis of breast cancer and BSE			
Willing	3.51 (0.54)	2.67 (0.83)	IN:0.000
Unwilling	3.03 (0.99)	2.40 (0.96)	SN:0.047
BSE			
Performed	3.47 (0.64)	2.75 (0.91)	IN:0.005
Did not perform	3.17 (0.87)	2.34 (0.82)	SN:0.001
Breast examination made by health personnel (doctors, nurses, midwives, etc.)			
Underwent (70)	3.56 (0.46)	2.87 (0.90)	IN:0.002
Did not undergo (129)	3.21 (0.87)	2.40 (0.84)	SN:0.000
Mammography			
Underwent (49)	3.50 (0.54)	2.74 (0.87)	IN:0.038
Did not undergo (150)	3.28 (0.83)	2.50 (0.89)	SN:0.095
Perceived cancer risk since has a primary relative with BC			
Yes (150)	3.48 (0.49)	2.55 (0.90)	
No (6)	3.27 (0.56)	3.37 (0.00)	IN:0.003
I do not know (43)	2.83 (1.09)	2.56 (0.87)	SN:0.326
Five-year risk			
No (76)	3.49 (0.48)	2.47 (0.77)	IN:0.060
Yes (31)	3.67 (0.42	2.71 (1.20)	SN:0.318
Lifetime risk			
No (97)	3.53 (0.48)	2.50 (0.94)	IN:0.755
Yes (10)	3.59 (0.32)	2.97 (0.48)	SN:0.164

IN: Information needs, SN: Support needs, BC: Breast cancer, FDFR: First degree female relatives

and in those who did not undergo breast examination by a healthcare professional (e.g., doctor, nurse, midwife) compared with those who did (p=0.000). These differences were found to be statistically significant (Table 2).

At the end of the multiple regression analysis in which importance was the dependent variable, the F value of 4,399, which tested the overall significance of the regression model, and the F statistic, which was calculated as p=0.000, were found to be significant. As the duration of BC diagnosis in their relatives increased, importance also increased among FDFRs; it was determined that importance decreased among FDFRs who were not in menopause and whose sisters and daughters were

diagnosed as having cancer. It could be stated that variables such as age, education and age of the relatives at diagnosis did not have a significant effect at the significance level of 0.05 (Table 3).

As a result of the multiple regression analysis using needs met as the dependent variable, the F value of 3,659, which tested the overall significance of the regression model, and the F statistic, which was calculated as p=0.002, were found to be significant. As the duration of BC diagnosis increased, needs met also increased. It could be stated that variables such as age, education, degree of kinship and the age of the relatives at diagnosis did not have a significant effect at the significance level of 0.05 (Table 3).

Discussion

The findings from this study determined the most important issues for the guidance of FDFRs with respect to their BC risk. The risk of the development of cancer was found to be increased 2-fold among the women whose first-degree relatives such as mother, sister or daughter had BC; moreover, the risk was increased 3-4 fold among women with two or more relatives diagnosed as having BC (14,19,20). The importance of the detection of the information and support needs of first-degree relatives of women with BC, and of meeting these needs during primary prevention has been previously reported (14,20-22).

Out of the 29 items of importance that were identified, the five most important subjects that were selected by the participants were: genetic counseling for themselves and their daughters, ways to help decrease the suffering of a relative with BC, treatments for BC, emotional reactions of women who were newly diagnosed as having BC, and side effects of BC treatments. Among the 29 items for needs met that were identified, the five most important items that were chosen by the participants were: treatments for BC, causes of BC, side effects of BC treatments, support for relatives with BC, and frequency of mammographic screening. In agreement with these results, it was understood that the majority of FDFRs in the study group knew that BC was inherited, and they wanted genetic counseling to learn their risk as well as their children's risk. They also wanted to be informed as to how to support their relatives during cancer treatment.

It was also determined that other topics for which information needs were not met were associated with the causes of BC, its treatment, side effects, ways to provide support to relatives and the frequency of mammography procedure. These results showed that FDFRs were aware but that they required a more accurate orientation. Previous studies have shown that FDFRs of patients with BC need information about the causes of breast cancer, diagnostic methods and behaviors that promote health. Another common theme is that their needs for information about the causes of BC, its treatment, side effects, ways to provide support to their relatives and the frequency of mammography have not been met (14,22-25). Nurses may help women understand their risks of BC more accurately by determining and meeting the information needs of FDFRs of patients with BC, providing relief to them through confidence and providing support for their care. FDFRs of patients with BC have a variety of needs. The results of one study indicated that nurses were not always capable of correctly assessing and meeting these needs (26). In Turkey, nurses generally spend their time on routine care in the wards, and no professional oncology nurses exist solely for this purpose. Oncology specialists or liaison-consultant nurses should be trained and employed in clinics as a guide just for these patients and their FDFRs.

In our study, the importance of information and support needs of FDFRs was evaluated based on sociodemographic characteristics, and it was found that the information and support needs were higher in those who were 40 years of age and older, were divorced or widowed, had a lower education level, were unemployed and were in the menopause period. In addition, the support needs were also higher in the middle-aged group (31-40 years of age) and in those who had a middle school education and above. In the literature, few studies have shown the relationship between the information and support needs of FDFRs of women with BC and their sociodemographic characteristics. In one study, Aslan and Ceber (15) reported no significant relationship between sociodemographic characteristics of FDFRs and their requirement for information and support. In our study, the informational support needs of women who were older than 40 years of age, had a low education level and were unemployed were found to be higher. This revealed that these women demonstrated a higher

Table 3. Result of multiple regression analysis when importance and needs met taken as dependent variables

Independent variable	Unstandardized coefficients		T		p	
	IN	SN	IN	SN	IN	SN
Age	.081	-.070	.930	-.725	.353	.470
Education	.062	-.217	.847	-1.949	.398	.053
Status of menopause	-.178	-.364	-2.153	-1.729	.033	.085
Family relationship	-.206	-.075	-2.681	-1.324	.008	.187
Age at diagnosis in relatives	-.121	-.124	-1.654	-.864	.100	.388
Relatives diagnosis time	.208	.504	2.721	3.616	.007	.000

SN: R^2=0.325 Adjusted R square :0.077 F=3.659 p=0.002
SN =16.193 + .208 (relatives diagnosis time).
IN: R^2=0.350 Adjusted R square :0.095 F=4.399 p=0.000
IN =14.46 +-.178 (menopause) + -.206 (relationship degree) +-.208 (diagnosis time)

awareness because they had a relative with BC. Moreover, these data suggest that middle-aged women and women who have a higher education level can obtain information more easily, which increases their requirement for support.

In our study, information needs were found to be higher among those who obtained education/information about BC and breast self-examinations, performed breast self-examinations, underwent breast examination by a healthcare professional, underwent mammography and stated that "having a relative with BC increases my personal risk". It was observed that awareness of FDFRs about early diagnostic methods of BC and its risk factors increased their requirement for information. The literature has primarily addressed the approaches to the early diagnosis of BC in women with a family history of BC. In previous studies, approaches to the early diagnosis of BC in FDFRs have shown differences. In the study by Cohen (27), it was determined that women with a family history of BC were more likely to perform regular breast self-examinations. In their study, Chalmers et al. (20) found no difference between women who did and did not have a family history of BC in terms of compliance to early diagnostic applications. In the study by Norman and Brain (28), it was reported that women with a family history of BC did not undergo regular clinical breast examinations. The reasons for these different diagnostic approaches were due to the inadequacy that the FDFRs felt regarding this subject matter, the lack of sufficient information about clinical breast examinations, their emotional obstacles and their concerns about experiencing BC (29,30). Concerns and fears of FDFRs of patients with BC may increase their tendency to seek more information (15,31,32). In the study by Dincel et al. (33) which evaluated the knowledge of FDFRs about their risk of BC, breast examinations and screening methods and which provided information to women who had certain risk factors, it was found that the needs of women regarding BC could be eliminated by providing information.

In our study, it was determined that the support needs of those whose relatives were diagnosed as having BC after one year, who did not undergo breast self-examinations and did not undergo breast examinations by a healthcare professional, were not met. In parallel to our results regarding information needs, the lack of early diagnostic applications among FDFRs has revealed the requirement for support. During early diagnostic applications, information and support should be provided together. No planned health program has been established for these support needs in our country, and individual approaches of healthcare professionals and support approaches that are accomplished privately are not sufficient.

Study Limitations

In our study, 5-year and lifelong cancer development risks were calculated and no significant difference was found between information and support needs of the ones who did and did not have risk. Rees and Bath (32) postulated that the acceptance of BC risk occurs in three phases. They observed that FDFRs of patients with BC share the experiences of the patient during the first phase. They accept that they are also at risk during the second phase, and during the third phase, they are completely aware of their risk (32). Researchers who have emphasized that information, support and communication are helpful during this process, have also indicated that information is effective in the correct perception of individual risk, the provision of individual control and minimizing fear (32,34). The lack of any information regarding risk calculations may not affect the level of information and support needs of FDFRs.

In the regression analysis, the increase in the duration of BC diagnosis in their relatives increased the need of FDFRs for information and support, whereas menopause and a BC diagnosis in mothers increased the information needs of FDFRs. In previous studies, no information was found regarding the year of diagnosis of the relative with BC and the needs of FDFRs with respect to information and support. It has been emphasized that women can negatively affect their family members by reflecting their fear and anxiety due to BC; thus, BC should be evaluated as a disease of the family (26). In our study, the duration of BC diagnosis might cause FDFRs to feel anxious about an advanced stage of cancer that cannot be treated.

Conclusion

An issue that should be considered is that relatives do not have the same individual needs as women with BC. Therefore, it is important to learn their special needs. However, it is difficult to reach these relatives. On the other hand, because of the priorities, nurses generally have to take their time to patient care on wards instead of support and talk with patients. For this purpose, oncology programs should be organized to reach primary relatives at risk through women with BC. It is recommended to increase training in primary relatives as much as possible through strategies such as increasing awareness by informing periodically, and planning to diminish unwanted stress-causing situations. The results of our study will guide health professionals working in both clinics and field. A liaison-consultant nurses or oncology nurses should be recruited. It should be make it possible for nurses to understand these needs is so important. We found out that FDFRs of patients with BC needed factual information and psychosocial support. This study supports the establishment of psycho-oncology units in oncology centers.

Ethics

Ethics Committee Approval: Pamukkale University Non-invasive Clinical Research Ethics Committee (date: 06/02/2017/ decision no: 60116787-020/8900).

Informed Consent: A total of 199 FDFRs agreed to participate and signed the informed consent before completion of the surveys.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: S.Ö., F.Ö., İ.Ö.Ç., S.G.T., Concept: S.Ö., F.Ö., İ.Ö.Ç., S.G.T., Design: S.Ö., F.Ö., İ.Ö.Ç., S.G.T., Data Collection or Processing: S.Ö., F.Ö., İ.Ö.Ç.,

S.G.T., Analysis or Interpretation: S.Ö., F.Ö., İ.Ö.Ç., S.G.T., Literature Search: S.Ö., F.Ö., İ.Ö.Ç., S.G.T., Writing: S.Ö., F.Ö., İ.Ö.Ç., S.G.T.

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Original Article

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The Evaluation of Multidrug Resistance-Related Protein 1 as a Prognostic Factor in the Pediatric B-cell Acute Lymphoblastic Leukemia: A Pilot Study

Pediyatrik B-hücreli Akut Lenfoblastik Lösemi Hastalığında Prognostik Faktör Olarak Multidrug Resistance-Related Protein 1 Değerlendirilmesi: Pilot Çalışma

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ABSTRACT

Objective: Acute lymphoblastic leukemia (ALL) is the most prevalent type of cancer in children. Minimal residual disease (MRD) is still the most important indicator of clinical results and relapse after chemotherapy. Multidrug resistance is the main obstacle to successful treatment. Multidrug resistance-related protein 1 (MRP1) may play a key role in throwing the chemical drug out of cells leading to therapy resistance. This study aims to detect MRP1 protein in the bone marrow cells of children with B-ALL and determine its value as a prognostic factor in comparison with other factors such as DNA index and MRD obtained by flow cytometric measurement.

Methods: Bone marrow samples were obtained from children who are diagnosed as B-ALL (n=20) at day 0 (diagnosis) and 15 of therapy. Risk groups' classification is based on discrimination of age and white cell count on day 0. The expressions of MRP1 levels and DNA index at diagnosis and MRD on the 15th day of treatment in the bone marrow were detected by using flow cytometry. The B-ALL blast cells were stained using anti-CD10, -CD19, -CD20, -CD34, -CD45 monoclonal antibodies. MRP1 content of cells was detected in an intracellular manner.

ÖZ

Amaç: Akut lenfoblastik lösemi (ALL), çocuklarda en sık görülen kanser türüdür. Kemoterapinin ardından, minimal kalıntı hastalık (MRD) hala klinik sonuçların ve nüksün en önemli göstergesidir. Çoklu ilaç direnci başarılı tedavinin önündeki ana engeldir. Çoklu ilaç direnci ile ilişkili protein 1 (MRP1) kimyasal ilacın hücrelerden dışarı atılmasında anahtar rol oynayabilir ve tedaviye dirence neden olabilir. Bu çalışmanın amacı, B-hücre ALL'li çocukların kemik iliğinden alınan hücrelerde MRP1 proteinini saptamak ve akan hücre ölçer ile elde edilmiş DNA indeksi ve MRD gibi diğer faktörlerle karşılaştırılarak MRP1'in prognostik bir faktör olarak değerini belirlemektir.

Yöntemler: B-ALL (n=20) tanılı çocuklardan tedavinin 0. (tanı anı) ve 15. gününde kemik iliği örnekleri alındı. Risk grupları tanı anındaki yaş ve beyaz hücre sayısına göre ayrılmıştır. Kemik iliğinde tanı anındaki MRP1 düzeyleri ve DNA indeksi ve tedavinin 15. gününde MRD düzeyi akan hücre ölçer kullanılarak tespit edildi. B-ALL blast hücreleri anti-CD10, -CD19, -CD20, -CD34, -CD45 monoklonal antikorlar kullanılarak boyanmıştır. Hücrelerinin MRP1 içeriği hücre içi boyama yöntemi ile belirlenmiştir.

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Results: There was no statistically significant difference in MRP1 expression between risk groups and the other prognostic factor as Flow MRD and DNA index.

Conclusion: The utilization of MRP1 as a predictive factor may not provide information on the B-ALL prognosis. Our results can help to better understand the nature of MRP1 in B-ALL patients.

Keywords: B-cell acute lymphoblastic leukemia, multidrug resistance-associated protein 1, multidrug resistance-related protein 1, minimal residual disease, DNA index, flow cytometry

Bulgular: Risk grupları ile diğer prognostik faktörler flow MRD ve DNA indeks arasında MRP1 ekspresyonu açısından istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: MRP-1'in protein seviyeleri B-ALL prognozu hakkında bilgi sağlamayabilir. Sonuçlarımız, B-ALL hastalarında MRP-1'in doğasını daha iyi anlamaya yardımcı olabilir.

Anahtar Sözcükler: B-hücreli akut lenfoblastik lösemi, çoklu ilaç direnci ile ilişkili protein 1, multidrug resistance-related protein 1, minimal kalıntı hastalık, DNA indeksi, akan hücre ölçer

Introduction

Leukemia is made up of two Greek words of "*leukos*" and "*emia*" and refers to the term "white blood" and describes its characteristics as a cancer of the blood that appears with high numbers of malignant white blood cells (leukocytes) and it is the most common pediatric cancer and the cause of 29% of all cancer-related deaths in children between 1 and 14 years (1). The acute form of childhood leukemia is the most frequent (2). Acute lymphoblastic leukemia (ALL) is present in over 80% of children with ALL and B-cell ALL comes from an uncontrolled expansion of anomalous immature B-lymphocytes (3). ALL is one malignant disease most prevalent in childhood with the greatest therapeutic progress in the last three decades. This progress was achieved by recognizing certain prognostic risk factors which enabled the treatment to be adjusted accordingly. Many major risk factors are used in diagnosis and treatment stratification. These include age and white cell count (WCC), DNA index, as well as treatment response (4). According to National Cancer Institute (NCI) stratification, a worse prognosis is associated in patients with age ≥10 years and/or initial WCC of ≥50,000 per mm^3 (5). In addition, the DNA index is an independent prognostic factor and gives valuable information for diagnostic and treatment stratification. Studies have demonstrated that hyperdiploidy (DNA index >1.16) is associated with a better or favorable prognosis in ALL (6,7). As well as, minimal residual disease (MRD), assessed by molecular or flow cytometric manner, is considered the most important indicator of clinical results and relapse in childhood. Researchers have demonstrated that a high level of MRD is associated with an unfavorable prognosis regardless of the other risk markers commonly used for risk stratification (8).

Chemotherapy is the main way of treating leukemia. Drug resistance in patients diagnosed as having leukemia is often considered the principal clinical obstacle to efficient chemotherapy. Nowadays, several resistance mechanisms are identified, and the most important and common mechanism for chemotherapy failure is multidrug resistance (MDR) (9). Many MDR drug efflux pumps belong to the superfamily of ATP-binding cassette (ABC) transporters. Multidrug resistance-related protein 1 (MRP1) also belongs to the ABC transporter family. Findings indicate that MRP1 plays a key role in eliminating and sequestrating chemotherapeutic drugs, which results in reduced levels in concentrations at their target locations (10). However, the precise clinical value of these MDR proteins in ALL childhood is not clear and the data available are contradictory.

The purpose of this paper is to assess the importance of MRP1 as a predictor for responsiveness to therapy of childhood B-ALL. It is questionable whether the detection of MRP1 at diagnosis is an independent prognostic factor for B-ALL or not. Depending on certain actual independent predictive factors like DNA index at diagnosis and the MRD risk evaluation on the 15th day of treatment, the correlation of MRP1 expression with these factors was investigated.

Methods

Patients

Bone marrow samples obtained from patients with newly diagnosed and untreated ALL (n=20; 10 females, 10 males; age mean: 7.83; age range 3-15 years) from 2017 through 2018 were enrolled in the study after their parents signed the informed consent forms. The study was approved by the local ethics committee (17.10.2017/1143).

The bone marrow samples were collected in a tube with EDTA obtained from patients. The samples were used at the time of diagnosis, for immunophenotyping of leukemia, MRP1 expression of different cell subsets, and DNA index of B-ALL patients. On day 15th day of treatment, MRD levels were analyzed. All parameters were examined by the flow cytometric method.

Blast Characterization

The B-ALL panels were selected concerning over-expressed markers and for the B-ALL phenotype. Blasts were labeled by anti-CD10 phycoerythrin (PE) or PE-Cyanine 7 (Cy7), -CD19 allophycocyanin, -CD20 fluorescein isothiocyanate (FITC), -CD34 PE and -CD45 peridinin chlorophyll protein complex labeled monoclonal antibodies with "stain, lyse and then wash" approach (all monoclonal antibodies from BD Biosciences, USA). Samples were directly stained with a four-color monoclonal antibody cocktail at room temperature for 15 minutes in the dark. After incubation, 2 mL of FACS Lysing Solution (Becton Dickinson; BD Bioscience, San Jose, USA) was added and incubated for 10 minutes at room temperature in the dark. Following the lysis step, cells were washed with 2 mL phosphate-buffered saline (PBS), resuspended in 500 mL PBS and subsequently, data were acquired by flow cytometry

(FACSCalibur, Becton Dickenson, San Jose, USA) with CellQuest software (Becton Dickenson, San Jose, USA).

MRP1 Staining

Intracellular staining protocol was used for the detection of MRP1 expression of bone marrow cells. After labeling surface markers with anti-CD10, -CD19, -CD45 monoclonal antibodies, started intracellular staining through two steps: fixation and permeabilization (FIX & PERM, Nordic Mubio, Netherlands). In both stages, the cell suspension was incubated for 15 minutes in the dark followed by washing steps with PBS. The last incubation step was performed with permeabilization reagent and anti-MRP1 FITC antibody (BD Bioscience, San Jose, USA) together. The detection of MRP1 florescence bone marrow-derived cells: lymphoid, monocytes, myeloid cells, and blasts ($CD10^{+}CD19^{+}CD45^{neg/dim}$) was done on flow cytometry regarding fluorescence minus one control.

DNA Index

Estimation of the cellular DNA content was performed by flow cytometry. Mononuclear cells from bone marrow mononuclear cells were isolated by density gradient centrifugation using Ficoll-Paque (Histopaque-1077; Biochrom, Cambridge, UK) at 2,100 x rpm for 30 min. The buffy coat was washed twice with PBS, then stained by propidium iodide after digestion with enzymes trypsin and ribonuclease A using the BD Cycletest Plus DNA Reagent Kit (BD Cycletest, San Jose, USA) according to Vindelov (11). The DNA content was analyzed with a computer program Modfit LT by histograms. DNA index is defined as the ratio of the mean channel of G0/G1 cells of an aneuploid population divided by the normal diploid of G0/G1 mean channel (12).

Minimal Residual Disease

The bone marrow samples obtained from the patients on the 15th day of treatment were identified with specific monoclonal antibodies (CD10, CD11a, CD19, CD20, CD34, CD45, and CD58) according to the phenotypic characteristics and ALL IC-BFM 2009 Standard Operating Procedure. The residual blast cells were determined in $CD19^{+}$ gate by displaying lymphoid-scattering properties and leukemia-associated immunophenotypic characteristics as a percentage by flow cytometry (13).

Statistical analysis

In conjunction with the IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp. USA) program, the data collection and management were performed using the Microsoft Office Excel package. Non-parametric methods such as Mann-Whitney U test were used and $p<0.05$ was considered statistically significant. The data that were continuous variables were presented as median (minimum-maximum).

Results

Clinical Features and Risk Group Stratification

The clinical and laboratory data of 20 patients with B-ALL are shown in Table 1. Patients were grouped according to standard-risk [(SR), n=8] and high-risk [(HR), n=12] ALL. This classification was based on discrimination of age and WCC. SR-ALL group characteristics were WCC less than 50,000/μL and age 1-9 years, and HR-ALL group characteristics were WCC ≥50,000/μL and/or age 10 years or older (5).

DNA index was measured as 1.15 (1-1.83) in 20 patients and it was ranging from 1 to 1.15 in 12 (60%) patients as hypodiploidy and ranging from 1.17 to 1.83 in 8 (40%) patients as hyperdiploidy (7). All HR patients had DNA index <1.16 and all SR patients were hyperdiploid (DNA index >1.16). According to this correlation, study groups were identified as SR and HR (Table 2).

MRP1 Expression of Bone Marrows Cells of SR and HR Risk Group B-ALL Patients

The expression MRP1 was investigated in 20 patients with B-ALL and determined by flow cytometry by direct immunofluorescence intracellular staining. MRP1 positive cells were determined by reference to isotype control (BD Bioscience, San Jose, USA). Lymphocytes, monocytes, and myeloid cells were gated on SSC/CD45 dot plot (Figure 1). Blast cells were gated on CD10/CD45 dot plot obtained from CD19 positive cells (Figure 2). There was no statistical difference between SR and HR risk groups in terms of bone marrow cells ($p>0.05$) (Figure 3).

Day 15 Minimal Residual Disease and MRP1 Expression

On the 15th day of therapy, the bone marrow samples obtained from patients with B-ALL were examined for MRD by flow cytometry. The percentages of leukemic cells were classified

Table 1. Clinical characteristics of patients with B-ALL

Demographic data [median (minimum-maximum)]		
Number of patients	20	
Age (year)	4.5 (3-15)	
Sex (male/female)	10/10	
Clinical features		**Normal ranges**
WCC ($x10^{9}$/L)	68.4 (12.4-186.4)	5.0-17.0
RCC ($x10^{12}$/L)	3.2 (0.1-3.9)	3.9-5.3
Hemoglobin (g/dL)	9.7 (0.3-13.0)	115-135
Platelet count ($x10^{3}$/uL)	339.0 (42.0-876.0)	150-400
Blast cells (%)	82.2 (63.9-95.3)	0.0-2.0

WCC: White cell count, RCC: Red cell count

Table 2. Distribution of patients with B-ALLaccording to DNA index and risk (based on age and WCC): High and standard risk groups were associated with hypodiploidy and hyperdiploidy groups, respectively

DNA index	Risk stratification		
	HR	SR	N
Hypodiploidy (<1.16)	12	0	12 (60%)
Hyperdiploidy (>1.16)	0	8	8 (40%)
Total	12 (7F/5M)	8 (3F/5M)	20 (10F/10M)

F: Female, M: Male

as flow low risk (FLR) with blast cells <0.1%, flow medium risk (FMR) with blast cells between 0.1-10%, and flow high risk (FHR) with blast cells >10% (13). One sample with a normoblast rate of less than 2% (1.6%) was excluded because of hemodilution. There were two patients with FHR, 16 patients with FMR, and one with a negative result that was considered as FLR. One patient who had negative MRD and FLR was HR at diagnosis and two patients who had weak early response to therapy had FHR and MRD >10% (15.9297 and 66.6032% MRD) and were SR at diagnosis. The majority of our patients (85%) had a medium response (FMR) (Table 3). Patients with FHR and FLR were excluded because of their low numbers. MRP1 expression of bone marrow cells of FMR-SR (n=5) and FMR-HR (n=11) groups were compared and there was no difference (p>0.05, Figure 3). Furthermore, patients with B-ALL with FMR were ranked depending on the CD34 phenotype of blasts as FMR-34 negative (n=3) and positive (n=13) (Table 3).

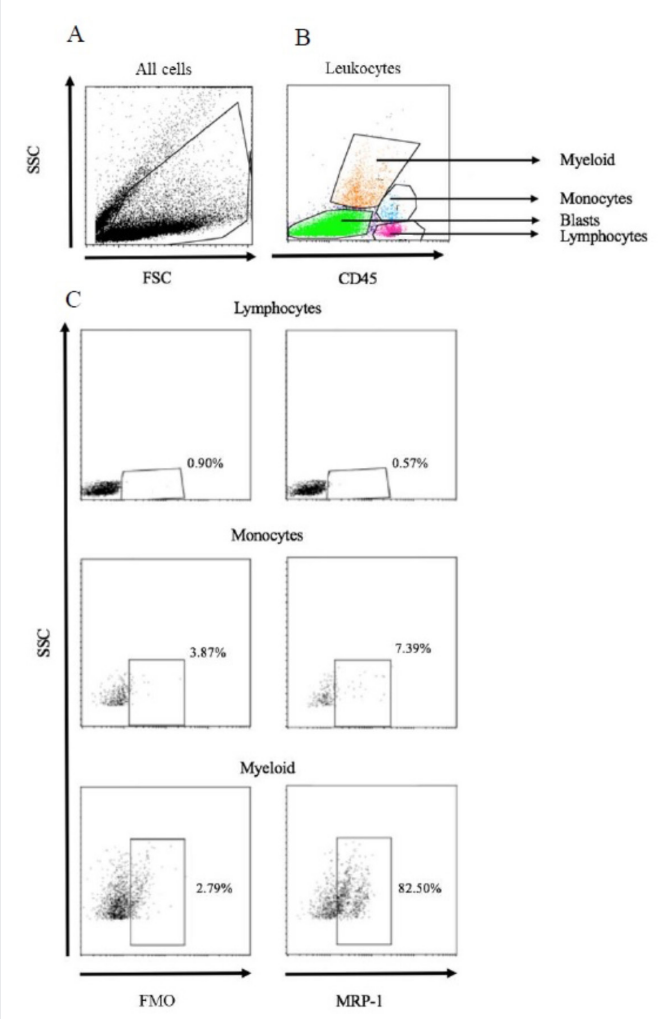

Figure 1. Gating strategy for detection of MRP1 in different bone marrow-derived cells: lymphocytes, monocytes, and myeloid cells. Leukocytes were gated on SSC versus FCS graph (A), the subdivision of bone marrow cells discriminated by their CD45 expression intensity (B), MRP1 expression of each subpopulation was determined regarding FMO (C). FMO: Fluorescence minus one control

Two subgroups were compared in terms of MRP1 expressions and there was no difference between them (p>0.05, Figure 3).

Discussion

MDR which is due to the overexpression of many efflux proteins such as MRP1 can traffic chemotherapeutic drugs from the cell. Although the role is not fully understood, the importance and the influence of MRPs on the strategy and outcome of clinical cancer treatment cannot be neglected (14). It has been suggested that nuclear MRP1 can be a better prognostic factor in human mucoepidermoid carcinoma (15). But, in breast tumor tissues, low level of MRP1 is considered as a predictive biomarker for the patients responding to chemotherapy (16). An investigation proposed an alternative pathway to understanding the failure of therapy after assessment of MRP1 content by quantitative polymerase chain reaction (PCR) in lymph node biopsy

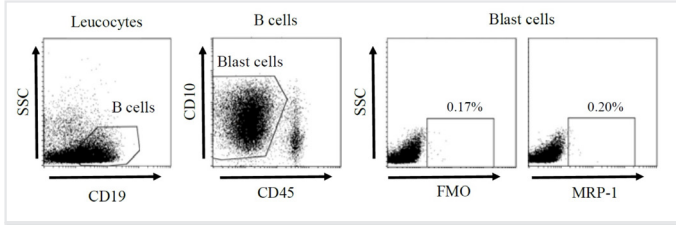

Figure 2. Gating strategy of MRP1 expression in blast cells. CD19 positive B cells were extracted from leukocytes and gated on SSC versus CD19 dot plot (A). B cells with CD10+CD45neg/dim phenotype were gated on CD10 versus CD45 scatter graph as Blast cells (B). And finally, blast cells' MRP1 expression was determined by SSC versus MRP1 regarding FMO. FMO: fluorescence minus one

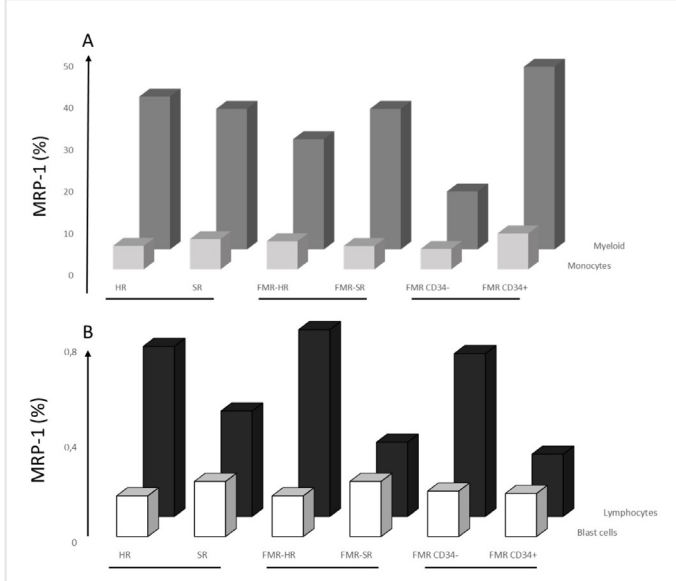

Figure 3. MRP1 expression (median %) of bone marrow cells of B-ALL patient HR (n=12) and SR (n=8), FMR-HR (n=11) and FMR-SR (n=5), FMR with (n=13) or without (n=3) CD34 blast phenotype expression. (A) Monocytes, myeloid cells, (B) blast cells, and lymphocytes

specimens from a group of patients with lymphoma (non-Hodgkin's lymphoma, non-malignant lymphadenopathy, and Hodgkin's lymphoma) (17). Many studies investigated expression levels of MRP1 mRNA and showed poor prognosis related to childhood ALL and reported significantly higher expression at relapse in association with age >15 years or age-independent or with poor 2-year survival (10,18-22). As concerning resistance to chemotherapy, MRP1 was suggested as one of the mechanisms responsible for induction failure, also in adult patients with ALL (23).

Many studies have not confirmed the correlation between overexpression of MRP1 and clinical drug resistance in hematologic malignancies. The expression of MRP1 mRNA shown by PCR appeared to have no prognostic importance and no association with risk factors such as phenotype, age, and leukocyte count in newly diagnosed patients with childhood ALL and it was not related to a worsening of event-free survival (24,25). It was also shown that the expression of Pgp, MRP1, and LRP was not related to WCC and age, and did not contribute to treatment failure (26-28). Also, MRP1 measurement at the protein level by FCM analysis appeared to have no concordance with the clinical aspect (29). In an *in vitro* study, cytotoxicity of 4-hydroxy-ifosfamide, daunorubicin, and prednisolone on peripheral blood lymphocytes was assessed by the MTT assay and a weak correlation was found with high MRP protein expression which suggested that MRP was unlikely to be involved in drug resistance (30).

MRD is a demonstration of drug resistance and is correlated significantly with the NCI risk group. HR patients are significantly more likely to be MRD-positive than SR patients (31). The DNA index measurement is important for treatment stratification. In the earlier studies it was shown that SR patients were associated with a DNA index greater than 1.16 (hyperdiploidy) and that it was a favorable factor such as age between one and ten years and WCC lower than $50x10^9$/L (7,32). We examined the baseline WCC, age and DNA index, and found that all HR patients were simultaneously hypodiploid and SR patients were hyperdiploid matching

Table 3. General aspects of patients with B-ALL in terms of risk stratification (regarding age/WCC, DNA index, and flow MRD), phenotype (CD10, CD20, CD34, and CD45 expressions), and MRP1 expression in bone marrow cells

Patient No	Age (year)	Sex	Bone marrow WCC ($x10^9$/L)	Risk	DNA index	CD10 (%)	CD19 (%)	CD20 (%)	CD34 (%)	CD45 (%)	MRP-1 (%) in gate: Blast	Lymphoid	Monocytoid	Myeloid	Norm (%)	MRD (%)	MRD risk
1	3	F	69.43	HR	1.07	98.48	98.57	5.37	73.60	49.42	0.17	0.26	10.97	46.65	3.86	0.9326	FMR
2	3	F	121.60	HR	1.08	97.53	97.54	94.78	97.70	6.64	0.45	1.67	1.97	17.95	2.52	0.1071	FMR
3	6	F	186.36	HR	1.09	98.45	98.49	1.39	0.49	97.74	0.12	1.59	3.01	6.31	18.44	0.8678	FMR
4	5	M	24.61	SR	1.19	75.89	85.24	2.83	0.44	28.19	0.20	0.25	1.19	2.79	3.10	1.5732	FMR
5	7	F	32.99	SR	1.83	5.83	95.05	0.93	2.81	100	0.15	0.50	15.38	1.51	8.20	15.9297	FHR
6	9	F	81.34	HR	1.12	42.52	96.88	30.83	42.14	99.51	0.19	1.82	6.73	6.28	2.64	4.3599	FMR
7	3	M	74.25	HR	1.15	97.70	98.11	3.85	84.82	65.05	0.17	0.22	4.29	65.31	58.00	0.0000	FLR
8	12	F	110.59	HR	1.15	81.84	99.50	6.00	94.44	99.66	0.18	0.91	8.57	63.46	61.10	6.8486	FMR
9	2	M	67.35	HR	1.15	98.64	98.66	26.58	98.53	3.75	0.14	0.64	2.69	8.81	9.16	0.1391	FMR
10	5	F	30.31	SR	1.23	93.26	93.59	38.80	62.26	26.16	0.25	0.67	8.88	58.51	4.80	66.6032	FHR
11	4	M	12.47	SR	1.17	70.89	98.46	10.51	74.10	70.58	0.24	0.24	9.80	43.56	14.84	0.7524	FMR
12	10	M	115.90	HR	1.11	98.96	99.21	3.41	3.51	80.02	0.12	0.60	16.15	89.33	19.74	1.4366	FMR
13	15	F	29.25	HR	1.14	85.88	98.10	4.28	98.86	68.8	0.23	1.45	14.46	55.69	19.50	9.2981	FMR
14	13	M	85.61	HR	1.13	7.11	97.95	2.01	98.05	97.8	0.09	0.10	1.11	1.77	18.40	7.547	FMR
15	3	M	30.45	SR	1.35	98.22	98.24	35.59	29.60	26.7	0.28	0.73	7.03	89.76	31.90	0.7942	FMR
16	4	F	26.45	SR	1.25	89.87	89.98	74.40	86.26	41.83	0.26	0.57	7.39	82.50	32.01	1.6212	FMR
17	6	M	28.05	SR	1.42	98.24	98.35	1.99	95.42	6.14	0.15	0.22	3.06	11.77	1.65	6.7534	FMR
18	3	F	132.88	HR	1.00	94.60	94.73	1.84	60.86	2.32	0.17	0.26	4.50	26.30	4.44	0.7064	FMR
19	3	M	40.36	SR	1.28	93.23	93.59	3.69	68.07	86.32	0.22	0.38	4.13	23.52	4.68	0.3396	FMR
20	3	M	99.46	HR	1.08	72.69	99.49	3.02	92.60	61.82	0.46	0.78	62.56	66.03	19.00	9.2005	FMR

	Age	Sex	Bone marrow WCC ($x10^9$/L)	Risk	DNA index	CD10, CD19, CD20, CD34, CD45 (%)	MRP-1	Normoblast	MRD Risk
	>10 years	Male	>50	HR	Hypodiploidy	100-80	>2 SD	Adequate	FHR
						80-60	>1 SD		
						60-40	Mean		FMR
						40-20	<1 SD		
	<10 years	Female	<50	SR	Hyperdiploidy	20-0	<2 SD	Hemodilution	FLR

F: Female, M: Male, HR: High risk, SR: Standard risk, FMR: Flow medium risk, FLR: Flow low risk, FHR: Flow high risk, SD: Standard deviation, WCC: White cell count

Lustosa de Sousaa et al's study (32). And we observed no relation with risk stratification criteria regarding WCC/age/DNA index and, MRP1 of which expression was examined by flow cytometry at the time of diagnosis of newly diagnosed patients. We evaluated the cell types of the bone marrow according to CD45 fluorescence characteristics as lymphoid, monocytes, myeloid, and $CD10^+CD19^+$ blast cells, and their MRP1 levels. On the 15th day of therapy which was administered according to initial risk, the response was determined. MRD in bone marrow was examined by flow cytometry and 85% of our patients had FMR. The number of patients with FLR (n=1) and FHR (n=2) were too small to compare, so only patients with FMR response were grouped according to WCC/age/DNA index risk at the time of diagnosis and MRP1 protein levels were compared. Thus, in our study, no difference was found in terms of baseline MRP1 expression of HR and SR patients who had FMR response on day 15th. Patients with relapse were not included in this study.

Drach et al. (33) and Chauhan et al. (23) showed the association of the MDR-1 gene expression with immature immunophenotype as $CD34^+$ cell lineage. On the other hand, there was an observation of the similarity of MRP gene expression levels in AML and normal $CD34^-$ and $CD34^+$ bone marrow cells (18). We found high MRP1 protein expression in myeloid cells of bone marrow samples from patients with B-ALL , but there was no significant difference between risk groups. Also, our findings did not determine the relation of MRP1 expression in blast cells with phenotype either $CD34^-$ or $CD34^+$.

From point of view of gender, our finding conflicted with an earlier study that considered male gender as an independent prognostic factor and associated it with poor prognosis (34). The number of male children in this study was equal to the number of female children. In the examination of the distribution of gender to WCC/age/DNA index, male subjects showed equal distribution and the majority (70%) of female subjects were in the HR group. We could not prove strongly that the male children had a poor prognosis because of their low number.

Study Limitations

Some established poor genetic factors such as IKZF1 deletion, KMT2A rearrangements, and the Philadelphia chromosome are considered molecular indicators of high risk for failure of treatment or resistance to treatment. Because of scarce data about the presence of this factor, we couldn't evaluate the relationship with MRP-1.

Conclusion

In this study, the expression of MRP1 protein at diagnosis whether to help or not as a predictive factor for prognosis of patients with B-ALL was investigated. Although the low number of patients made it difficult to compare subgroups, our study focused on the level of MRP1 protein expression in different bone marrow cell subsets by comparing risk factors: WCC, age, DNA index, and CD34 positive/negative phenotype groups regarding their therapy response. We think that the cause of conflicting results in the literature is the disease phenotype ($CD34^-$ and $CD34^+$) or cell subgroups (lymphocytes, monocytes, myeloid and blast cells) cannot be evaluated separately. Measuring MRP1 by flow cytometry is advantageous than western blot and PCR because it is easily analyzed in individual different cell groups including blast cells, although intracellular staining has disadvantages as fixation and permeabilization procedures that can damage cell integrity. In conclusion, our findings do not support MRP1 protein expression as a prognostic factor and as a predictor for drug resistance.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (17.10.2017/1143).

Informed Consent: The study after their parents signed the informed consent forms.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: A.A., S.Ç., Design: A.A., S.Ç., Data Collection or Processing: A.A., M.Y.G., I.T., Analysis or Interpretation: A.A., M.Y.G., I.T., G.A., G.D., S.Ç., Literature Search: A.A., M.Y.G., I.T., G.A., G.D., S.Ç., Writing: A.A., G.D., S.Ç.

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

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Original Article

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Relationship Between Toilet Type and Hemorrhoids

Tuvalet Tipi ve Hemoroid Arasındaki İlişki

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ABSTRACT

Objective: The aim of the study is to investigate the relationship between the development of hemorrhoid disease and toilet habits.

Methods: This cross-sectional study was conducted in the digestive endoscopy unit of the University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital between January 2022 and March 2022. Presence of constipation was evaluated according to Rome IV criteria and the patients were asked to fill out the short questionnaire after the procedure.

Results: There was a total of 142 patients in the study. The mean age was 53.6±11.8 years. The female to male ratio was 1.33. The seated toilet was reported by more than half of the patients (58.5%) as the preferred toilet type. Constipation was detected in 70 patients (49.3%). Hemorrhoidal disease was detected in 60 (42.3%) of the patients. We did not find a significant difference between hemorrhoid disease and the type of toilet at home, preferred toilet type and constipation (p>0.05). There were significantly more male patients with hemorrhoids (56.7% vs. 32.9%, p=0.008).

Conclusion: We concluded that there was no relationship between the preferred toilet type and hemorrhoid disease in the current study.

Keywords: Hemorrhoid, constipation, toilet type

ÖZ

Amaç: Çalışmanın amacı hemoroid hastalığının gelişimi ile tuvalet alışkanlıkları arasındaki ilişkiyi araştırmaktır.

Yöntemler: Bu kesitsel çalışma Sağlık Bilimleri Üniversitesi, Gaziosmanpaşa Eğitim ve Araştırma Hastanesi Sindirim Endoskopi Ünitesi'nde Ocak 2022 ile Mart 2022 tarihleri arasında yapıldı. Konstipasyon varlığı Roma IV kriterlerine göre değerlendirildi ve işlem sonrasında hastalardan kısa anketi doldurmaları istendi.

Bulgular: Çalışmaya toplam 142 hasta alındı. Ortalama yaş 53,6±11,8 yıldı. Kadın/erkek oranı 1,33 idi. Oturaklı tuvalet hastaların yarısından fazlası tarafından (%58,5) tercih edilen tuvalet tipi olarak bildirilmişti. Yetmiş hastada (%49,3) kabızlık saptandı. Hastaların 60'ında (%42,3) hemoroidal hastalık saptandı. Hemoroidal hastalık ile evdeki tuvalet tipi, tercih edilen tuvalet tipi ve kabızlık arasında anlamlı bir fark saptanmadı (p>0,05). Hemoroidli erkek hasta sayısı anlamlı olarak daha fazlaydı (%56,7'ye karşı %32,9, p=0,008).

Sonuç: Bu çalışmada tercih edilen tuvalet tipi ile hemoroid hastalığı arasında bir ilişki olmadığı sonucuna vardık.

Anahtar Sözcükler: Hemoroid, kabızlık, tuvalet tipi

Introduction

Hemorrhoid disease is the third most common gastrointestinal system disease diagnosed in outpatient visits to the doctor in the USA (1). Hemorrhoids are diagnosed in one-third of patients undergoing colonoscopy (2). It is predicted that the demand for hemorrhoid treatment will increase even more in the coming years (3).

Hemorrhoids are structures consisting of smooth muscle, connective tissue and vascular tissue that extend around the anal canal (4). Hemorrhoids are actually cushions that provide continence in healthy people (5). However, the concept of hemorrhoids is generally used for swollen blood bags and symptomatic hemorrhoid disease detected on examination, rather than a normal anatomical structure (4). The etiology of

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hemorrhoid disease is unclear (6). The most common view is that hemorrhoidal disease is caused by the hydrostatic pressure of the blood under the influence of gravity. The second opinion is that hemorrhoid disease occurs as a result of the reflection of arterial blood pressure in the anorectal region through the connections to the veins. Thirdly, it is thought that hemorrhoid disease occurs as a result of weakening of the anal mucosa due to recurrent irritation and inflammation (7).

Hemorrhoid disease is thought to be more common in developed countries, and it is assumed that this may be related to the low fiber diet and the use of seated toilets (8-10). It has been shown that the rectoanal angle is straightening in the squat posture (9), evacuation is easier and takes a shorter time (10). Turkey is a developing country between the West and the East. Both seated and squat toilets are used. According to our hypothesis, we think that hemorrhoids may be significantly less in those who have the habit of using squat toilets.

Methods

The study which was organized as a prospective cross-sectional study was approved by the local ethics committee.

Patients Selection

Consecutive outpatients who were admitted to University of Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital Endoscopy Unit for colonoscopy between January 2022 and March 2022 were included in the study. Hemorrhoids were diagnosed with colonoscopy by specialists in general surgery. Presence of constipation was evaluated according to Rome IV criteria (11). Toilet types at home were learned from all patients.

A questionnaire was applied to 161 patients whose ages ranged between 21 and 80 years. We excluded 19 patients with missing data or with the presence of anorectal tumor, ileo-anal/rectal anastomosis, anal stenosis, anal fistula, anal fissure, pelvic floor disorders, any previous history of anorectal surgery, pelvic irradiation, neurologic diseases, diabetes, chronic use of opioid medications, Chron's disease, ulcerative colon or rectum tumors, because we thought that they would directly affect toilet habits. As a result, 142 patients were included in the study.

The relationship between the presence of hemorrhoids and age, gender, type of toilet at home, preferred toilet type and constipation was investigated.

Study Design

According to the pilot study results, which included a randomly selected 10 patients from the database, 15.0% of this population had a hemorrhoidal disease. To estimate a 5% difference in effect size, with an error rate of 5% and 80% power, at least 53 patients were needed. Considering the possibility for incomplete or incorrect questionnaires by adding 10% loss to this figure resulted in a total of 58 patients needed.

Quantitative Variables

Etiologically, age, gender, fiber-poor diet, constipation, diarrhea and posture during defecation are thought to be associated with hemorrhoid disease (6). Patients with hemorrhoid disease were included in the research group, and patients without hemorrhoid disease were included in the control group.

Statistical Methods

Descriptive statistics were given as mean ± standard deviation and median with minimum-maximum values for continuous variables depending on their distribution. Numbers and percentages were used for categorical variables. The normal distribution of the numerical variables was analyzed by the Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests. The Independent Samples t-test was used to compare two independent groups where numerical variables had a normal distribution. Pearson chi-square and Fisher's Exact tests were used to compare the differences between categorical variables in 2x2 tables. For statistical analysis, Jamovi project (2021), Jamovi (Version 2.2.2.0) and JASP (Version 0.16) were used. In all statistical analyses, the significance level (p-value) was set at 0.05.

Results

There were a total of 142 patients in the study. The mean age was 53.6±11.8 years. The female to male ratio was 1.33. The demographic and clinical characteristics of the study group are given in Table 1. The seated toilet was reported by more than half of the patients (58.5%) as the preferred toilet type. Constipation was detected in 70 patients (49.3%). This rate was 43% in patients with hemorrhoids and 53.7% in patients without hemorrhoids. Other clinical characteristics of the patients are summarized in Table 1.

Of them, 60 patients (42.3%) reported the presence of hemorrhoidal pathologies. The mean ages of the patients with and without hemorrhoids were similar (p=0.654). There were significantly more male patients with hemorrhoids (56.7% vs. 32.9%, p=0.008). Sex distribution and preferred toilet type of the patients with and without hemorrhoids are shown in Figure 1 and 2. We detected no significant differences in the type of toilet present at their homes, preferred toilet type and constipation (p>0.05).

Discussion

Most studies in the literature on hemorrhoids are therapeutic, but studies on risk factors are few. There are studies showing relationship between age, gender, fiber diet, defecation posture, chronic constipation, portal hypertension, spinal cord injury and hemorrhoids (5). According to the study of Johanson and Sonnenberg (12) the prevalence of hemorrhoids peaked between the ages of 45 and 65 and declined after the age of 65. The effect of age and gender on anal pressure is known (13). Also, the relationship between anal pressure and hemorrhoids has been shown in many studies (14). In addition, anorectal mean resting pressure has been shown to decrease with age in 40 percent of studies (13). When we think logically, we expect the incidence of hemorrhoids to decrease with age. However, in the current study, no significant relationship was found between age and hemorrhoids. In women, on the other hand, it was found

Table 1. Demographic and clinical characteristics of the study group

	Patients Overall (n=142) Mean ± SD	With hemorrhoids (n=60) Mean ± SD	Without hemorrhoids (n=82) Mean ± SD	p value
Age (year)	53.6±11.8	54.1±11.0	53.2±12.4	0.654**
	n (%)	**n (%)**	**N (%)**	
Sex				
Female	81 (57.0)	26 (43.3)	55 (67.1)	**0.008***
Male	61 (43.0)	34 (56.7)	27 (32.9)	
Type of toilet in home				
Squat	71 (50.0)	30 (50.0)	41 (50.0)	0.999*
Seated	107 (75.4)	41 (68.3)	66 (80.5)	0.143*
Preferred toilet type				
Only squat + usually squat	55 (38.7)	26 (43.3)	29 (35.4)	0.154*
Squat and seated	4 (2.8)	0 (0.0)	4 (4.9)	
Only seated + usually seated	83 (58.5)	34 (56.7)	49 (59.8)	
Constipation				
Positive	70 (49.3)	26 (43.3)	44 (53.7)	0.296*
Negative	72 (50.7)	34 (56.7)	38 (46.3)	

*: Independent Samples t-test, **: Pearson chi-square or Fisher's Exact test, SD: standard deviation

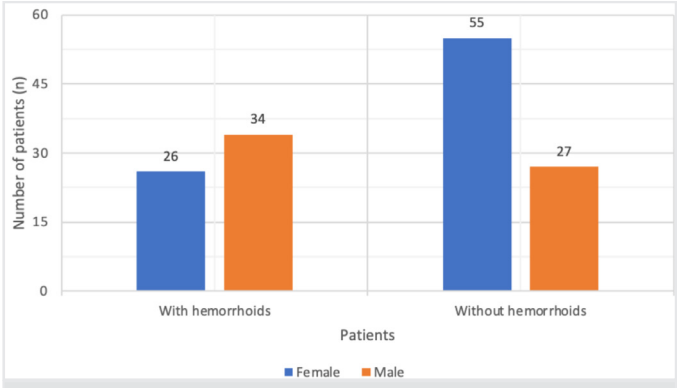

Figure 1. Sex distribution of the patients with and without hemorrhoids

Figure 2. Preferred toilet types of the patients with and without hemorrhoids

that the mean anal squeeze pressure was lower in most of the studies (15,16). However, no significant relationship was shown between external hemorrhoids and gender in adults (17,18). But, it was shown that boys had significantly more hemorrhoids (19). In our study, we found that hemorrhoids were significantly more common in men, as in boys.

There are few studies in the literature examining the relationship between toilet type and hemorrhoids, and the findings are controversial (9,10,19,20). In the literature, we did not find a study similar to the current study in adults. It was shown that the rectoanal angle was straightened during squat (9), in one study, defecation was more comfortable and less strained in the squat position, and in another study, complaints were reduced in patients with hemorrhoid diseaseusing the seated toilet (10). For this reason, it has been suggested that hemorrhoids can be seen less frequently in those who have squat toilet habits. According to a study by Yildiz et al. (19), external hemorrhoids were significantly more common in children with seated toilet habits. But, in the current study, it was observed that there was no significant relationship between toilet type and hemorrhoid disease. However, we included both adults and patients with internal and external hemorrhoids in our study. Therefore, for

only symptomatic hemorrhoid disease, a seated toilet may be a risk factor.

Chronic constipation is thought to be a risk factor for hemorrhoid disease (12). However, in our study, no significant relationship was found between the presence of constipation and hemorrhoids. In this study, it was thought that such a result was obtained because acute constipation was questioned, not chronic constipation. In addition, it would be more accurate to use scores such as KESS scoring in the evaluation of constipation (21).

Study Limitations

The patients included in the current study usually had a gastroenterological complaint, so it would be better to perform a similar study on the general population. This was a cross-sectional study and it would be more appropriate to conduct a long-term cohort study. The grade and types of hemorrhoids were not included in the study, only the presence of hemorrhoids was examined.

Conclusion

As a result, studies on the etiology of hemorrhoids are insufficient, controversial and scarce in terms of evidence level. In the literature, the relationship between anal pressure and hemorrhoid disease has been revealed more clearly. Although we concluded that there was no relationship between the preferred toilet type and hemorrhoid disease in the current study, we thought that this should be clarified by multicenter randomized cohort studies with long-term follow-up. It is thought that future studies on the causes of hemorrhoid disease will be designed by using the classification of hemorrhoids, body mass index measurement, diet questioning, family history, anal manometry and chronic constipation scoring.

Acknowledgement: I would like to offer my special thanks to Associate Professor Deniz Öğütmen Koç for her help and contribution to this paper.

Ethics

Ethics Committee Approval: In this prospective study, ethics committee approval was obtained from the Gaziosmanpaşa Training and Research Hospital Ethics Committee (approval number: 49 and date: 22/12/2021).

Informed Consent: Informed consent was obtained from all patients participating in the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Comparison of the Physicochemical Properties and Release Profiles of Metformin Tablets of Eight Different Brands Available in the Northern Cyprus Pharmaceutical Market

Kuzey Kıbrıs İlaç Pazarında Mevcut Sekiz Farklı Markaya Ait Metformin Tabletlerin Fizikokimyasal Özelliklerinin ve Salım Profillerinin Karşılaştırılması

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ABSTRACT

Objective: In the study, it was aimed to compare the physicochemical and *in vitro* dissolution parameters of metformin hydrochloride (MET) tablet brands from Northern Cyprus to evaluate the pharmaceutical equivalence.

Methods: Seven brands of MET tablets which were bought from community pharmacies were compared and evaluated with the innovative product Glucophage®. The impurity of MET contained in the sample tablets was determined using Fourier transform infrared spectroscopy. Pharmacopoeia tests were used to evaluate the physicochemical equivalence of the tablets. *In vitro* dissolution test was performed and dissolution data were analyzed including dissolution difference (f_1) and similarity factors (f_2) were evaluated. In addition, the release kinetics of selected MET tablets were examined with a release kinetics software (KinetDS3).

Results: All the tablet brands complied with the official specifications for uniformity of weight hardness and disintegration. Brand MF failed the friability test (>1%); while brands MC, MF and MG failed the content uniformity (assay) test (<95%). Difference (f_1) and similarity factors (f_2) of all brands were calculated in pH 6.8 buffer medium and evaluated with reference to the innovative brand. The facts that MB's f_1 value (15.45) was greater than 15 and that the f_2 values of MB and MF (48.57, 47.13, respectively) were less than 50 indicated that the dissolution profiles of MB and MF formulations were different from the dissolution profile of the innovative brand.

ÖZ

Amaç: Çalışmada, Kuzey Kıbrıs'ta bulunan metformin hidroklorür (MET) içeren farklı markalardaki tabletlerin, fizikokimyasal ve *in vitro* çözünme parametrelerinin karşılaştırılması ve farmasötik eşdeğerliğinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Eczaneden satın alınan farklı firmalara ait yedi MET, yenilikçi ürün Glucophage® ile karşılaştırılmış ve değerlendirilmiştir. Örnek tabletlerde bulunan MET'nin safsızlığı, Fourier transform kızılötesi spektroskopisi kullanılarak belirlenmiştir. Tabletlerin fizikokimyasal eşdeğerliğini değerlendirmek için farmakope testleri kullanılmıştır. *In vitro* çözünme testi yapılmış ve çözünme farkı (f_1) ve benzerlik faktörü (f_2) dahil olmak üzere analiz edilen çözünme verileri değerlendirilmiştir. Ek olarak, seçilen MET tabletlerinin salım kinetikleri, KinetDS3 yazılımı ile incelenmiştir.

Bulgular: Tüm tablet markalarının ağırlık sapması, sertlik ve dağılma özelliklerinin resmi spesifikasyonlara uyduğu saptanmıştır. MF markası friabilite testinde (>%1) başarısızken; MC, MF ve MG markalarının içerik tekdüzeliği (etkin madde miktar tayini) testinde (<%95) başarısız olduğu tespit edilmiştir. pH 6,8 tampon ortamında tüm markaların fark (f_1) ve benzerlik faktörü (f_2) hesaplanmış ve yenilikçi marka referans alınarak değerlendirilmiştir. Buna göre MB'nin f_1 değeri (15,45) 15'ten büyük; MB ve MF'nin f_2 değerlerinin (sırasıyla 48,57, 47,13) 50'den küçük olması MB ve MF formülasyonlarının çözünme profillerinin yenilikçi markanın çözünme profilinden farklı olduğunu belirlemiştir.

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Conclusion: Five of the eight tablet brands passed all the official tests and could be regarded as pharmaceutically equivalent but f_2 analysis showed only five brands were similar to the reference brand. The study has shown that all the MET tablet brands sampled in Northern Cyprus are not pharmaceutically equivalent.

Keywords: Metformin tablets, comparison, dissolution, pharmaceutical equivalency, physicochemical properties

Sonuç: Sekiz tablet markasından beşinin tüm farmakope testlerini geçtiği ve farmasötik açıdan eşdeğer olarak kabul edilebilir olduğu saptanmıştır. Ancak f_2 analizi, yalnızca beş markanın referansa benzer olduğunu göstermiştir. Çalışma, KKTC'de örneklenen tüm MET tablet markalarının farmasötik açıdan eşdeğer olmadığını ortaya koymuştur.

Anahtar Sözcükler: Metformin tabletleri, karşılaştırma, çözünme, farmasötik eşdeğerlik, fizikokimyasal özellikler

Introduction

Diabetes mellitus (DM) is a disease of fat and carbohydrate metabolism characterized by chronic hyperglycemia as a result of inadequate insulin secretion and activity (1). In the first diabetes screening conducted in the Cyprus in 1996; 7.3% of the population had previously known DM. In addition, 4% of the participants were found to have previously unrecognized DM. The same study was repeated in 2008, and DM was found in 11.5% of the population aged 20-80 years (2).

Metformin hydrochloride (MET) (1,1-dimethyl biguanide hydrochloride) works as an antihyperglycemic medication by inhibiting gluconeogenesis and improving insulin action in skeletal muscles. Thus, it can be said that MET is a commonly prefered drug in the treatment of type II DM (3,4).

In Northern Cyprus and in many other countries, numerous brands of generic metformin tablets are available in the pharmaceutical markets. Generic drug products are often more widely available and less expensive than innovator ones. Since many generic products are available on the pharmaceutical market, the question of whether the products are bioequivalent becomes important. Especially, considering that the product is a drug, choosing products that have formulated the same active ingredient in the same dosage form but have different trade names should not cause health problems (5,6). In addition, there has been an increasing number of drug counterfeiting incidents in recent years. When adequate safe precautions are not taken, many pharmaceutical products imitated in primitive conditions can be found in the drug market (7). Therefore, drug products are expected to have comparable quality properties before they become clinically modifiable (8).

As a result, in order to be acceptable alternatives, generics must have pharmacological and therapeutic qualities comparable to innovator drugs. The determination of whether a product is chemically and biopharmaceutically identical is a key step in determining therapeutic equivalence.

Physicochemical properties of metformin tablets, which were available on the Northern Cyprus pharmaceutical market, were determined in this study. Additionaly, in order to provide information on the differences and similarities in their dissolving patterns, f_1 and f_2 factors of these tablets were calculated.

Methods

MET was gifted by Abdi İbrahim İlaç, Turkey. On the other hand, eight brands (generic and innovator brands) of commercial uncoated MET tablets having 500 mg of MET were purchased from community pharmacies in Northern Cyprus. The general characteristics of the tablets are shown in Table 1. In addition, all chemicals were purchased from Sigma-Aldrich.

Isolation of Metformin Hydrochloride from Tablets

A powdered tablet containing 50 mg of MET was mixed with 50 mL of ethyl alcohol and filtered. The filtrate was dried, and the residue was dried for 1.5 hours at 100 °C. The method was repeated for other brands of tablets, and the residues were used for infrared examination (9).

Recognition of Isolated Metformin Hydrochloride Using FTIR

A sufficient amount of powder mass was taken and placed in the device and the IR spectrum was determined in the range of 500-4,000 cm^{-1}. The process was repeated for the mass of powder from each brand and compared with the reference metformin spectrum.

Weight Variation Test

Twenty tablets of each brand were randomly selected and weighed with Shimadzu balance and standard deviation values were calculated. The process was repeated for all brands (10).

Friability Test

Ten tablets were chosen at random from the each brand, weighed together, then placed in a friabilator for 100 rpm. They were weighed again, and the % weight reduction was measured (10,11).

Hardness Test

Ten randomly chosen tablets from each brand were tested with an Erweka tester, determining the hardness of the tablets in Newton. For each brand, the standard deviation value of hardness was computed (9).

Diameter and Thickness Test

Ten metformin tablets were chosen at random from each brand to be tested with an Erweka tester and diameter and thickness of the tablets were measured. For all groups of tablet, the standard deviation values of diameter and thickness were computed (9,10).

Disintegration Test

Disintegration is the operate of breaking the tablet into granules and is the first step in dissolution, therefore it is part of the *in*

vitro and *in vivo* correlation, and the disintegration test indicates the time required to break the tablet. The disintegration period of the tablets in purified water at 37±0.5 °C was calculated (9).

Content Uniformity Test (Assay Test)

The UV spectrophotometric method was used to determine the amount of metformin in solution. For this purpose, first of all, the standard metformin solution was prepared by using 100 mg MET in 1,000 mL of 6.8 buffer solution in a volumetric flask with vigorous shaking. This solution was further diluted to get a set of solutions containing MET in vary concentrations. Absorbance values of the solutions at 235 nm were determined UV spectrophotometrically. Analytical parameters were determined by ANOVA.

The amount of MET in a tablet was determined by using UV spectrophotometric method. A standard MET solution was prepared into 6.8 phosphate buffer solution and sample solutions were prepared dissolving MET tablets from each batch in the same medium. The MET amount in each solution was determined spectrophotometrically (10).

***In vitro* Dissolution Test**

The dissolution studies on metformin tablets were performed according to USP paddle method at 100 rpm. The dissolution medium was selected as 1,000 mL of 6.8 buffer at 37±0.5 °C. The samples were taken at definite time and assayed spectrophotometrically at 235 nm. The % released of MET from the tablets were determined (12,13).

Comparison of the Dissolution Data

According to the dissolution data obtained, the f_1 and f_2 of the other brands were calculated by comparing them to the reference drug (Glucophage®).

The difference factor ranges from 0 to 15. If f_1 ≤15, the two dissolution profiles are identical or similar, and the two products can be changed. If f_1 >15, it indicates that the dissolution profiles are different and so the products can not be interchanged.

The similarity factor ranges from 0 to 100. If f_2 ≥50, the two dissolution profiles are identical or similar, and the two brands can be changed. If f_2 <50, it indicates that the dissolution profiles are different and so the brands can not be interchanged (9,12).

Results

Each of the eight brands of metformin tablets bought had at least three months of expire dates left, and all analytical measurements were performed prior to the expire dates. While one brand was produced in Northern Cyprus, seven brands were imported. All metformin tablets were uncoated tablets. Details of the selected tablets are shown in Table 1.

The FTIR spectrum obtained from metformin isolated from each metformin brand represented absorption bands at 1,520, 1,630 and 3,458 cm^{-1} similar to FTIR spectrum of pure MET (10). FTIR spectrums are shown in Figure 1.

The amount of metformin HCl in the tablets was determined by UV spectrophotometric method (14). Method validation details are shown in Table 2.

The metformin tablet brands generally had acceptable characteristics. The tablet's weight uniformity and friability test results were suitable except brand MF (1.05%). All eight uncoated tablet brands disintegrated in the medium <15 minutes. The MET amount rate of the tablet brands was in the range of 90.08-99.15%. Five metformin tablet brands passed the content uniformity test (Assay test), but three (MC, MF, MG) had lower doses. The dissolved drug amount in 30 min, in all the tablet brands was higher than 80% (15).

Figure 2 depicts the dissolution profiles of MET tablets in pH 6.8 buffer. The tablets represented counterpart dissolution profiles and achieved higher than 80% release in 30 minutes in the dissolution medium. f_1 and f_2 of the MET tablet brands are shown in Table 4. All metformin tablets exhibited high dissolution properties (>90%) in pH 6.8 phosphate buffer. For all metformin brands in buffer pH 6.8, f_2 values were not higher than 50. While the f_2 value of the MB coded metformin tablet was 48.57, the f_2 value of the MF coded tablet was 47.13.

Table 1. Some details of studied metformin hydrochloride tablets

MET tablet code date	Origin	Lot number	Expiry
MA	Northern Cyprus	112	08/2021
MB	Turkey	064	01/2022
MC	United Kingdom	082	09/2021
MD	Turkey	099	09/2021
ME	Turkey	108	01/2022
MF	United Kingdom	096	06/2021
MG	United Kingdom	820	07/2021
MH	France	306	04/2021

Table 2. Analytical method validation parameters for the assay of MET by UV spectrophotometric method

Parameter	Result
Linearity range (µg/mL)	1-12
Slope (m)	0.0738
RSD of m (%)	0.21
SE of m	0.022
Intercept (n)	0.1839
RSD of n (%)	3.5
SE of n	0.004
Determination coefficient (r^2)	0.9978
LOD (µg/mL)	0.0198
LOQ (µg/mL)	0.06
RSD for precision (%)	2.03
RSD for accuracy	0.38

RSD: Relative standard deviation, SE: Standard error

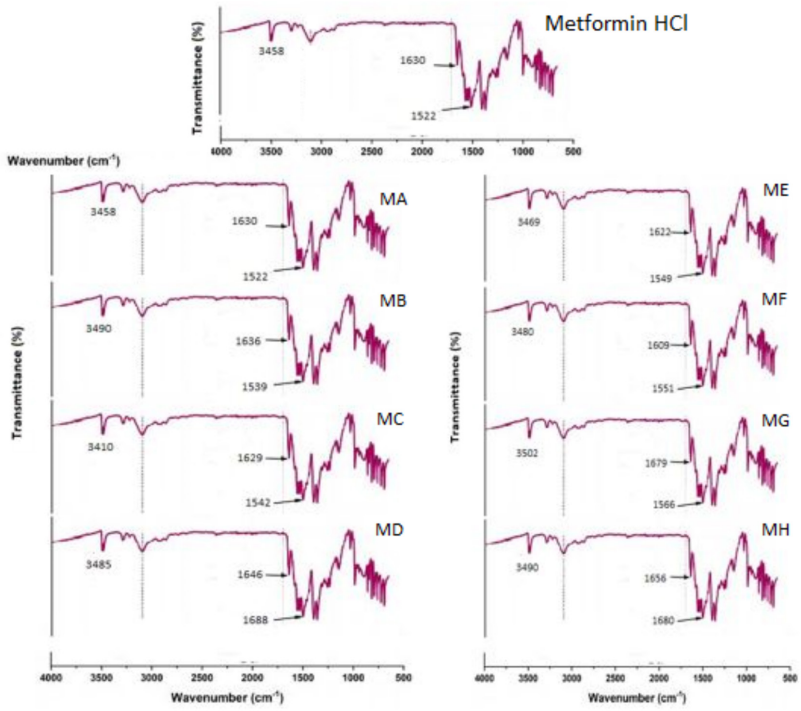

Figure 1. FTIR spectrums of pure metformin HCl and metformin tablets

Table 3. Results of physicochemical tests of metformin hydrochloride tablet brands

MET tablet	Weight (mg) (Xmean ± SD)	Diameter (mm) (Xmean ± SD)	Thickness (mm) (Xmean ± SD)	Hardness (N) (Xmean ± SD)	Friability (%) (Xmean ± SD)	Disintegration time (min.sec) (Xmean ± SD)	Content uniformity
MA	535.37±2.84	11.06±0.02	5.93±0.03	445.7±1.09	0.01±0.001	8.42±0.001	99.01±0.32
MB	589.37±4.21	11.10±0.02	5.57±0.02	423.6±1.23	0.05±0.001	8.34±0.006	95.67±0.55
MC	544.43±3.92	11.05±0.01	5.68±0.02	408.4±1.09	0.04±0.018	7.59±0.002	90.12±0.48
MD	550.41±1.92	11.12±0.01	5.55±0.04	474.7±1.55	0.05±0.002	8.42±0.001	97.11±0.22
ME	541.32±2.52	10.92±0.02	5.47±0.02	445.7±1.01	0.11±0.003	6.18±0.002	96.14±0.37
MF	560.87±1.23	11.02±0.02	5.71±0.05	446.1±0.88	1.05±0.009	5.70±0.001	90.08±0.62
MG	552.88±2.12	11.32±0.03	5.48±0.01	445.7±0.92	0.02±0.001	8.01±0.001	92.77±0.44
MH	538.44±1.55	11.05±0.01	5.38±0.01	408.5±0.71	0.01±0.001	5.45±0.003	99.15±0.19

SD: Standard deviation, min: Minimum

Table 4. Difference (ƒ1) and similarity (ƒ2) factors for reference (MH) versus test brands

	MA	MB	MC	MD	ME	MF	MG
ƒ1 values	7.81	15.45	6.17	5.59	8.11	9.14	10.05
ƒ2 values	81.14	48.57	78.26	85.45	79.33	47.13	88.21

When the release kinetics of the tablets were examined, it was determined that the highest r^2 values were observed in the first order kinetic and Hixson-Crowell release models. Table 5 shows the release kinetic details for each tablet.

Discussion

To determine the identity of the active pharmaceutical ingredient, 8 MET tablets were exposed to FTIR analysis. Identification testsensured that all brands of metformin tablets contained MET as active ingredient and were not imitation products. MET tablets were tested for quality and pharmaceutical equivalency using pharmacopoeia and other methods. Weight variation, friability, thickness, disintegration time, hardness, dissolution and drug content were evaluated. During production, these criteria are used to measure the uniformity of quality across multiple batches of tablets (16). The quality factors are interconnected and have a significant impact on bioavailability (17). The majority of the

Table 5. Kinetic parameter results of dissolution data for metformin tablets

		MA	MB	MC	MD	ME	MF	MG	MH
First order kinetic	RMS	1.921	1.690	2.614	1.815	1.922	1.754	1.669	1.915
	k	0.013	0.006	0.021	0.014	0.016	0.039	0.022	0.028
	r^2	0.984	0.996	0.985	0.982	0.979	0.991	0.975	0.999
Hixson-crowell	RMS	4.659	3.226	4.202	4.442	4.521	3.996	4.205	4.336
	k	0.190	0.002	0.159	0.080	0.140	0.208	0.184	0.121
	r^2	0.929	0.903	0.955	0.901	0.916	0.892	0.902	0.896

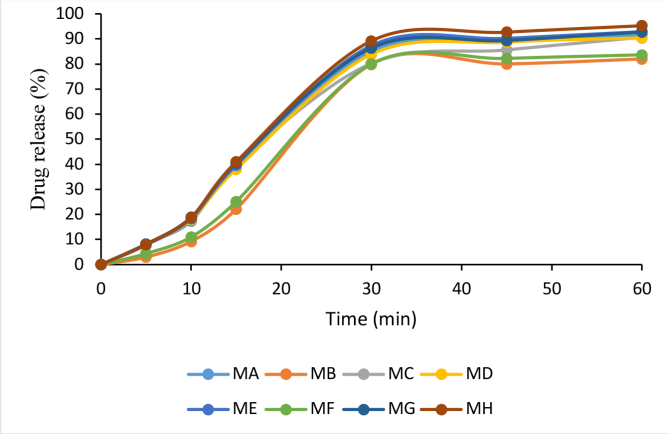

Figure 2. Dissolution profiles of metformin tablets

quality evaluation tests performed on the tablet brands under consideration were passed. Because of differences in granulation density and compression force applied to the tablets, the thickness of a tablet may fluctuate while the weight remains constant.

The weight uniformity test is used to ensure consistent dosing among tablets within a batch, preventing overdosage or underdosage. The change in drug content of a tablet is directly affected by its weight variation. Because all MET tablets weigh higher than 500 mg, no more than two individual tablet weights should differ from the average weight by higher than 5%, and no tablet should differ by more than twice the permissible percentage deviation (14). All MET tablets evaluated met this requirement and hence passed the weight uniformity test.

The friability test is performed to determine a tablet's ability to tolerate abrasion during packaging and transportation. The nature and amount of binder used in tablets influence this feature (18). For pharmaceutical items, friability should be lower than 1% of the tablets (14,15). All of the brands passed the friability test, with the exception of MF, which had a 1.05 friability. The failure of MF could be attributed to the use of inadequate binder or the use of not enough amount of compaction force.

Tablet hardness measurements are used to detect whether or not tablet machines require pressure adjustments. A highly hard tablet can not disintegrate in the requisite time in an aqueous solution, whereas a very soft tablet can not resist handling activity (9). The minimum crushing force for a good tablet is 400 N (14). All MET tablet brands performed well in terms of fracture resistance, exceeding the minimum of 400 N.

Disintegration is a critical process before medication release from immediate release dosage formulations. Uncoated tablet disintegration time should not exceed 15 minutes (14). According to the findings, all tablets disintegrated at suitable time. MET tablets should contain 95-105 % of the drug's label claim upon assay, according to the British and American pharmacopoeias (14,15). The spectrophotometric measurements (Table 3) revealed that all brands, with the exception of MC, MF, and MG, met this pharmacopoeia standard. Brands MC, MF, and MG had drug content percentages lower than the minimal level of 95% and could be deemed of inferior quality. The failure of the MC, MF, and MG assay tests could be attributable to inaccuracies in API weighing and inadequate mixing during the tableting process.

Oral solid dose forms can be absorbed after they have been disintegrated and dissolved. Therefore, the dissolution test is used to predict product behavior *in vivo*. The dissolution test can be considered an *in vitro* bioequivalence test to examine whether solid dosage forms are equivalent (19). In 30 minutes, immediate release formulations should release 80% of the specified dosage (14). The study's findings demonstrated that all of the MET tablets had good dissolving profiles as instant release tablets.

Study Limitations

According to FDA, f_1 (difference factor) value, which is one of the parameters used to express that the dissolution profiles are not different, should be 0-15. In our study, when we compared the dissolution profiles of the metformin containing tablets of different manufacturers with the innovative company's product, it was determined that the f_1 value (15.45) of only the MB formulation was more than 15. On the other hand, the two brands (MB and MF) did not have f_2 values in the dissolution media within the range specified by the FDA (50-100) (20). Therefore, it should be considered that these brands do not have the same drug release bioequivalence as the MH (reference brand) in pH 6.8 phosphate buffer medium. Two oral dosage forms are accepted as bioequivalent if they release drugs at the same rate. *In vivo* bioequivalence studies are typically used to determine a product's bioequivalence. These *in vivo* bioequivalence studies, on the other hand, are typically costly and involve the use of intrusive methods. The most significant benefits of *in vitro* dissolution studies include lower costs and a more accurate assessment of product performance. Because the drug is soluble in physiological settings, generic metformin tablets with varying dissolving characteristics may nonetheless provide equal therapeutic efficacy *in vivo*.

Conclusion

Eight MET tablets were determined to comply with pharmacopoeia specification for disintegration, weight variation, dissolution test and hardness for uncoated tablets. One brand failed the friability test, while three failed the assay test. Two of the sampled MET tablet brands showed that dissolution profiles were not similar to the reference brand (MH) in pH 6.8 buffer medium in terms of similarity factors. Based on the results of the study, it can be said that in order to ensure the quality and therapeutic efficacy of metformin tablets on the market, the drug regulatory authorities in Northern Cyprus should intensify post-market inspection and surveillance.

Ethics

Ethics Committee Approval: Ethics committee approval was not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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Biological Activities and Chemical Composition of Turkish Sweetgum Balsam (Styrax Liquidus) Essential Oil

Türk Sığala Balzamı (Styrax Liquidus) Uçucu Yağının Biyolojik Aktiviteleri ve Kimyasal Bileşimi

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ABSTRACT

Objective: The purpose of this present study was to make a chemical analysis of the composition of essential oil obtained from sweetgum balsam and to examine its antimicrobial, anticholinesterase, α-glucosidase inhibition, and antioxidant activities.

Methods: The essential oil obtained by the hydrodistillation method was analyzed by gas chromatography-mass spectrometry systems. The antimicrobial activities of the essential oil were evaluated by disc diffusion and resazurin microplate methods, the anticholinesterase effect was determined by using *in vitro* AChE and BChE enzymes inhibition assays, and the antioxidant effect was evaluated by ABTS and CUPRAC methods.

Results: The main components of the essential oil were determined as styrene (92.6%) and α-pinene (2.2%). The essential oil showed weak antimicrobial activity against *K. pneumoniae*, *S. epidermidis* but strong antimicrobial activity against *A. baumannii, C. glabrata*. It showed moderate inhibitory activity to AChE and BChE enzymes, and IC_{50} values were calculated as 36.5 μg/mL and 69.5 μg/mL, respectively. It also showed low inhibition of α-glucosidase (IC_{50} value was 637.2 μg/mL) and a similar antioxidant effect in the CUPRAC and ABTS method ($A_{0.5}$ value was 637.2 μg/mL and IC_{50} value was 632.2 μg/mL, respectively).

Conclusion: Styrax Liquidus essential oil can be considered a natural antimicrobial agent due to its strong antimicrobial activity capacity against *A. baumannii* and *C. glabrata* strains.

ÖZ

Amaç: Bu çalışmada, sığala balzamından elde edilen uçucu yağın bileşiminin kimyasal analizinin yapılması, antimikrobiyal, antikolinesteraz, α-glukozidaz inhibitörü ve antioksidan aktivitelerinin incelenmesi amaçlanmıştır.

Yöntemler: Hidrodistilasyon yöntemiyle elde edilen uçucu yağ, gaz kromatografisi kütle spektrometresi sistemleri ile analiz edilmiştir. Uçucu yağın antimikrobiyal aktiviteleri disk difüzyon ve resazurin mikroplak yöntemleri ile; antikolinesteraz etkisi *in vitro* AChE ve BChE enzimleri inhibisyon testi ile; antioksidan etkisi ise ABTS ve CUPRAC yöntemleri ile değerlendirilmiştir.

Bulgular: Uçucu yağın ana bileşenleri stiren (%92,6) ve α-pinen (%2,2) olarak tespit edilmiştir. Uçucu yağ, *K. pneumoniae, S. epidermidis* kökenlerine karşı zayıf, *A. Baumannii, C. glabrata* kökenlerine karşı güçlü antimikrobiyal aktivite göstermiştir. AChE ve BChE enzimlerine orta derecede inhibitör aktivite gösterip IC_{50} değerleri sırasıyla 36,5 μg/mL ve 69,5 μg/mL olarak hesaplanmıştır. Ayrıca düşük oranda α-glukozidaz inhibisyonu (IC_{50} değeri 637,2 μg/mL) ve CUPRAC ve ABTS yönteminde benzer bir antioksidan etki (sırasıyla $A_{0,5}$ değeri 637,2 μg/mL ve IC_{50} değeri 632,2 μg/mL) göstermiştir.

Sonuç: Styrax liquidus uçucu yağı, *A. baumannii* ve *C. glabrata* suşlarına karşı güçlü antimikrobiyal aktivite kapasitesi nedeniyle doğal bir antimikrobiyal ajan olarak değerlendirilebilir.

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Keywords: Styrax liquidus, essential oil, antimicrobial, anticholinesterase, α-glucosidase, antioxidant

Anahtar Sözcükler: Styrax liquidus, uçucu yağ, antimikrobiyal, antikolinesteraz, α-glukozidaz, antioksidan

Introduction

Styrax liquidus is a resinous exudate (sweetgum balsam) obtained from the wounded trunk of *Liquidambar orientalis* Mill. from the Altingiaceae family. The species is found only in southwestern Turkey and on the island of Rhodes. *L. orientalis* is an endangered relict species (1,2). In history, essential oil of Styrax liquidus was known as Egyptian Queen Cleopatra's "love elixir" and used as perfume oil. It has been used as a medicine since the Hippocrates. Ancient Egyptians used this oil during mummification. Amphoras filled with balsam extracted from sunken Phoenician ships indicated that the Styrax liquidus had an important place in the Mediterranean trade in the past (3).

The Styrax liquidus has been used for the treatment of various ailments in Turkish folk medicine. It is used to treat wounds, asthma, bronchitis, upper respiratory tract diseases, skin diseases like scabies and it is used as expectorant and antifungal. It used as fumigation, in the form of powder and pastille, and in the form of pomade and plaster (4-7). The essential oil of Styrax liquidus has been used in the pharmaceutical and cosmetic industry particularly in perfumery. The essential oil constitutes 0.5-1% of the balsam (8).

In the literature review, it was determined that balsam had antibacterial, antioxidant and antiulcerogenic effects (9-11). Styrax liquidus essential oil has been reported to be inhibitory on the central nervous system as well as having an antimicrobial activity (12,13).

The present study was performed to evaluate the antimicrobial, antioxidant, anticholinesterase, and α-glucosidase inhibitory activities of Styrax liquidus essential oil and investigate its chemical composition.

Methods

Extraction of Essential Oils

Styrax liquidus was obtained from local people in Marmaris, Muğla. The essential oil of Styrax liquidus was obtained by hydrodistillation in the Clevenger apparatus for 3 hours. Essential oil, thus obtained was stored in sealed vials at +4 °C until analyzed and tested.

Gas Chromatography (GC) and Gas Chromatography-mass Spectrometry Analysis (GC/MS)

The composition of the essential oil was determined by using gas chromatography (GC) and GC/mass spectrometry (GC/MS).

The GC analysis was performed on the Agilent 6890N GC system at an FID detector temperature of 300 °C. To achieve the same elution order as GC/MS, the simultaneous automatic injection was performed on a replicate of the same column, applying the same operating conditions. The relative percentage amounts of the separated compounds were calculated from the FID chromatograms. The analysis results are given in Table 1.

The GC/MS analysis was performed on an Agilent 5975 GC/MSD system on an Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) using helium (0.8 mL/min) as carrier gas. The GC oven temperature was held at 60 °C for 10 minutes and programmed at 220 °C at 4 °C/min, and kept constant at 220 °C for 10 minutes, then programmed to 240 °C at 1 °C/min. The division ratio was set to 40:1 and the injector temperature was set to 250 °C. Mass spectra were recorded at 70 eV. The mass range was m/z 35 to 450.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to a series of n-alkanes. Commercial (Wiley GC/MS Library, MassFinder Software 4.0) (14,15) and in-house (Başer Library of Essential Oil Constituents) mass spectral libraries built up by genuine compounds and components of known oils were used.

Antimicrobial Activities

The essential oil was screened for their antimicrobial activities against *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *Candida glabrata ATCC 2001* microorganisms by disc diffusion and resazurin microplate methods.

Disk Diffusion Test

Fresh passages from the microorganisms were taken first to be tested using Disk Diffusion Tests in our study. The next day, suspensions were obtained from standard strains in saline water with a turbidity level of McFarland 0.5 (10^8 microorganisms/mL). The Mueller Hinton Agar plate was seeded with a sample taken from these suspensions using a sterile swab.

The essential oil of sweetgum balsam was first passed through a 0.22 μm filter for sterilization. Then, the paper discs obtained from Whatman paper that we would prepare were impregnated with the essential oil of sweetgum balsam placed on the plates. Moreover, various antibiotic discs with known sensitivity were also placed in Petri dishes for comparison. During this process, care was taken to keep a 22 mm space between the discs and a 14 mm distance from the edge of the Petri dish to ensure that the zones formed did not overlap. The inhibition zones were then measured by incubating the media for 18-24 hours at 37 °C.

Resazurin Microplate Assay (REMA)

Resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide) microplate method was used to determine the antibacterial activities and minimum inhibitory concentrations (MIC) of the essential oil of sweetgum balsam *in vitro* against the standard

origins of *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606 *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853 *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *C. glabrata ATCC 2001*. The activity determination was planned as two replicates. Streptomycin and fluconazole were used as the standard drugs. Stock solutions of the studied substances were prepared with DMSO at a concentration of 1,024 µg/mL and sterilized by passing through a 0.22 µg membrane filter. To begin, each well was filled with 100 µL of Muller Hinton Broth medium. Serial dilutions of the prepared solutions were made by adding 1,000 µg/mL to the first well of 96-well microplates (MIC range of chemicals 0.3-1,000 µg/mL). The final concentration of the standard drug streptomycin was adjusted to 83 µg/mL and other standard drug fluconazole was adjusted to 30 µg/mL, and serial dilutions were made by adding 50 µL to the first well. Only DMSO was placed in one column of the plate as a negative control, and only standard bacteria as a positive control in the other column, both of which were 50 µL, and serial dilutions were made. A suspension of McFarland 0.5 turbidity was prepared from 1-2 day old colonies of microorganisms and then diluted 1:100. 10 µL of these final suspensions were added to the plate wells. Plates were covered with parafilm and incubated in a normal atmosphere for 24 hours at 37 °C. After the incubation, 10 µL each of 33.75 mg resazurin and 20% Tween 80, which were dissolved in 5 mL distilled water, were added to all wells, and plates were left to incubate for 2-4 hours, and the results were evaluated visually. The MIC value was determined to be the lowest concentration value preventing the color change from purple to pink.

Inhibitory Activities Against AChE and BChE

The anticholinesterase activity of essential oil was determined by using *in vitro* AChE and BChE enzymes inhibition assays. AChE and BChE inhibition activities were determined using the method found by Ellman et al. (16). Galantamine is used as reference compound. The IC_{50} was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of seven different concentrations. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates in the reaction, and 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used as a reagent. Stock solutions of essential oils and galantamine were prepared with methanol at a 4,000 µg/mL concentration. Acetylthiocholine iodide at 7.085 mM concentration and butyrylthiocholine iodide at 0.785 mM concentration were prepared. Hundred and fifty µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution, and 20 µL of AChE (2,476x10-4 U/µL) (or 3,1813x10-4 U/µL of BChE) solution were mixed. It was incubated at 25 °C for 15 minutes. Ten µL of DTNB solution at a concentration of 5 mM was added. The reaction was initiated by the addition of 10 µL acetylthiocholine iodide (or butyrylthiocholine iodide). In this method, the activity was measured by following the yellow color produced as a result of the presence of thio anion which was produced by the enzymatic hydrolysis of the substrate with DTNB. Also, methanol was used as a control solvent. The hydrolysis of the substrates was monitored using a BioTek Power Wave XS at 412 nm (17). All experiments were done in triplicate and inhibition activity was calculated as;

Inhibition (%) = ($Absorbance_{Control}$ - $Absorbance_{Sample}$)/ $Absorbance_{Control}$ x100

IC_{50} values were calculated using Graphpad Software.

α-Glucosidase Inhibition Assay

Commercially available α-glucosidase from *Saccharomyces cerevisiae* (Sigma, G5003) was selected as the target protein using p-nitrophenyl-α-D-glucopyranoside (pNGP, Sigma, N1377) as substrate. The essential oil and genistein were dissolved in DMSO, and the enzyme and substrate were dissolved in potassium phosphate buffer (0.05 M, pH 6.8). The enzymatic reaction mixture consisting of α-glucosidase (0.02 U, 20 µL), substrate (1.25 mM, 30 µL), essential oil solution (10 µL), and potassium phosphate buffer (140 µL) was incubated at 37 °C for 30 minutes. Next, the absorbance of the yellow color produced due to p-nitrophenol formation was measured at 405 nm using a Synergy H1 (BioTek, USA) 96-well microplate reader (18). All experiments were done in triplicate. α-glucosidase inhibition activity was calculated as;

Inhibition (%) = ($Absorbance_{Control}$ - $Absorbance_{Sample}$)/ $Absorbance_{Control}$ x100

IC_{50} values were calculated using Graphpad Software.

Antioxidant Activity Assay

ABTS Cation Radical Scavenging Assay

ABTS cation radical scavenging activities of the essential oil were determined using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (19). A stock solution of 1,000 µg/mL of essential oil was prepared. Two, 5, 10, and 20 µL of this stock solution were taken and their volume was made up to 40 µL with ethanol, and 160 µL of 7 mM ABTS cation radical solution was added to them. After the reaction was kept in the dark for 6 minutes, absorbance was measured at 734 nm. ABTS cation radical scavenging activity was calculated by evaluating the absorbance values of the essential oil sample against the control. The percent inhibition was calculated from the following equation;

Inhibition (%) = ($Absorbance_{Control}$ - $Absorbance_{Sample}$)/ $Absorbance_{Control}$ x100

IC_{50} values were calculated using Graphpad Software.

CUPRAC Assay

In the presence of antioxidant compounds in the essential oil, Cu(II)-Neocuproin (Nc) complex was reduced to colored Cu(I)-Nc chelate, and the absorbance of this chelate was measured at 450 nm. Butylated hydroxytoluene (BHT) was used as standard. $CuCl_2$, neocuproin, and NH_4OAc buffer were added to the essential oil and BHT with final concentrations of 10, 25, 50,

100 µg/mL, and absorbance was measured at 450 nm after 1 hour (20). The absorbance values of the essential oil sample were evaluated against the standard. The study was performed in three replications.

Results

Composition of the Essential Oils

The analysis of essential oil obtained by the hydrodistillation method was carried out with GC/FD and GC/MS systems. Comparative analysis results are given in Table 1.

Antimicrobial Activities

The essential oil was screened for its antimicrobial activities against *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 49461, *Acinetobacter lwoffii* ATCC 19002, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeuroginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans ATCC 66027* and *Candida glabrata ATCC 2001* microorganisms by disc diffusion method and resazurin microplate methods. According to our results; the essential oil showed weak antimicrobial activity against *K. pneumoniae,* and *S. epidermidis* but strong antimicrobial activity against *A. baumannii,* and *C. glabrata* (Table 2).

Table 1. The essential oil composition of Styrax liquidus

No	RRI	Compound	(%)
1	1032	*α*-Pinene	2.19
2	1035	*α*-Thujene	0.06
3	1076	Camphene	0.01
4	1118	*β*-Pinen	0.5
5	1203	Limonene	0.07
6	1218	*β*-Phellandrene	0.05
7	1272	Styrene	92.59
8	1466	*α*-cubebene	0.18
9	1497	*α*-Copaene	0.15
10	1541	Benzaldehyde	0.08
11	1549	*β*-cubebene	0.18
12	1611	Terpinen-4-ol	0.02
13	1612	*β*-caryophyllene	0.03
14	1671	Acetophenone	0.43
15	1704	*γ*-Murolen	tr
16	1740	*α*-Murolen	tr
17	1773	*δ*-Cadinene	0.19
18	1804	Myrtenol	tr
19	2049	4-Ethylguaiacol	0.06
20	2065	Benzenepropanol	0.87
21	2068	*(E)*-Cinnamaldehyde	0.09
22	2113	Cumin alcohol	0.48
23	2157	*(E)*-Ethyl cinnamate	0.05
24	2195	4-Ethyl phenol	0.67
25	2219	*α*-Muurolol	0.24
26	2308	Cinnamyl alcohol	tr
Total		**99.19**	
Oil yield		**0.57**	

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data, and tr: Trace (<0.01%).

AChE and BChE Inhibition

The essential oil moderately inhibited cholinesterase enzymes. Galantamine was used as the standard substance. The calculated IC_{50} values are given in Table 3.

α-Glucosidase Inhibition

Styrax liquidus essential oil showed a low rate of α-glucosidase inhibition compared to genistein, which had strong inhibitory properties against α-glucosidase. Calculated IC_{50} values are given in Table 4.

Antioxidant Activity

Antioxidant effect was examined by CUPRAC and ABTS methods. Activity results were evaluated by comparison with standard BHT. It was observed that essential oil had a very low antioxidant effect when compared to standard (Table 5).

Table 2. Investigated microorganisms for antimicrobial effects

Microorganisms	Minimal inhibitory concentration of the essential oil of Styrax liquidus (µg/mL)
Enterococcus faecalis ATCC 29212	125
Streptococcus pneumoniae ATCC 49619	250
Stapyhlococcus aureus ATCC 29213	125
Stapyhlococcus epidermidis ATCC 49461	62.5
Escherichia coli ATCC 25922	125
Pseudomonas aeruginosa ATCC 27853	125
Klebsiella pneumoniae ATCC 70063	62.5
Acinetobacter baumannii ATCC 19606	31.25
Acinetobacter lwoffii ATCC 19002	125
Candida albicans ATCC 66027	125
Candida glabrata ATCC 2001	<3.9

Discussion

Styrax Liquidus is known to have various biological effects due to its use in folk medicine and scientific studies. A limited number of studies has reported the biological effects of essential oil Styrax liquidus. On GC-GC/MS analysis of the essential oil, 26 compounds representing 99.19% of the total oil were identified where styrene (92.59%) and α-pinene (2.2%) were identified as the major components (Table 1).

There are several previous publications with different results in the literature regarding the chemical content of Styrax liquidus. Analysis by GC-MS resulted in cinnamyl cinnamate (21.5%), phenyl propyl cinnamate (7.5%), cinnamic acid (4.0%), cinnamyl alcohol (2.0%), styrene (0.5%) and phenyl propyl alcohol (0.5%). However, in this study, approximately 60% of the content was analyzed and different results were thought to be due to this (21). Another study reported that the main components of essential oil were styrene (89.5%), α-pinene (7.2%), and β-pinene (1.1%) (22). These results are very close to our findings.

In a different study, among 58 components representing more than 99.4% of the essential oil, styrene (70.4%), α-pinene (19.4%), and β-pinene (4.3%) were determined as the main components (23).

The benefits of Styrax liquidus have been known for years, especially by local people. It has been used to treat skin problems, peptic ulcers, parasitic infections (4,7,24). The antibacterial, antiulcerogenic, antioxidant, and cytotoxic effects of styrax were investigated by researchers (10,25,26).

Our aim in this study was to reveal the potential for antioxidant, antidiabetic, anticholinesterase, and antimicrobial effects of the essential oil of sweetgum balsam.

Table 3. *In vitro* inhibition IC_{50} for AChE and BChE activities

Test sample	AChE IC_{50} (μg/mL)	BChE IC_{50} (μg/mL)
Styrax liquidus essential oil	36.5	69.5
Galantamine	0.55	3.56

Table 4. *α*-Glucosidase inhibition IC_{50} values

Test sample	IC_{50} (μg/mL)
Styrax liquidus essential oil	637.2
Genistein	12.279

Table 5. CUPRAC and ABTS values

Test sample	CUPRAC $A_{0.5}$ (μg/mL)	ABTS IC_{50} (μg/mL)
Styrax liquidus essential oil	637.2	632.2
BHT	3.275	1.242

BHT: Butylated hydroxytoluene

Anti-α-glucosidase compounds have received great attention due to their potential use in treating diabetes (27). Our study is the first report on anticholinesterase and α-glucosidase activities of essential oil of Styrax liquidus. The IC_{50} values against AChE and BChE were 36.5 μg/mL and 69.5 μg/mL, respectively. The IC_{50} values of enzyme inhibition results of galantamine were AChE =0.55 μg/mL and BChE =3.56 μg/mL, respectively. The obtained essential oil appeared to inhibit cholinesterase enzymes at a moderate level. It showed a low rate of α-glucosidase inhibition (IC_{50} value was 637.2 μg/mL, Genistein = IC_{50} value of was 12,279 μg/mL) and similar antioxidant effect on CUPRAC and ABTS method ($A_{0.5}$ value was 637.2 μg/mL, BHT was 3,275 μg/mL, and IC_{50} value was 632.2 μg/mL, BHT was 1.242 μg/mL, respectively).

A previous study investigated the antibacterial potential of Styrax liquidus against several bacteria strains by using the agar diffusion method. Different concentrations of balsam are potent on some bacterial strains. In one of the most comprehensive studies, its antibacterial activitywas studied. The concentration of 10% balm was effective against *Bacillus brevis, B. cereus, B. subtilis, Corynebacterium xerosis, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumoniae, Micrococcus luteus, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescent,* and *Staphylococcus aureus.* The concentration of 0.4% balm was effective against *E. aerogenes, P. vulgaris*. The concentration of 0.2% balm was effective against *E. aerogenes,* and *P. vulgaris*. Besides, the concentration of 0.1% did not show any antibacterial effect reported (11). In our study, the essential oil of Styrax liquidus showed weak antimicrobial activity against *Klebsiella pneumoniae, Staphylococcus epidermidis* but strong antimicrobial activity against *Acinetobacter baumannii, Candida glabrata* (Table 2).

Due to their strong antimicrobial activities, plant-derived secondary metabolites are known to be important in the treatment of various diseases (28). In a study investigating the antiadhesive efficacy of coated with Styrax liquidus surgical silk sutures against common oral pathogenic microorganisms, the highest antiadhesive efficacy was observed against *S. aureus* (29).

Styrax liquidus is obtained from *L. orientalis.* This species is considered endangered due to threats such as agricultural activities, fires, pollution, polluted water, heavy tourism investments, and overgrazing. It was therefore recorded as "Critically Endangered" on the 2017 European Red List (30).

In order to ensure continuity in the supply of natural products in pharmaceutical research, it is necessary to protect and cultivate medicinal plants.

Study Limitations

Strong antimicrobial effects of Styrax liquidus essential oil were determined. However, evaluation of its use as an antimicrobial agent was limited, since studies on cytotoxic effects could not be performed.

Conclusion

As in all balsams, the content of Styrax liquidus includes resin, essential oil, and free cinnamic acid. The resin containing "cytorezinol" constitutes 30-40% of the balm and 20-25% of the essential oil. The traditional medicinal use of the balm in various diseases and the low biological activity of the essential oil alone show that the components in the balm have a synergistic effect. In this study, the most effective biological activity of essential oil is found as the antimicrobial effect. It is important to search for alternative antimicrobial agents because of resistance to existing antimicrobial drugs over time. Styrax liquidus essential oil can be considered a natural antimicrobial agent due to its strong antimicrobial activity against *A. baumannii* and *C. glabrata* strains.

Ethics

Ethics Committee Approval: Our study does not require ethics committee approval.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: B.B.A., G.I., B.D., Design: B.B.A., Data Collection or Processing: B.B.A., Analysis or Interpretation: B.B.A., G.I., B.Z.K., B.D., Literature Search: B.B.A., Writing: B.B.A., G.I., B.Z.K., B.D.

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Original Article

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The Color Change of a Novel Single-shade Composite Immersed in Different Beverages

Farklı İçeceklere Batırılma Sonrası Yeni Nesil Tek Renk Kompozitin Renk Değişimi

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ABSTRACT

Objective: The purpose of this study was to evaluate the effects of different beverages on the color change of a novel single-shade composite and two different composite resins.

Methods: Three different resin composites were used: Microhybrid (MH), single- shade supra-nanohybrid (SS) and nano-ceramic composite resin (NC). Totally, 120 disc-shaped samples were prepared (N=40) and subdivided into 4 groups according to the immersion beverages:tea, coffee, cola and distilled water (control) (n=10). CIELAB coordinates were obtained using a spectrophotometer device and color change (ΔE) before and after immersion in beverages were calculated. For statistical analysis, two-way ANOVA and Bonferroni tests were used ($p<0.05$).

Results: Coffee showed significantly the highest ΔE values compared to other tested beverages for all resin composites. Besides, tea exhibited significantly higher ΔE values than distilled water. Tea showed significantly higher ΔE values than cola for SS and MH. Regarding the resin composites, SS showed significantly the highest ΔE values than other tested composites after immersion in coffee. SS showed significantly higher ΔE values than MH after immersion in tea. NC showed significantly higher ΔE values than MH after immersion in cola ($p<0.05$).

Conclusion: Coffee caused the highest color change in all tested composites. Besides, SS supra-nanohybrid composite showed significantly higher color change than MH composite after immersion in coffee and tea.

Keywords: Color, composite, microhybrid, single-shade, nanohybrid, nano-ceramic

ÖZ

Amaç: Bu çalışmanın amacı, farklı içeceklerin yeni nesil tek renk kompozitin ve iki farklı kompozit rezinin renk değişimi üzerine etkisini incelemektir.

Yöntemler: Üç farklı rezin kompozit kullanılmıştır: Mikrohibrit (MH), tek renk supra-nanohibrit (SS) ve nano-seramik kompozit rezin (NC). Toplam 120 disk şeklinde örnek hazırlanmış (N=40) ve bekletilme içeceklerine göre 4 gruba ayrılmıştır: Çay, kahve, kola ve distile su (kontrol) (n=10). CIELAB koordinatları, spektrofotometre cihazı kullanılarak elde edilmiş ve renk değişimleri (ΔE) içeceklere batırılma öncesi ve sonrası hesaplanmıştır. İstatistiksel analizler, iki yönlü ANOVA ve Bonferroni testleri kullanılarak yapılmıştır ($p<0,05$).

Bulgular: Kahve, tüm kompozit rezinler için istatistiksel olarak en yüksek ΔE değerleri göstermiştir. Ayrıca, çay, distile suya göre istatistiksel olarak daha yüksek ΔE değerleri sergilemiştir. SS ve MH için çay, kolaya göre istatistiksel olarak daha yüksek ΔE değerleri göstermiştir. Rezin kompozitler kıyaslandığında, kahvede bekletme sonrası SS, diğer test edilen kompozitlere kıyasla istatistiksel olarak en yüksek ΔE değerleri göstermiştir. Çayda bekletme sonrası SS, MH'ye göre istatistiksel olarak daha yüksek ΔE değerleri göstermiştir. Kolada bekletme sonrası NC, MH'ye göre istatistiksel olarak daha yüksek ΔE değerleri göstermiştir ($p<0,05$).

Sonuç: Tüm test edilen kompozitler için kahve en yüksek renk değişimine neden olmuştur. Ayrıca, çay ve kahvede bekletilme sonrası SS-nanohibrit kompozit, MH kompozite göre belirgin olarak daha fazla renk değişimi göstermiştir.

Anahtar Sözcükler: Renk, kompozit, mikrohibrit, tek renk, nanohibrit, nano-seramik

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Introduction

In recent years, microhybrid (MH) composite resins are frequently preferred in either anterior or posterior restorations due to their good esthetic properties and high wear resistance (1). With the developments in nano technology, nano-composite resins are comprised of nano-sized particles which result in better polishability and reduced filler size and increased filler amount and wear resistance (2,3). Especially, new generation nano-ceramic composite resins with superior abrasion and fracture resistance and good polishability have been popular (4).

The different color options of composite resins can provide a restoration that is esthetically similar to natural tooth tissue (5). Composite resins have been launched in multiple enamel and dentin colors with different opacities and translucencies. Therefore, shade matching procedure may be complicated with the inventory, and results in increased cost and chairside time (6). As a result, the tendency to simplify the shade options can ensure the development of universal composite resins which have a universal opacity (7). Recently, a novel, universal composite with a single shade matching all colors from A1 to D4 has been developed, in accordance with the manufacturers' claims (7,8). This property can be obtained from the inclusion of regular spherical fillers, which can produce red to-yellow color as ambient light passes through the restorative material (7). Color shifting of resin composites originates from two main factors: the blending effect (primarily perceptual, subjective, and not measurable) and a quantifiable, physical translucency associated with composition (9). The term "chameleon effect" describes blending effect, color assimilation and color induction (10). This unique property of "blending effect" or "chameleon effect" defines the capability of a restorative material to achieve a color similar to that of its environmental tooth tissues (11).

It was reported that filler particles of resin composites were separated from the organic matrix and that their matrix structure showed signs of deteriorations when restorative materials wereexposed to temperature changes and low pH in the oral conditions (12). There are various studies in the literature on the effects of beverages consumed in daily life on the color change of restorative materials (13,14). However, in the literature, there is limited data on the color change of single-shade composites in beverages such as tea, coffee and cola that are frequently consumed in daily life. Therefore, the aim of this study was to evaluate the effects of different beverages on the color change of a novel single-shade composite and two different types of resin composites. The tested null hypothesis of this in vitro study was that the beverages would not affect the color change of different composite resins.

Methods

The sample size calculation was determined on the estimated effect size between groups according to the literature (12). It was stated that 10 samples were required per each group to obtain a medium effect size (d=0.50) at 90% power and 5% type 1 error rate. Composite resin materials and compositions employed in this in vitro study are shown in Table 1. In this study, three different types of composite resins were used: MH (Filtek Z250, 3M ESPE, USA), single-shade supra-nano hybrid = SS (Omnichroma, Tokuyama Corp., Japan) and nano-ceramic = NC composite resins (CeramX, Dentsply Sirona, Germany). A total of 120 disc-shaped composite resin samples was prepared

Table 1. Material brand names/manufacturers, batch numbers and chemical compositions

Filtek Z250 (A2 shade) **microhybrid=MH**	3M ESPE, St. Paul, MN, USA	NA23939	***Organic matrix composition*** Bis GMA, UDMA, Bis EMA Inorganic Filler Particulate (82 wt%/60 vol%) 0.01-3.5 µm Zirconia-Silica filler
Omnichroma **single-shade supra-nanohybrid=SS**	Tokuyama Dental Corp. (Tokyo, Japan)	038M1	***Organic matrix composition*** 1.6-bis(methacryl-ethyloxycarbonylamino) trimethyl hexane (UDMA), triethylene glycol dimethacrylate (TEGDMA), mequinol, dibutyl hydroxyl toluene and UV absorber. ***Inorganic filler particulate*** (79 wt%, 68 vol%) spherical silica-zirconia filler (mean particle size: 0.3 µm, particle size range: 0.2 to 0.4 µm)
Ceram X SphereTEC one (A2 shade) **nano-ceramic=NC**	Dentsply Sirona (York, USA)	180500110	***Organic matrix composition*** methacrylate modified polysiloxane(organically modified ceramic), dimethacrylate resins, Camphorquinone, Ethyl-4(dimethylamino) benzoate, Bis(4-methyl-phenyl)iodonium hexafluorophosphate ***Inorganic filler particulate*** (72-73 wt%/48-50 vol%) Barium glass, pre-polymerized filler, ytterbium fluoride, 0.1-3 µm filler size (0.6 µm)

BIS-GMA: Bisphenol A glycidyl methacrylate, UDMA: Urethane dimethacrylate, BIS-EMA: Bisphenol A diglycidyl methacrylate ethoxylated, TEGDMA: Triethylene glycol dimethacrylate, µm: Micrometer, Bis-MPEPP: Bis-methacryloxyethoxy phenyl propane, wt%: Weight percentage, vol%: Volume percentage, µm: Micrometer, nm: Nanometer

using teflon molds (diameter: 4 mm, thickness: 2 mm) (N=40). Transparent polyester matrix strip and glass slides were used on both surfaces of samples, and finger pressure was applied to these glass coverslips to obtain a flat sample surface. Then, all samples were polymerized (B.O.) for 20 s using a LED light curing unit (Valo, Ultradent, USA) (1,000 mW/cm^2 power). Light intensity was checked periodically with a radiometer (Demetron LED Radiometer, Kerr Corp., USA). Subsequently, the samples were removed from the teflon molds and kept in distilled water for 24 h at 37 °C in a dark vial. The samples were allocated into 4 subgroups according to the immersion beverages: tea (Lipton, Glasgow, Scotland), coffee (Nescafe Classic, Vaud, Switzerland), cola (Coca Cola, Atlanta, United States) and distilled water (control) (n=10). They were immersed in different beverages for 24 hours every day during 6 days, and the beverages were renewed daily (15). Coffee and tea solutions were prepared by dissolving 12 g coffee powder or 3 g tea powder in 250 mL boiled distilled water (16). After each immersion, samples were washed and stored in distilled water.

Color values were measured from the upper surfaces of the samples using a spectrophotometer device (Vita Easy Shade Advance 4.0, VITA Zahnfabrik, Germany) before and after immersion in beverages. Measurements were carried out under D65 standard lighting conditions (14). The spectrophotometer device was calibrated before each measurement. Initial measurements were recorded as CIE (L0, ao, b0) and 6th day measurements as CIE (L1,a1,b1). Measurements were repeated 3 times for each sample and average CIE (L*a*b*) values were calculated. The formula was used to calculate the differences (ΔE) between the acquired measurements:

$$\Delta E^* = [(L1^* - L0^*)^2 + (a1^* - a0^*)^2 + (b1^* - b0^*)^2]^{1/2}$$

All measurements were carried out by a second operator (L.F.) who was unaware of the resin composites and beverages employed in this study.

Statistical Analysis

Statistical analysis was performed using the IBM Statistical Package for Social Sciences 22.0 software (SPSS Inc., Chicago, IL, USA) for Windows. The normality of the variances was analyzed with Shapiro-Wilk test and the homogeneity of the variances were analyzed with Box's M test. The data were normally distributed. Two-way analysis of variance (ANOVA) was used to compare within- and between-group differences. All pairwise comparisons were done using Bonferroni test. Statistical significance was considered at a confidence level of 0.05 for all analyses.

Results

Mean color change values and standard deviations of all tested groups are shown in Table 2. When comparing the beverages, coffee showed significantly the highest color change than other tested beverages for all resin composites ($p<0.05$). Tea exhibited significantly higher color change than distilled water for all composites ($p<0.05$). There were no significant differences in terms of color change between tea and cola and cola and distilled water for NC ($p>0.05$). Tea showed significantly higher color change than cola ($p<0.05$), whereas no significant differences were found in terms of color change between cola and distilled water ($p>0.05$) for SS and MH. When comparing the composite resins, SS showed significantly the highest color change than other tested composite resins after immersion in coffee ($p<0.05$). SS showed significantly higher color change than MH after immersion in tea ($p<0.05$). NC showed significantly higher color change than MH after immersion in cola ($p<0.05$). There were no significant differences in terms of color change among composite resins after immersion in distilled water ($p>0.05$).

Discussion

In the current study, the effects of beverages on the color change of single-shade composite and two different types of composite resins were evaluated. The tested null hypothesis that the beverages would not affect the color change of different types of composite resins, was partially rejected.

When composite resin restorations are exposed to fluids in the oral cavity, the organic matrix structure absorbs fluids (17). The sorption and solubility may produce deleterious effects such as swelling, plasticization and softening, oxidation and hydrolysis on the structure and function of a resin matrix (18,19). Discoloration is one of the common reasons of deterioration of resin composites (20). Microcracks or voids at the filler and matrix interface may lead to penetration of dyes and discoloration (21).

There are two main mechanisms regarding the color change of resin composites: intrinsic and extrinsic factors (15). Internal

Table 2. Mean color change values and standard deviations of all tested groups (ΔE ± SD)

	NC	SS	MH	p
Tea	5.84±2.01^{ABa}	7.12±1.8^{Aa}	4.4±1.18^{Ba}	0.003
Coffee	9.56±1.98^{Ac}	18.45±2.7^{Bb}	8.1±1.51^{Ab}	<0.001
Cola	3.88±2.38^{Aab}	2.56±1.4^{ABc}	1.4±0.81^{Bc}	0.006
Distilled water	2.47±1.46^{b}	2.35±1.45^{Ac}	1.78±0.48^{c}	0.625
p	<0.001	<0.001	<0.001	

*Different capital letters in the same row indicate statistically significant differences.
Different lowercase letters in same column indicate statistically significant differences ($p<0.05$)
SD: Standard deviation

discoloration depends on the structure of the resin composites, the properties of the filler particles, the chemical properties such as water sorption and degree of polymerization (21). External discoloration may occur due to poor oral hygiene, eating habits, and cigarette consumption (22).

Guler et al. (23) indicated that immersion in coffee for 24 h would imitate approximately 30 days of regular consumption. In this study, the similar time-simulation protocol was carried out for the other beverages and the composite resin samples were immersed for 24 h at 37 ^{0}C during 6 days, which corresponded to 6 months of daily use.

Digital color measurement devices have been used to determine the color properties of restorative materials (24). Vita Easyshade is the most preferred spectrophotometer device, as it is reproducible and reliable in terms of color detection (25). In this study, spectrophotometer (Vita Easyshade Advance 4.0) device was used for color measurement of the samples. Subjective errors are thus eliminated, and more importantly, it is more sensitive in measuring small differences than with the naked eye (24). The two systems used for spectrophotometric analysis are CIELAB ve CIEDE 2000 (26). In this study, CIE lab color difference formula was preferred since it was stated in the literature that this formula was widely used.

The CIELAB for evaluating color change measurement; using the equation $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$ is highly recommended compared to the 3 color vector (L *, a * and b *). ΔE is summarized as the difference in 3 color vectors and presents their combined effect on color change (27). In this system, clinically acceptable and desirable ΔE value is considered to be below 3.3. (28). In this study, when the effect of beverages on the color changes of different composite resins were evaluated, ΔE values were determined above the clinically acceptable value for all tested composite resins after immersion in tea and coffee. This finding was in line with Ertaş et al. (29), who indicated that tea and coffee caused significant discoloration on MH and nanohybrid composite resins with color change above acceptability threshold. In this study, with respect to the beverages, coffee caused significantly the highest color change for all tested composite resins. This finding was in line with Domingos et al. (30), who indicated that coffee caused higher color change compared to tea and cola for nanofilled composite resins. Tavangar et al. (31), who evaluated the color change of nanohybrid and MH composites, indicated that coffee and tea caused more color change than cola. Besides, in this study, tea caused significantly higher color change than cola for MH and single-shade supra-nanohybrid composite resins. The sorption of yellow colorants of the coffee and tea may occur due to the organic phase of the restorative material (32). Silva et al. (32) reported that tea and coffee could produce water sorption and color degradation due to hot temperatures (65-80 ^{o}C), which might also cause to the surface degradation of the polymeric matrix. Besides, in this study, tea caused significantly higher color change than distilled water for all tested composite resins. However, no significant color changes were observed between cola and distilled water. Ertaş et al. (29) reported that coffee and tea had a greater influence on the staining of nanofilled composite than cola and water.

The contents of the restorative materials and the characteristics of the filler particles are notable in the tendency of discoloration (33). The hydrophilicity of the resin matrix and the degree of water absorption affect the susceptibility of the resin composites to staining. After immersion in coffee, single-shade supra-nanohybrid composite resin exhibited significantly higher color change than other composites. A previous study revealed that resins formulated with Bis-EMA tended to be less susceptible to dissolution when compared with those with Bis-GMA and UDMA (34). Bis-GMA is usually combined with low viscosity monomers like TEGDMA (35). However, the addition of TEGDMA increases water sorption and hinders color stability (36). Filtek Z250 contains three major components: Bis-GMA, UDMA, and Bis-EMA while Omnichroma consist of UDMA and TEGDMA. This finding can be contributed to the hydrophilic monomer, TEGDMA of this restorative material. Increased filler content could lead to lower water absorption and less surface degradation (37). This could explain why Filtek Z250 exhibited the best overall results. Besides, this composite contains Bis-EMA monomer with high molecular weight in its organic matrix and it is more resistant to degradation (19). Besides, parameters such as the amount, size, distribution and type of filler could affect the water absorption of the resin composite (38). In this study, single-shade supra-nanohybrid composite resin, which had a small filler size, had significantly higher color change than MH composite resin with a larger filler size after immersion in tea and coffee. This finding is in accordance with the results of the study by Gönülol and Yılmaz (39), who concluded that composites with smaller filler size showed higher discoloration. Besides, Villalta et al. (40) reported that nano-filled composite resin showed higher color change than micro-filled composite. In this study, after immersion in cola, nano-ceramic composite resin exhibited significantly higher color change than MH composite. No significant differences in terms of color change were found between nano-ceramic and single-shade supra-nanohybrid composite resin. Llena et al. (41) also reported that nano-ceramic composite resin showed similar color change to nano- hybrid composite after immersion in cola.

In this study, poorly polymerized resin-rich layer referred to as oxygen inhibition layer was not removed because the polishing procedures were not performed to the composite resin samples. In addition, the samples were not subjected to thermocycling aging. Consequently, these two factors might influence the color change of composite resin materials. Different parameters such as the properties of surface roughness and hardness were not investigated. Therefore, further studies should focus on the effects of these factors on color stability, surface roughness and microhardness change of single-shade composite resins. Besides, SEM images could be beneficial to determine the surface morphology of the composite resins.

Conclusion

Within the limitations of this study,

The following conclusions can be drawn:

1) For all tested composite resin, coffee caused the highest color change than other beverages. Besides, tea caused higher color change than distilled water. No significant differences in color change were found between cola and distilled water

2) After immersion in tea and coffee, single-shade supra-nanohybrid composite resin showed higher color change than MH composite.

3) After immersion in cola, nano-ceramic composite resin showed higher color change than MH composite resin.

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Original Article

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Anticholinesterase and Anti-inflammatory Activities of Essential Oils of Naturally Grown *Daucus* L. Species in Turkey

Türkiye'de Doğal Olarak Yetişen *Daucus* L. Türlerinin Uçucu Yağlarının Antikolinesteraz ve Anti-enflamatuvar Aktiviteleri

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ABSTRACT

Objective: This present study was conducted to determine the interspecific chemical variability and evaluate the biological effects of the essential oils of *Daucus* L. species growing naturally in Turkey. The species were *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* and *D. conchitae* (endemic).

Methods: The essential oils were obtained from fruit samples by distillation method and were analyzed both by GC-FID and GC-MS. The anti-inflammatory and anticholinesterase effects of the essential oils were investigated. The anti-inflammatory effect was evaluated by *in vitro* LOX enzyme inhibition activity. The anticholinesterase effect was tested on AChE and BChE enzymes.

Results: The components, ratios, and yields of *D. carota* essential oils differed depending on the locations where the samples were collected. The main components were detected as carotol (1-74.6%), β-bisabolen (0.9-62.4%), 11αH-himachal-4-en-1β-ol (0.3-49.4%), *trans*-methylisoeugenol (1-45.7%). The main volatile compounds were found in *D. broteri, D. guttatus, D. involucratus, D. littoralis, D. conchitae* as β-sinensal (30.4%), methyleugenol (30.5%), methyleugenol (40.9%), α-humulene (29.4%), methyleugenol (29.6%) respectively. The essential oils didn't exhibit anti-inflammatory activity. Two essential oil samples of *D. carota* showed high anticholinesterase effects compared to the standard. The AChE IC_{50} was calculated as 6.04±0.30 μg/mL, 2.15±0.10 μg/mL (Galantamine IC_{50} 1.13±0.02 μg/mL) and BChE

ÖZ

Amaç: Bu çalışma, Türkiye'de doğal olarak yetişen *Daucus* L. türlerinin uçucu yağlarının türler arası kimyasal değişkenliğini belirlemek ve biyolojik etkilerini değerlendirmek amacıyla yapılmıştır. Türler şunlardır: *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* ve *D. conchitae* (endemik).

Yöntemler: Meyve örneklerinden distilasyon yöntemiyle elde edilen uçucu yağlar GK-AİD ve GK-KS sistemleri ile analiz edilmiştir. Uçucu yağların anti-enflamatuvar ve antikolinesteraz etkileri araştırılmıştır. Anti-enflamatuvar etki, *in vitro* LOX enzim inhibisyon aktivitesi ile değerlendirilmiştir. Antikolinesteraz etkisi AChE ve BChE enzimleri üzerinde test edilmiştir.

Bulgular: *D. carota* uçucu yağlarının bileşenleri, oranları ve verimleri, örneklerin toplandığı yerlere bağlı olarak farklılık göstermiştir. Ana bileşenler; karotol (%1-74,6), β-bisabolen (%0,9-62,4), 11αH-himakal-4-en-1β-ol (%0,3-49,4), trans-metilizoöjenol (%1-45,7) olarak tespit edilmiştir. *D. broteri, D. guttatus, D. involucratus, D. littoralis, D. conchitae* türlerine ait uçucu yağ ana bileşenleri sırasıyla; β-sinensal (%30,4), metilöjenol (%30,5), metilöjenol (%40,9), α-humulen (%29,4) ve metilöjenol (%29,6) olarak belirlenmiştir. Uçucu yağlar, anti-inflamatuvar aktivite göstermemiştir. *D. carota* türüne ait iki uçucu yağ numunesi, standarda kıyasla yüksek antikolinesteraz etki göstermiştir. AChE IC_{50} 6,04±0,30 μg/mL, 2,15±0,10 μg/mL (Galantamin IC_{50} 1,13±0,02 μg/mL) ve BChE IC_{50} 11,32±0,20 μg/mL, 31,03±0,02 μg/mL (Galantamin

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IC_{50} 11.32±0.20 μg/mL, 31.03±0.02 μg/mL (Galantamine IC_{50} 12.15±0.36 μg/mL). These essential oils contained high levels of 11αH-himachal-4-en-1β-ol (25.04%, 49.42%).

Conclusion: Because of their anticholinesterase potential, some *D. carote* essential oils can be evaluated in the preparation of pharmaceutical or nutraceutical products as a complementary therapy for Alzheimer's disease by standardizing their components.

Keywords: *Daucus* spp., essential oil, anti-inflammatory, anticholinesterase

IC_{50} 12,15±0,36 μg/mL) olarak hesaplanmıştır. Bu uçucu yağlar yüksek seviyelerde 11αH-himachal-4-en-1β-ol (%25,04, %49,42) içermektedir.

Sonuç: Antikolinesteraz potansiyelleri nedeniyle bazı *D. carota* uçucu yağları, bileşenleri standardize edilerek Alzheimer hastalığında tedaviye destek amaçlı farmasötik veya nutrasötik ürünlerin hazırlanmasında değerlendirilebilir.

Anahtar Sözcükler: *Daucus* spp., uçucu yağ, anti-enflamatuvar, antikolinesteraz

Introduction

Research on plants for protection of health and treatment of diseases has come for ages and increasingly continues. Wild plants are an important resource for new drug discoveries. Essential oils are mixtures of natural terpenes with a wide range of pharmacological activities and their preparation from various plant species has become increasingly popular in recent years. Antimicrobial, sedative, antispasmodic, anthelmintic, anti-inflammatory, expectorant and diuretic effects are some of them (1). *Daucus* L. belongs to the essential oil content rich Apiaceae family. In the Flora of Turkey and the East Islands, 6 species including *D. carota* L., *D. broteri* Ten., *D. guttatus* Sibth Sm., *D. littoralis* Sm., *D. involucratus* Sm. and *D. conchitae* W Greuter (endemic) are registered (2,3). *D. carota* L. (carrot) is the best-known species of the genus and is cultured widely around the world. Carrot root is used as food and essential oil derived from fruits is used in perfumery (4). *D. carota* fruits are used in folk medicine as stomachic, carminative, diuretic, anthelmintic, emmenagogue, contraceptive and aphrodisiac. Young aerial parts of wild types are also eaten as vegetables (5).

Phytochemical and biological activity studies on *Daucus* species other than *D. carota* are very limited. Several studies have described that essential oils and extracts obtained from *D. carota* show a wide range of biological activities, such as antifungal, antibacterial, antioxidant, anti-inflammatory, hepatoprotective, antihyperlipidemic and antitumour activities against human oesophageal cancer cell (6-14). *D. guttatus* essential oil showed significant antibacterial activity against *Corynebacterium pyogenes* (15). When examined for use in the treatment of tuberculosis, the essential oil obtained from the aerial parts of *D. littoralis* showed antibacterial effect against *Mycobacterium tuberculosis* (16).

In this paper, the chemical compositions of the fruit essential oils of all *Daucus* species grown in Turkey were comparatively analyzed for the first time. The volatile compounds, extracted using hydrodistillation, were analyzed by gas chromatography/flame ionization detection (GC/FID) and GC-mass spectrometry (GC/MS). Also, *in vitro* anticholinesterase and anti-inflammatory activities of *Daucus* species' essential oils were investigated. .

Examination of plants with ethnomedicinal use in drug research makes important contributions to the development of new drugs. Turkey has a rich floristic structure because experiences different geological periods and covers ecologically different regions. Many plants in Turkey have traditional medicinal uses for various diseases and one of them is *D. carota*. The purpose of this study was to evaluate the biological effects and to determine the interspecific chemical variability of the essential oils of *Daucus* species growing naturally in Turkey. Since *D. carota* is the most common species of the genus, samples taken from different locations were examined and compared. On the other hand, other species of the genus had limited growing areas, so they were collected from only one location. The essential oils, which gave the most effective results in biological activity studies, were divided into sub-fractions to determine the effective components that caused the activity. Content analysis and activity studies of the obtained fractions were performed again.

Methods

Plant Material

The following 6 species belonging to *Daucus* genera were collected by Betül Büyükkılıç-Altınbaşak in the fruiting period from the Marmara, Aegean, and Mediterranean regions: *D. carota*, *D. broteri*, *D. guttatus*, *D. littoralis*, *D. involucratus* and *D. conchitae* (endemic). Plant samples were collected from İstanbul, Denizli, Muğla, and Antalya between June and September in 2016, 2017 and 2018 (Figure 1). Taxonomical determinations of the collected specimens were made using the Flora of Turkey and the Aegean Islands by Dr. Gülay Ecevit-Genç and Betül Büyükkılıç-Altınbaşak (3,17). The voucher specimens were kept at the Herbarium of the Faculty of Pharmacy, İstanbul University (ISTE). Nine samples of *D. carota* species were collected from different locations. Due to the large number of samples examined, a code was given to each sample to avoid confusion. This is presented in Table 1. These scientific names were verified for each of the species using the International Plant Name Index (18).

Extraction of Essential Oils

The dried fruits of *Daucus* species were cut into small pieces and immediately subjected to hydrodistillation in Clevenger apparatus for 3 hours. Essential oils, thus obtained were stored in sealed vials at +4 °C until analyzed and tested.

Gas Chromatography Analysis

The GC analysis was performed on the Agilent 6,890N GC system at an FID detector temperature of 300 °C. To achieve

Table 1. List of *Daucus* species

Scientific name	ISTE herbarium number	Location and collection dates	Code
D. carota	ISTE 115524	A2(A), İstanbul, 119 m, 29.08.2016	D1
D. carota	ISTE 115525	A2(E), İstanbul, 5 m, 30.08.2016	D2
D. carota	ISTE 115526	A2(E), İstanbul, 47 m, 30.08.2016	D3
D. carota	ISTE 115527	A2(E), İstanbul, 119 m, 30.08.2016	D4
D. carota	ISTE 115528	C2, Denizli, 483 m, 10.09.2016	D5
D. carota	ISTE 115529	C2, Denizli, 976 m, 10.09.2016	D6
D. carota	ISTE 115530	C1, Muğla, 50 m, 14.09.2016	D7
D. carota	ISTE 115532	C1, Muğla, 16 m, 15.09.2016	D8
D. carota	ISTE 115533	C2, Denizli, 1158 m, 27.08.2017	D9
D. broteri	ISTE 115534	C2, Denizli, 735 m, 27.08.2017	D10
D. guttatus	ISTE 115531	C1, Muğla, 50 m, 14.09.2016	D11
D. littoralis	ISTE 115790	C2, Antalya, 52 m, 08.06.2018	D12
D. involucratus	ISTE 115792	C3, Antalya, 21 m, 09.06.2018	D13
D. conchitae	ISTE 115791	C2, Antalya, 360 m, 08.06.2018	D14

the same elution order as GC/MS, the simultaneous automatic injection was performed on a replicate of the same column, applying the same operating conditions. The relative percentage amounts of the separated compounds were calculated from the FID chromatograms. The analysis results are given in Table 2.

The GC/MS analysis was performed on an Agilent 5,975 GC/MSD system on an Innowax FSC column (60 m x0.25 mm, 0.25 µm film thickness) using helium (0.8 mL/min) as carrier gas. The GC oven temperature was held at 60 °C for 10 minutes and programmed at 220 °C at 4 °C/min, for 10 minutes, then 240 °C at 1 ratio. °C/min. The division ratio was set to 40:1 and the injector temperature was set to 250 °C. Mass spectra were recorded at 70 eV. The mass range was *m/z* 35 to 450.

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to series of *n*-alkanes. Commercial (Wiley GC/MS Library, MassFinder Software 4.0) (19,20) and in-house libraries ("Başer Library of Essential Oil Constituents" which was built up by genuine compounds and components of known oils) were used.

Anti-inflammatory Activity

In the present study, the essential oils were evaluated for possible anti-inflammatory activity by *in vitro* soybean lipoxygenase (Soy LOX) (1.13.11.12, Type I-B, 7.9 unit/mg) enzyme inhibition which was performed spectrophotometrically. Based on the study conducted by Baylac and Racine (21) in 2003, the method updated in microscale was applied (21,22). 1.94 mL of potassium phosphate buffer (100 mM; pH: 8.80), 40 µL essential oil samples at a concentration of 100 µg/mL and 20 µL lipoxygenase enzyme were incubated for 10 min at 25 °C. Three hundred µL of this mixture was added to each well of quartz microplate. The reaction was then initiated by the addition of 7.5 µL linoleic acid solution. The experiments were carried out in 3 replicates. The results were calculated by recording the changing absorbance values every minute for 10 minutes in the ELISA reader (ELx808IU) at 234 nm. nordihydroguaiaretic acid (NDGA) was used as a positive control at 20, 12, 4, 3, 2 µg/mL concentrations.

Determination of AChE and BChE Inhibitory Activities

The essential oils' anticholinesterase activity was determined by using *in vitro* AChE and BuChE enzymes inhibition assays. AChE and BuChE inhibition activities were determined using the method found by Ellman et al. (23). Galantamine is used as reference compound. The IC_{50} was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of seven different concentrations. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates in the reaction, and 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used as a reagent. Stock solutions of essential oils and galantamine were prepared with methanol at a 4,000 µg/mL concentration. Hundred and fifty µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution, and 20 µL of AChE (2.476x10-4 U/µL) (or 3.1813x10-4 U/µL of BuChE) solution were mixed. It was incubated at 25 °C for 15 minutes. Ten µL of DTNB solution at a concentration of 2 mg/mL was added.The reaction was initiated by the addition of 10 µL acetylthiocholine iodide (or butyrylthiocholine iodide). In this method, the activity was measured by following the yellow color produced as a result of the presence of thio anion which was produced by the enzymatic hydrolysis of the substrate with DTNB. Also, methanol was used as a control solvent. The hydrolysis of the substrates was monitored using a BioTek Power Wave XS at 412 nm (24).

Fractionation by Column Chromatography

The essential oils effective in biological activity studies were divided into 2 sub-fractions by column chromatography to increase the proportion of their major components. Silica gel (7733; Merck) was used as column adsorbent in fractionation. It was first eluted with n-hexane and then with ethanol. Accumulated fractions were checked by TLC (thin layer

Table 2. Chemical composition of the essential oils of *Daucus* species

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1032	α-Pinene	0.3	0.2	0.4	0.1	2.3	0.8	0.2	0.4	0.5	2.6	0.1	4.6	2.0	3.0
1035	α-Thujene	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1
1076	Camphene	-	-	-	tr	0.2	0.1	-	-	tr	0.1	-	0.9	0.1	0.2
1118	β-Pinene	0.2	0.1	0.2	0.1	0.3	0.6	0.1	0.1	0.1	22.3	0.1	0.2	0.1	0.3
1132	Sabinene	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	1.4	0.1	0.2	0.9	1.3
1174	Myrcene	0.1	tr	tr	tr	0.2	0.1	tr	0.1	0.1	1.7	0.1	1.5	0.2	0.4
1188	α-Terpinene	-	-	-	-	-	-	tr	-	-	0.1	-	-	tr	-
1203	Limonene	0.1	0.2	0.1	0.1	-	0.3	0.1	0.2	0.2	0.7	0.1	1.6	0.2	0.7
1218	β-Phellandrene	-	-	-	-	tr	tr	tr	tr	tr	0.3	-	0.5	tr	0.1
1255	γ-Terpinene	tr	-	-	-	-	tr	tr	tr	tr	0.7	tr	0.1	0.2	tr
1266	(E)-β-Ocimene	tr	-	tr	-	-	-	tr	-	-	tr	-	-	0.1	0.2
1290	Terpinolene	-	-	-	-	tr	-	-	-	-	0.1	-	tr	0.1	0.1
1280	p-Cymene	tr	tr	tr	tr	tr	tr	tr	tr	tr	1.7	0.1	0.1	0.4	0.3
1398	3-Octanol	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-
1443	Trans-Sabinene hydrate	-	-	-	-	-	-	-	-	tr	0.1	-	-	0.1	-
1452	1-Octen-3-ol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1466	α-Cubebene	-	-	-	-	-	-	-	-	-	0.4	-	-	0.1	-
1482	Longipinene	1.4	0.3	0.7	-	2.4	-	13.5	5.6	tr	-	-	0.1	-	-
1493	α-Ylangene	-	-	-	-	-	-	0.1	tr	-	-	-	-	-	-
1497	α-Copaene	-	-	-	-	-	-	-	-	-	0.6	-	0.2	0.1	0.2
1504	Daucene	2.1	0.1	-	-	1.8	4.8	-	-	3.3	-	0.4	-	-	-
1513	Longicyclene	-	-	-	-	0.1	-	0.2	0.1	-	-	-	-	-	-
1532	Camphor	-	-	-	-	-	-	-	-	-	-	0.2	7.9	-	0.5
1535	β-Bourbonene	-	-	-	-	-	-	-	-	-	0.2	-	-	tr	-
1549	β-Cubebene	0.4	-	-	-	0.4	1.0	-	-	0.7	1.2	-	0.1	0.3	0.3
1553	Linalool	0.3	0.3	-	-	0.1	0.2	0.3	0.4	0.1	-	0.3	1.9	0.1	0.3
1568	Trans-α-Bergamotene	0.9	0.3	0.4	0.1	0.9	2.0	1.8	2.4	1.2	-	-	-	-	-
1570	Trans-Myrtanal	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1583	Junipene (Longifolene)	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1586	Pinocarvone	tr	-	-	-	0.1	-	-	-	-	0.6	-	-	-	-
1597	Bornyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	tr	0.1	-
1599	β-Copaene	-	-	-	-	-	0.3	-	-	-	-	-	tr	-	-
1600	β-Elemene	-	-	-	-	-	-	0.1	-	-	-	0.2	0.2	0.1	0.2
1601	Nopinone	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1607	Thymol methyl ether	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
1611	Terpinen-4-ol	-	-	-	-	-	-	-	-	-	0.4	-	-	0.2	-
1612	β-Caryophyllene	0.3	-	0.5	-	0.5	0.6	1.6	1.0	0.6	2.2	10.1	4.4	4.1	3.4
1613	β-Cedrene	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
1613	Acora-2,4-diene	-	-	-	-	-	-	-	-	-	0.1	-	-	-	0.1
1639	Cadina-3,5-diene	0.2	-	-	-	0.2	0.4	-	-	tr	-	-	-	-	-
1648	Myrtenal	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-
1661	α-Himachalene	0.1	-	-	-	0.1	-	0.6	0.3	-	-	-	-	-	-
1661	Trans-Pinocarvyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
1664	Trans-Pinocarveol	-	-	-	-	0.2	-	-	-	-	0.8	-	-	tr	-
1664	(Z)-β-Santalene	-	-	-	-	-	-	-	-	tr	-	-	-	-	-

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1669	Sesquisabinene	0.1	0.4	0.5	0.4	0.1	-	0.2	0.1	tr	-	-	-	-	-
1668	(Z)-β-Farnesene	1.4	0.2	0.2	0.2	1.1	2.8	0.8	0.5	2.5	0.8	0.2	-	-	-
1674	Sesquisabinene-B	-	-	-	0.2	0.1	-	-	-	tr	-	-	-	-	-
1677	Epizonarene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
1683	Trans-Verbenol	0.2	-	0.1	-	0.6	0.1	-	-	-	0.2	0.2	-	0.2	tr
1687	α-Humulene	-	-	0.1	-	0.1	0.1	0.2	0.1	0.4	0.2	1.8	29.4	3.1	8.7
1687	Methyl chavicol (Estragole)	-	-	-	-	-	-	-	-	-	-	-	10.9	-	-
1688	Selina-4,11-diene	-	-	-	-	-	-	-	-	-	-	2.5	0.1	3.8	3.0
1690	α-Acoradiene	-	1.8	-	-	-	-	-	-	-	0.1	-	-	-	-
1693	β-Acoradiene	0.8	-	0.3	1.3	0.2	-	-	-	-	-	-	-	0.1	-
1695	(E)-β-Farnesene	0.1	-	-	-	-	0.2	0.4	0.4	-	-	-	-	-	-
1698	Myrtenyl acetate	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
1704	γ-Muurolene	-	-	-	-	-	-	-	-	-	-	-	-	0.1	1.0
1706	α-Terpineol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1719	Borneol	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
1726	Germacrene D	-	-	-	-	-	0.1	-	-	-	0.3	-	5.3	-	1.5
1726	α-Zingiberene	0.1	-	-	-	0.1	0.4	-	-	0.3	-	-	-	-	-
1729	β-Himachalene	0.5	0.1	0.2	-	0.8	-	5.6	2.3	0.1	-	-	-	-	-
1740	α-Muurolene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
1741	β-Bisabolene	15.0	47.7	62.4	48.0	5.6	0.9	1.0	8.9	6.0	5.9	-	0.1	1.5	2.3
1742	β-Selinene	-	-	-	-	-	0.4	-	-	-	-	10.4	0.5	10.2	6.5
1743	α-Cadinene	-	-	-	-	-	-	-	-	-	-	0.3	0.1	-	-
1740	α-Selinene	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-
1751	Carvone	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1755	Dauca-8,11-diene	0.3	-	-	-	0.5	1.1	-	-	0.9	-	-	-	-	-
1755	Bicyclogermacrene	0.4	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1755	β-Curcumene	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-
1765	Geranyl acetate	-	0.1	-	-	-	-	-	-	-	-	-	-	1.0	0.7
1773	δ-Cadinene	-	-	-	-	-	-	-	-	-	0.3	0.4	0.3	tr	-
1776	γ-Cadinene	-	-	-	-	-	-	-	-	-	-	1.9	0.5	0.2	-
1783	β-Sesquiphellandrene	-	-	-	-	-	0.4	-	-	0.4	-	-	-	-	-
1784	(E)-α-Bisabolene	0.8	1.5	1.8	1.8	0.2	-	0.6	0.5	-	-	-	-	-	-
1785	7-epi-α-Selinene	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1786	ar-Curcumene	-	-	-	-	-	0.1	-	-	0.1	-	-	-	-	-
1804	Myrtenol	-	-	-	-	0.1	-	-	-	-	0.7	-	-	-	-
1854	Germacrene B	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
1857	Geraniol	2.1	0.1	-	-	-	-	-	-	-	-	-	-	0.6	0.4
1868	Neryl isovalerate	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
1882	α-Dehydro-ar-himachalene	-	-	-	-	-	-	0.1	tr	-	-	-	-	-	-
1888	ar-Himachalene	0.1	tr	0.3	-	1.0	-	0.6	1.1	-	-	-	-	-	-
1900	epi-Cubebol	-	-	-	-	-	-	-	-	-	0.3	-	-	tr	-
1924	γ-Dehydro-ar-himachalene	-	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-
1957	Cubebol	-	-	-	-	-	-	0.3	0.1	-	0.3	-	-	0.1	-
2001	Isocaryophyllene oxide	-	-	-	-	-	-	-	-	-	0.3	0.9	tr	0.1	0.2
2004	Oxido himachalene	-	-	-	-	-	-	tr	-	-	-	-	-	-	-

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
2008	Caryophyllene oxide	0.1	-	0.5	-	0.5	-	0.1	0.2	-	2.1	10.8	0.5	0.7	0.9
2025	Perillyl alcohol	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2030	Methyl eugenol	0.2	-	-	-	-	-	tr	0.3	-	-	30.5	0.2	40.9	29.6
2045	Carotol	42.8	1.7	1.9	1.0	51.4	74.6	-	-	63.1	-	5.8	-	-	-
2045	Humulene epoxide I	-	-	-	-	-	-	-	-	-	-	-	0.4	0.2	0.7
2048	6,7-Epoxy-himachalene (β-himachalene oxide)	-	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-
2071	Humulene epoxide II	-	-	-	-	-	-	-	-	-	0.2	1.0	3.0	0.5	3.8
2080	1,10-di-epi-Cubenol	-	-	-	-	-	-	-	-	-	-	0.5	0.2	0.1	0.3
2081	Humulene epoxide III	-	-	-	-	-	-	-	-	-	-	-	0.2	tr	0.1
2088	1-epi-Cubenol	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2089	6-Methyl-5-(3-methylphenyl) heptan-2-one	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2096	cis-Sesquisabinene hydrate	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-
2104	Viridiflorol	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-
2109	cis-Methylisoeugenol	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-
2131	Hexahydrofarnesyl acetone	-	-	-	-	-	-	-	-	-	0.4	0.3	-	-	-
2131	11αH-himachal-4-en-1β-ol	5.4	1.8	2.7	0.5	11.5	-	49.4	25.0	0.3	-	0.3	-	-	-
2144	Spathulenol	0.2	-	-	-	-	-	-	-	-	0.2	0.2	0.2	-	0.2
2173	6-epi-Cubenol	-	-	-	-	0.5	-	0.8	0.5	-	-	0.4	-	-	-
2187	T-Cadinol	-	-	-	-	-	-	-	-	-	-	8.1	3.5	0.8	6.1
2200	trans-Methylisoeugenol	3.2	5.7	1.0	15.1	-	-	6.6	45.7	-	-	-	0.1	0.1	-
2205	Thymol	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-
2219	2-Himachalen-7-ol	-	-	-	-	-	-	0.5	0.4	-	-	-	-	-	-
2219	Acorenone	-	-	-	0.5	-	-	-	-	-	3.2	-	-	2.9	2.0
2226	Methyl hexadecanoate	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
2228	Acorenone B	-	-	-	0.5	0.4	-	-	-	-	1.2	-	-	1.0	0.8
2231	Torilenol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
2232	α-Bisabolol	0.1	-	-	-	0.6	-	0.3	0.2	0.2	-	-	-	-	-
2237	β-Sinensal	-	-	-	-	-	-	-	-	-	30.4	-	-	-	-
2246	Elemicin	4.8	14.9	1.7	1.9	-	-	-	-	-	-	-	-	2.9	2.1
2248	γ-Asarone	-	-	-	-	-	-	-	-	-	-	-	-	18.5	-
2255	α-Cadinol	-	-	-	-	-	-	-	-	-	-	0.6	0.4	-	0.5
2265	Longiverbenone (Vulgarone B)	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-
2269	Guaia-6,10(14)-diene-4β-ol	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-
2289	cis-Isoelemicine	-	-	-	-	-	-	-	-	-	-	-	-	-	13.7
2296	Myristicin	-	-	-	-	-	-	-	-	0.1	5.7	-	-	-	-
2300	Cryptomerione	0.3	0.7	1.0	0.7	-	-	-	-	-	-	-	-	-	-
2312	Daucol	0.9	-	-	-	4.1	0.4	-	-	0.2	-	-	-	-	-
2316	Caryophylladienol I	-	-	-	-	-	-	-	-	-	0.2	0.4	0.1	0.1	-
2320	Juniper camphor	0.4	-	0.3	-	-	-	-	0.1	0.1	-	-	-	-	-
2324	Caryophylladienol II	-	-	-	-	-	-	-	-	-	0.4	0.7	-	-	-
2349	Cadina-4,10(15)-dien-3-one	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-
2361	β-Asarone	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-
2369	Eudesma-4(15),7-dien-4β-ol	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
2392	Caryophyllenol II	-	-	-	-	-	-	-	-	-	0.4	1.0	-	-	0.5

Table 2. Continued

RRI	Compound	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
2404	trans-Isoelemicin	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
2431	Methyl stearate	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-
2467	Methyl elaidate	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
2478	α-Asarone	2.0	15.2	13.9	18.8	1.1	0.2	0.5	-	-	-	-	-	-	-
2500	Pentacosane	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2509	Methyl linoleate	-	-	-	0.9	-	-	-	-	-	-	-	-	-	-
2607	14-Hydroxy-δ-cadinene	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2622	Phytol	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2670	Tetradecanoic acid	-	-	-	-	-	-	-	-	-	0.3	0.5	-	-	-
2700	Heptacosane	-	-	-	-	-	-	-	-	-	0.3	-	-	-	-
2822	Pentadecanoic acid	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2931	Hexadecenoic acid	-	-	0.5	0.5	tr	-	-	-	-	2.8	2.7	0.7	tr	-
	Total	88.8	94.8	91.6	94.4	90.8	93.0	97.1	98.5	83.6	98.0	95.3	82.3	98.7	97.7
	Oil yield %	**1.7**	**2.5**	**1.7**	**1.3**	**1.1**	**2.0**	**2.4**	**1.8**	**1.3**	**0.02**	**0.1**	**0.1**	**0.8**	**0.1**

RRI: Relative retention indices calculated against n-alkanes, % calculated from FID data, and tr: Trace (<0.1%). **D1-9:** *D. carota*, **D10:** *D. broteri*, **D11:** *D. guttatus*, **D12:** *D. littoralis*, **D13:** *D. involucratus,* **D14:** *D. conchitae.*

chromatography) method (Figure 2). The fractions obtained were concentrated by rotavapor. After analyzed with GC/FID and GC/MS systems, biological activity studies were carried out.

Results

Composition of the Essential Oils

The analysis of essential oils obtained by the hydrodistillation method were carried out with GC/FID and GC/MS systems. Comparative analysis results are given in Table 2.

The essential oil samples (D7, D8) belonging to *D. carota* species with high anticholinesterase effects were separated into 2 sub-fractions by column chromatography. As a result of GC/FID and GC/MS analysis, the ratios of the components belonging to *n*-hexane and ethanol fractions are given in Table 3.

LOX inhibition

The essential oils at 100 µg/mL concentration didn't exhibit any anti-inflammatory activity while the positive control, NDGA showed strong anti-inflammatory activity. The IC_{50} value of NDGA was calculated as 9.00±0.01 µg/mL.

AChE and BChE Inhibition

An anticholinesterase effect was observed in three essential oil samples of *D. carota* species. With two of these, we achieved results close to the standard substance galantamine. Essential oils considered to be effective were divided into sub-fractions and cholinesterase inhibition was re-examined using the same method. Calculated IC_{50} values are given in Table 4 and Table 5.

Discussion

The components, ratios, and yields of 9 essential oil samples of *D. carota* species collected from different locations differ according to the regions (Table 2). The main components of the examined *D. carota* essential oils were carotol (1-74.6%), β-bisabolen (0.9-62.4%), 11αH-himachal-4-en-1β-oil (0.3-49.4%) and *trans*-methylisoeugenol (1-45.7%). In previous studies, the main component of the essential oil found in *D. carota* fruits was determined as carotol (66.78%) (25). Also, the main component of essential oil obtained from the aerial parts of *D. littoralis* was determined as *cis*chrysanthenyl acetate (46.8%) (16). In this study, the main component of the essential oil obtained from *D. littoralis* fruits was α-humulen (29.4%). In another study conducted in Turkey, the main components were found as carotol (27.7%), elemicin (18.1%), and limonene (16.0%) in aerial parts of *D. carota* essential oils' (26). The reason for this is thought to be a feature of the Apiaceae family of which members contain different aromatic compounds in their different organs.

The main volatile compound was β-sinensal (30.4%) in *D. broteri,* methyleugenol (30.5%) in *D. guttatus,* methyleugenol (40.9%) in *D. involucratus,* α-humulene (29.4%) in *D. littoralis,* methyleugenol (29.6%) in *D. conchitaeas.* In the literature, the main component of *D. guttatus* fruit essential oil was indicated as β-pinene (18.8%) (27). Such differences are thought to be due to subspecies, because the *Daucus* genus includes systematically problematic species due to its high hybridization rate (28). More studies are needed on its taxonomic status.

The fruit morphology of *Daucus* species is generally similar. Differences in the chemical composition of essential oils support the identification of *Daucus* species.

The chemical composition and biological activity of essential oils can be affected by many factors, such as harvest time and which part of the plant will be used for the essential oil. Significant differences are also found, especially in the composition of *D. carota* fruit essential oils, depending on geographical origin (8).

Our results reinforce previous data on the variability in fruit

Table 3. Chemical composition of *n*-hexane and ethanol fractions of the essential oils

RRI	Compounds	D7-H	D7-E	D8-H	D8-E
1400	Tetradecane	0.4	-	0.5	-
1482	Longipinene	33.5	-	16.1	-
1493	α-Ylangene	0.2	-	tr	-
1513	Longicyclene	0.5	-	0.2	-
1530	β-Longipinene	1.9	-	-	-
1568	Trans-α-Bergamotene	5.4	-	13.1	-
1600	Hexadecane	1.0	-	1.1	-
1610	Calarene	0.2	-	-	-
1612	β-Caryophyllene	3.1	-	2.4	-
1654	1-Hexadecene	0.2	-	-	-
1661	α-Himachalene	1.8	-	-	-
1669	Sesquisabinene	0.2	-	0.4	-
1668	(Z)-β-Farnesene	2.1	-	1.3	-
1687	α-Humulene	0.4	-	0.2	-
1695	(E)-β-Farnesene	1.1	-	2.0	-
1729	β-Himachalene	10.1	-	3.8	-
1741	β-Bisabolene	21.9	-	49.2	-
1744	Eremophylene	5.9	-	1.5	-
1755	β-Curcumene	1.1	-	-	-
1784	(E)-α-bisabolene	0.9	-	1.0	-
1800	Octadecane	1.1	-	1.1	-
1882	α-Dehydroarhimachalene	0.4	-	0.4	-
1888	ar-Himachalene	2.6	-	1.4	-
1924	γ-Dehydroarhimachalene	0.2	-	0.2	-
2000	Eicosane	0.5	-	0.5	-
2008	Caryophyllene oxide	-	0.9	-	0.9
2048	6,7-Epoxy-himachalene (β-Himachaleneoxide)	-	1.2	-	0.7
2131	11αH-himachal-4-en-1β-ol	-	72.2	-	52.0
2173	6-Epicubenol	-	1.4	-	0.8
2200	trans-Methylisoeugenol	-	9.6	-	29.8
2219	2-Himachalen-7-ol	-	-	-	0.8
2232	α-Bisabolol	-	1.0	-	0.5
2300	Tricosane	-	-	0.2	-
2415	Methyl vanillin (veratraldehyde) 3,4-dimethoxybenzaldehyde	-	1.1	-	3.5
2471	Veratrryl acetone (3,4-dimethoxy fenil aceton)	-	-	-	0.8
2478	α-Asarone	-	0.52	-	
	Total	96.9	87.9	96.8	89.8
	Fraction yield %	**41.8**	**58.2**	**33.8**	**66.2**

RRI: Relative retention indices calculated against n-alkanes, % calculated from FID data, and tr: Trace (<0.1%). **D7, D8:** *D. carota* essential oil, **-H:** *n*-Hexane fraction, **-E:** Ethanol fraction
Chromatograms of GC analysis are given in Figures 3-20.

essential oils, depending on the geographical origin of the samples. Differences were observed in essential oils components and ratios of samples collected from different locations. Two samples collected from nearby locations showed strong anticholinesterase activity. Unlike other samples, high levels of 11αH-himachal-4-en-1β-ol (25.04%, 49.42 %) were found in the content of these essential oils.

These essential oils withhighest anticholinesterase activity were divided into sub-fractions to determine the effect of the main component on the activity. The ratios of 11αH-himachal-4-en-1β-ol in the sub-fractions were measured as 72.2% and 52.0%. However, it was observed that the anticholinesterase effect of the fractions decreased. Therefore, the activity is thought to be due to the synergistic effect of the components in the essential oil.

In the literature, it has been reported that the extract prepared with petroleum ether and ethanol from *D. carota* fruits significantly reduces brain acetylcholinesterase activity and cholesterol levels in young and old mice (29). Ethanol extract of *D. carota* seeds has been shown to have a memory-enhancing effect on rats. (30). It has been stated that essential oils can be developed as nutraceuticals in the prevention and improvement of neurodegenerative diseases such as Alzheimer's disease since they have the ability to cross the blood-brain barrier and reach the central nervous system (12). Our findings are in agreement with these results. The essential oil of *D. carota* characterized by high amounts of 11αH-himachal-4-en-1β-ol can be a natural anticholinesterase agent and can be regarded for the management of Alzheimer's disease.

Table 4. AChE and BChE IC50 values of essential oils

Test sample	AChE (µg/mL)	BChE (µg/mL)
D7	6.04±0.30	11.32±0.20
D8	2.15±0.10	31.03±0.02
D9	31.1±0.35	76.8±0.06
Galantamine	1.13±0.02	12.15±0.36

D7-9: *D. carota* essential oil

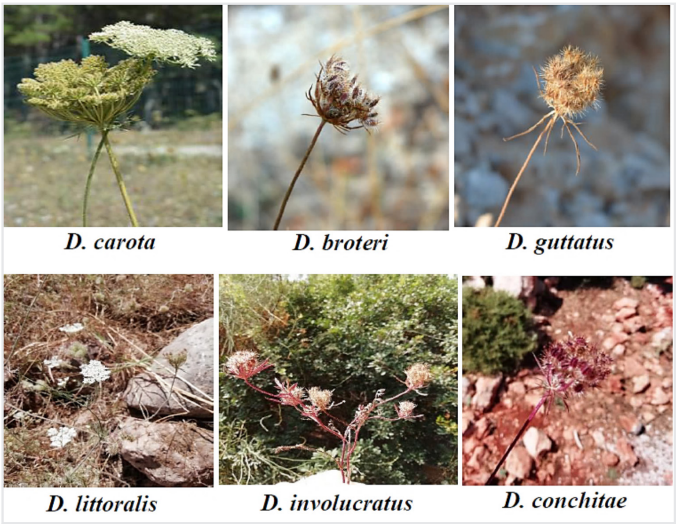

Figure 1. *Daucus* species collected in field studies

Figure 2. Preparation of fractions by column chromatography

Study Limitations

The fact that *D. carota* essential oil compositions and anticholinesterase effects are different increases the possibility that the samples belong to different subspecies. However, there was no taxonomic study on *D. carota* subspecies in Turkey, which limited the chemotaxonomic evaluation of the results. Since different methods were not tested in the anti-inflammatory activity study, the evaluation of this effect was limited.

Table 5. AChE and BChE IC50 values of fractions

Test sample	AChE (µg/mL)	BChE (µg/mL)
D8-H	71.417±0.02	>200
D8-E	181.551±0.30	123.42±0.40
D7-H	132.478±0.14	>200
D7-E	152.267±0.01	111.315±0.56
Galantamine	2.41±0.01	17.38±0.12

>200: IC_{50} value is greater than 200 µg/mL. **-H:** *n*-Hexane fraction of *D. carota* essential oil, **-E:** Ethanol fraction of *D. carota* essential oil

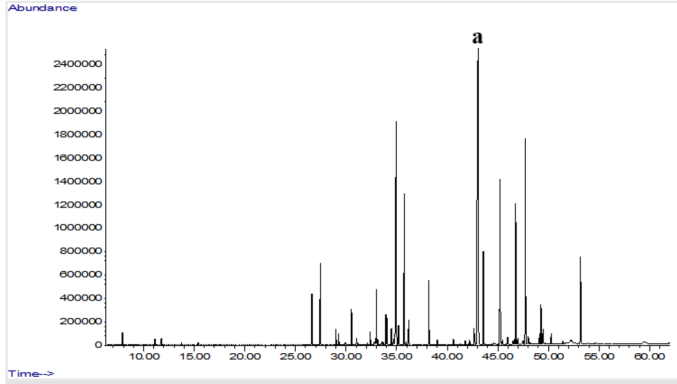

Figure 3. GC chromatogram of essential oil extracted from fruit of *D. carota* (D1) **a:** Carotol (42.8%)

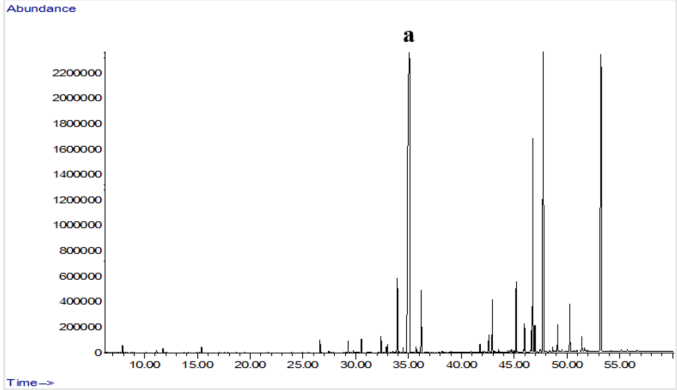

Figure 4. GC chromatogram of essential oil extracted from fruit of *D. carota* (D2) **a:** β-Bisabolene (47.7%)

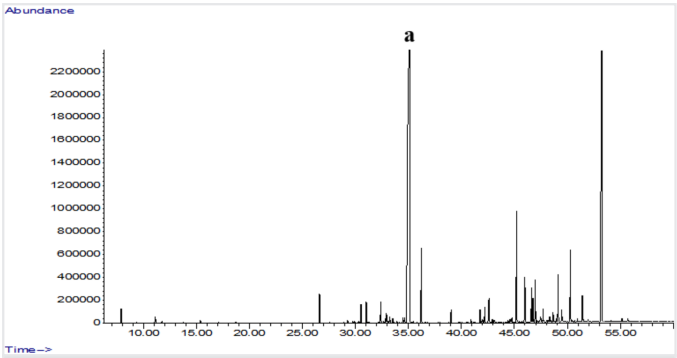

Figure 5. GC chromatogram of essential oil extracted from fruit of *D. carota* (D3) **a:** β-Bisabolene (62.4%)

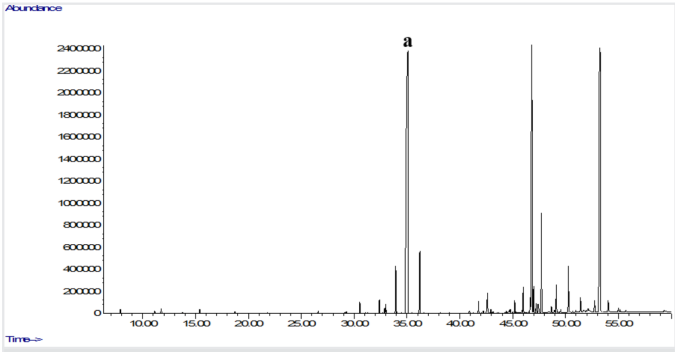

Figure 6. GC chromatogram of essential oil extracted from fruit of *D. carota* (D4) **a:** β-Bisabolene (48.0%)

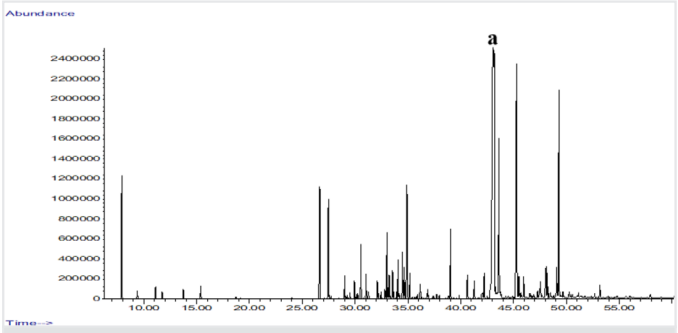

Figure 7. GC chromatogram of essential oil extracted from fruit of *D. carota* (D5) **a:** Carotol (51.4%)

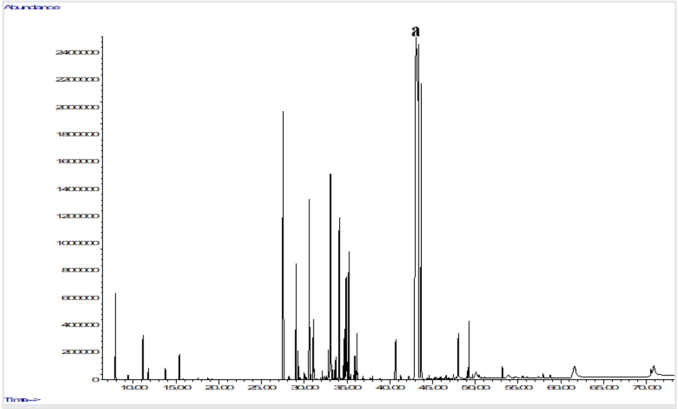

Figure 8. GC chromatogram of essential oil extracted from fruit of *D. carota* (D6) **a:** Carotol (74.6%)

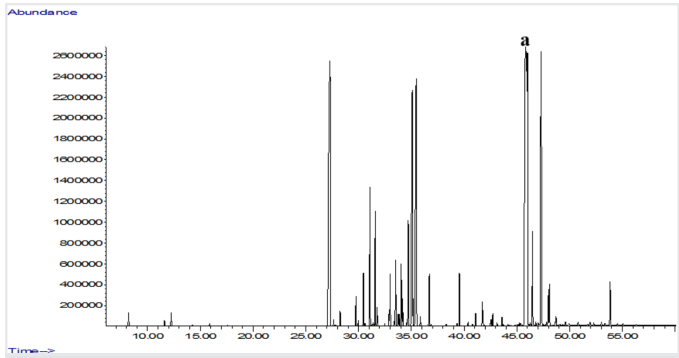

Figure 9. GC chromatogram of essential oil extracted from fruit of *D. carota* (D7) **a:** 11αH-himachal-4-en-1β-ol (49.4%)

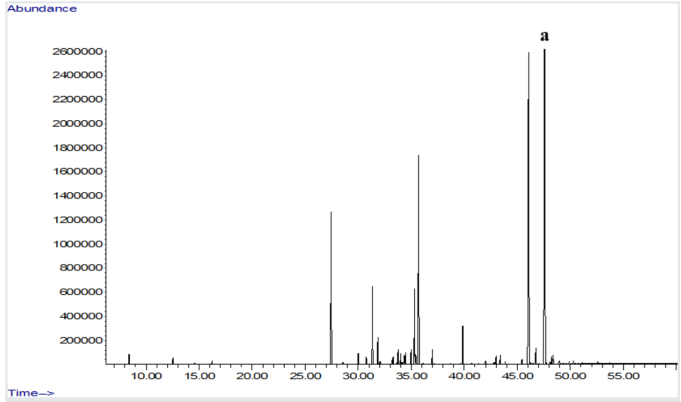

Figure 10. GC chromatogram of essential oil extracted from fruit of *D. carota* (D8) **a:** *Trans*-Methylisoeugenol (45.7%)

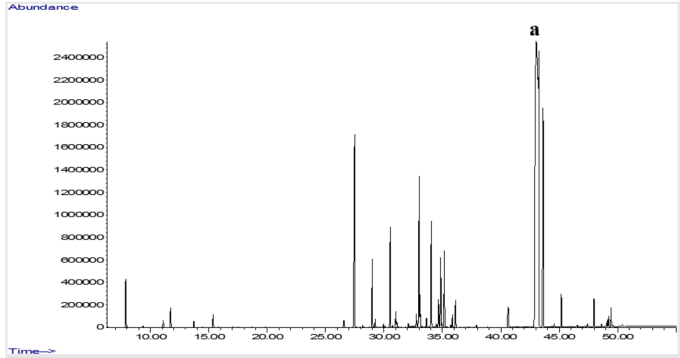

Figure 11. GC chromatogram of essential oil extracted from fruit of *D. carota* (D9) **a:** Carotol (63.1%)

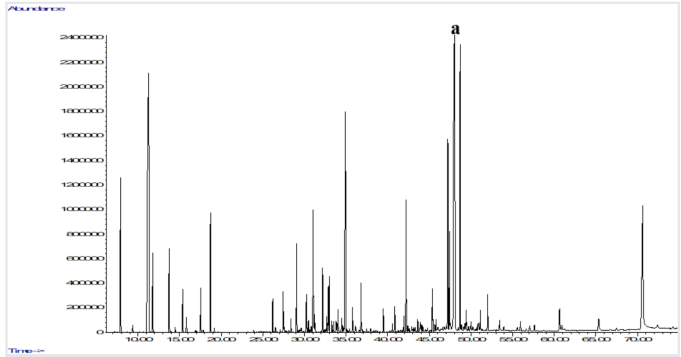

Figure 12. GC chromatogram of essential oil extracted from fruit of *D. carota* (D10) **a:** β-Sinensal (30.4%)

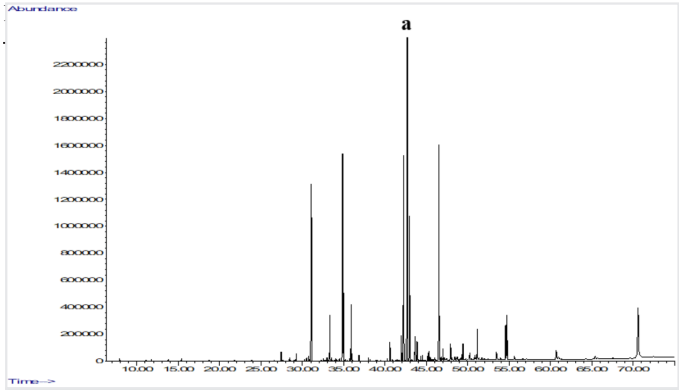

Figure 13. GC chromatogram of essential oil extracted from fruit of *D. carota* (D11) **a:** Methyl eugenol (30.5%)

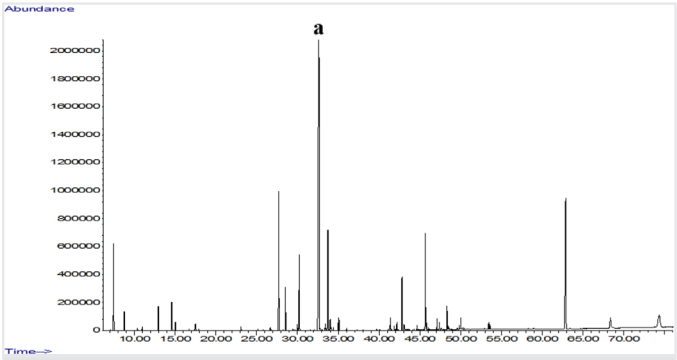

Figure 14. GC chromatogram of essential oil extracted from fruit of *D. carota* (D12) **a:** α-Humulene (29.4%)

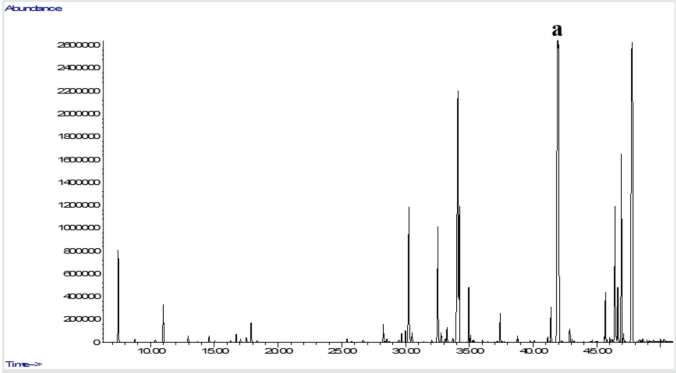

Figure 15. GC chromatogram of essential oil extracted from fruit of *D. carota* (D13) **a:** Methyl eugenol (40.9%)

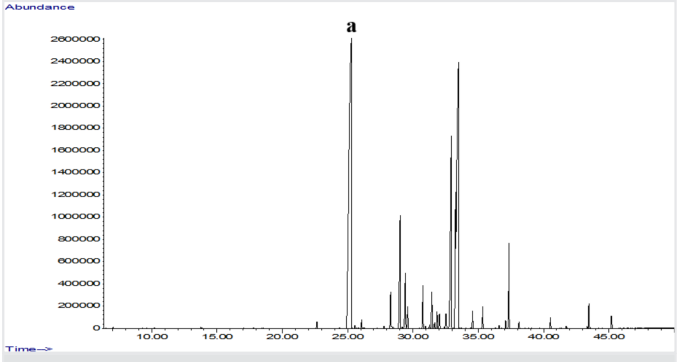

Figure 16. GC chromatogram of essential oil extracted from fruit of *D. carota* (D14) **a:** Methyl eugenol (29.6%)

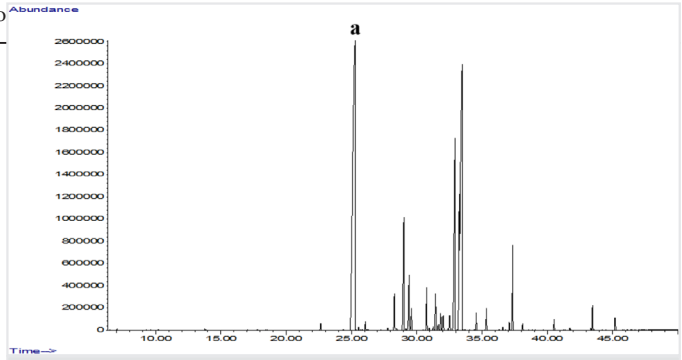

Figure 17. GC chromatogram of *D. carota* essential oil' *n*-hexane fraction (D7-H) **a:** Longipinene (33.5%)

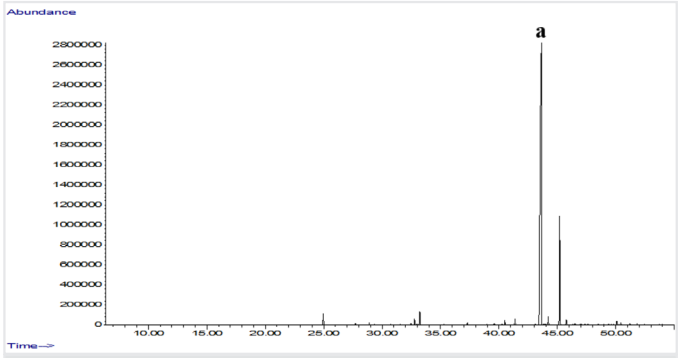

Figure 18. GC chromatogram of *D. carota* essential oil' ethanol (D7-E) **a:** 11αH-himachal-4-en-1β-ol (72.2%)

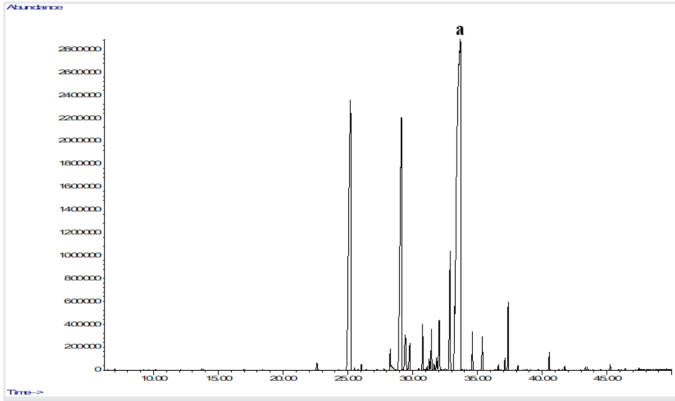

Figure 19. GC chromatogram of *D. carota* essential oil' *n*-hexane fraction (D8-H) **a:** β-Bisabolene (49.2%)

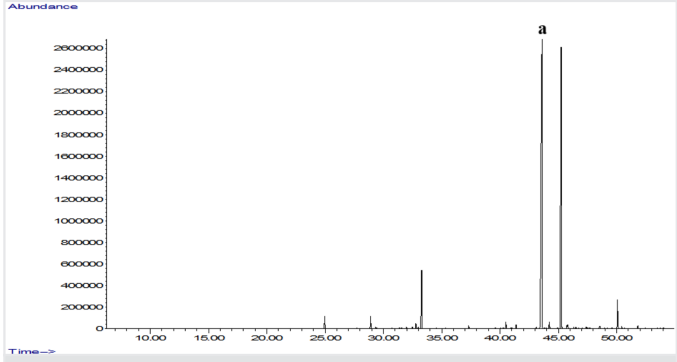

Figure 20. GC chromatogram of *D. carota* essential oil' ethanol fraction (D8-E) **a:** 11αH-himachal-4-en-1β-ol (52.0%)

Conclusion

In this study, the chemical composition of the fruit essential oils of *Daucus* species grown in Turkey was comparatively analyzed for the first time. The anti-inflammatory and anticholinesterase effects of the essential oils were investigated. Although there wasn't any anti-inflammatory activity in the samples, the anticholinesterase effect was observed in three samples of *D. carota* species' essential oils in our study. According to the results, it was seen that if the main components of the essential oil were standardized, it could be used in the preparation of potential pharmaceuticals and nutraceuticals. It is thought to be useful for complementary therapy, especially in neurodegenerative diseases.

Ethics

Ethics Committee Approval: Since there is no study related to teeth, ethics committee approval is not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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

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Original Article

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Evaluation of Drug Release Kinetics of Temozolomide Loaded Plga Nanoparticles in Pluronic® F-127 Hydrogel

Pluronic® F-127 Hidrojel İçinde Temozolomid Yüklü PLGA Nanopartiküllerinden İlaç Salım Kinetiklerinin Değerlendirilmesi

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ABSTRACT

Objective: Controlled local release of temozolomide (TMZ) at the tumor site is a new strategy in the treatment of glioblastoma. Localized delivery systems, based on biodegradable polymers, are capable of slowing and controlling the drug release for a certain period of time. Therefore, the main objective of the study was to investigate a new approach for encapsulating TMZ in a poly(lactic-co-glycolic acid) nanoparticle (NP) system which was then formulated in 18% Pluronic® hydrogel matrix which would provide a sustained and local delivery of TMZ.

Methods: Hydrogels are investigated as local drug delivery methods due to their tunable characteristics and capacity to retain labile pharmaceuticals. The lack of established procedures for describing and evaluating drug release, on the other hand, offers considerable problems, impeding reliable evaluation of systems for defining drug release characteristics.

Results: In this part our study, we aimed to research drug release kinetics of TMZ NPs which had an encapsulation efficiency and particle size ranging between 52-69.67% and 164.4-235.5 nm from a novel hydrogel drug delivery system.

Conclusion: The application of mathematical modeling proves to be extremely beneficial for estimating the release kinetics before the release systems are implemented. The release mechanism was found to be diffusion controlled and not accompanied by dissolution of matrix.

Keywords: Hydrogel, Pluronic F-127, temozolomide, nanoparticles, drug release kinetics

ÖZ

Amaç: Tümör bölgesinde temolozomid'in (TMZ) kontrollü lokal salınımı, glioblastomanın tedavisinde yeni bir stratejidir. Biyobozunur polimerlere dayanan lokalize dağıtım sistemleri, belirli bir süre boyunca ilaç salınımını yavaşlatabilir ve kontrol edebilir. Bu nedenle, çalışmanın ana amacı, TMZ'nin sürekli ve yerel bir dağıtımını sağlayacak olan %18 Pluronic® hidrojel matrisinde formüle edilen bir poli(laktik-ko-glikolik asit) nanoparçacık (NP) sisteminde TMZ'yi kapsüllemek için yeni bir yaklaşımı araştırmaktı.

Yöntemler: Hidrojeller, ayarlanabilir özellikleri ve kararsız farmasötikleri tutma kapasiteleri nedeniyle yerel ilaç dağıtım yöntemleri olarak hızla araştırılmaktadır. Öte yandan, ilaç salımını tanımlamaya ve değerlendirmeye yönelik yerleşik prosedürlerin olmaması, ilaç salım özelliklerini tanımlamaya yönelik sistemlerin güvenilir bir şekilde değerlendirilmesini engelleyen önemli sorunlar ortaya çıkarmaktadır.

Bulgular: Yeni bir hidrojel ilaç taşıma sisteminden 164,4-235,5 nm arasında değişen partikül boyutu ve %52-69,67 kapsülleme etkinliğine sahip TMZ NP'lerin *in vitro* ilaç salım kinetiğini araştırmayı amaçladık.

Sonuç: Serbest bırakma sistemleri uygulanmadan önce serbest bırakma kinetiğini tahmin etmek için matematiksel modellemenin kullanılmasının çok faydalı olduğu ortaya çıkmıştır. Salım mekanizmasının difüzyon kontrollü olduğu ve matriksin çözünmesinin eşlik etmediği bulundu.

Anahtar Sözcükler: Hidrojel, Pluronic F-127, temozolomid, nanopartiküller, ilaç salım kinetiği

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Introduction

Temozolomide (TMZ) is an anticancer agent with alkylating properties, included in the group of imidazotetrazine derivatives, developed in Aston University in the 1980s. TMZ was found to have antitumor activity in intracranial and extracranial tumors. It has been used for glioblastoma multiforme treatment (GBM) (1). However, treatment of GBM remains a challenge, largely due to the fast degradation of TMZ, inability to deliver an effective dose of TMZ to tumors, and lack of target specificity which may cause systemic toxicity. Nanoparticles can be a solution for the rapid degredation of TMZ and can specifically deliver TMZ to GBM cells.

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) have been used as efficient delivery vehicles for therapeutic agents to the brain. PLGA NPs can be produced in any desired shape and size and can trap molecules of any size (2). PLGA has been evaluated for encapsulating small anticancer agents used in nanoparticular cancer chemotherapy (3). Although NPs offer a number of positive properties for drug delivery, NPs have often been combined with hydrogels to further improve the therapeutic index, particularly in localized administration.

Hydrogels, like Pluronic®, excel in controlled release applications because of their tissue compatibility and ease of dispersion in the matrix (4). Hence, we hypothesized that a hydrogel, containing NPs loaded with TMZ, would provide a sustained and local delivery of the drug and destroy cancer cells that might remain in the areas that could not be reached by tumor resection during surgical intervention in the treatment of GBM.

Pluronic F-127 is a hydrophilic polymer which is capable of holding a large amount of water and showing sol-gel transition near 37 °C and the unique thermo-responsive property of the polymer is directed towards a wide area of drug delivery applications (5). In addition to Pluronics inertness and good biocompatibility, its ability to release entrapped drug in aqueous media makes Pluronics particularly suitable as drug carriers in the controlled release of pharmaceuticals (6).

The first part of the study was conducted to formulate TMZ loaded NP formulations with PLGA by emulsion-solvent evaporation method. The PLGA NP formulations were evaluated for their particle size and size distrubution, entrapment efficiency, zeta potential and *in vitro* drug release studies. Then, the optimum NP formulation was chosen to develop a thermo-responsive hydrogel formulation with Pluronic F-127. Also, hydrogel formulations were tested for their rheological and drug release properties (7).

Many manufacturing process variables affect drug release from dosage forms. To contemplate the TMZ release mechanism from Pluronic F-127 hydrogel and PLGA NPs, different kinetic models were considered to fit the experimental data (8).

After giving a brief information about the aim of the study, it was planned to focus on the kinetics of TMZ release from NPs from a Pluronic F-127 hydrogel matrice. Caccavo reviewed the trend in mathematical models used in the field of hydrogel-based drug delivery system and found that the main fit model equations used were: Zero order kinetics, first order kinetics, Korsmeyer-Peppas kinetics, Weibull and The Higuchi kinetics (9).

Methods

Materials

Temozolomide, PLGA (acid terminated; Mw =24,000-38,000; copolymer ratio 50:50), Mowiol 18-88 (Mw =130,000), and Pluronic® F-127 were supplied by Sigma-Aldrich. Ultrapurified water was obtained from the Milli-Q system. All other reagents and solvents used in this study were of analytical grade.

Methods

Formulation of TMZ Loaded PLGA Nanoparticles

The formulation method of TMZ NPs was modified from the study of Ananta et al. (7,9,10). NPs were prepared using emulsion (w/o) solvent evaporation method. TMZ and PLGA were dissolved in 1 mL dimethylformamide. The resulting solution was added in drops to 5 mL of PVA 5% solution under homogenization. The organic solvent used was evaporated. NPs were collected by centrifugation and washed with deionized water to remove free TMZ. And then NPs were collected from the resulting emulsion.

Particle Size Measurement of Nanoparticles

The mean particle size of the NPs was measured by Malvern Zeta Sizer (Malvern Instrument Ltd.). The dispersions were diluted with nanopure water and the sample was placed in a disposable cuvette at a count rate for 20 seconds. The particle size measurment data are reported in Table 1.

Determination of Encapsulation Efficiency (%)

Entrapped TMZ amount in the NPs was determined with our validated HPLC assay and calculated as equation below (7).

$$\text{Encapsulation efficiency \%} = \frac{\text{TMZ amount in formulation}}{\text{Added TMZ amount}} \times 100$$

Formulation Design of Nanoparticle Loaded Pluronic F-127 Thermoreversible Gel

The "cold method" was used in the preparation of hydrogels. Pluronic® F-127 was weighed, added to 50 mL of ultrapure water, and mixed on magnetic stirrer at 900 rpm in an ice bath until Pluronic® F-127 was homogeneously dissolved (7). Gel formulations containing 18-20 and 25% (w/v) Pluronic® F-127 were prepared.

Physicochemical Evaluation of Nanoparticle Loaded Pluronic® F-127 Hydrogels

pH Levels of Produced Hydrogels

The pH levels of the hydrogel formulations were measured with Eutech Instruments pH 700 (n=3) (9).

Sol-gel Transition Temperature

The gelation temperatures of developed formulations were determined by a modified version of the reverse-tube method (n=3) (9).

Viscosity

Sol and gel viscosities of the formulations produced were measured by running the viscometer at speeds of 5-100-5 rpm. Viscosities in gel form were measured with T96 spindle in Brookfield DVII device. Measurements were repeated three times and graphs were plotted as viscosity (cP) vs. shear rate (rpm) (9).

Mechanical Properties

Texture profile analysis (TPA) was performed in the TPA mode of the TA-XT2 Texture Analyzer (Surrey, UK). The formulations were transferred in gel form into 25 mL beakers. Measurements were recorded by puncturing the samples (11).

In vitro TMZ Release Studies

In vitro drug release experiments for all formulations were performed in a shaker bath at a temperature of 37 °C ±1 °C and pH 5 buffer containing 0.1% ascorbic acid. The amount calculated according to the data obtained from the loading capacity and which would provide the target dose was weighed and suspended in buffer with 0.1% ascorbic acid, and for hydrogel formulations, they were suspended in Pluronic® F-127 hydrogel and attached with two-end weighted clips in the dialysis membrane tube placed in a beaker. Twenty mL of buffer solution was placed in each beaker. Samples were taken at various time intervals and the release medium was replaced with new medium to prevent degradation of the active substance after 24 hours.

Kinetic Evaluation of *In Vitro* Drug Release Studies

Dissolution rate profiles were obtained by determining the amount of active substance released by conducting an *in vitro* release study from the formulations. In order to determine which mathematical model the active substance release profiles fit into, the equations of the zero-order, first-order, Higuchi, Weibull and Korsmeyer-Peppas kinetic models were applied in Excel®. Statistical evaluations were made using GraphPad Prism 6® and the findings obtained were evaluated (12).

Results and Discussion

NPs were prepared by emulsion solvent diffusion method. All formulations were produced with two different TMZ amounts, respectively 5 and 10 mg, so as to observe the effect of TMZ amount on the size and encapsulation efficiency of nanoparticles. NPs were discrete, nearly spherical with a size range of 164.4-235.5nm. NPs of 10-200 nm size are suitable for a nano-carrier systems designed for local application that will cross the blood-brain barrier in the brain and reach the tumor site. Because, nanoparticles smaller than 10 nm are excreted renally and NPs larger than 300 nm are removed from the body by RES (13,14). A2-10 formulation with least particle size had the maximum entrapment efficiency (Table 1). It was determined that increasing the amount of TMZ correlated to a higher encapsulation efficiency in all the formulations (15). This effect is explained by the fact that an excess of drug leads in a more viscous dispersed phase, making mutual dispersion of the phases problematic and resulting in the formation of large particles (16). Based on these findings A2-10 formulation was selected in the preparation of hydrogel formulations.

The Zeta potential indicates that it can generate enough repulsion to overcome the gravitational attraction between NPs and suspension dispersion has better stability (17). If formulations were observed to evaluate the effect of the amount of TMZ on the particle properties in Table 1, it was seen that particle size, polydispersity index (PDI) and zeta potential increased where the active substance was adsorbed to the terminal carboxyl groups of PLGA NPs. This is because terminal carboxyl groups are located on the surface of NPs (14). In addition, this phenomenon can be explained by the production of larger particles by causing more active substances to a more viscous oil phase and making it difficult for phases to disperse into each other (16).

The PDI value of polymeric NPs is required to be less than 0.3. According to the PDI data obtained in our study (Table 1), it

Table 1. Formulation design and particle size measurement data of TMZ nanoparticles prepared by emulsion solvent diffusion method

	Formulation code		
Component	A2	A2-5	A2-10
PLGA	2:20	5:20	10:20
DMF (mL)	1	1	1
Z-average (mV)	-13.10±0.416	-13.10±1.05	-4.16±0.337
Size (nm)	169.30±4.053	235.50±24.96	164.40±4.236
PDI	0.255±0.014	0.362±0.1	0.143±0.046
Entrapment efficiency (%)	52.63	60.69	69.67

TMZ: Temozolomide, PLGA: Poly(lactic-co-glycolic acid), PDI: Polydispersity index

was seen that the PDI value of all formulations was within the acceptable range.

Smart hydrogels are attractive because of their unique sol-gel phase transitions at body temperature, biocompatibility, safety, and injectability as a solution in the body before transforming into gel matrices. In formulations containing 18%, 19% and 20% Pluronic® F-127, which showed sol-gel transition, it was observed that the pH level increased the with increasing concentration (18). According to statistical calculations, the difference between pH levels was found to be significant ($p<0.0001$), but since the pH level of the tumor microenvironment was close to the pH level, the obtained pH level was suitable for local drug administration to the tumor site (19). In our study, increased concentration resulted with a lower transition temperature (Table 2). Sol-gel phase transition temperatures of 18%, 19%, 20% and 25% Pluronic F127 containing solutions were 32 °C, 30 °C, 26 °C and 20 °C, respectively. Elasticity and cohesiveness of gels were close for all formulations.

The importance of the sol-gel transition temperature is that the formulation remains in fluid sol state at room temperature, leaving the package or injector and transforming into an *in situ* gel and maintaining its shape. It was observed that the gelation temperature decreased with the increase of Pluronic® F-127 concentration in the formulation, and this was confirmed by the literature (18).

It was observed that the formulations were at the bottom of the tubes in the left state at room temperature (25 °C). Concentrations of 18% and above appeared to be a non-flowing gel when the gels were turned upside and incubated (37 °C). It was observed that the formulation containing 17% Pluronic® F-127 returned to its sol form, and formulations containing 15 to 16% Pluronic® F-127 remained in its left form. According to the results which was shown in Table 2, H18 was chosen as a proper injectable system with a gelation temperature of 32 °C which led to be a solution in the room and transited into a gel in the body (5). pH values of gels rangingbetween 6.80-6.94 are suitable for brain application. Also, Persi revealed that acidic pH disabled hypoxia adaptations of cancer cells and compromised tumor cell growth which indicated that pH values of formulations between 6.80-6.94 were suitable for brain cancer treatment with less damage to brain tissue (20).

The A2, A2-5, and A2-10 formulations showed a triphasic profile. In all formulations, a burst effect was observed in the first 4 hours and a plateau was detected in the following 72 hours (Figure 1). The reason for the difference in the percentage of active substance released from the formulations is thought to be proportional to the decrease in the percentage of active substance released per unit time as the encapsulation rate of the active substance increases (21). In Figure 2, the drug release of NPs from the hydrogel formulations reached approximately 25% of the total TMZ in 60 days. The *in vitro* release profile of the active substance in the hydrogel from the NP system showed an average of 10% of immediate release in the first 12 hours, then reaching a plateau in the next 60 days.

The *in vitro* release profile of TMZ in hydrogel showed an immediate release of 46% in the first 6 hours and reached a plateau in the next 18 hours. The release profile of the active substance from the NP system in the hydrogel allowed the release of TMZ at lower doses over a long period of time.

Dissolution rate profiles were obtained by determining the amount of active substance released by conducting an *in vitro* release study from the formulations. In order to determine which mathematical model the active substance release profiles fit into, the equations of the zero-order, first-order, Higuchi, Weibull and Korsmeyer-Peppas kinetic models were applied in Microsoft Office Excel and the findings obtained were evaluated (22). The applied mathematical equations used to describe release characteristics of TMZ NP from Pluronic F-127 gels are given in Table 3.

The mathematical models include: (a) zero-order model, $Q=Q_0+k_0\ t$, where Q is the cumulative release percentage, k_0 zero release rate constant, and t time; (b) first-order model, Log C_t=Log C_0-k_1t/2.303, where Log C is the cumulative release percentage, k_1 first release rate constant, t time (c) Higuchi model, $F_t=k_{Ht}^{1/2}$, where F_t is the cumulative release percentage, k_H Higuchi release rate constant, and t time (11). (d) The formula for Weibull function is F=1-exp (-atb). Where F is the drug fraction released at time t, and a and b are constants. b, as a shape parameter (18). (e) Korsmeyer - Peppas model= M_t/M_∞ = kp tn where M_t/M_α is the proportion of drug released at time t, k is the rate constant (Table 3) (11).

As can be seen in Table 4, *in vitro* release kinetics of the active substance show compatibility with the Weibull kinetic model in A2, A2-5, and A2-10 formulations. The fact that all of the calculated β values were less than 1 indicated a kinetic profile where the active substance release rate occured faster at first, then

Table 2. Properties of TMZ nanoparticles loaded Pluronic® F-127 gels

Hydrogel	Viscosity of hydrogel cP	Pluronic concentration %	pH	Gelation temperature °C	Elasticity	Cohesiveness
H18	22000.00±1833.03	18	6.80±0.01	32-33	0.9874±0.0171	0.9523±0.0030
H19	11600.00±529.15	19	6.94±0.01	28-30	0.9960±0.0148	0.9176±0.0000
H20	12666.67±665.83	20	6.94±0.01	25-26	0.9920±0.0041	0.9721±0.0000
H25	32966.67±3194	25	6.80±0.00	20-21	0.9960±0.0467	0.7668±0.0000

TMZ: Temozolomide

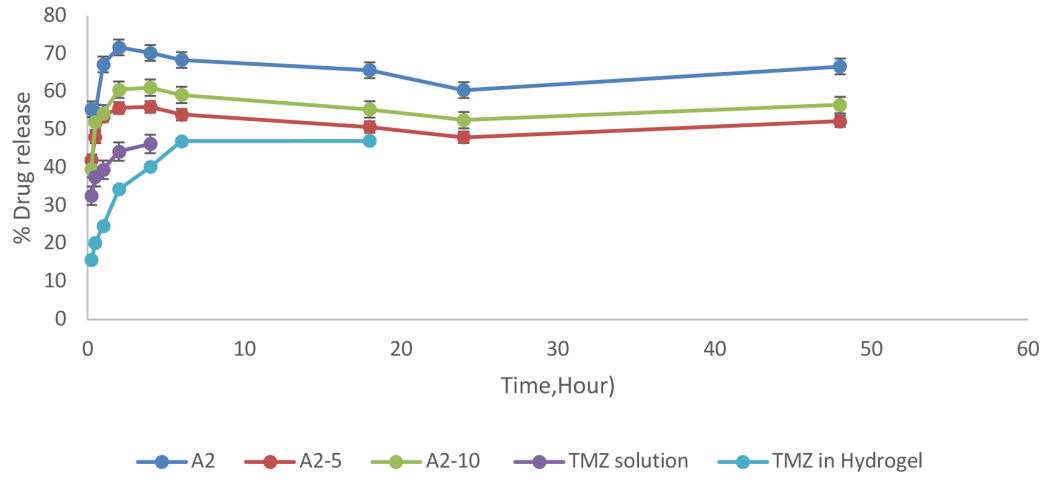

Figure 1. *In vitro* release of TMZ from nanoparticles
TMZ: Temozolomide

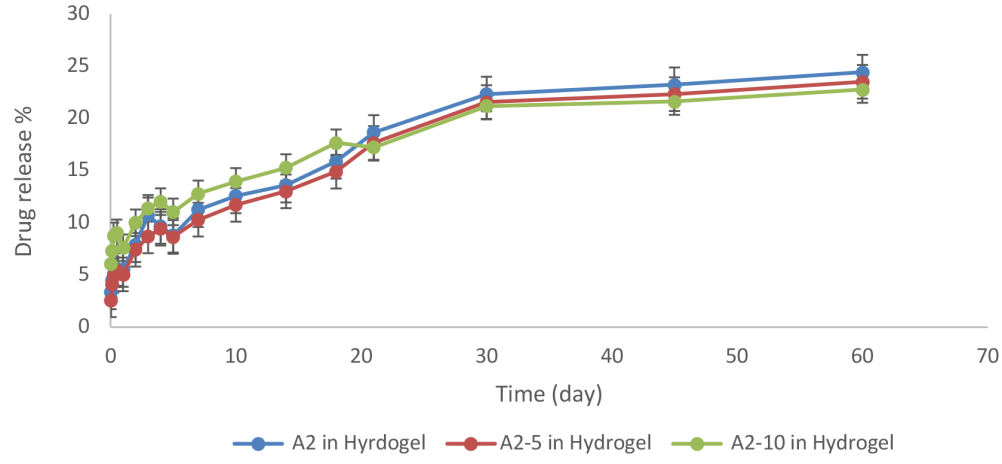

Figure 2. *In vitro* release of TMZ from nanoparticle loaded hydrogels
TMZ: Temozolomide

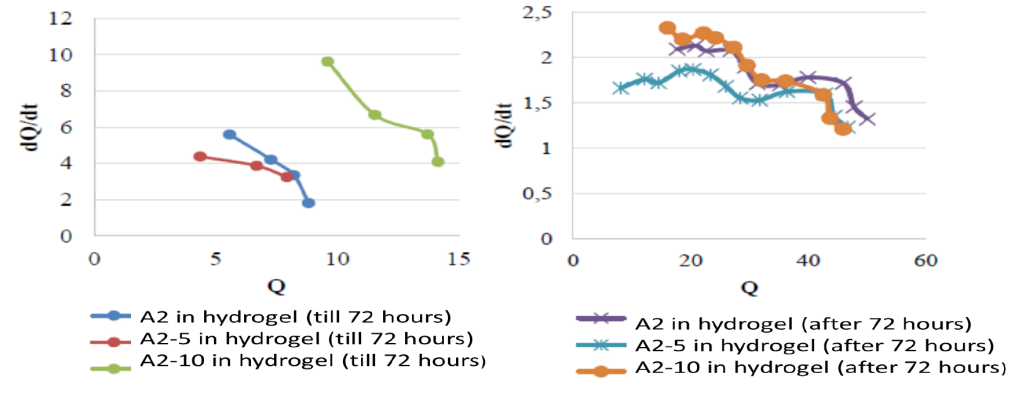

Figure 3. dQ/dt-Q plots of nanoparticle formulations in hydrogel

Table 3. Mathematical equations for the models used to describe release characteristics of TMZ from Pluronic® F-127 gels

Model	Equation
Zero-order	$Q=Q_0+k_0 t$
First order	$Log C_t=Log C_0-k_1 t /2,303$
Higuchi	$Ft=k_H t^{1/2}$
Weibull	F=1-exp (-atb)
Korsmeyer-Peppas	Mt/M∞=kp tn

TMZ: Temozolomide

Table 4. Results of mathematical model fitting of TMZ release of nanoparticle loaded hydrogel

		A2-2	A2-5	A2-10	A2-2 in hydrogel	A2-5 in hydrogel	A2-10 hydrogel
Zero order	**k_0**	-10.433	-3.004	-0.412	-0.032	-0.030	-0.025
	r^2	0.804	0.580	0.723	0.794	0.816	0.794
	RMS	30.924	20.283	67.400	50.383	39.287	32.877
First order	**k_1**	-0.292	-0.061	-0.013	-0.001	0	0
	r^2	0.844	0.601	0.876	0.853	0.866	0.841
	RMS	0.018	0.008	0.026	0.007	0.005	0.005
$Q \rightarrow t^{1/2}$	**k**	23.344	8.637	4.122	1.326	1.243	1.042
	r^2	0.871	0.736	0.813	0.960	0.969	0.960
	RMS	20.334	12.714	45.476	9.605	6.666	6.424
Peppas	**n**	0.197	0.105	0.113	0.332	0.341	0.226
	r^2	0.915	0.872	0.904	0.961	0.967	0.959
	RMS	0.001	0.001	0.001	0.004	0.004	0.002
Weibull	**β**	0.339	0.151	0.195	0.375	0.379	0.260
	r^2	0.931	0.881	0.952	0.955	0.961	0.949
	RMS	0.002	0.001	0.002	0.006	0.005	0.004

TMZ: Temozolomide, RMS: Rhabdomyosarcoma

resembled the 1st degree kinetic profile and reached a plateau (23).

The release of active substance by diffusion and/or relaxation in the polymeric system is explained by the Korsmeyer-Peppas kinetics. According to the R^2 values obtained by the calculation made from the A2, A2-5 and A-10 formulations in the hydrogel, the release kinetics of the active substance were in accordance with the Korsmeyer-Peppas kinetics. This finding confirms the fact that Pluronic® F-127 hydrogel swells by absorbing water and the active substance is released as diffuse within the hydrogel system (24). The fact that the calculated n values were less than 0.45 indicated the Fickian diffusion profile (25).

Since the formulations showed compatibility with the Higuchi and 1st order kinetic model at the same time, a graph of the amount of active substance released (Q) versus the amount of active substance released (d_Q/d_t) in a certain time period was plotted to determine which one was compatible.

The release of water-soluble drugs from anhydrous hydrogel matrices involves simultaneous absorption of water and desorption of drug via a swelling-controlled diffusion mechanism (26). The fact that the calculated n values were less than 0.45 indicated the Fickian diffusion profile (25).

Study Limitations

Since the formulations showed compatibility with the Higuchi and First order kinetic model at the same time, a graph of the amount of active substance released (Q) versus the amount of active substance released (dQ/dt) in a certain time period was plotted to determine which one was compatible. For graphs showing biphasic characteristics, both phases and the entire profile were applied to the graph, and it was observed that the dQ/dt and Q values in all three graphs showed inverse proportion (Figure 3). This showed that the release was in accordance with the Higuchi kinetics (27).

Conclusion

A modified release drug delivery system of TMZ developed as a NP hydrogel served as a depot for sustained drug release and provided a rate-limiting barrier for modulation of drug release. Drug release from the hydrogel system was evaluated by means of mathematical modelling. The use of mathematical modeling turned out to be very useful for estimating the release kinetics before the release systems were implemented. The release mechanism was found to be diffusion controlled and not accompanied by dissolution of matrix. The release kinetics in H18 followed Korsmeyer-Peppas model.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Peer-review: Externally peer reviewed.

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Original Article

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Analysis of Capsaicinoids in Chilli Sauce with Ultra Fast Liquid Chromatography

Ultra Hızlı Sıvı Kromatografisi ile Biber Sosunda Kapsaisinoidlerin Analizi

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ABSTRACT

Objective: In current study, quantification of the capsaicinoids in chilli sauces based on a sensitive ultra fast liquid chromatography method and derivatization with dansyl chloride (DNS-Cl) was described. Capsaicinoids are biosynthesized as secondary metabolites by chilli sauces. The major components of capsaicinoids are capsaicin (CPS) and dihydrocapsaicin (DCPS).

Methods: Phenol groups within the CPS and DCPS are suitable for derivatization reaction using DNS-Cl (chemically named as 5-(dimethylamino) naphthalene-1-sulfonyl chloride) at pH 10 with 0.5 M sodium bicarbonate which leads formation of a derivative highly fluorescent properties that can be measured at 520 nm following excitation at 360 nm wavelength. Separation of the compounds was conducted on a chromatographic system having a mobile phase formed by a combination of acetic acid (0.5 M, pH 7.0 with NaOH) solution and acetonitrile under solvent programming on a consistent flow rate of 0.4 mL. min-1 using a C18 column.

Results: Method validation was evaluated as per the regulations described in International Conference on Harmonization Guidelines. The calibration graph for CPS and DCPS was linear between 0.2 and 200 µg mL-1.

Conclusion: The proposed analytical procedure represents a simple, time and cost effective method with a suitable selectivity regarding quantification of capsaicinoids in chilli sauces.

Keywords: Capsaicin, chilli sauces, dansyl chloride, dihydrocapsaicin, UFLC, validation

ÖZ

Amaç: Mevcut çalışmada, hassas bir ultra hızlı sıvı kromatografisi yöntemine ve dansil klorür (DNS-Cl) ile türevlendirmeye dayalı olarak acı biber soslarındaki kapsaisinoidlerin miktar tayini tanımlanmaktadır. Kapsaisinoidler, biber sosları tarafından sekonder (ikincil) metabolitler olarak biyosentezlenir. Kapsaisinoidlerin ana bileşenleri kapsaisin (CPS) ve dihidrokapsaisindir (DCPS).

Yöntemler: CPS ve DCPS içindeki fenol grupları, pH 10'da DNS-Cl (kimyasal olarak 5-(dimetilamino) naftalen-1-sülfonil klorür olarak adlandırılır) kullanılarak 0,5 M sodyum bikarbonat ile türevlendirme reaksiyonu için uygundur, bu da yüksek floresan özelliklere sahip bir türevin oluşumuna yol açar ve 360 nm dalga boyunda uyarmayı takiben 520 nm'de ölçülebilir. Bileşiklerin ayrılması, asetik asit (0,5 M, NaOH ile pH 7,0) çözeltisi ve asetonitrilin bir kombinasyonu ile oluşturulan bir mobil faza sahip kromatografik bir sistem üzerinde C18 kolonu kullanılarak, 0,4 mL dk-1 akış hızında solvent programlaması altında tutarlı bir şekilde gerçekleştirildi.

Bulgular: Metot validasyonu, Uluslararası Harmonizasyon Topluluğu Konferansı'nda açıklanan düzenlemelere göre değerlendirildi. CPS ve DCPS için kalibrasyon grafiği 0,2 ve 200 µg mL-1 arasında doğrusaldı.

Sonuç: Önerilen analitik prosedür, biber soslarında kapsaisinoidlerin miktar tayini ile ilgili olarak uygun bir seçiciliğe sahip, basit, zaman ve maliyet açısından etkin bir yöntemi temsil etmektedir.

Anahtar Sözcükler: Kapsaisin, biber sosları, dansil klorür, dihidrokapsaisin, UFLC, doğrulama

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Introduction

Chilli peppers are commonly preferred food additives due to high spicy flavor allowing preparation of tasty foods (1). Commercial food processing for chilli pepper became critical as there were a number of chilli pepper subtypes and high level of demand for chilli pepper. Capsaicinoids, as hydrophobic alkaloids, are the major component in chilli pepper (2,3). The majority, as 90%, of capsaicinoids are represented as capsaicin (trans-8-methyl-N-vanillyl-6nonenamide) (CPS) and dihydrocapsaicin (8-methyl-N-vanillyl-nonanamide) (DCPS) (4) (Figure 1). The recent update in regulations governing food industry suggests the recommended daily average and highest intake of CPS as 0.77 mg day-1 and 2.64 mg day-1, respectively, limiting CPS content of industrial food as 5 µg g-1 (5). According to literature, capsaicinoids can be use in the therapy due to their following therapeutic properties such as being analgesic (6) and anti-inflammatory (7), and having gastroprotective properties against the gastrointestinal adverse effects of drugs (8,9), high level antioxidant effects (10,11) and anti-tumoral (12,13) properties.

Various analytical procedures were defined in literature for quantification of capsaicinoids as follows; high performance liquid chromatography (HPLC) (14-16), HPLC coupled with mass spectrometry (HPLC-MS) (17) gas chromatography coupled with MS (GC-MS) (18) and capillary electrophoretic methods (19). Sensitivity provided by HPLC method using ultraviolet detection is not adequate. Separation quality is better with LC-MS and GC-MS methods with high sensitivity however highly costed equipment and qualified operator are required. In this study, capsaicinoids were analyzed with UFLC method based on fluorescence detection through derivatization of CPS and DCPS with dansyl chloride (DNS-Cl). Detection based on fluorescence measurement enabled adequate sensitivity for the analytical procedure. Weberl was the first researcher using DNS-Cl, (5-(dimethylamino) naphthalene-1-sulfonyl chloride) for the formation of fluorescent derivatives of albumin

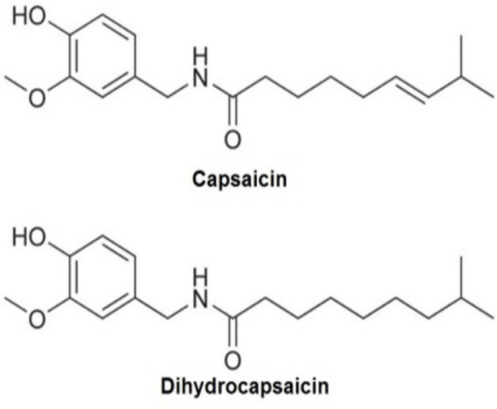

Figure 1. Chemical structures of capsaicin and dihydrocapsaicin

(20). DNS-Cl was used as a fluorescent derivatization agent in determination of several pharmaceutical active ingredients such as primary amines, secondary amines, imidazoles and phenols in their chemical structure (21-25). As the authors of the proposed study, we also used derivatization procedures carried out with DNS-Cl for some pharmaceutical analysis (26-28). In all these analysis, we trialed various conditions to gain the most effective derivatives. The pH values should be in alkaline range and temperatures of the mediums were supposed to be at about 40-60 °C with requiring short reaction durations.

In current study, DNS-Cl was selected as an efficient fluorescent labelling reagent for quantification of CPS in chilli hot sauces. The proposed method based on derivatization with DNS-CI, has the advantages of being simple with faster sample preparation, having sensitivity and selectivity resulting from fluorescence spectra, use of widely available equipment. In addition to the advantages of the derivatization procedure, UFLC also provided advantageous separation procedure such as shorter chromatographic process, reduced mobile phase consumption, facility to study with trace amounts of sample and more sensitive assays than conventional HPLC applications. As per the results, currently developed analytical UFLC procedure was suitable for quantification of capsaicinoids in chilli sauce.

Method

Reagents and Solutions

The CPS and DCPS standards and DNS-Cl were procured through Sigma (St. Louis, MO). All reagents and chemical substances were of analytical-reagent grade. CPS and DCPS were solvated in methanol at 1 mg mL-1 concentration as the stock solution which was the basis for preparation of working solutions through dilutions from this stock solution. Concentration of freshly prepared DNS-Cl solution in acetonitrile was 2.0 mg mL-1 (0.02%). The sodium bicarbonate was dissolved in water at 0.5 M concentration and pH was adjusted with addition of 0.5 M sodium hydroxide to pH 10 by a pH meter. The prepared solution was stored in the fridge and available for use for approximately seven days.

Apparatus

Shimadzu LC 20A UFLC (Shimadzu, Kyoto, Japan) was used for chromatographic separations. Elements of the system were LC 20AB Binary pump, CTO-10As column oven. Data gathered from chromatographic system was analyzed by system software of LC Solution. Excitation wavelength of 360 nm and an emission wavelength of 520 nm was specifically set by the fluorescence detector. Inertsustain C18 column (4.0x100 mm, 3 µm) procured from GL Sciences, Tokyo-JAPAN was used for chromatographic separation. A mobile phase was consisted of the combination of acetonitrile (Mobile phase A) and solution of acetic acid at 0.5 M concentration at pH 7.0 (adjusted using

NaOH) (Mobile phase B) under gradient elution with a constant flow rate of 0.4 mL min-1. The column temperature was kept constant at 25 °C.

General Procedure

The aliquots taken from the standard solution involving CPS and DCPS corresponding to the concentration interval of 0.02 -200.0 µg mL-1 were taken into a series of test tubes and a 250 µL bicarbonate solution at pH 10 and 750 µL of DNS-Cl solution were added to every test tube. The reaction solution mixture was kept at 40 °C during 10 min. They were left to cool at ambient temperature in air and then the formed drug fluorescent derivative was extracted using 5 mL of dichloromethane during 1.0 min. The extract was subjected to evaporation under nitrogen gas at 40 °C. The 0.5 mL of the mobile phase was used for dissolving of remaining residue. A 20 µL aliquots of the resulting solution was used for quantification by UFLC.

Sample Preparation

Three samples of daily-used chilli hot sauces (one red pepper-based, one jalapeno pepper-based and one cayenne pepper-based sauce) were procured from local market in Turkey. First step covered the transfer of 10 mL of hot sauce into a volumetric flask of 50 mL volume having absolute ethanol of 25 mL. Secondly, the resulting mixture was kept in an ultrasonic bath for sonification during 1 h. Following second step, the mixture was stirred during 2 h via a magnetic stirrer. Centrifugation of the resulting mixtures was performed at 10,000 rpm during 10 min followed by addition of absolute ethanol to complete the total volume to 50 mL. A vacuum rotatory evaporator was used for evaporation of the ethanol phase at 40 °C. The residue was dissolved with addition of 1.0 mL methanol solution and vortexed. Upon completion of solvation, a 750 µL of DNS-Cl solution and a 250 µL of bicarbonate solution at pH 10 were added to every tube. The reaction mixture was kept at 40 °C during 10 min. They were then left to cool down at ambient temperature in air. Extraction of the resulting derivative was performed with 5 mL of dichloromethane during 1.0 min. A 0.5 mL of the mobile phase was used for solvation of the remaining residue. A 20 µL aliquots of the resulting solution was analyzed by UFLC for determination of capsaicinoids.

Results

Chromatographic Conditions

A C18 column (4.0x100 mm, 3 µm) was used for chromatographic separation under gradient elution and the column temperature was kept at 25 °C during the analysis. A mobile phase formed by the mixture of acetonitrile (Mobile phase A) and acetic acid solution at 0.5 M concentration (pH adjusted to 7.0 with NaOH) (Mobile phase B) was used with 0.4 mL min-1 flow rate. The optimum chromatographic separation was performed by using acetic acid solution at 0.5 M concentration and with adjusting pH to 7 by NaOH additions with acetonitrile. Fluorimetric detection was carried out at emission wavelength of 520 nm following excitation at 360 nm excitation wavelength. Table 1 represents the gradient elution program. Figure 2 represents the typical chromatograms.

Optimization of Derivatization Reaction Parameters

The CPS and DCPS were derivatized with DNS-Cl to yield derivatives with fluorescence properties. According to the results of investigation for identification of optimum reaction conditions, a 750 µL of DNS-Cl in acetonitrile solution was found to be sufficient to achieve the highly effective derivatives. The effect of various pH values on fluorescent intensity were investigated in pH mediums changing between 9 and 11, using bicarbonate solution and borate buffer. As evidenced in literature, DNS-Cl has a tendency to yield reaction under

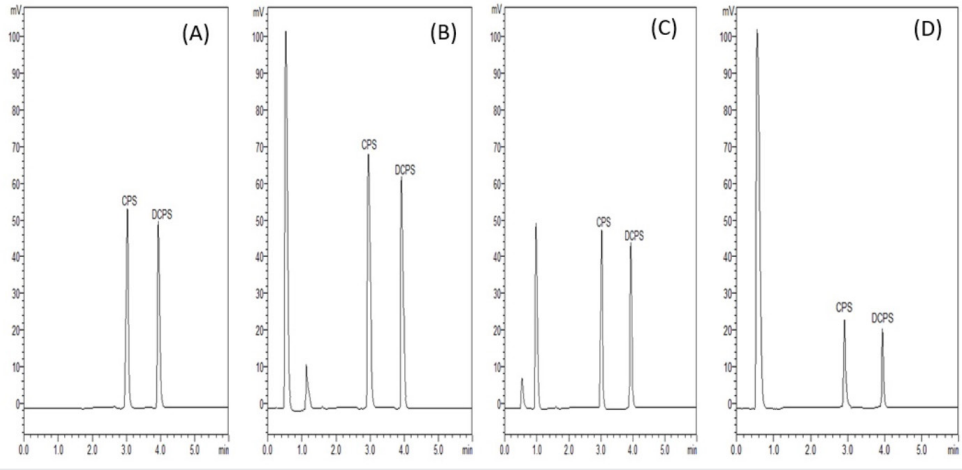

Figure 2. Representative chromatograms of (A) standard chromatogram of CPS (100 µg mL-1) and DCPS (100 µg mL-1), (B) red pepper-based sauce sample (C) jalapeno pepper-based sauce sample and (D) cayenne pepper-based sauce sample

CPS: Capsaicin, DCPS: Dihydrocapsaicin

alkaline conditions. The bicarbonate solution of pH 10 with a volume of 250 µL enabled the maximum fluorescence intensity for this derivatization reaction.

The effects of temperature and various durations on the intensity of fluorescence resulting from the formed derivatives were also examined between 40-60 ºC. The optimum fluorescence measurements were achieved and remained stable in 40 °C water bath during 10 min. The effect of different solvents on fluorescence intensity was investigated using benzene, diethyl ether, toluene, ethyl acetate, chloroform, dichloromethane. It was observed that dichloromethane, as a solvent, allowed the highest level of fluorescence intensity. The derivatives synthesized according to the pre-defined conditions, were stable during at least 2 h. Table 2 lists optimum values for the derivatization parameters.

Method Validation

Method validation was evaluated in line with ICH Guidelines (29).

Linearity

Linearity test was performed with solution involving CPS and DCPS prepared at six different concentration values covering the range of 0.2 to 200.0 µg mL-1. The calibration curve was established by the plotting the substance peak area against the concentration. The correlation coefficients, y-intercept and slope values of the calibration curves were computed and presented (Table 2). Calculation of the detection limit (LOD) and the quantitation limit (LOQ) was performed according to the pre-defined equation: LOD or LOQ = κSDa/b. Standard deviation of the intercept is represented as SDa and the slope as b. Constant k is assigned as 3 for calculation of LOD and as 10 for calculation of LOQ. Data for LOD and LOQ are represented in Table 3.

Table 1. Gradient elution program

Time (min)	%A	%B
0	80	20
2	50	50
5	60	40
8	60	40
10	80	20

min: Minute

Table 2. Optimum values for the derivatization parameters

Reagent concentration	pH	Temperature	Duration	Extraction solvent
0.02%	10	40 °C	10 min	CH_2Cl_2

min: Minute

Precision

The precision investigation was performed during five consecutive days by quantitation of CPS and DCPS (each n=5). The within the day and between the days precisions (relative standard deviations) were <2%, indicating good level of precision for the method. The results of the precision are presented in Table 4.

Accuracy

The accuracy of the proposed method was proved using the standard addition technique. Calculation of the percentage recovery regarding the added standard in each assay sample was performed according to the following equation:

Percentage recovery %, %=[(Ct–Cu)/Ca]x100

Ct standing for the total concentration of the analyte measured, Cu representing the concentration of the unknown analyte amount actually existing in the sauce, and Ca expressing the known concentration of the pure analyte which is deliberately added to the sauce. The findings are presented in Table 3. The percentage recovery values for the measured were quantitatively 88.08-93.64% for CPS, 84.15-94.78% for dihydrocapsacin, leading to a high level of accuracy of the method. The findings of the recovery study have been presented in Table 5.

Robustness

The method was found to be robust based on the observed changes in the flow rate of the mobile phase (±0.1 mL min-1) and the determined organic phase composition (±2%). During the analyses, the mobile phase pH (7.0±0.5) and column oven temperature (25±5 °C) were measured and noted. The study demonstrated that minor variations in the variable parameters of the method did not have significant impact on the results, proving the robustness of the currently proposed method.

The chemical stability of the stock solutions, which were composed of the study compounds in mobile phase mixture, was assessed after storing the solutions at room temperature for 48 hours. All of the studied compounds were found out to be stable in the mobile phase for 48 hours at room temperature and in the refrigerator (at 4 °C). The stability studies yielded no further peaks in the chromatograms.

Table 3. Calibration and sensitivity data of capsaicinoids

Capsaicinoids	Linear range (µg mL^{-1})	Regression equation	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Capsaicin	0.2-200	y=417.5x + 4795	0.9961	0.0029	0.0099
Dihydrocapsaicin	0.2-200	y=374x + 4193	0.9952	0.0040	0.0133

LOD: Detection limit, LOQ: Quantitation limit

Determination of CPS and DCPS in Chilli Sauces

Application of the described methods to samples and the procedure for extraction were detailed at Determination of CPS and DCPS in Chilli sauces. The CPS and DCPS concentration values in the examined pepper samples and the pungency level defined as Scoville Heat Units (SHU) are presented in Table 5 (1 ppm CPS measured being roughly equivalent to 16 SHU) (30). Classification as per SHU is as follows: non-pungent (0-700 SHU) - mildly pungent (700-3,000 SHU) - moderately pungent (3,000-25,000 SHU) - highly pungent (25,000-70,000 SHU) - very highly pungent (>80,000 SHU). The mean concentration of capsaicinoids in daily-used chilli hot sauce (one red pepper-based, one jalapeno pepper-based and one cayenne pepper-based sauce) was 288.08, 180.57 and 81.5 ppm, respectively. According to the findings of the current study, the pungency level of the examined samples could be ranked as follows, as presented in Table 6: red pepper-based (moderately pungent) > jalapeno pepper-based (mildly pungent) > cayenne pepper-based sauce.

Conclusion

Development and validation of a cost and time effective and selective method aiming the quantification and separation of CPS and DCPS in different kind of chilli sauces were successfully completed. The currently developed method had good repeatability and selectivity. The quantitative analysis of capsaicinoids in chilli sauces was performed satisfactorily with this method. UFLC method could be readily implemented in the analysis of chili sauce extracts. DNS-Cl derivatization improved the selectivity of capsaicinoids. Two capsaicinoids were well separated from each other within 4 min. Capsaicinoid composition of chilli sauce samples were accurately determined. The observed pungency level from the highest to the lowest as expressed in SHU could be ranked as follows; highest SHU level with red pepper-based, while jalapeno pepper-based, cayenne pepper-based sauce had lower SHU values. In conclusion, the proposed method is faster, more sensitive and cost effective than the previously reported methods for the determination of CPS and DCPS in food samples.

Table 4. Intra-day and Inter-day precision results of capsaicinoids

	Intra-day (n=5)			Inter-day* (n=5)		
	Added concentration (μg mL^{-1})	Found concentration (μg mL^{-1}) ± SD	RSD %**	Added concentration (μg mL^{-1})	Found concentration (μg mL^{-1}) ± SD	RSD %*
Capsaicin	0.2	0.201±0.0015	0.75	0.2	0.203±0.003	1.38
	100	100.25±0.890	0.89	100	100.34±1.240	1.24
	200	201.32±1.270	0.63	200	202.45±1.700	0.84
Dihydrocapsaicin	0.2	0.203±0.0018	0.89	0.2	0.204±0.0024	1.18
	100	100.18±0.900	0.90	100	100.48±0.984	0.98
	200	201.26±1.251	0.75	200	203.02±1.865	0.92

*Five consequtive day
**RSD: Relative standard deviation

Table 5. Accuracy results of capsaicinoids

Capsaicin*			Dihydrocapsaicin*		
Spiked amount (μg mL^{-1})	Recovery	RSD %*	Spiked amount (μg mL^{-1})	Recovery	RSD %*
0.2	88.08	1.25	0.2	84.15	0.45
100	93.31	1.53	100	94.78	0.23
200	93.64	1.41	200	94.28	0.26

*Sample amount 10.0 mL; Concentration of capsaicin and dihydrocapsaicin in the initial sample was 150.04 and 120.17 μg/mL, respectively

Table 6. Concentrations of capsaicin, dihydrocapsaisin and Scoville heat units (SHU) in analyzed samples

Pepper type	Capsaicin (ppm)*	Dihydrocapsaicin (ppm)*	Total capsaicinoids (ppm)	Scoville heat units (SHU)	Pungency level
Red pepper-based sauce	152.21±1.017	135.87±1.1287	288.08	4609	moderately pungent
Jalapeno pepper-based sauce	95.36±0.9541	85.21±0.8365	180.57	2889	mildly pungent
Cayenne pepper-based sauce	41.36±0.2547	40.14±0.3698	81.5	1304	mildly pungent

*n=5

Ethics

Ethics Committee Approval: This article does not contain any studies with human participants or animal performed by any of the authors.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: B.C., C.Ö., A.Ö., Design: B.C., C.Ö., A.Ö., Data Collection or Processing: B.C., C.Ö., A.Ö., Analysis or Interpretation: B.C., C.Ö., A.Ö., Literature Search: B.C., C.Ö., A.Ö., Writing: B.C., C.Ö., A.Ö.

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

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Original Article

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The Effect of Hypermobility on Pain and Quality of Life in Young Adults

Genç Yetişkinlerde Hipermobilitenin Ağrı ve Yaşam Kalitesi Üzerine Etkisi

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ABSTRACT

Objective: Hypermobility is the excessive range of motion of joints, and related to various musculoskeletal and extra-articular problems that may significantly impair quality of life (QoL) by causing pain. The aim of the study is to examine the prevalence of hypermobility in young adults, and its relationship with pain in various regions of body and QoL.

Methods: Two hundred and twenty five volunteers, aged between 17 and 23, were classified as subjects-with-hypermobility or subjects-without-hypermobility according to the Beighton Criteria. Chronic pain was identified by using Nordic Pain Questionnaire, QoL was identified by Short Form-36 (SF-36) Questionnaire. Pain presence in 9 body regions and SF-36 scores were compared between groups using chi-square test and Independent Samples T-test, respectively.

Results: Of the participants, 164 (64%) were female, 91 (36%) were male, 119 (46.7%) had hypermobility. Upper back was the body region with the highest pain prevalence where 79% of hypermobile and 74% of non-hypermobile subjects reported pain at least once in past 12 months. Pain prevalence in body regions did not differ between groups ($p>0.05$). In terms of QoL, physical and mental component scores of SF-36, as well as all subgroup scores except social function were significantly lower in hypermobile subjects ($p<0.05$).

Conclusion: Pain prevalence in different body regions did not differ between subjects with and without hypermobility whereas the QoL

ÖZ

Amaç: Hipermobilite eklem hareket açıklığının normal olan değerlerden fazla olması olarak tanımlanmaktadır. Çeşitli muskuloskeletal yaralanmalar ve ekstra-artiküler problemlerle ilişkilidir ve vücudun farklı bölgelerinde kronik ağrılara sebep olarak yaşam kalitesini (YK) belirgin şekilde etkileyebilir. Bu çalışmanın amacı, genç yetişkinlerde hipermobilitenin görülme sıklığını incelemek ve hipermobilitenin vücudun çeşitli bölgelerindeki ağrı prevelansı ve YK üzerindeki etkisini araştırmaktır.

Yöntemler: Araştırmaya yaşları 17-23 arası olan toplam 255 gönüllü katıldı. Katılımcılar Beighton Hipermobilite Kriterleri'ne göre hipermobilitesi olan veya olmayan bireyler olarak sınıflandırıldı. Nordic Ağrı Anketi ile kronik ağrının varlığı ve lokalizasyonu; Kısa Form-36 (KF-36) anketi ile de YK değerlendirildi. Gruplar vücudun 9 bölgesi için bildirilen ağrı açısından ki-kare testiyle, KF-36 anket skorları açısından ise Bağımsız Örneklem t-testiyle karşılaştırılarak değerlendirildi.

Bulgular: Katılımcıların 164'ü kadın (%64) 91'i erkek (%36) idi. Katılımcıların 119'unda (%46,7) hipermobilite saptandı. Sırt bölgesi katılımcıların en sık ağrı hissettiği bölgeydi ve son 12 ay içerisinde hipermobilitesi olan olguların %79'unun, hipermobilitesi olmayan olguların ise %74'ünün bu bölgede ağrı hissettiği saptandı. Gruplar arasında vücut bölgelerindeki ağrı prevalansları açısından anlamlı fark bulunmadı ($p>0,05$). YK açısından hipermobil bireylerde KF-36 anketinin fiziksel ve mental total skorları ile sosyal fonksiyon haricindeki alt grup skorları daha düşüktü ($p<0,05$).

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was significantly impaired in hypermobile subjects. Hypermobility is a substantial anatomical finding in young adults that should not be disregarded. Education, emotional support and encouraging about strengthening and proprioception exercises may contribute to their quality of life.

Keywords: Joint laxity, hypermobility, pain, quality of life, anatomy

Sonuç: Hipermobiliteye sahip genç yetişkinlerde hipermobilitesi olmayan bireylere kıyasla vücudun farklı bölgelerindeki ağrı prevalansları açısından fark saptanmamıştır; ancak YK'sinde belirgin bir etkilenme mevcuttur. Hipermobilite, bu yaş grubunda göz ardı edilmemesi gereken bir anatomik bulgudur. Bireylerin bu konu ile ilgili eğitimi, emosyonel yönden desteklenmesi ve güçlendirme ve propriosepsiyona yönelik egzersiz programı açısından cesaretlendirilmesi ile YK'sine katkı sağlanabilir.

Anahtar Sözcükler: Eklem laksitesi, hipermobilite, ağrı, yaşam kalitesi, anatomi

Introduction

Joint hypermobility is defined as the range of motion of the joint that is greater than normal values according to age, gender and ethnicity. It is called Generalized Joint Hypermobility (GJH) when joint hypermobility is asymptomatic and Benign Joint Hypermobility Syndrome (BJHS) in cases accompanied by musculoskeletal symptoms (1). Therefore, joint hypermobility can be considered as a descriptive statement rather than a diagnosis (2). The primary cause of hypermobility is ligamentous laxity which is determined by genes encoding collagen, elastin, and fibrillin (3).

The prevalence of hypermobility varies according to age, gender and ethnicity. It is more common in females, Asians and Africans, and its prevalence is higher in childhood and tends to decrease towards adulthood (4,5). In addition to this genetic predisposition, it can be acquired as a result of intense training and stretching. Recently, it has been considered that it may overlap with or be a mild form of Heritable Connective Tissue Disorders (HCTDs) such as Ehlers-Danlos syndrome (EDS), Marfan Syndrome and Osteogenesis Imperfecta, in which connective tissue matrix proteins are affected. It is even thought that BJHS is the same as EDS type III (hypermobile type) (3). There are various scoring systems in diagnosis. Beighton scoring system and the revised Brighton criteria are commonly used for GJH (6) and BJHS (7), respectively. Lately, updates have been made emphasizing that the Beighton score should have different cut-off values according to the different ages (8). The results regarding the prevalence of GJH in the literature are highly variable due to the different evaluation methods, cut-off values, populations and age groups chosen in the studies.

Hypermobility may not cause any symptoms. Moreover, it may be advantageous for musicians and individuals who is engaged in gymnastics, martial arts, ballet (9-11). However, changes in the connective tissue matrix lead to joint and soft tissue injuries, and an inability of fast and adequate recovery through the instability of the joint capsule and decreased flexibility of ligaments and tendons (1,12). Besides, it has been observed to be associated with a wide variety of extra-articular clinical conditions such as muscle weakness, decreased motor abilities, skin problems, rectal-uterine prolapsus, pan intestinal dysmotility, fibromyalgia, low bone density, anxiety and panic disorders (13-19). Hypermobility is also associated with chronic pain which leads to avoidance of exercise to prevent pain. This sedentary life causes functional disability, chronic fatigue, sleep disorders, deterioration in work and social lives, anxiety, depression, and a decrease in quality of life (QoL) all of which make the individual more inactive as a vicious circle (1,5). The aim of this study is to investigate the prevalence of hypermobility in young adults, and evaluate its impact on pain and QoL, which is often disregarded.

Methods

This observational and cross-sectional study was conducted with a total of 255 participants aged between 17-23 years. Participants were selected among the university students by using the university notice board, and volunteers were included in the study. Those with a diagnosis of orthopedic or neurological diseases, and a history of musculoskeletal surgery that might affect mobility were excluded. Participants were informed about the purpose of the study and the evaluations to be applied. An informed consent form was obtained from each participant. This study was approved by the Non-interventional Clinical Research Ethics Committee and conducted in accordance with the Declaration of Helsinki.

Demographic data of participants including age, gender, height, weight, body mass index, smoking status, sports status, and the department they studied were recorded. Participants were classified as "subjects with hypermobility" and "subjects without hypermobility" according to the Beighton criteria. Presence and localization of chronic pain were evaluated with the Nordic Pain Questionnaire (NPQ). QoL was evaluated with the Short Form-36 questionnaire (SF-36). Participants with and without hypermobility were compared in terms of chronic pain and QoL.

The Beighton criteria examines the presence of hypermobility with 9 movements involving the extremities and trunk (Table 1). Items about extremities are evaluated symmetrically as right and left, while the trunk is evaluated with only one item. Each item is scored as "1" if the movement can be done, "0" if it cannot be done. In this evaluation, which is made out of 9 points in total, individuals with a score of 4 and above are considered to have joint hypermobility (6).

The NPQ is used to assess the localization and severity of chronic pain. "Did you feel pain in the last 12 months?", "Did the pain in that area prevent you from doing your job in the last 12 months?", "Did you feel pain in the last 7 days?" are asked to

Table 1. Beighton criteria

	Right	Left
Passive dorsiflexion of the little fingers beyond ≥90˚		
Passive apposition of the thumbs to the flexor aspects of the forearm		
Hyperextension of the elbow beyond ≥10˚		
Hyperextension of the knee beyond ≥10˚		
Forward flexion of the trunk with knees fully extended so that the palms of the hands rest flat on the floor		
Total score (9 points)		

evaluate the pain in neck, shoulders, upper back, elbow, wrists-hands, lower back, hips-thighs, knees, ankles-feet (20). The validity and reliability in Turkish language were demonstrated (21).

The SF-36 is used to assess health-related QoL physically and mentally. It evaluates 8 contents of health with 36 items: physical function, limitation due to physical and emotional problems (physical and emotional role limitations), vitality (energy/fatigue), mental health, social function, bodily pain and general perception of health. Scores vary between 0-100, with 100 points indicating the best and 0 points the worst health situation. In addition to giving a score for each scale, two separate total scores can be calculated as physical and mental component scores (22). Turkish validity and reliability of SF-36 were demonstrated (23).

Statistical Analysis

The SPSS v.20 program (IBM Inc. USA) was used for data analysis. The distribution characteristics of the data were analyzed with the Kolmogorov-Smirnov test. Independent Samples t-test was used for numerical variables with normal distribution. Mann-Whitney U test was used for non-normally distributed or ordinal variables to compare pain and QoL scores of participants with and without hypermobility. Chi-square test was used to compare categorical (nominal) variables between groups. The level of significance was accepted as $p<0.05$ for all analyses.

G-Power 3.1 (Universitat Dusseldorf, Germany) computer program was used to determine the sample size (24). It was shown that the QoL score measured by SF-36 was 55±25 in individuals with hypermobility and 87±18 in healthy controls (25). In order to detect a similar difference in this study with 95% confidence level and 80% power, it was calculated that at least 10 patients should be included in each of the "subjects with hypermobility" and "subjects without hypermobility" groups. Considering the prevalence of hypermobility was at least 5% in the healthy population (5), it was calculated that including at least 200 participants in the study would be appropriate to determine hypermobility in at least 10 participants among the total participants.

Results

Of the participants, 164 (64%) were female, 91 (36%) were male. The mean age of the participants was 19.81±1.41 years. According to the Beighton Criteria, 119 (46.7%) of the participants were hypermobile, while 136 (53.3%) were not hypermobile. Hypermobility was present in 50% of females and 40.7% of males. The demographic characteristics of the participants are shown in detail in Table 2.

Painful regions of the participants in the last 12 months were examined according to NPQ and it was observed that 79% of the hypermobile subjects felt pain in the upper back and 70.6% in the lower back region. Similarly, in the group without hypermobility, the most affected regions were upper back (74.3%) and lower back (69.9%). There were no statistically significant differences between participants with and without hypermobility in terms of pain prevalence in all regions ($p>0.05$) (Table 3).

When the QoL of the participants was evaluated with SF-36 (Table 4); subgroup scores of physical function, role limitation (physical-emotional), energy, mental health, bodily pain, general health and total scores of physical and mental component were statistically lower in hypermobile individuals compared to individuals without hypermobility ($p<0.05$). There was no

Table 2. Demographic characteristics of the participants

Age (years) (mean ± SD)	19.81±1.41
Body mass index (kg/m²) (mean ± SD)	21.93±2.88
Gender	n (%)
Female	164 (64.3)
Male	91 (35.7)
Smoking	n (%)
Smokers	35 (13.7)
Non-smokers	220 (86.3)
Regular physical exercise	n (%)
Doing regular physical exercise	68 (26.7)
Not doing regular physical exercise	187 (73.3)
Body mass index (kg/m²)	n (%)
Underweight (<18.5)	21 (8.2)
Normal weight (18.5-24.9)	203 (79.6)
Overweight (25-29.9)	26 (10.2)
Obesity class I (30-34.9)	5 (2)
Hypermobility status	n (%)
Subjects with hypermobility	119 (46.7)
Subjects without hypermobility	136 (53.3)

SD: Standard deviation

Table 3. Pain presence reported in last 12 months

	Normal subjects (n=136)	Hypermobile subjects (n=119)	p value
Neck	94 (69.1%)	83 (69.7%)	1.000
Shoulder	75 (55.1%)	75 (63%)	0.251
Upper back	101 (74.3%)	94 (79%)	0.376
Elbow	20 (14.7%)	16 (13.4%)	0.858
Wrist	56 (41.2%)	54 (45.4%)	0.528
Lower back	95 (69.9%)	84 (70.6%)	1.000
Hip	36 (26.5%)	39 (32.8%)	0.275
Knee	37 (27.2%)	40 (33.6%)	0.277
Ankle	41 (30.1%)	33 (27.7%)	0.681

Table 4. Quality of life outcomes based on SF-36

	Normal subjects (n=136)	Hypermobile subjects (n=119)	p value
Physical function	93.78±8.15	90.84±13.78	0.036
Role limitation-physical	81.06±30.19	72.47±35.71	0.039
Role limitation-emotional	50.73±42.16	38.65±44.04	0.026
Energy	54.08±19.70	47.94±20,26	0,015
Mental health	67.58±14.59	63.36±18.65	0.044
Social function	74.54±21.37	70.75±23.94	0.184
Bodily pain	80.11±12.54	74.40±17.67	0.003
General health	66.83±16.17	61.47±19.86	0.018
Physical component score	80.45±11.75	74.79±16.32	0.002
Mental component score	61.73±19.21	55.11±20.31	0.008

Short form-36: SF-36

statistically significant difference between the groups in terms of social function (p=0.184).

Discussion

In present study, hypermobility was detected in 46.7% of the participants. Pain prevalence in different body regions in the past 12 months did not differ between participants with and without hypermobility. In terms of QoL; physical function, role limitation due to physical and emotional problems, energy, mental health, bodily pain, general health, physical and mental component scores of hypermobile participants were lower than the participants without hypermobility.

The prevalence of GJH was reported between 10-30% in some studies examining the adult population (26,27). This rate was 43% in Nigeria (28), and 38.5% in females and 25.4% in males in Iraq (29). In UK, it was reported as 34% between the ages of 20-30 and 18.4% in those aged 60 and above in Caucasian female twins (30). In American university students; Reuter and Fichthorn (12) reported two different rates as 12.5% and 18.2% with two separate cut-off values, 5 and 4, respectively, and Russek and Errico (31) reported as 26.2% when the cut-off value was approved as 5. In Turkey, the prevalence was shown between 12.4% and 22% in females; between 6.1% and 7.7% in males in pediatric and adult populations (32-35). In the only study conducted in Turkey with different cut-off values, the prevalence in university students was shown as 25.9% and 34.9% when cut-off values were approved as 5 and 4, respectively (36). Conspicuously, it was observed that different results were obtained with different cut-off values in the same populations. Considering the decrease in range of motion of the joints with aging, it has been thought that using different cut-off values for adults and children when diagnosing GJH can prevent false positive and false negative results. Due to the diagnostic complexity and different demographic characteristics, the results regarding the prevalence of GJH are quite different in the literature. In present study, the cut-off value of Beighton criteria was determined as 4. Accordingly, the prevalence of GJH was 46.7%, and its prevalance was higher in females (50%) than males (40.7%), similar to the literature. The prevalence of GJH detected was higher than the studies conducted with similar age groups in the literature. This may be due to the fact that the majority of the population in this study is women. Also, the determined cut-off value may also have increased the calculated prevalence levels.

Hypermobile individuals are thought to be more prone to musculoskeletal diseases, such as subluxations, dislocations, meniscal and muscle tears, degenerative joint diseases, synovitis, arthralgia, myalgia, spondylolysis/spondylolisthesis, due to the increased joint laxity with the changes in neuromuscular reflexes and the decrease in proprioception (5,37,38). The most dominant complaint of these individuals is pain. Pain may be acute, localized and recurrent as a result of the musculoskeletal problems mentioned above, or it may be presented as chronic widespread pain (39). Its relationship with chronic pain is thought to be related to the possible pathogenic mechanisms, such as repetitive microtrauma, sensitization of pain receptors, amplification of pain signals, and central hyperexcitability (5). Musculoskeletal pains are observed more frequently in weight-bearing joints, such as knees and ankles due to biomechanical loading, impaired proprioception, decreased muscle strength and endurance (40,41). In adolescents, hypermobility was reported to be associated with shoulder, knee, foot-ankle pain, while not associated with neck, upper back, upper arm, elbow, wrist-hand and hip pain (42). Seçkin et al. (34), reported that 30.7% of hypermobile high school students had low back pain, 16.8% had arthralgia, and 13.9% had sprain complaints. In patients with BJHS, Albayrak et al. (43), observed low back (32.2%) and knee pain (27.8%) most frequently. In another study on BJHS; upper back pain, sprain/strain, and muscle pain ratios were reported 60-74%, 66-74% and 54-72%, respectively (44). When the patients with BJHS and hypermobile type EDS were evaluated, the most common sites of pain were reported as neck (90.4%), shoulder (80.8%), knee and ventral side of the leg (76.9%) (25). Russek and Errico (31) reported that upper back pain, sprain,

and stress fractures were more common in those with BJHS than in those without BJHS, but there was no difference in the injury prevalence between those with and without GJH, and so hypermobility alone was not associated with symptoms. In present study, upper and lower back were observed as the regions that pain was mostly localized rather than weight-bearing lower extremity joints in hypermobile individuals. However, compared with healthy individuals, no statistically significant relationship was found between joint hypermobility and painful areas in whole body. The frequencies of upper back pain (74.3%) and lower back pain (69.9%) were also found to be high in healthy participants. This may be related to the situation that lower and upper back are frequently observed pain regions in normal populations regardless of hypermobility as reported in epidemiological studies (45). Besides, the studies in the literature were mostly conducted with BJHS and EDS types, while the present study was conducted with the participants from the healthy population rather than the patients with HCTDs, and this might also affect the results obtained. In addition, recall bias should be considered, due to the retrospective questioning of pain.

Hypermobility is associated with acute and chronic pain as well as various extra-articular problems, such as autonomic and psychiatric problems that affect physical functions, and cause chronic fatigue, sleep problems, and various psychosocial problems. This situation affects the QoL negatively (1). Voermans et al. (46), emphasized that pain was more common in the patients with hypermobile type EDS, and associated with deterioration in sleep quality and functional loss in daily living activities. Studies evaluating QoL with SF-36 generally yielded lower scores than the normal population. In a study on hypermobile type EDS, all scores of SF-36 were found to be lower than the normal population (47). Albayrak et al. (43), found the scores of physical function, physical and emotional role, and mental component lower in patients with BJHS. In the same study, it was observed that lower physical and mental component scores were associated with higher fatigue level and decreased sleep quality (43). Castori et al. (48), detected a lower bodily pain score in those with classical and hypermobile type EDS compared to the normal population which might be associated with chronic pain. In the same study physical function, physical role, general health, vitality and social function scores also created significant differences compared to the normal population (48). In a study conducted with a group, consisting of patients with BJHS and hypermobile type EDS, all physical scores except mental health and mental component scores were found to be lower (25). It is observed that the studies evaluating the QoL in the literature mostly focus on patients with a diagnosis of EDS and BJHS. In present study, the QoL of individuals with GJH was evaluated. Consistent with the literature, lower physical and mental scores were remarkable in the results. The major differences were found in bodily pain and physical component scores. Hypermobile participants did not differ from the participants without hypermobility only in terms of social function. Although there were no differences between the groups in terms of painful areas, the fact that bodily pain and physical component scores of SF-36 were affected more prominently suggested that pain and accompanying physical limitations had a significant effect on hypermobile individuals' QoL. In addition, low mental scores may support that hypermobility is not only a physical problem. In the literature, it was presented that various psychiatric problems could accompany with hypermobility. Therefore, the effects of chronic fatigue, sleep problems, and psychiatric problems on QoL should be evaluated in hypermobile individuals. The fact that social functions were not affected by hypermobility might be due to the selection of the participants from healthy university students rather than a group of patients with EDS subtypes.

Study Limitations

The most important limitations of this study were that; it was limited to a specific population, and the majority of the population was already female participants where hypermobility is common. The prevalence of hypermobility in young adult population may be overestimated due to the significantly higher number of female participants. The results obtained mostly reflected young adults and could not be generalized to the whole population. Therefore, longitudinal studies with wider age ranges are needed. In addition to these, especially considering the updates in 2017, age-based assessments in the diagnosis of hypermobility can reflect the society better. Besides, evaluating the sleep quality, fatigue, and physical activity level in hypermobile individuals may be essential in investigating the underlying causes of deterioration in QoL.

Conclusion

Although hypermobility is a common condition in the population, it can be neglected due to the wide symptom scale, the nonspecific nature of some symptoms, and the belief that it is a benign condition that does not cause any problems. Therefore, it is important to recognize hypermobile individuals and be aware of the clinical importance of the problems that may be encountered in the future. Hereby, there can be an opportunity to support them with education and exercise programs, including strengthening and proprioception. Early recognition of hypermobility can prevent the inveteracy of pain. Since hypermobility is a common feature of HCTDs, it is important to evaluate the family history and other accompanying symptoms for the definition and prognosis of BJHS and other HCTDs. These individuals, for whom we often seek solutions to their pain problems in daily practice, should also be evaluated for sleep quality, physical activity status, and participation in daily life activities. Thus, it can be aimed not only to solve the pain and other symptoms, but also to increase their QoL.

Ethics

Ethics Committee Approval: Bezmialem Vakıf University Non-Interventional Research Ethics Committee (date: 12/07/2018/ no: 11200).

Informed Consent: An informed consent form was obtained from each participant.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: E.Z., Y.A., Design: E.Z., Y.A., Data Collection or Processing: E.Z., F.M., M.P., Analysis or Interpretation: Ç.A.K., M.Z., Literature Search: Ç.A.K., E.Z., F.M., M.P., Ö.K., H.B., Y.A., Writing: Ç.A.K., E.Z., M.Z.

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Original Article

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Investigation of Short and Long Term Effects of Various Mouthwashes on the Color Stability of Hybrid Composites

Çeşitli Ağız Gargaralarının Hibrit Kompozitlerin Renk Stabilitesi Üzerine Kısa ve Uzun Dönem Etkilerinin İncelenmesi

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ABSTRACT

Objective: The color stability of dental composite restorations is an important criteria for clinical success. This study aimed to investigate the long-term effects of various mouthwashes on the staining of direct composites.

Methods: Disc-shaped samples were prepared by using 4 different commercially available hybrid composites (Clearfil Majesty, Kuraray; Charisma Smart, Heraeus Kulzer; Quadrant Universal, Cavex; Brilliant EverGlow, Coltene) and divided randomly into five groups according to mouthwashes: Sensodyne, Oral B 3D White Luxe Glamorous Shine, Listerine, Colgate Plax, Meridol. Initial colors of specimens were measured by using a spectrophotometer (Vita Easyshade V, Germany). Specimens were immersed in the mouthwashes and stored in an incubator set at 37 °C for 12, 60, and 120 hours, equivalent to daily use of mouthwash for 1, 5, and 10 years, respectively. Subsequently, the color change value of different materials was calculated as ΔE*ab. The data were analyzed by ANOVA and paired sample t-tests.

Results: The mouthwash type and application time affected the color change values (p=0.00). The most color change (ΔE*) was observed in Colgate Plax, followed by Meridol. The least ΔE* was observed in Sensodyne after 1 year and in Oral B after 5 and 10 years. The materials showing the least and most ΔE* after 1-year mouthwash application were Brilliant and Quadrant, respectively. The least and most ΔE* after 5 and 10 years were observed in Brilliant and Charisma groups, respectively.

ÖZ

Amaç: Dental kompozit restorasyonların renk stabilitesi klinik başarı için önemli bir kriterdir. Bu çalışmanın amacı çeşitli gargaraların direkt kompozitlerin renk stabilitesi üzerindeki uzun dönem etkilerini araştırmaktır.

Yöntemler: Disk şeklidenki örnekler 4 farklı hibrit kompozit (Clearfil Majesty, Kuraray; Charisma Smart, Heraeus Kulzer; Quadrant Universal, Cavex; Brilliant EverGlow, Coltene) ile hazırlanarak, gargaralara göre rastgele beş gruba ayrıldı: Sensodyne, Oral B 3D White Luxe Glamorous Shine, Listerine, Colgate Plax, Meridol. Numunelerin başlangıç renkleri bir spektrofotometre (Vita Easyshade V, Almanya) kullanılarak ölçüldü. Numuneler 37 °C'ye ayarlanmış bir inkübatörde 1, 5 ve 10 yıl boyunca günlük gargara kullanımına eşdeğer olan 12, 60 ve 120 saat süreyle gargaralar içerisinde saklandı. Ardından farklı materyallerin renk değişim değeri ΔE*ab olarak hesaplandı. Veriler ANOVA ve eşleştirilmiş örnek t-testleri ile analiz edildi.

Bulgular: Gargara çeşidi ve uygulama süresi renk değişim değerlerini etkilemiştir (p=0,00). En fazla renk değişimi (ΔE*) değerleri Colgate Plax'ta gözlendi ve bunu Meridol izledi. Bir yıllık uygulama sonrası en düşük ΔE* değerleri Sensodyne grubunda, 5 ve 10 yıllık uygulama sonrasında ise Oral B grubunda gözlendi. Bir yıllık gargara uygulamasından sonra en düşük ve en yüksek ΔE* değerleri gösteren materyaller sırasıyla Brilliant ve Quadrant'tır. Beş ve 10 yıllık uygulama sonrası en düşük ve en yüksek ΔE* değerleri ise sırasıyla Brilliant ve Charisma gruplarında gözlendi.

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Conclusion: The staining in composite restorations caused by mouthwashes varies depending on the structural properties of the resin composite, the pH of the mouthwashes, and exposure time.

Keywords: Coloration, color measurement, composite resins, mouthwashes, spectrophotometry

Sonuç: Kompozit restorasyonlarda ağız gargaralarının neden olduğu renklenme, rezin kompozitin yapısal özelliklerine, gargaraların pH değerine ve gargaraya maruz kalma süresine bağlı olarak değişmektedir.

Anahtar Sözcükler: Renklenme, renk ölçümü, kompozit rezin, ağız gargaraları, spektrofotometri

Introduction

The increase in aesthetic expectations has resulted in the development of various tooth-colored restorative material compositions for clinical use. Therefore, various direct resin composites with different particle size, shape, and distribution of fillers have been developed and available on the market (1). The filler particles in the resin composite directly affect the properties such as surface roughness, gloss, wear resistance, and polymerization shrinkage (2). Despite all the improvements, concerns about color stability, longevity, and durability of resin composite restorations still remain.

Dental biofilm formation is the main factor for the initiation and progression of oral infectious diseases such as gingivitis, periodontal inflammation, and caries (3). Mechanical methods such as tooth brushing and interdental cleaning are effective for plaque removal but are directly dependent on personal skills. Besides, it is difficult to provide oral hygiene with effective brushing in disabled or traumatized patients. Various studies have shown that the use of auxiliary methods such as mouth rinsing can be effective in preventing plaque accumulation (4,5). However, frequent usage of mouthwashes can have detrimental effects on dental tissues and restorative materials (6).

Despite the constant improvements in the composition of resin composites, substances such as saliva, food, liquids, and mouthwashes can result in increased solubility (7). Additionally, mouthwashes trigger a decrease in oral pH associated with an increase in sorption and solubility, causing surface degradation and thus discoloration of the composite resin material (2). Previous studies stated that mouthwashes and antiseptics used for oral infection control and antimicrobial activity can cause external discoloration of dental hard tissues and restorations (8-12). However, only a few focused on the newly developed mouthwashes and the discoloration of hybrid composites. Therefore, the amount of discoloration that may occur as a result of exposure to different types of resin composite restorations to different antimicrobial agents is still an issue that needs to be investigated. Accordingly, this study aimed to examine the effects of five mouthwashes on four different aesthetic restorative materials during different periods of time by analyzing color stability. The tested null hypotheses were that: sustainable color stability of different restorative materials after immersion in numerous mouthwashes (1) would not be affected by increasing exposure time (2) and would not demonstrate a difference between the different composite materials or mouthwashes.

Methods

The direct composites used in the current study were included in four hybrid resin composites and presented in Table 1. A3 shade was selected for each brand. A total of 100 disk-shaped specimens were prepared in polytetrafluoroethylene molds

Table 1. The restorative materials used in the present study and their compositions

Material (manufacturer)	Composition	Type
Clearfil Majesty Esthetic (Kuraray Medical Inc., Tokyo, Japan)	Silanated barium glass filler (40% by volume) Pre-polymerized organic filler Bis-GMA* Hydrophobic aromatic dimethacrylate di-Camphorquinone	Nano-hybrid composite
Charisma Smart (Heraeus Kulzer GmbH, Hanau, Germany)	BIS-GMA* matrix and contains approximately 59% filler by volume with a particle size of 0.005-10 µm Barium Aluminum Fluoride glass, highly dispersive silicon dioxide	Micro-hybrid composite
Quadrant Universal LC (Cavex, Holland BV, Netherlands)	Methacrylate-based monomers (24.5% by volume) Silica, silicate glass and fluoride containing fillers (75.0% by volume) Polymerisation catalysts Inorganic pigments	Hybrid composite
Brilliant EverGlow (Coltene/Whaledent AG Altstatten, Switzerland)	Methacrylates, dental glass, amorphous silica, zinc Oxide (range of dimensions of inorganic filler particles: 0.02-1.5 µm)	Submicron hybrid

*Bis-GMA: Bisphenol A-glycidyl methacrylate

(thickness: 2 mm and diameter: 5 mm) and divided into four groups according to the restorative material they were prepared from (n=25 from each material).

Resin composites were packed into these molds according to the manufacturer's instructions. Each mold was located on a flat glass surface separated with a mylar strip that was positioned on the glass to prevent the resin composite from adhering to the surface. Following condensation, another mylar strip was placed on the top surface to avoid oxygen inhibition layer formation and then gently pressed with a glass plate to extrude excess material. Then, each specimen was light-cured for 40 s on each side with a power density of 1,000 mW/cm^2 (Valo LED, Ultradent Products Inc., South Jordan, UT, USA) from the nearest distance perpendicular to the surface. The light output was measured with a radiometer (SDI LED Radiometer, Bayswater, Australia) to provide standardization. To obtain equal thickness in each group, the thickness was checked with a digital caliper (Powerfix Electronic Digital Caliper, Padget Services, London, England).

After polymerization, the specimens surfaces were polished with polishing discs in a decreasing gradient (SwissFlex, Coltene, Altstatten, Switzerland). At the end of these procedures, the debris was removed from surface with ultrasonic cleaning and then specimens were stored in distilled water for 24 h at 37 °C.

The initial colors were measured on a CIE L*a*b* color scale with a spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Bad Sackingen, Germany). Before measurement, the specimens were thoroughly dried and placed in contact with the measuring probe of the spectrophotometer. Color measurements were repeated three times on a standard white background and averaged. Prior to each measurement, the device was calibrated.

The pH of five different mouthwash types was recorded by using a digital pH meter (Hanna HI 83141, USA). Three measurements were taken from each mouthwash and averaged. Subsequently, 25 specimens from each group were randomly divided into five subgroups (n=5) and different mouthwashes were applied to each subgroup (Table 2). The specimens were then packed in 20 mL of the mouthwashes in capped containers to prevent evaporation and were stored in an incubator set at 37 °C for 12 hours, equivalent to daily use of mouthwash for one year (1,12). At the end of the one-year test period, the specimens were immersed in distilled water and the color measurements were repeated. The color change value ΔE*ab was calculated according to the following formula:

$$\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where L* stands for lightness, a* for green-red (-a=green; +a=red) and b* for blue-yellow (-b=blue; +b=yellow). Values of ΔE >3.3 were considered clinically unacceptable.

The same measurements were repeated after 60 and 120 hours which were equivalent to daily use of mouthwash for 5 and 10 years, respectively. The data were collected and subjected to statistical analysis.

Before the sampling procedure, a power analysis was conducted for sample size calculation. When 80% power and error probability α=0.05 were accepted, and the losses of specimens were taken into consideration, it was determined that 5 specimens in each subgroup were required.

Statistical Analysis

The results of color measurements were analyzed by using statistical software, SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The normality of the distributions was confirmed by Skewness, Kurtosis, and the Shapiro-Wilk test. Means and standard deviations were given as descriptive statistics and analysis of variance (ANOVA) was used to evaluate the effect of mouthwashes and material type on color change. Besides, paired sample t-test was used for intragroup comparisons of the different time results. The significance limit was set at $p<0.05$.

Table 2. The mouthwashes used in the present study

Mouthwashes	Composition	Alcohol content	PH	Manufacturer
Sensodyne Cool Mint	Aqua, Glycerin, Sorbitol, Potassium Nitrate, PEG-60 Hydrogenated Castor Oil, Poloxamer 407, Sodium Benzoate, Aroma, Disodium Phosphate, Methylparaben, Propylparaben, Sodium Phosphate, Sodium Fluoride, Sodium Saccharin, CI 42090.	Alcohol free	6.82	GlaxoSmithKline, Brasil
Oral B 3D White Luxe Glamorous Shine	Aqua, Alcohol, Glycerin, Disodium Pyrophosphate, Tetrasodium Pyrophosphate, Polysorbate 80, Aroma, Poloxamer 407, Sodium Saccharin, Sodium Fluoride, Sucralose, CI 42090.	Containing alcohol	6.88	Procter & Gamble, Weighbridge, UK
Listerine Cool Mint	Aqua, Propylene Glycol, sorbitol, poloxamer 407, benzoic acid, sodium saccharin, eucalyptol, methyl salicylate, aroma, thymol, menthol, sodium benzoate, sodium fluoride.	Alcohol free	5.82	Johnson&Johnson Inc., USA
Colgate Plax	Aqua, Glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Flavor, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Fluoride, Sodium Saccharine, Menthol, CI 42051.	Alcohol free	4.5	Colgate-Palmolive, New York, NY, USA
Meridol	Aqua, Xylitol, PVP, PEG-40 Hydrogenated Castor Oil Olaflur, Aroma (mint-aniseed-eucalyptus), Stannous Fluoride, Sodium Saccharin, CI 42051.	Alcohol free	4.2	GABA Group, Basel, Switzerland

Results

The means and standard deviations of the color change (ΔE*) values of the various hybrid composites in five different mouthwash types were shown in Table 3. As a result of the variance analysis, it was detected that the material, the mouthwash type, and application time affected the color change values (p=0.00). In general, it was observed that the color changes caused by mouthwashes, except for Colgate Plax, were within the limits accepted (ΔE<3.3). When the exposure time to mouthwashes was prolonged, the color change values increased mostly, although there was no statistically significant difference in Sensodyne and Oral B subgroups.

In all material groups, the most color change was observed in the Colgate Plax group, followed by Meridol. While the mouthwashes causing the least color change after 1 year of application were Sensodyne and Oral B after 5 and 10 years of application, respectively. The material showing the least color change after 1, 5, and 10 years of mouthwash application was Brilliant, whereas the material that showed the most color change after 1-year application was the Quadrant. On the other hand, the most color change after 5 and 10 years of mouthwash application was observed in the Charisma group.

Discussion

Preservation of the color stability of aesthetic restorative materials is one of the most important features in terms of durability. This property indicates inconsistency between various restorative materials and the color instability is one of the main reasons for the replacement of aesthetic restorations (13).

Intrinsic factors including the matrix, filler composition and size, addition of minor pigments, and the photoinitiator system can affect the color stability of resin composite restorations (5,12). Besides, incomplete polymerization causes a significant influence on color stability (14,15). The increase in particle size of aesthetic restorative materials results in increased water absorption through the polymer chains, affects the bonds between the matrix and

Table 3. The means and standard deviations of the color change (ΔE*) values of the restorative materials in five mouthwashes

Mouthwashes	Restorative materials	1 year colour change (ΔE*) mean ± SD	5 years colour change (ΔE*) mean ± SD	10 years colour change (ΔE*) mean ± SD
Sensodyne Cool Mint	Clearfil	0.36±0.23^{A,a}	1.24±0.93 ^{A,a}	1.76±0.95 ^{A,b}
	Charisma	0.59±0.35 ^{A,a}	0.82±0.27 ^{A,a}	1.09±0.22 ^{A,a}
	Brillant	0.42±0.23 ^{A,a}	1.12±0.45 ^{A,b}	1.33±0.33 ^{A,b}
	Quadrant	0.88±0.33 ^{A,a}	1.12±0.38 ^{A,a}	1.00±0.35 ^{A,a}
	p	0.050	0.681	0.161
Oral B 3D White Luxe Glamorous Shine	Clearfil	0.81±0.28^{A,a}	1.06±0.25 ^{A,ab}	1.24±0.26 ^{A,b}
	Charisma	0.77±0.17 ^{A,a}	1.17±0.25 ^{A,b}	1.18±0.31 ^{A,b}
	Brillant	0.54±0.11 ^{A,a}	0.99±0.27 ^{A,b}	1.01±0.31 ^{A,b}
	Quadrant	0.76±0.16 ^{A,a}	0.85±0.21 ^{A,a}	0.92±0.17 ^{A,a}
	p	0.136	0.247	0.244
Listerine Cool Mint	Clearfil	0.76±0.25^{A,a}	1.40±0.43 ^{A,b}	1.94±0.44^{A,c}
	Charisma	0.96±0.41 ^{A,a}	1.13±0.31 ^{A,a}	1.34±0.53 ^{AB,a}
	Brillant	0.83±0.40 ^{A,a}	1.33±0.35 ^{A,b}	1.27±0.14 ^{B,b}
	Quadrant	0.82±0.30 ^{A,a}	1.06±0.46 ^{A,a}	0.88±0.18 ^{B,a}
	p	0.815	0.489	**0.003***
Colgate Plax	Clearfil	2.99±1.29 ^{A,a}	3.24±0.46 ^{A,a}	3.20±0.67 ^{A,a}
	Charisma	3.91±0.76 ^{A,a}	5.91±0.70 ^{B,b}	5.50±0.69 ^{B,b}
	Brillant	2.76±0.93 ^{A,a}	3.47±0.90 ^{A,a}	3.91±1.92 ^{AB,a}
	Quadrant	3.60±0.31 ^{A,a}	4.69±0.29 ^{C,b}	5.07±0.51 ^{AB,c}
	p	0.194	**0.000***	**0.017***
Meridol	Clearfil	1.43±0.54 ^{A,a}	2.07±0.10 ^{A,b}	2.18±0.14 ^{A,b}
	Charisma	1.16±0.38 ^{A,a}	1.01±0.57 ^{B,a}	2.69±0.41 ^{AB,b}
	Brillant	1.38±0.70 ^{A,a}	1.75±0.46 ^{AB,a}	1.83±0.45 ^{AC,a}
	Quadrant	2.22±0.88 ^{A,a}	2.11±0.45 ^{A,a}	2.38±0.59 ^{A,a}
	p	0.095	**0.003***	**0.038***

Different capital letters indicate statistically significant differences (*p<0.05) between different resin composites for the same mouthwash. Different small letters indicate statistically significant differences (*p<0.05) between different time intervals for the same material.

filler particles, and leads to an uneven surface during polishing and susceptibility to external staining (8,16). Additionally, it has been reported in various studies that the color stability depends on the brand and shade of the material, the radiation time and intensity, and the finishing technique (8). Therefore, the same color shades of different resin composite materials were included in the present study, and standard polishing procedures were followed after equal time polymerization with the same light-curing device.

Color stability can be determined both visually and by specific instruments such as colorimeter or spectrophotometer (16). The methodology used in the current study was similar to previous studies using spectrophotometry (10,15). CIE L*a*b* system is used to investigate color change (ΔE) since it has advantages such as sensitivity, objectivity, and repeatability. Few studies stated that ΔE values higher than 2 could be detected clinically (17). On the other hand, according to most studies, a ΔE value of 3.3 is the limit and higher values are considered clinically unacceptable (18,19). However, values between 2.2 and 4.4 are clinically acceptable for the Healthy Lifestyles Program of the Commissioned Corps of the United States Public Health Service and higher values can also be acceptable depending on the study design (20).

External discoloration may occur due to poor oral hygiene, diet, and smoking habits, and the use of mouthwashes is also considered one of the external factors that threaten the color stability of aesthetic restorations (21). The usage of mouthwashes to control caries and periodontal disease has become popular. Consequently, the current study aimed to assess the color stability of various aesthetic composite materials after subjected to 1, 5, and 10 years of mouth rinsing. It has been reported in the literature that storing resin composites in mouthwash for 12 hours is equivalent in time to 1 year of 2 minutes daily use (1). Therefore, the specimens were stored in mouthwashes for 12, 60, and 120 hours and it was aimed to provide an effect equivalent to the 1, 5, and 10-years exposure. The mouthwashes were changed every 4 hours to maintain their effectiveness.

Previous studies have stated that the smoothest surface of restorations is achieved by polymerizing the resin composites in direct contact with a mylar strip and any additional polishing procedures can lead to an increase in surface roughness (22,23). Therefore, in some studies, polishing was not preferred (14,24). However, finishing and polishing may be required in clinical conditions even if mylar strips are used. In this study, the same polishing processes were applied to all specimens in order to imitate the clinical conditions appropriately.

As is known, the presence of alcohol and low pH of the mouthwashes can affect the surface integrity of the composite materials, promote organic degradation and affect stain resistance (25). Sensodyne caused the least color change after 1 year period, as expected due to its high pH and alcohol-free content (26). In the present study, Oral B 3D White Luxe Glamorous Shine was the only alcoholic mouthwash and caused a similar effect on color change (ΔE) to Sensodyne and Listerine. This situation was explained by Oral B 3D White Luxe Glamorous Shine had the highest pH value among the studied mouthwashes. Even though Listerine did not contain alcohol, it was reported in several studies that due to its low pH (5.82), it could cause biodegradation of resin composites and erosion resulting in staining (1,27). However, in the present study, the color stability of resin composites was not affected by alcohol content, and there was no significant difference among the Sensodyne, Listerine and Oral B 3D White Luxe Glamorous Shine ($p=0.537$ and $p=0.910$, respectively). Colgate Plax and Meridol, which had the lowest pH values, showed significantly higher color change than the other mouthwashes. Considering the mouthwashes that caused the most and the least color change were alcohol-free, it could be stated that alcohol content was not the only factor that had a softening effect on resin composites.

The effect of mouthwashes on the color change of resin composites can also be material-dependent, and the discoloration susceptibility of the material can be attributed to the degradation caused by water sorption (17). Water sorption of a resin composite material is dependent on the quality of the bond between matrix and filler. Extra water sorption may expand the resin component, hydrolyze the silane and result in microcrack formation. The mouthwash solutions can cause staining by promoting microcrack formation at the interface between the filler and matrix (28). In addition, resin composites containing fewer fillers are more prone to staining. Besides, it was reported that the materials containing urethane dimethacrylate in the resin matrix presented higher color stability than materials containing other dimethacrylate types due to the low viscosity and the water sorption properties (29). Since all composite materials used in this study were hybrids and their contents and particle sizes were similar, no statistically significant difference was observed between materials in 1-year of application, even though Brilliant showed the lowest, and Quadrant showed the highest color change. However, after 10-years of Meridol and Colgate Plax application, the Clearfil Majesty Esthetic group exhibit significantly lower color change values than the Charisma Smart group. This situation can be explained by the nano-hybrid structure of Clearfil Majesty Esthetic as well as the micro hybrid structure of Charisma Smart. Thereby, both null hypotheses were partially rejected. The most discolored material after 5 and 10 years of mouthwash application was Charisma smart which contained the bigger filler particles among the composite materials used in the present study, proving the importance of the particle size.

In previous studies, the short-term effects of mouthwashes on composite resins have been widely investigated (1,5,8). However, owing to the developments in restorative materials and adhesive dentistry, the longevity of resin composites has increased. Previous studies have shown that at least 60% of resin composite restorations can last for more than 10 years when the appropriate materials are applied correctly (30,31). On the other hand, studies showing the long-term effects of mouthwashes are very limited (32). Studies have shown that the long term use of mouthwashes containing high concentrations of alcohol may have detrimental

oral effects such as epithelial detachment, keratosis, mucosal ulceration, petechiae, and oral cancer (33). However, it has been controversial if the use of alcohol-based rinsing increases the risk of oral cancer, oropharynx or other head and neck cancers (34). In previous studies, there was no consensus on whether it was a risk factor for cancer. The results of the studies in the literature were inconsistent. Therefore, alcohol-free mouthwashes were mostly preferred in the present study. Even though long-term use of mouthwashes is not recommended, the fact that these products are commercially available on the market and patients can easily buy and use without a prescription have created difficulties against control. In the present study, the effects of mouthwashes on the color stability of restorative materials after 1, 5, and 10 years of application were investigated and it was concluded that increased exposure time of mouthwashes also increased color change.

It has been reported that the washing effect of saliva, water, and different beverages consumed can reduce the staining caused by mouthwashes. Within the limitations of the present *in vitro* study, the relationship between mouth rinsing-induced staining, nutrition, and aging on resin composites could not be examined. Therefore, further *in vivo* studies are required to evaluate discoloration potential of different mouthwashes on various restorative materials.

Conclusion

The findings of the present study indicate that mouthwashes, which play an important role in maintaining periodontal health, may cause staining in resin composite restorations. The amount of this effect may vary depending on the structural properties of the resin composite, factors such as the color, consistency, and pH of the mouthwashes, and exposure time. All resin composites used in the present study showed color difference after immersion in mouthwashes but these differences were not visually perceptible after 1 year of application. However, after 10 years of application, clinically unacceptable staining was observed in some groups. The clinician should consider this situation, examine the color compatibility of existing resin composite restorations with dental tissues during routine controls, and replace discolored restorations when necessary.

Ethics

Ethics Committee Approval: Since the present study named as "investigation of short and long term effects of various mouthwashes on the color stability of hybrid composites" was not conducted on humans or animals, ethics committee approval is not required as only experimental studies were conducted on the material.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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COVID-19 and Vaccine Hesitancy: Could Health Literacy be the Solution?

COVID-19 ve Aşı Tereddüdü: Sağlık Okuryazarlığı Çözüm Olabilir mi?

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ABSTRACT

Objective: Practices such as the use of masks, cleaning measures, and social distancing have come to the fore to prevent the Coronavirus disease-2019 (COVID-19) pandemic. In addition to this, the most important way to fight the pandemic seems to be vaccination. However, "vaccine hesitancy" is seen as an important obstacle to attempts to control the pandemic. With this study, we aimed to evaluate the effects of having inadequate or incorrect information, one of the possible determinants of attitudes towards the COVID-19 vaccine.

Methods: The data of this descriptive study were collected via an online questionnaire from patients (N=496) involving Sociodemographic Data Form, Turkey Health Literacy Scale-32 and Anti-Vaccine Scale.

Results: The data revealed that 7.5% of the participants would not be vaccinated and 14.3% were indecisive. We found a negative correlation between vaccine refusal and health literacy, thus confirming the main hypothesis of our study. Also, an increase in education years was a negative predictor of vaccine hesitation.

Conclusion: Currently, the most important approach in fighting the pandemic is the vaccination of society. Having the right information is extremely important to fight vaccine refusal attitudes. The fight against vaccination requires joint efforts from governments and media resources, including social media.

Keywords: COVID-19, COVID-19 vaccine, health literacy, vaccine hesitancy

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) pandemisini önlemek için maske kullanımı, temizlik önlemleri ve sosyal mesafe gibi uygulamalar ön plana çıkmıştır. Bunun yanı sıra, salgınla mücadelenin en önemli yolunun aşı olduğu görülmektedir. Ancak "aşı tereddütü" pandemiyi kontrol altına alma girişimlerinin önünde önemli bir engeldir. Bu çalışmanın amacı COVID-19 aşısına yönelik tutumların olası belirleyicilerinden biri olan yetersiz veya yanlış bilgiye sahip olmanın etkilerinin değerlendirilmesidir.

Yöntemler: Tanımlayıcı tipteki bu çalışmanın verileri hastalardan (N=496), Sosyodemografik Veri Formu, Türkiye Sağlık Okuryazarlığı Ölçeği-32 ve Aşı Karşıtlığı Ölçeğini içeren çevrimiçi anket yoluyla toplanmıştır.

Bulgular: Veriler, katılımcıların %7,5'inin aşı olmayacağını ve %14,3'ünün kararsız olduğunu ortaya koymuştur. Aşı tereddüdü ile sağlık okuryazarlığı arasında negatif bir ilişki bulunmuş ve çalışmanın ana hipotezi doğrulanmıştır. Ayrıca eğitim yılındaki artışın aşı tereddüdünün olumsuz bir yordayıcısı olduğu saptanmıştır.

Sonuç: Şu anda pandemi ile mücadelede en önemli yaklaşım toplumun aşılanmasıdır. Doğru bilgiye sahip olmak, aşı reddi tutumlarıyla mücadele etmek için son derece önemlidir. Aşı tereddütü ile mücadele, hükümetlerin ve sosyal medya dahil medya kaynaklarının ortak çabalarını gerektirir.

Anahtar Sözcükler: COVID-19, COVID-19 aşısı, sağlık okuryazarlığı, aşı tereddütü

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Introduction

The Coronavirus disease-19 (COVID-19) pandemic not only affected the health of individuals but also brought many social and economic problems. Works to control the pandemic and reduce all these negative effects are continuing worldwide (1). Since the beginning of the pandemic, false information and fake news about COVID-19 started to spread rapidly, confusing people. Beliefs in the prevention and treatment of COVID-19 were negatively affected, as well. In Iran, for instance, misinformation about alcohol intake to eradicate the COVID-19 virus has resulted in the deaths of hundreds (2). Previous studies have reported that fake news may be at the center of vaccine hesitancy (3,4). Many conspiracy theories have been put forward with the rapid spread of fake news and unidentified information in society. Exposure to COVID-19 vaccine refusal conspiracy theories also affects vaccination intention (5).

Health literacy (HL), a way of preventing the spread of misinformation in society, affects people's ability to access reliable information and make informed decisions (6). HL is generally known to help distinguish fake news (7). The World Health Organization (WHO) defines HL as "personal characteristics and social resources that enable individuals and societies to access, understand, evaluate, and use the information to make health-related decisions" (8). The HL status of individuals plays a very important role in the context of seeking health information (9). In today's world, where access to information has become easier with the effect of technology, individuals with sufficient HL can more easily reach the correct health information they need among unverified health information from different sources (10). It is known that poor HL in chronic diseases is associated with increased healthcare expenditures and mortality (11). Due to the complex nature of chronic diseases, prevention and successful management can be achieved by increasing the level of HL of individuals and taking an active role in their health (12). According to a meta-analysis evaluating the relationship between HL and infectious diseases, a low level of HL also negatively affects protective behaviors such as vaccination and hand hygiene (13). With the emergence of the COVID-19 pandemic, it has emerged that HL is also important in communicable diseases. Low HL scores are associated with "vaccine hesitancy" (6,14).

Practices such as the use of masks, cleaning measures, and social distancing have come to the fore to prevent the COVID-19 pandemic. In addition to this, the most important way to fight the pandemic seems to be vaccination. However, "vaccine hesitancy" is seen as an important obstacle to attempts to control the pandemic. While many studies in the scientific world have focused on the effectiveness of the vaccine recently, the concepts of vaccine hesitancy and vaccine rejection appear to be an important public health problem. WHO has identified "vaccine hesitancy" as one of the top ten threats to global health. Vaccination programs can only be effective when they are accepted by large sections of the population (15). Discussions about vaccination applications have come up all over the world recently (16). In addition to the current vaccine refusal attitudes, concerns about the safety and effectiveness of the vaccine have arisen due to reasons such as the emergence of the disease and the rapid production of the vaccine (17). Regarding previous vaccination practices, studies examining anti-vaccine websites have found that the information on these sites underestimates the risk and severity of diseases (18,19). It is seen that these vaccine refusal campaigns are increasingly continuing in the COVID-19 pandemic (20). Hence, it is of great importance that people have access to correct and sufficient information in order to cope with the problems related to vaccine hesitancy and vaccine refusal.

Although it is known that a high level of HL is a basic requirement for the protection and development of an individual's health, very little information has been found about how it affects vaccine acceptance, which is the most important weapon in the fight against COVID-19. Therefore, to fill this gap in the literature, with this study, we aimed to evaluate the effects of having inadequate or incorrect information, one of the possible determinants of attitudes towards the COVID-19 vaccine. For this reason, we measured the "HL" levels and "vaccine refusal" attitudes of people and put forward the hypothesis that people with incomplete or incorrect information would have more negative attitudes towards vaccination.

Methods

Sample and Procedure

A snowball sampling method was used to determine the participants and the data collection tools were sent to 750 people via instant messaging apps by the researchers' personal contacts. All participants were informed about the study and gave informed consent via an online questionnaire. A total of 512 people participated in the study. The results of 12 people due to random marking and four people due to short survey completion times (less than 15 minutes) were not taken into consideration, and analyzes were conducted with 496 people in total.

Data Collection Tools

Sociodemographic Data Form: It was specially prepared for this study by the research team. It was a form in which the demographic data of the participants such as age, gender, occupation, and the preliminary opinions the people had about the vaccination application were asked.

Turkey Health Literacy Scale-32 (THLS-32): The scale was developed by the Republic of Turkey Ministry of Health in 2016 in line with the "European HL Survey-HLS-EU" (21). It consists of 32 questions in total and consists of two dimensions, "prevention from diseases/health promotion and treatment/service", and four processes "accessing health information, understanding health information, evaluating health information and applying/using health information". High scores indicate high HL.

Anti-Vaccine Scale: It was created to evaluate the factors related to vaccine refusal (22). The scale includes 21 items and four factors: "vaccine benefit and protective value, vaccine refusal, solutions not to be vaccinated and legitimization of vaccine hesitancy". High scores indicate high vaccine refusal.

The Ethical Aspect of the Research

In order to conduct the study, ethics committee approval was obtained from the Non-Interventional Research Ethics Committee of the faculty of health sciences of a university (date: 05.04.2021, number: 13). Besides, permission was obtained from the authors who developed the scales by e-mail. On the first page of the data collection form, participants were presented with an information form describing the study objectives and procedure (if the participants checked the "I understand the study and want to participate" box at the bottom of the information form), and those who wanted to participate were enabled to answer the survey questions.

Statistical Analysis

All data were analyzed using the Statistical Package for Social Science Windows version 22.00 (SPSS) web software. Quantitative data were evaluated as percentage, mean and standard deviation. Participants were grouped according to their attitudes towards the vaccine, and the normal distribution condition was evaluated using the Kolmogorov-Smirnov test. Categorical variables were evaluated with the Pearson chi-square test and mean scores of independent variables between groups were evaluated with the Independent Sample t-test. The Pearson Correlation test was used to evaluate the correlations between scale scores. Multiple linear regression analyzes were applied while evaluating the precursor factors of vaccine refusal. For all analyzes $p<0.05$ was taken as a basis for significance.

Results

A total of 135 male and 361 female participants was included in the study. The socio-demographic characteristics of the participants are shown in Table 1. Interestingly, while it was their turn to vaccine in 63.7% of all participants, only 5.2% stated that they were not vaccinated even though it was their time of vaccination, and 7.5% stated that they would not be vaccinated when their time of vaccination would come. Nearly half of the participants (42.1%) stated that they trusted the effects of the vaccine and that they would be vaccinated.

The ages of the patients ($p<0.001$) and their years of education ($p=0.002$) were found to be significantly higher in those who were accepted to be vaccinated. Furthermore, it was found that the intention of vaccination was lower in women than in men ($p=0.013$). Lastly, it was determined that the group with the low intention for the vaccine had higher scores on vaccine refusal scales ($p<0.001$) and lower HL scores ($p=0.008$) (Table 2).

The relationships between the total and sub-dimension scale mean scores of the participants against vaccination and the total and sub-dimension mean scores of HL are shown in Table 3.

In the linear regression analysis, it was determined that education year and age negatively predicted vaccine refusal scores. The model explains the 18% variance with the effect of only 2 of the 4 variables including demographic data and HL (Table 4).

Table 1. Distribution of sociodemographic and medical characteristics of participants (n=496)

	Mean ± SD	
Age (years)	39.30±12.59	
Number of children	1.12±0.97	
Education year	16.95±3.94	
	n	**%**
Sex		
Male	135	27.2
Female	361	72.8
Marital status		
Single	155	31.2
Married	341	68.8
COVID-19 history		
Yes	60	12.1
No	436	87.9
COVID-19 treatment story		
No cure	436	87.9
Outpatient treatment	53	10.7
Hospital treatment	5	1.0
Intensive care treatment	2	0.4
COVID-19 in a first-degree relative		
Yes	174	35.1
No	322	64.9
Death from COVID-19 in a first degree relative		
Yes	76	15.3
No	420	84.7
Did you schedule for the COVID-19 vaccine?		
Yes	180	36.3
No	316	63.7
Were you vaccinated?		
Yes	159	32.1
I choose not to get the vaccine	26	5.2
I was not scheduled	311	62.7
Are you going to get the vaccine when it's your turn to vaccinate?		
Yes	388	78.2
No	37	7.5
Unstable	71	14.3
What is your attitude towards the vaccine?		
I trust the effects of the vaccine and I will be	209	42.1
I am indecisive for the effects of the vaccine and I will be	198	39.9
I am completely indecisive	51	10.3
I am indecisive for the effects of the vaccine and I will not be	16	3.2
I think the vaccine is negative/ineffective and I will not be	22	4.4
Childhood vaccinations		
I've had all of childhood vaccinations	455	91.7
I haven't had all of the childhood vaccinations	6	1.2
I do not remember	35	7.1

SD: Standard deviation, COVID-19: Coronavirus disease-2019

Table 2. Groups by vaccine intention

		Accept to be vaccinated (n=388)		Group with low intention to vaccinate (n=108)		p
		Mean ± SD		Mean ± SD		
Age (years)		40.66±12.18		34.41±12.90		<0.001
Education year		17.16±3.99		16.18±3.66		0.022
Vaccine refusal		38.13±9.76		55.06±12.29		<0.001
HL total score		35.34±8.22		32.93±8.56		0.008
		n	**%**	**n**	**%**	
Sex	Male	115	29.6	20	18.5	0.013
	Female	273	70.4	88	81.5	

HL: Health literacy, SD: Standard deviation

Table 3. Correlations between scales

		HL accessing information	HL understanding information	HL evaluating information	HL using knowledge	HL total score
Vaccine benefit and protective value	r	-0.192**	-0.167**	-0.149**	-0.158**	-0.179**
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Vaccine refusal	r	-0.314**	-0.316**	-0.278**	-0.300**	-0.325**
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Solutions for not getting vaccinated	r	-0.261**	-0.242**	-0.208**	-0.225**	-0.252**
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Legitimation of vaccine hesitation	r	-0.294**	-0.255**	-0.184**	-0.212**	-0.253**
	p	<0.001	<0.001	<0.001	<0.001	<0.001
Vaccine refusal total	r	-0.311**	-0.291**	-0.247**	-0.269**	-0.301**
	p	<0.001	<0.001	<0.001	<0.001	<0.001

HL: Health literacy

Table 4. Regression for vaccine refusal

	Adjusted R^2	B	SE	β	95% CI (LL/UL) for β	p
Vaccine refusal	0.181					
Age		-0.045	0.042	-0.045	-0.128/0.038	0.286
Sex		2.002	1.166	0.071	-0.290/4.293	0.087
Education year		-0.901	0.132	-0.284	-1.159/-0.642	<0.001*
HL total score		-0.435	0.061	-0.291	-0.555/-.0315	<0.001*

HL: Health literacy, CI: Confidence interval, LL: Lower level, UL: Upper level
*: p<0.001

Discussion

According to the results of our study, we found a negative correlation between vaccine refusal and HL, thus confirming the main hypothesis of our study. In addition, we found that the negative predictors of vaccination opposition were not only HL, but also education year.

In a study examining the articles between 2007 and 2017, it was found that HL and vaccine hesitancy were associated with age, country, and vaccine type (13). In this review, it was reported that most of the studies originated from the USA and high-income European countries, data on low-income countries were scarce, hence geographical representation might be weak. Therefore, it is important to conduct such studies in different countries. In fact, vaccine hesitancy is also an important problem in the pre-pandemic period. A study conducted in Italy in 2016 reported that the rate of vaccine hesitancy-vaccine refusal among parents was 16% (23). Studies evaluating the relationship between the frequency of pneumococcal and influenza vaccinations and HL indicated that as the level of HL increased, the vaccination rates increased (24,25). The significant relationship between low HL and vaccine hesitancy has also been demonstrated by COVID-19

studies (6). This finding is in line with the findings of our study, in which we have found a negative relationship between HL scores and vaccine refusal attitudes.

Currently, the most important approach in fighting the pandemic is the vaccination of society. However, vaccine hesitancy-vaccine refusal is a major obstacle to this situation. In the study conducted with 7664 people from 7 European countries, 18.9% of the participants stated that they were not sure about being vaccinated and 7.2% of them did not want to be vaccinated (26). Despite the intervening period of nearly one year, according to the results of our study, 7.5% of the participants stated that they would not be vaccinated and 14.3% were indecisive. In a previous study, 31% of the participants in Turkey stated that they were ambivalent or negative about vaccination administration (27). In the same study, this rate was found to be 14% for the participants in the UK. In a study conducted with 745 students in Italy, 13.9% of the participants stated that they would not be vaccinated or were indecisive (16). When the studies in the literature were analysed, it could be considered that the vaccine hesitancy-vaccine refusal attitudes in Turkey were higher than in other European countries. Hence, it is extremely significant to reveal the reasons for this attitude. In a study in which COVID-19 vaccine hesitancy was examined comparatively in Turkey and England, it was evaluated that vaccine hesitancy was higher in Turkey and this result was associated with the belief that the virus did not have a natural origin (27). In particular, individuals who were exposed to false news about the disease and vaccine on social media increased their anxiety and risk perception towards vaccination and its harms (28). In this context, it is important to improve the HL of individuals to gain the ability to distinguish the right information, inform the public about the origin of the virus, to reduce vaccine hesitancy and support vaccination campaigns.

Having the right information is extremely important to fight vaccine refusal attitudes. For instance, previous studies have shown relationships between believing that the coronavirus is an artificial virus produced in the laboratory and vaccine refusal (27). People's interest in such conspiracy scenarios negatively affects the prevention or treatment strategies. In particular, conflicting news in the media regarding the effectiveness, reliability and side effects of the COVID-19 vaccine may cause vaccine hesitancy or vaccine refusal in individuals. In this context, it is important to share clear and reliable information about the vaccine in the media, which is the source that individuals frequently use to access vaccine-related data. In a recent study conducted with 1,153 people in Germany, only 49.9% of the participants were found to have sufficient HL (29). In the study, it was reported that the lowest scores of the participants were related to the capacity to "decide on the reliability of the information in the media". Having the right information is a very important factor affecting the vaccination decision of individuals.

Another important finding of our study was the negative correlation between age and vaccine refusal. This finding is consistent with the results of the studies evaluating the relationship between age and vaccine acceptance, resulting in lower vaccine hesitancy in the older age group (30). This situation can be interpreted as the elderly group prefer to be vaccinated with the risks rather than getting the disease, due to the frequency/severity of getting COVID-19 and complications as the age increases. In addition, it is evaluated that the fatalistic and submissive attitudes of elderly individuals result in their not being inquisitive about their health and high vaccination acceptance. Therefore, for a successful vaccination program, it should include non-formal education programs on the safety and efficacy of the vaccine, especially for the untrained and young age groups with high vaccine hesitancy (31). In addition to this, it should be taken into consideration that it is important to inform the public correctly; however, HL skills should also be developed in order for the information to provide attitude change (32). As the level of HL increases, it will be possible for individuals to become aware of the reasons behind medical advice and to evaluate the consequences of their actions (33).

The concept of vaccination literacy, which is built on the idea of HL, affects individuals' intention to be vaccinated. Besides, a specific emphasis on the concept of HL is vital to understanding the determinants of attitudes towards vaccination and enabling change of attitude (34). The Erice Declaration, which was prepared in Italy to address issues related to vaccine attitudes before the pandemic, emphasized the promotion of the concept of HL and vaccine literacy and the inclusion of the media in this movement (35). Given the uncertainty created by the pandemic and the confusion of information in the media, the concept of HL can be a fundamental basis for a way out of the pandemic (36).

Study Limitations

The present study had several limitations in interpreting the results. First, using an online survey might be limited to people who had smartphones and could access the internet. However, given the situation, this was the best possible methodology for reaching people. Additionally, responses were self-reported and might be subject to self-report bias. Despite these limitations, our findings were considered to contribute greatly to assessing COVID-19 vaccine hesitancy and its relationship with HL level.

Conclusion

Currently, the most important approach in fighting the COVID-19 pandemic is seen as the vaccination of society. It has been reported that 60-75% of the individuals in society should be vaccinated in order to prevent the transmission and spread of the virus. Based on the conclusion that low HL increases vaccine hesitancy, it may be possible to reduce vaccine hesitancy by improving the HL level of individuals. It is significant to provide accurate, comprehensive, reliable, and transparent information among the public through reliable channels that defend the safety and effectiveness of currently available vaccines, and to improve HL for individuals to distinguish correct information.

Ethics

Ethics Committee Approval: Ankara Medipol University Non-invasive Clinical Research Ethics Committee (date: 05.04.2021/ decision no: 13).

Informed Consent: All participants were informed about the study and gave informed consent via an online questionnaire.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: F.K., F.Y., Design: F.K., F.Y., Data Collection or Processing: F.K., F.Y., N.Ü., Analysis or Interpretation: F.Y., Literature Search: F.K., F.Y., N.Ü., Writing: F.K., F.Y., N.Ü.

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Original Article

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Ramelteon Protects Intestinal Tissue Against Injury Caused by Methotrexate Via Showing Anti-apoptotic, Anti-inflammatory and Antioxidant Properties

Ramelteon, Anti-apoptotik, Anti-enflamatuvar ve Antioksidan Özellikler Göstererek Barsak Dokusunu Metotreksatın Neden Olduğu Hasara Karşı Korur

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ABSTRACT

Objective: Methotrexate (MTX), a drug used in the treatment of autoimmune diseases and cancers, is a folic acid antagonist, but it has toxic effects on the gastrointestinal system (GIS). In this study, we examined the anti-inflammatory, antioxidant and anti-apoptotic effects of Ramelteon (RAM), a melatonin receptor agonist, on the MTX-induced toxicity in the intestinal tissue of rats.

Methods: Thirty-two male Wistar albino rats were randomly divided into 4 groups; Control group, MTX group, MTX + RAM group, and RAM group. Single-dose 0.1 mL 20 mg/kg MTX, saline or 0.1 mL 10 mg/kg RAM orally was administered for 7 days. Animals were sacrificed at the end of 7 days after the last drug administration. Then, intestinal tissues were collected for biochemical, histopathological and immunohistochemical analyses.

Results: While normal histological findings and biochemical parameters were observed in the control and RAM groups, in the MTX group, mononuclear cell infiltrations, hemorrhagic areas, degenerations in the submucosa and Lieberkuhn crypts were observed in the intestinal sections. Caspase-3 (Cas-3) and tumor necrosis factor-alpha (TNF-α) expressions, total oxidant status (TOS) and oxidative stress index (OSI) increased and total antioxidant status (TAS) decreased in the MTX group. RAM treatment decreased Cas-3 and TNF-α expressions, TOS, OSI levels and increased TAS levels.

ÖZ

Amaç: Otoimmün hastalıklar ve kanserlerin tedavisinde kullanılan bir ilaç olan metotreksat (MTX), bir folik asit antagonistidir ancak gastrointestinal sistem (GİS) üzerinde toksik etkileri vardır. Bu çalışmada, bir melatonin reseptör agonisti olan Ramelteon'un (RAM) sıçanların barsak dokusunda MTX ile indüklenen toksisite üzerindeki anti-enflamatuvar, antioksidan ve anti-apoptotik etkilerini araştırdık.

Yöntemler: Otuz iki erkek Wistar albino rat rastgele 4 gruba ayrıldı: Kontrol grubu, MTX grubu, MTX + RAM grubu ve RAM grubu. Yedi gün boyunca tek doz 0,1 mL 20 mg/kg MTX, salin veya 0,1 mL 10 mg/kg RAM oral yoldan uygulandı. Hayvanlar, son ilaç uygulamasından sonra, yani 7. günün sonunda sakrifiye edildi. Daha sonra, barsak dokuları biyokimyasal, histopatolojik ve immünohistokimyasal analizler için toplandı.

Bulgular: Kontrol ve RAM gruplarında normal histolojik bulgular ve biyokimyasal parametreler gözlemlenirken, MTX grubunda mononükleer hücre infiltrasyonları, hemorajik alanlar, submukozada dejenerasyonlar ve barsak kesitlerinde Lieberkuhn kriptleri gözlendi. MTX grubunda kaspaz-3 (Cas-3) ve tümör nekroz faktör-alfa (TNF-α) ekspresyonları, total oksidan seviyesi (TOS) ve oksidatif stres indeksi (OSI) arttı ve total antioksidan seviyesi (TAS) azaldı. RAM tedavisi Cas-3 ve TNF-α düzeylerini, TOS, OSI seviyelerini azaltırken TAS seviyelerini arttırdı.

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Conclusion: In this study, RAM showed anti-apoptotic, antioxidant and anti-inflammatory effects on MTX-induced toxicity in intestinal tissue. Therefore, it was suggested that RAM might be used in MTX-like toxicities to alleviate the side effects on the GIS.

Keywords: Intestinal injury, inflammation, methotrexate, oxidative stress, ramelteon

Sonuç: Bu çalışmanın sonucunda RAM, barsak dokusunda MTX kaynaklı toksisite üzerinde anti-apoptotik, antioksidan ve anti-enflamatuvar etkiler gösterdi. Bu nedenle, GİS üzerindeki yan etkileri hafifletmek için MTX benzeri toksisitelerde RAM'nin kullanılabileceği öne sürülmüştür.

Anahtar Sözcükler: Barsak hasarı, enflamasyon, metotreksat, oksidatif stres, ramelteon

Introduction

Methotrexate (MTX), is a folic acid synthesis inhibitor that is widely used as a chemotherapeutic and immunomodulatory agent (1). It is used in the treatment of rheumatological diseases like arthritis as a Disease-Modifying Anti-Rheumatic Drug (DMARD) and several types of childhood and adult cancers such as osteosarcoma, non-Hodgkin lymphoma (2,3). However, it is a cytotoxic agent and can cause toxic effects on liver, kidney, bone marrow and gastrointestinal system (GIS) (4).

MTX, which inhibits DNA synthesis and cell proliferation by binding to dihydrofolate reductase, causes an increase in the production of reactive oxygen species (ROS) (6). This reduces the amount of antioxidants that protect against damage and causes an inadequate antioxidant response. It is known that ROS-induced oxidative damage may result from the inflammation process (7). Oxidative stress in the gastrointestinal tract may lead to the progression of inflammatory disorders. Disruption of the mucosal barrier in the gut may activate the innate immune system and lead to the expression of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) (8,9). This progression may result in the activation of apoptotic signaling pathways inside the cell depending on increased caspase-3 (Cas-3) levels (9,10).

The common side effects of MTX that limit its therapeutic usage on the GIS can be classified as diarrhea, nausea, vomiting, ulceration, enterocolitis, and mucositis (5). Therefore, elucidating the molecular mechanism of MTX is important for eliminating these side effects and improving the therapeutic efficacy of this agent.

Ramelteon (RAM) is a potent and selective melatonin receptor-1 and 2 (MT1 and MT2) agonist drug and is generally used in the treatment of insomnia (11,12). Studies revealed that this agent exhibited anti-inflammatory effects by reducing interleukin-6, TNF-α, interleukin-1beta, and transforming growth factor-β cytokine levels (13,14). Moreover, Kandezi et al. (15), demonstrated that RAM showed anti-apoptotic properties by inhibiting JNK/Bcl-2-Beclin-1 or JNK/Bcl-2/Bax signaling pathways and showed antioxidant effect via suppressing ROS production.

In this study, we aimed to investigate the underlying mechanisms of RAM's protective effects against MTX-induced intestinal toxicity through antioxidant, anti-inflammatory, and anti-apoptotic pathways.

Method

Animals

Adult male Wistar albino rats (n=32; 250-300 g) were purchased from the Animal Research Laboratory of Süleyman Demirel University. The animals were accommodated in standard laboratory conditions to acclimatize for at least seven days before experimentation. Then, they were group-housed under a 12:12-hour light: dark cycle with constant temperature (24±1 °C) and humidity (50±10%) with access to food and water ad libitum.

Experimental Procedures

All animal procedures were conducted following the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research at Süleyman Demirel University, Isparta (ethic no: 11.09.2020 06/15).

Thirty-two rats were randomly divided into 4 groups with 8 rats in each group: Control group: 0.1 mL of saline was administered by oral gavage (p.o.) for 7 days and intraperitoneal (i.p.) 0.1 ml of saline was administered on day 2. MTX group: 0.1 mL saline p.o. for 7 days and single dose 0.1 mL 20 mg/kg MTX i.p. (MTX 50 mg/mL vial, Koçak, Turkey) was administered on day 2 (16). MTX + RAM group: 0.1 mL 10 mg/kg RAM (Ramelda, Abdi İbrahim,Turkey) p.o. for 7 days and single dose 0.1 mL 20 mg/kg MTX i.p. was administered on day 2. RAM group: 0.1 mL 10 mg/kg RAM p.o. for 7 days and 0.1 mL of saline i.p. was applied on day 2 (17).

At the end of the 7 days after the last drug administration, animals were sacrificed under anesthesia with solution mixture containing ketamine (80-100 mg/kg) (Alfamin, Alfasan IBV) and xylazine bio 2% solution (8-10 mg/kg) (Bioveta, Czech Republic). Then, intestinal tissues were collected. One-half of the tissue was stored at (-20 °C) for biochemical measurements. The remaining part of the tissue was fixed in 10% buffered formaldehyde for histopathological and immunohistochemical analyzes. All these procedures are shown in Figure 1.

Histopathological Analysis

Intestinal tissues taken from rats were washed in water overnight, 10% neutral formaldehyde solution was used to fix them and embedded in paraffin. Samples from the prepared paraffin blocks were sectioned with a thickness of 3-4 mm with a sliding microtome (Leica SM2000R, Germany) and Hematoxylin-Eosin staining was performed, then covered with mounting medium. A

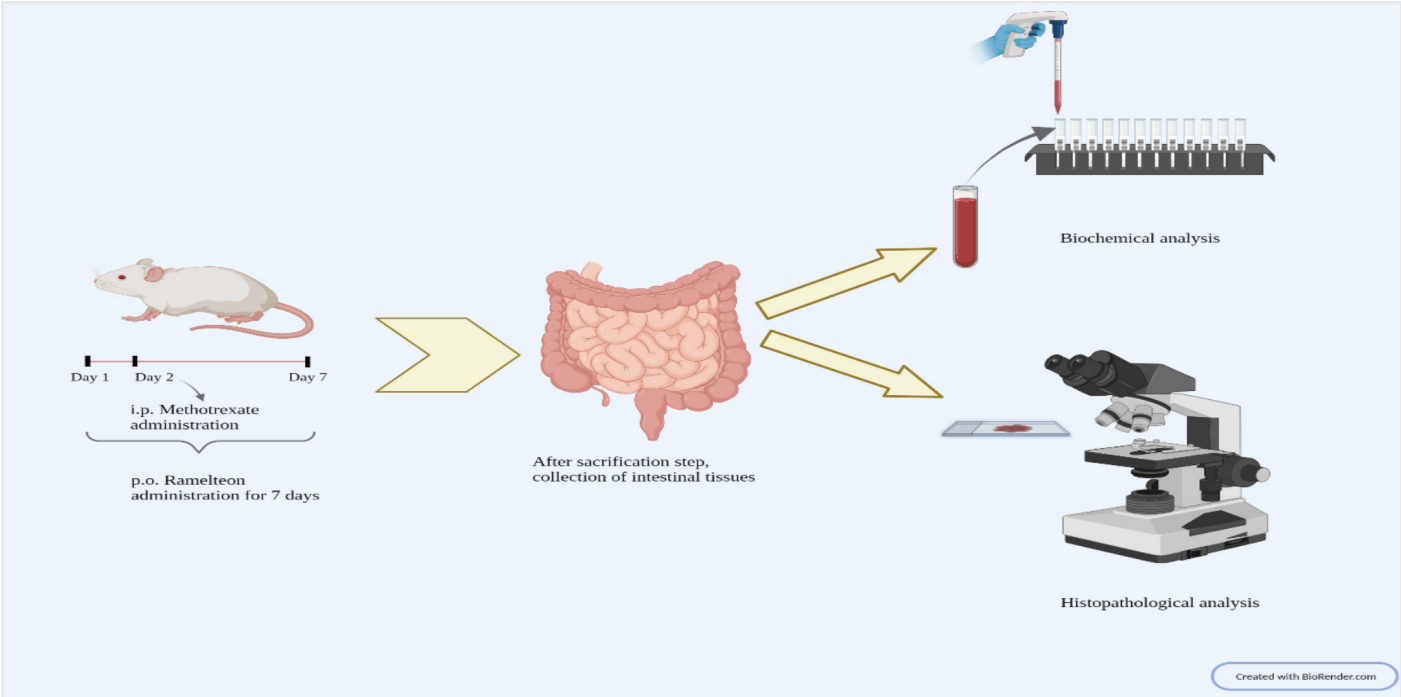

Figure 1. Graphical abstract for experimental procedures

semi-quantitative analysis of histopathological findings was then calculated to allow comparison between the groups. All groups were analyzed and evaluated with a photomicroscope according to scoring by Refaiy (18).

Immunohistochemical Analysis

Intestinal tissues with 3-4 µm thicknesses were fixed in 10% neutral formaldehyde solution and embedded in paraffin before histological methods were utilized. Tissues were stained with TNF-α (rabbit TNF-α antibody, Abcam, Cambridge, USA) and Cas-3 (rabbit Caspase-3 antibody, Abcam, Cambridge, USA) primary antibodies and were covered with mounting medium. Then, immunohistochemical methods were applied and results were evaluated by a semi-quantitative method (16).

Biochemical Analysis

Biochemical analyses included measurements of TAS, TOS, and OSI levels. Homogenization of intestinal tissue samples was carried out with the Ultra Turrax Janke & Kunkel T-25 homogenizer (IKA® Werke, Germany). By using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey), the total antioxidant status (TAS) and total oxidant status (TOS) were measured spectrophotometrically (Beckman Coulter AU5800, Beckman Coulter, USA), and according to results, OSI values were calculated using the formula OSI = TOS/TAS [Erel (19)]. In the TAS analysis which determined the antioxidative effect of the sample against the potent free radical reactions triggered by the produced hydroxyl radical; antioxidants were reduced in the sample from a dark blue-green colored 2,2'-azino-bis (3-ethylbenzthiazoline-6 sulphonic acid; ABTS) radical to a colorless ABTS form. The changing of absorbance at 660nm was related with TAS level of the sample. The results are presented with millimolar Trolox equivalents per liter (mmol Trolox Eq/L) unit (19).

In the TOS analysis, oxidants found in the sample oxidized the ferrous ion-dianisidine complex to the ferric ion. The oxidation reactions were raised with glycerol molecules of the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The intensity of the color is related to TOS levels in the samples. TOS was measured spectrophotometrically at 530 nm by using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). Hydrogen peroxide (H_2O_2) was used in the calibration of this assay. Results were presented with micromolar H_2O_2 equivalents per liter (µmol H_2O_2 Eq/L) unit (20).

Statistical Analysis

Data were analyzed using Graphpad Prism software (Prism5, San Diego, California, US). One-way ANOVA followed by Bonferroni multiple comparison test was performed for analyzing statistical significance of differences between control and experimental groups. Differences were considered significant for $p<0.05$. All results were expressed as mean ± standard deviation.

Results

Total Oxidant Status (TOS), Total Antioxidant Status (TAS), and Oxidative Stress Index (OSI)

The TAS levels decreased significantly compared to the control group in the MTX administered group ($p<0.05$). In the MTX + RAM and RAM groups, TAS levels elevated compared to the MTX group ($p<0.01$ for both). TOS levels significantly elevated in the MTX group compared to the control group ($p<0.001$), decreased both in MTX + RAM and RAM groups

compared to the MTX group (p<0.001, p<0.05, respectively). OSI levels elevated in the MTX group compared to the control group (p<0.001). In MTX+RAM and RAM groups, OSI levels attenuated compared to the MTX group (p<0.01) (Figure 2).

Histopathological Findings

Significant difference was observed between the control group and the MTX, MTX + RAM groups in HE staining of intestinal tissue sections (p<0.05). Histopathological findings demonstrated mononuclear cell infiltration, hemorrhagic areas, degeneration in the submucosa and crypts of Lieberkuhn were detected in the MTX group and RAM treatment reversed these findings (p<0.05) (Figure 3, Table 1).

Immunohistochemical Findings

According to findings of immunohistochemical staining in intestinal tissue sections, TNF-α and Cas-3 expressions were found significantly higher in MTX, MTX + RAM groups compared to the control group (p<0.01, p<0.05, respectively, for both). TNF-α and Cas-3 expressions decreased significantly in the MTX + RAM group, compared to the MTX group (p<0.05, for both). No significant difference was examined between the control and RAM groups (Figure 4, Table 2).

Discussion

The MTX is already a widely used drug in the treatment of malignant and nonmalignant diseases. It is used in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (3). Although it is preferred as a chemotherapeutic agent, hepatic and renal toxicity that causes complications limit its usage. Due to the fact that it causes DNA damage, side effects are not limited to hepatic and renal toxicity, and it can also lead to harmful conditions in other tissues. MTX also affects human gut microbiota resulting in alteration of host immunity (21). These circumstances can cause gastrointestinal complications in patients. The findings from histopathological examinations might be consequences of microbiota changes depending on MTX administration. As a result of histopathological examination, mononuclear cell infiltration in intestinal tissue

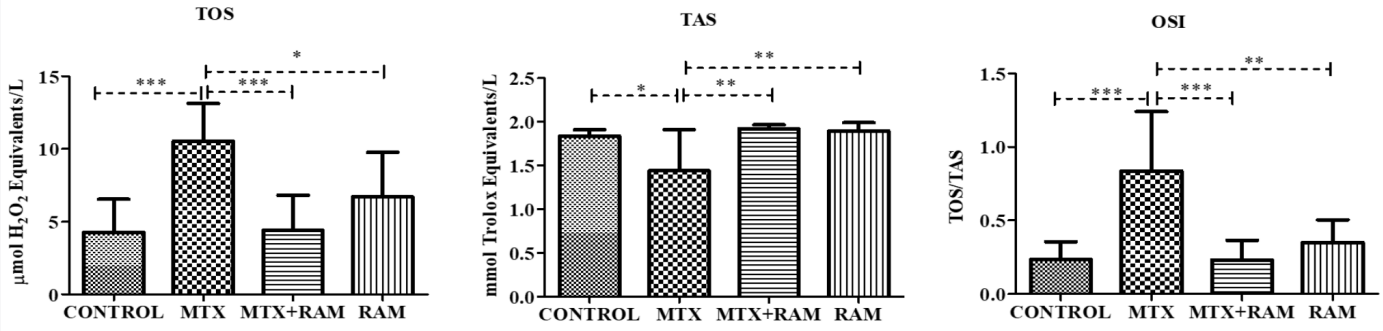

Figure 2. Values are represented as means ± SD. Comparison between groups and results of oxidative stress markers were assessed by one-way ANOVA test followed by post hoc Bonferroni multiple comparison test

MTX: Methotrexate, RAM: Ramelteon, TOS: Total oxidant status, TAS: Total antioxidant status; OSI: Oxidative stress index, SD: Standard deviation

***p<0.001 **p<0.01 * p<0.05

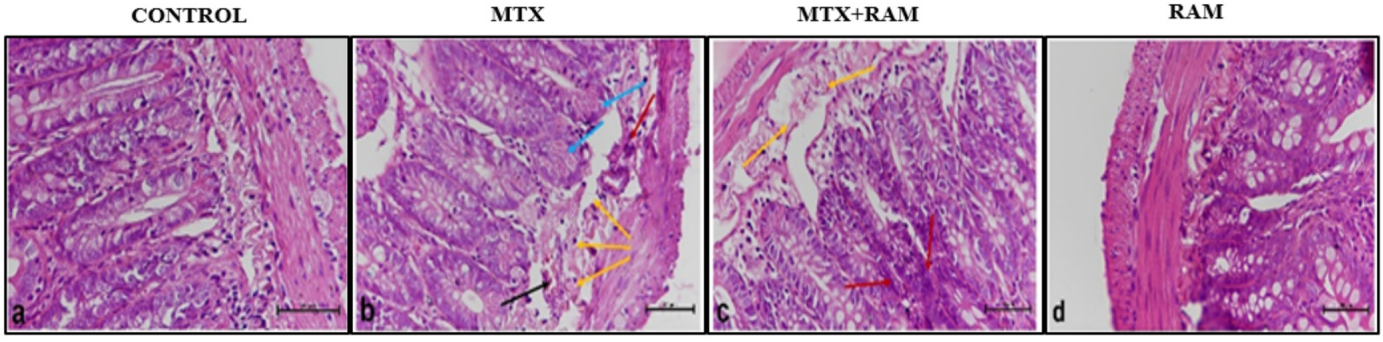

Figure 3. Histopathological findings in intestine tissues belonging to control and experimental groups: **a-** CONTROL group, no histopathological findings were found. **b-** MTX group, **c-** MTX + RAM group, **d-** RAM group. Red arrows; mononuclear cell infiltration, black arrows; hemorrhagic areas, yellow arrows; sinusoidal degeneration in the submucosa and blue arrows; degeneration in Lieberkuhn crypts, HE, x40

MTX: Methotrexate, RAM: Ramelteon

Table 1. Average score of histopathological findings between all groups

Groups	Mononuclear cell infiltrations	Hemorrhagic areas	Degenerations of submucosa	Degenerations of lieberkuhn crypts
Control	-	-/+	-	-/+
MTX	+++	++/+++	++	+++
MTX + RAM	+/++	++	-/+	++
RAM	-	-	-	-/+

(-), negative score: No structural changes; **(+)**, 1 positive score: Light structural changes; **(++)**, 2 positive score: Mild structural changes; **(+++)**, 3 positive score: Serious structural changes
MTX: Methotrexate, RAM: Ramelteon

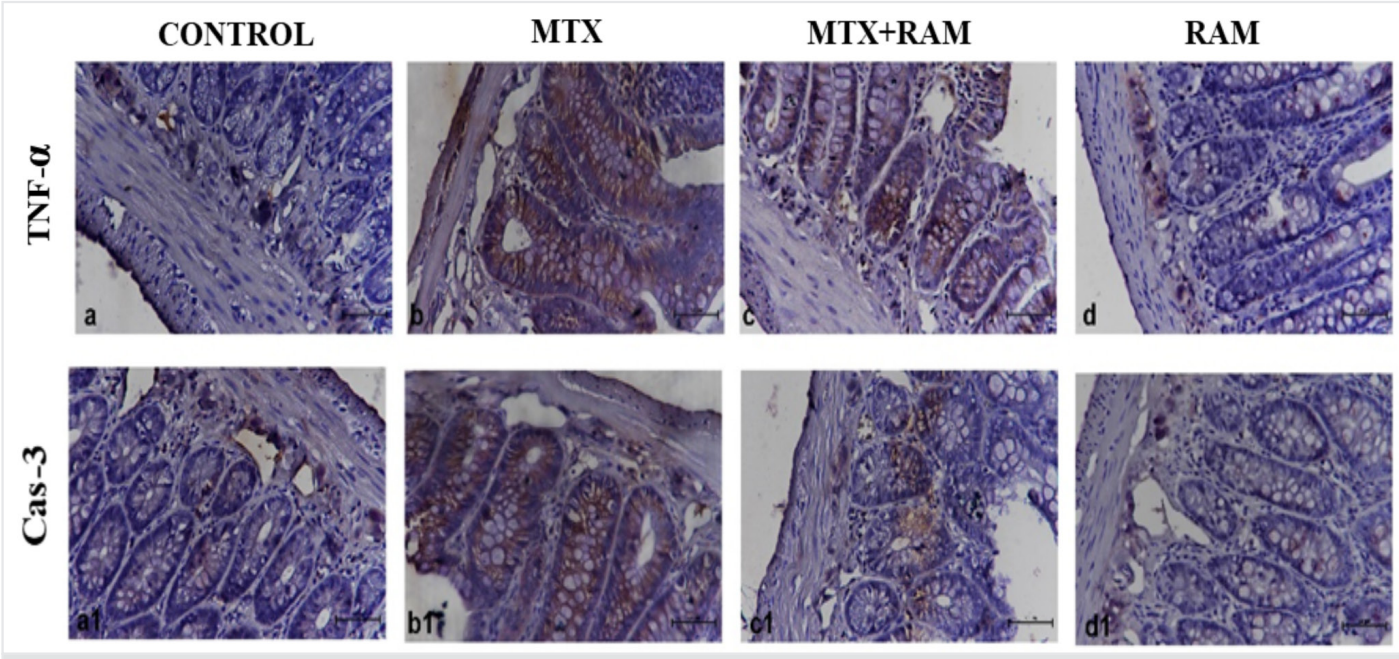

Figure 4. TNF-α, and Cas-3 immune staining in intestine tissues belonging to control and experimental groups: **a-a1**; CONTROL group, **b-b1**; MTX group, **c-c1**; MTX + RAM group, **d-d1**; RAM group. **a-a1**; CONTROL group, no positive staining, **b-b1**; MTX group, positive staining, **c-c1**; MTX + RAM group, mild positive staining, **d-d1**; RAM group, no positive staining, x40.
MTX: Methotrexate, RAM: Ramelteon, Cas-3: Caspase-3, TNF-α: Tumor necrosis factor-alpha

Table 2. TNF-α and cas-3 marking average degrees between all groups

	Control	MTX	MTX + RAM	RAM
TNF- α	0.19±0.463[a]	2.53±0.51[b]	1.65±0.453[c]	0.23±0.374[a]
Cas-3	0.15±0.354[a]	2.78±0.344[b]	1.78±0.364[c]	0.21±0.443[a]

Values are expressed as means ± SD. The comparison between groups and results are evaluated by one-way ANOVA. a, b, c; different characters indicate statistically significant differences in the same column, $p<0.05$, $p<0.01$, $p<0.001$.
TNF-α: Tumor necrosis factor-alpha, Cas-3: Caspase-3, MTX: Methotrexate, RAM: Ramelteon

was a clue for an inflammatory pathway that resulted in the occurrence of hemorrhagic areas, degenerations of the submucosa and formation of Lieberkuhn crypts.

Damaged intestinal tissue indicates that there may be blood flow from the hemorrhagic areas into the intestinal lumen. In addition, disruptions may occur in functions such as fluid absorption and mucus secretion due to degeneration in the submucosa layer and Lieberkuhn layers. As a result of all these, GIS complications such as diarrhea, blood in the urine or stools, vomiting, nausea and indigestion can be seen (22,23).

Moreover, MTX can lead to side effects due to both long and short-term usages. Myelosuppression, abnormalities of liver enzymes and toxicity in the male-reproductive system are possible adverse effects of long-term usage of MTX (24,25).

It is also known that MTX induces overexpression of ROS which initiates mucositis, followed by up-regulation of nuclear factor kappa B pathway-mediated pro-inflammatory cytokine production such as TNF-α which may stimulate apoptosis (26). Morsy et al. (27), studied the toxic effects of MTX on the intestine of rats and found that this agent increased oxidative and

nitrosative stress marker levels in the intestinal mucosa by causing up-regulation of nuclear factor kappa B, cyclooxygenase-2, and increasing TNF-α and Cas-3 levels. In this study, according to biochemical analyses and immunohistochemical examinations, increments in TNF-α, Cas-3 expressions and TOS and OSI levels in the MTX group confirmed this view.

The RAM is a widely used drug in the treatment of insomnia showing a high affinity for MT receptors; MT1 and MT2. Antioxidant effects of melatonin are thought to be the result of its radical-scavenging ability by the MT receptor (28). Moreover, Wang et al. (17), showed that RAM treatment improved dysfunction of brain endothelial, oxidative stress, and inflammation via activating nuclear factor erythroid 2-related factor 2 pathway in traumatic brain injury. In the isoflurane-induced in vitro cell culture model of brain microvascular endothelial cells, RAM downregulated p38 MAPK/NF-κB signaling pathway activation and exhibited an anti-inflammatory effect (29). In the present study, our results confirmed anti-inflammatory, antioxidant, and anti-apoptotic properties of RAM on short-term MTX-induced toxicity in intestinal tissue by diminishing TOS, OSI levels and TNF-α, Cas-3 expressions. In parallel to these results, it also decreased mononuclear cell infiltrations, hemorrhagic areas, degenerations in the submucosa and formation of Lieberkuhn crypts in the intestinal tissues. In line with these observations, to support conceivable molecular mechanisms of RAM on MTX-induced toxicity, additional studies are required.

Conclusion

The RAM, which was a melatonin receptor agonist, showed anti-apoptotic, antioxidant, and anti-inflammatory effects on MTX-induced toxicity in intestinal tissue. Although, MTX is a one of widely used chemotherapeutic agent in the treatment of many diseases, it has toxic effects on the tissues in dose dependent manner. This study showed that RAM could be a preferable drug candidate in MTX-like toxicities to alleviate the side effects on the GIS and might increase patient compliance during the treatment period. However, molecular studies are required to support histochemical, immunohistochemial and biochemical analyzes, therefore our studies will be continuing towards this goal.

Ethics

Ethics Committee Approval: All animal procedures were conducted following the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research at Süleyman Demirel University, Isparta (ethic no: 11.09.2020 06/15).

Informed Consent:

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: D.Ç., M.A.S, S.C., O.İ., E.K., Y.S.S., M.Ö., Design: D.Ç., M.A.S., O.İ., M.Ö.,

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

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Original Article

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Bezmialem Science 2022;10(6):777-85

Identification of Drug-Related Problems and Investigation of Related Factors in Patients with COVID-19: An Observational Study

COVID-19'lu Hastalarda İlaçla İlişkili Sorunların Belirlenmesi ve İlişkili Faktörlerin İncelenmesi: Gözlemsel Bir Çalışma

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ABSTRACT

Objective: Clinical prognosis of coronavirus disease-19 (COVID-19) may be severe and unexpected. Patients may quickly progress to respiratory failure, infections, multiple organ dysfunction, and sepsis. The main objective of this study is to investigate the drug-related problems of patients with COVID-19 and related factors.

Method: A prospective observational study was conducted on patients with COVID-19 between September 2020 and May 2021. Patients' demographics, comorbid diseases, prescribed medicines and laboratory findings were recorded. Drug-related problems (DRPs) were identified by a clinical pharmacist according to recent guidelines, UpToDate® clinical decision support system and evidence-based medicine.

Results: The median age of 107 patients was 64 and 50.46% of them were male. The median number of comorbidities was 3 (2-4) per patient. The majority of the patients had at least one comorbidity (88.79%) other than COVID-19 and the most frequent comorbidities were hypertension, diabetes mellitus and coronary artery disease. The total number of DRPs was recorded as 201 and at least one DRP was seen in 75 out of 107 patients. The median number of DRPs was 2 (0-8). In multivariate model, number of

ÖZ

Amaç: Koronavirüs hastalığı-19 (COVID-19) ağır ve beklenmedik şekilde seyredebilmektedir. Hastalarda solunum yetmezliği, ikincil enfeksiyonlar, çoklu organ yetmezliği ve sepsis tablosu görülebilmektedir. Bu çalışmanın amacı, COVID-19'lu hastalarda ilaçla ilgili sorunları (İLİS) ve ilişkili faktörleri araştırmaktır.

Yöntemler: Eylül 2020 ile Mayıs 2021 tarihleri arasında COVID-19'lu hastaların katılımıyla prospektif gözlemsel bir çalışma tasarlanmıştır. Hastaların demografik özellikleri, komorbid hastalıkları, kullandıkları ilaçlar ve laboratuvar bulguları kayıt altına alınmıştır. İLİS'lerin belirlenmesi; klinik eczacı tarafından güncel kılavuzlara ve UpToDate® klinik karar destek sistemlerine göre yapılmıştır.

Bulgular: Toplamda 107 hasta çalışmaya dahil edilmiştir. Yaşların medyanı 64 (54,5-76,0) idi ve hastaların %50,46'sı erkek olarak kayıt altına alınmıştır. Komorbiditelerin ortanca sayısı 3 (2-4) idi. Hastaların çoğunluğunda COVID-19 dışında en az bir komorbidite (%88,79) mevcuttu ve en sık görülen komorbiditeler hipertansiyon, diabetes mellitus ve koroner arter hastalığı olarak belirlenmiştir. Toplam İLİS sayısı 201 olarak belirlenmiş ve 107 hastanın 75'inde en az bir İLİS görüldü. Medyan İLİS sayısı 2 (0-8) olarak kaydedilmiştir. Çok değişkenli regresyon modeline göre

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comorbidities (odss ratio (OR)=1.952; 95% confidence interval (CI)=1.07-3.54, p<0.05, number of medications (OR=1.344; 95% CI=1.12-1.61, p<0.001), and serum potassium levels (OR=5.252; 95% CI=1.57-17.56, p<0.001) were the factors related with DRP.

Conclusion: This study highlights the DRPs and related factors in patients with COVID 19 in hospital settings. Considering unknown features of the infection and multiple medication use, DRPs are likely to occur. It would be beneficial to consider the related factors in order to reduce the number of the DRPs.

Keywords: COVID-19, hospital settings, clinical pharmacist, drug-related problems, pharmaceutical care need

komorbidite sayısı (olasılık oranı (OO)=1,952; %95 güven aralığı (GA)=1,07-3,54, p<0,05), ilaç sayısı (OO=1,344; %95 GA=1,12-1,61, p<0,001) ve serum potasyum seviyeleri (OO=5,252; %95 GA=1,57-17,56, p<0,001) ile ilişkili faktörler olarak belirlenmiştir.

Sonuç: Bu çalışma COVID 19'lu hastalarda İLİS ve ilişkili faktörleri incelemektedir. Enfeksiyonun bilinmeyen özellikleri ve ilaç kullanım ihtiyacı göz önüne alındığında İLİS'lerin ortaya çıkması muhtemeldir. İLİS sayısını azaltmak için ilgili faktörleri göz önünde bulundurmanın faydalı olacağı kanaatindeyiz.

Anahtar Sözcükler: COVID-19, hastane ortamı, klinik eczacı, ilaçla ilgili sorunlar, farmasötik bakım ihtiyacı

Introduction

Coronavirus disease-19 (COVID-19) is a viral infection causing severe acute respiratory syndrome in humans. Suspicion of COVID-19 is firstly reported as pneumonia with unknown etiology on December 31th in China (1). An increase in the number of patients with pneumonia was identified on 31 December 2019 and then it was identified as the new Coronavirus on January 7th, 2020 which was not detected in humans before. After this date, the number of patients including infected health care professionals increased rapidly. The first case with COVID-19 in Turkey was confirmed on March, 11th, 2020 (2,3). Current situation in Turkey on January 13rd, 2022 involved more than 9.4 million confirmed cases and 82,361 deaths. The numbers are increasing each day (3). The rapid global spread of COVID-19 continues, which remains a danger throughout the world however, an accurate and certain treatment is still being investigated by the scientists.

The treatment of COVID-19 is complex and requires different groups of drugs and combinations. However, despite all effort and clinical studies, a conclusive consensus on treatment is still lacking (4). Individualized treatment measures were preferred according to to clinical severity and conditions. In many cases, multiple medication use was inevitable. The most prescribed drugs for the treatment of COVID-19 were antivirals, antibiotics, analgesics and antipyretics, corticosteroids, tocilizumab, anakinra, and convalescent plasma, etc. (4).

Since the concept of pharmaceutical care has emerged, one of the primary services of pharmacists is to ensuring optimal drug use and minimize adverse events occuring as a result of medications (5). The pharmacist-led cognitive services, which are defined as *"the use of specialized knowledge by the pharmacist for the patient or health professionals for the purpose of promoting effective and safe drug therapy"* aims to optimize pharmacotherapy (6).

The pharmacist should evaluate the medication therapies using their skills about pharmacotherapy as their daily routine (7). As an expert on drug use and pharmacotherapy pharmacist has an essential role in identifying and resolving the Drug-Related Problems (DRPs). The term DRPs could be defined as any events or circumstances related to pharmacotherapy that could interfere with the health outcomes (8). According to the definition of the Pharmaceutical Care Network Europe, DRP is *"An event or circumstance involving drug therapy that actually or potentially interferes with desired health outcomes*" (9). Potential or actual DRPs may be harmful for the patient and increase the healthcare costs (10). In a recent study held by Liew et al. (11), up to 60 % potentially inappropriate prescribing observed in patients above the age of 65 years.

The prevention and resolving of DRPs requires a professional experience and collaboration among the healthcare professionals. The pharmacists are one of the most qualified healthcare professionals to identify and prevent DRPs due to their pharmacotherapy knowledge, and regular communication with patients (5). During the medication review process, DRPs should be identified and classified by a clinical pharmacist. To optimize the drug therapy, an evaluation of the indications, dosage, adherence, adverse events, and therapeutic effects of all drugs should be assessed and recorded (7). Despite increased attention and identification of DRPs, management and prevention of this issue is still a challenge.

Aim of the Study

The main objective of this study is to investigate the pharmaceutical care need of patients with COVID-19 in hospital settings. The primary outcomes of the present study were the identification of DRPs and influencing factors.

Methods

Study Design and Sample Size

A prospective observational was study conducted on patients with COVID-19 who were admitted to pulmonology service at a tertiary-care university hospital in Istanbul, Turkey between September 2020, and May 2021. The patients in need of intensive care were excluded. The patients with COVID-19 indication enrolled in this study were diagnosed according to the World Health Organization and Turkish Ministry of Health Interim Guidance (12). A sample size of 85 was required within a 5% margin of error and confidence intervals (CI) of 95% (13).

Data Collection

The patients' demographic factors (age, gender, body weight etc.), comorbid diseases, prescribed medicine (dosing, frequency, and treatment duration) and duration of hospital stay were recorded. In addition, blood pressure, heart rate, oxygen saturation, respiratory rate, and laboratory findings (e.g., creatinine, uric acid, fasting blood glucose, hemogram, LACE index, Quick COVID-19 severity index, COVID-GRAM Critical Illness Risk Score) on admission were recorded (14-16). Meanwhile, the number of prescribed medicine or over-the-counter medications during hospital stay were collected. The identification of DRPs was made by the clinical pharmacist according to recent guidelines, UpToDate® and Medscape® clinical decision support system, and evidence-based medicine. Potential drug-drug interactions (pDDI) were determined by using UpToDate®. Among detected pDDIs, only X (avoid combination), D (consider therapy modification) and C (monitor therapy) categories were taken into consideration.

All assessments about DRPs which had clinical significance were performed by clinical pharmacists. The DRPs and clinical significance were evaluated using the Hepler and Strand DRPs classification system (17). Data were collected using convenience sampling methods. This study were reported according to recommendation of Strengthening the Reporting of Observational Studies in Epidemiology standards (Figure 1) (18).

Statistical Analysis

Descriptive statistics, mean, median, standard deviation, and interquartile range (IQR) or counts and percentages were given for continuous variables. The frequency, percentage were given for categorical variables. The normality of continuous variables was tested using the Kolmogorov-Smirnov test. The difference among groups was analyzed with an independent t-test or Mann-Whitney U test. Chi-square tests were used to investigate the relationship between categorical variables. The univariate logistic regression analysis was used to determine which variables were significant by using $p<0.20$. The significant variables were included in the binary logistic regression analysis. The missing data were excluded from the analysis. All the data were analyzed by using SPSS version 26® and Jamovi version 1.6.

Ethical Approval

The study protocol was approved by the Ministry of Health and the Local Ethics Committee of Bezmialem Vakıf University's clinical research ethics committee (approval number of 5/42, 06.05.2020). Informed consent was obtained from all individual participants included in the study.

Results

Demographics, Medications, and Drug-Related Problems (DRPs)

The total number of patients were 107, the median (IQR) age of patients was 64 (54.5-76.0) and 50.46% of them were male (Table 1). The median (IQR) score of body mass index was 25.4 (23.7-27.7). The median number of comorbidities (IQR) was 3 (2-4) per patient. The majority of the patients had at least one comorbidity (88.79%) other than COVID-19. The median (IQR) number of medicines prescribed, and the median number of days of hospital stay were 12.8 (8-16) and 9 (6-15) days, respectively (Table 1). The most frequently prescribed medicines were favipiravir, enoxaparin, pantoprazole, paracetamol, and dexamethasone (Figure 2). The total number of DRP was recorded as 201, and at least one DRP was seen in 75 out of 107 patients (Table 2). The baseline vital signs of the patients were given in Table 3. The mean and median (IQR) numbers of DRPs were 1.93±1.91/patient and 2 (0-8), respectively (Table 4). According to our results, 140 of the DRPs consisted of pDDIs. One the most abundant pDDIs was increased bleeding due to concomitant use of dexamethasone and enoxaparin.

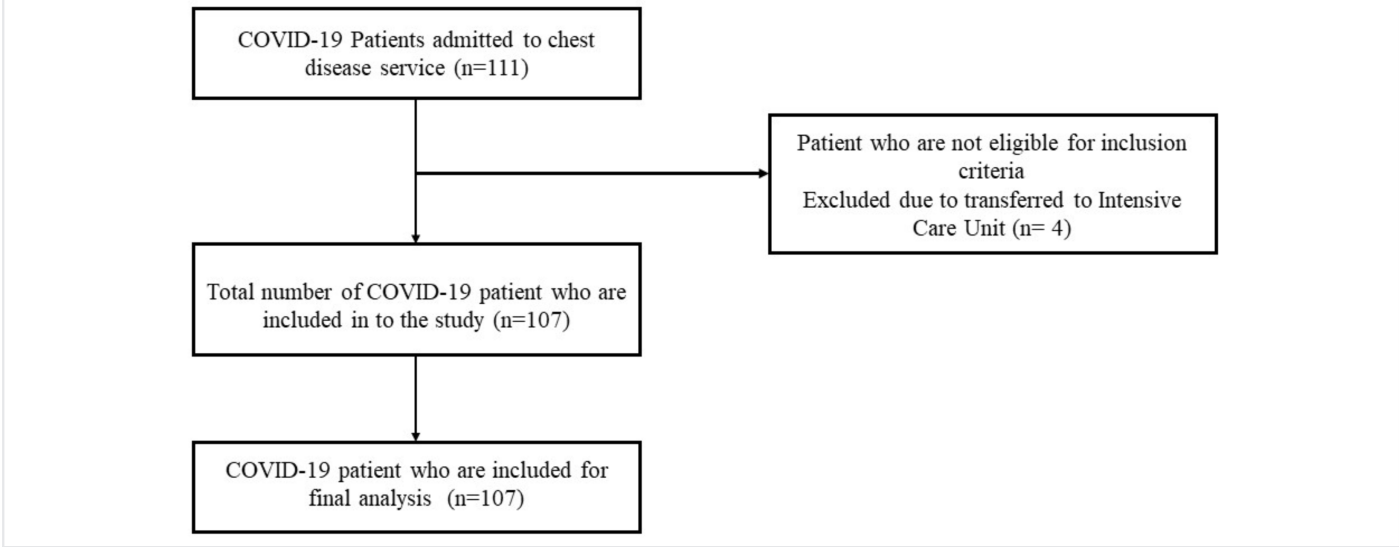

Figure 1. STROBE flow chart

STROBE: Strengthening the reporting of observational studies in epidemiology, COVID-19: Coronavirus disease-2019

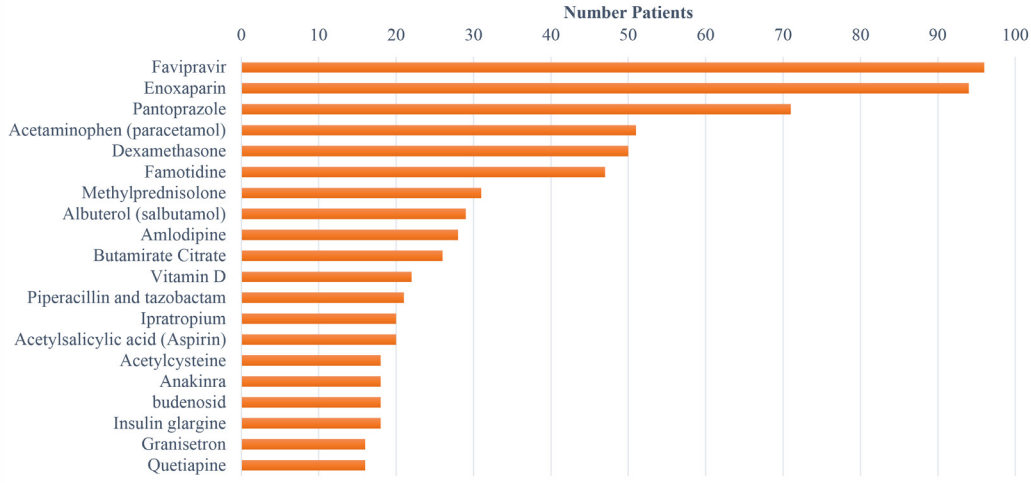

Figure 2. Number of prescribed medications

Medical conditions of the participants are summarized in Table 1. Among 107 patients, nearly half of them were diagnosed with hypertension (HT) (n=54, 50.47%). The second and the third most common comorbidities were Diabetes Mellitus (DM) (n=30, 28.47%) and coronary artery diseases (CAD) (n=25, 23,365), respectively (Table 1). Among 75 patients with detected DRP, 44 patients had HT, 25 patients had DM and 24 patients had CAD (Table 1).

Vital Signs and Biochemical Findings

Baseline clinical and vital findings of the participants were recorded and presented in Table 3 and 4. The median (IQR) of body temperature of patients was recorded as 37 (36.4-37.5). Among our sample, median (IQR) values of heart beats per minute and oxygen saturation (spO_2) were 87 (78-96.5) and 93 (88-95), respectively. The median (IQR) systolic and diastolic blood pressure values were 131 (119-145) and 76 (65-82), respectively. The median (IQR) respiratory rate of the participants was 20/min (16-22). Participants' basal biochemical values are given in Table 4.

Factors associated with the Drug-Related Problems

Univariate analysis exploring the factors associated with DRP are presented in Table 5. The number of comorbidities [odds ratio (OR)=2.097, 95% confidence interval (CI)=1.43-3.08; p<0.001)], number of medications (OR=1.32, 95% CI=1.17-1.49; p<0.001), serum urea mg/dL levels (OR=1.035, 95% CI=1.01-1.06; p<0.05), serum blood urea nitrogen levels (OR=1.00, 95% CI=1.00-1.00; p<0.05), serum potassium levels (OR=2.409, 95% CI=1.16-5.00; p<0.05), serum lymphocyte ratio (OR=0.967, 95% CI=0.936-1.00; p<0.05), Quick COVID severity index score (OR=1.23, 95% CI=1.01-1.353; p<0.05), and LACE index score (OR=1.23, 95% CI=1.07-1.42; p<0.001) were associated with the DRPs. Binary logistic regression analysis for DRPs is presented in Table 5.

A binomial logistic regression was performed to ascertain the effects of the number of medications, the number of comorbidities, BUN, serum potassium levels, lymphocytes ratio, LACE index, score, Quick COVID severity Index, serum urea level, on the likelihood that participants had a DRP. The logistic regression model was statistically significant, $\chi^2(8)=57.842$, p<0.0001. The model explained 59.7% (Nagelkerke R^2) of the variance in DRPs and correctly classified 84.1% of cases. Sensitivity was 92.1%, specificity was 64.52%, positive predictive value was 61.5% and negative predictive value was 74.3%. Of the 8 predictor variables, only 4 were statistically significant: number of comorbidities, number of medications, serum potassium levels and lymphocyte ratio (Table 5). Increased number of comorbidities (OR=1.952; 95% CI=1.07-3.54, p<0.05), number of medications (OR=1.344; 95% CI=1.12-1.61, p<0.001), and serum potassium levels (OR=5.252; 95% CI=1.57-17.56, p<0.001) were associated with an increased likelihood of exhibiting DRP. But, increasing lymphocyte ratio (OR=0.953; 95% CI=0.91-0.99, p<0.05) was associated with a reduction in the likelihood of exhibiting DRPs.

Discussion

In this study, we investigated the DPRs in patients with COVID-19 and related factors in hospital settings. Hospitalized patients with COVID-19 have multiple comorbidities and need multiple medications (4). Currently, many scientists are investigating alternative medicine for COVID-19. However, there is no exact treatment alternative for COVID-19 approved by different authorities. On the other hand, the ethical aspects of these options are still questionable (19).

In this study, many of the participants were prescribed with antivirals, antibiotics, analgesics, antipyretics, and anti-thrombotic drugs. Based on the primary findings, the number of comorbidities, serum potassium levels, lymphocyte ratio, and the number of medications were associated with the number of DRPs. The identified risk factors should be assessed by pharmacist to prevent DRPs in patients with COVID-19.

According to Pradhan and Olsson (20) and Imam et al. (21), HT and DM were the most common comorbid diseases among patients with COVID-19. Another study held in Turkey

Table 1. Patient characteristics

Total number of patients	Total n=107	DRP present (n=75)	DRP not present (n=32)	p
Gender n (%)				N.S
Female	53 (49.53%)	38 (35.51%)	15 (14.02%)	
Male	54 (50.46%)	37(34.58%)	17(15.88%)	
BMI [median (IQR)]	25.4 (23.7-27.7)	24.9 (23.7-28.2)	25.9 (23.7-27.4)	*N.S*
Weight (kg)	75 (15, 65.0-80.0)	72 (65-80)	75 (70-80)	
Height (m)	1.66 (0.135, 1.62-1.75)	1.65 (1.60-1.75)	1.68 (1.65-1.75)	
Age [median (IQR)]	64 (54.5-76.0)	68 (58-76.0)	60 (50.5-67.0)	***p<0.05***
No. of comorbidities [median (IQR)]	3 (2-4)	4 (2-5)	2 (1.75-3)	***p<0.001***
1 (n, %)	12 (11.21%)	4 (5.33%)	8 (5.33%)	
2 (n, %)	29 (27.10%)	19 (25.3%)	10 (31.25%)	
3 (n, %)	20 (18.69%)	11 (14.6%)	9 (28.12%)	
4 (n, %)	25 (23.36%)	22 (29.3%)	3 (9.37%)	
5 (n, %)	13(12.14%)	12 (16.0%)	1 (3.12%)	
6 (n, %)	8 (7.47%)	8 (10.6%)	0 (0%)	
Comorbidities				NA
COVID-19	107 (100%)	75 (100%)	32 (100%)	
Hypertension	54 (50.47%)	44 (58.6%)	10 (31.25%)	
Diabetes mellitus	30 (28.04%)	25 (33.3%)	5 (15.6%)	
Coronary artery disease	25 (23.36%)	24 (32.0%)	1 (3.12%)	
Cancer	23 (21.50%)	23 (30.6%)	7 (21.87%)	
Chronic obstructive lung disease	20 (18.70%)	18 (24.0%)	2 (6.25%)	
Cerebrovascular disease	11 (10.28%)	10 (13.3%)	1 (3.12%)	
Chronic kidney disease	8 (7.47%)	7 (9.33%)	1 (3.12%)	
Neurological diseases	8 (7.47%)	7 (9.33%)	1 (3.12%)	
Psychiatric diseases	7 (7.65%)	7 (9.33%)	0 (0%)	
Others	32 (29.90%)	20 (26.6%)	12 (37.5%)	
Hospital stay in days [median, (IQR)]	9 (6-15)	10 (6-17.5)	7 (5-11)	***p<0.001***
Number of medication [median, (IQR)]	12.8 (8-16)	14 (10.5-19)	7 (5-10)	***p<0.001***
Quick COVID severity index	2 (0-5)	2 (0-5.5)	0 (0-2)	***p<0.001***
COVID GRAM critical index	140 (117-164)	144 (127-164)	125 (108-164)	***p<0.001***
Charlson comorbidity index	4 (0-5)	4 (2-6)	2 (1-4)	***p<0.001***
LACE index	13 (10-16)	14 (12-16)	10.5 (9-13.3)	***p<0.05***

DRP: Drug related problem, BMI: Body mass index, IQR: Interquartile range

pointed out that DM, HT, and CAD were the most frequent comorbidities among patients with COVID-19 (22). Our findings showed a correlation with their statements. The most common comorbid disease was recorded as HT (50.47%), which was followed by DM (28.04%), and CAD (23.36%) (Table 1). Therefore, detailed medical history should be taken and preventive measures for DRPs should be underlined by the clinical pharmacist.

A retrospective cohort study held by Imam et al. (21) pointed out that older age and increased number of comorbidities were associated with mortality. Older age (>60 years) (OR=3.66, 95% CI=2.57-5.20) and increased number of comorbidities (>3) (OR=4.11, 95% CI=3.00-5.62) were independent predictors of mortality (21). Similarly, our investigation pointed out that increased number of comorbidities (OR=1.952; 95% CI=1.07-3.54, p<0.05) were associated with the increased number of the DRPs. However, in terms of age, there was not statistically difference between the groups (Table 5). Many patients, especially elders, have multiple comorbidities. Knowledge of pharmacist should be implemented into practice to improve healthcare services for patients with COVID-19. A detailed medication review process might be useful to detect and prevent

Table 2. Features drug related problems in patients with COVID-19

	Total	Median	(Min-max)
Total no of DRP	201	2	(0-8)
Drug interactions	140	1	(1-7)
Improper drug selection	40	1	(1-3)
Overdosage	21	1	(1-4)
Untreated indications	-	-	
Adverse reactions	-	-	
Failure to receive drugs	-	-	
Subtherapeutic dosage	-	-	
Drug use without indication		-	

DRP: Drug related problem, COVID-19: Coronavirus disease-2019, Min: Minimum, max: Maximum

Table 3. Baseline vital signs of patients

n=107	Median	(IQR 25-75)
Body temperature, °C	37	(36.4-37.5)
Heartbeat (HBM)	87	(78-96.5)
Oxygen saturation (SpO_2)	93	(88-95)
Systolic blood pressure (mmHg)	131	(119-145)
Diastolic blood pressure (mmHg)	76	(65-82)
Respiratory rate (BPM)	20	(16-22)

HPM: Heartbeat per minute, BPM: Breath per minute, IQR: Interquartile range

Table 4. Baseline biochemical values of participants

n=107	Reference range	Median	(IQR, 25-75)
Acute phase reactants			
CRP mg/dL	*0-5*	63	(97.3, 24.7-122)
Procalcitonin ng/mL	*0-0.5*	0.157	(0.318, 0.02-0.34)
Ferritin ng/mL	*21-274*	369	(649, 142-791)
D-dimer ng/mL	*0-300*	253	(391, 187-578)
Albumin g/dL	*3.2-4.6*	3.60	(0.50, 3.30-3.80)
Renal function parameters			
Urea (mg/dL)	*17-49*	41	(32.5,28.5-61)
Blood urea nitrogen, BUN (mg/dL)	*8-23*	19.2	(15.2, 13.3-28.5)
Creatinine (mg/dL)	*0.7-1.3*	0.88	(0.46, 0.74-1.15)
GFR (mL/min/1.73)	*>90*	78	(36, 60-96)
Hepatic function parameter			
Lactate dehydrogenase, LDH (U/L)	*125-220*	316	(169, 260-429)
Aspartate aminotransferase, AST (U/L)	*5-34*	29	(19.5, 24-43.6)
Alanine aminotransferase, ALT (U/L)	*0-55*	24	(24.5, 14.5-39)
Alkalen phosphatase ALP (U/L)	*40-150*	55	(36.5, 39.3-75.8)
Gamma-glutamyl transferase, GGT (U/L)	*12-64*	31.5	(18.3, 24-42.3)
Total bilirubin (mg/dL)	*0.3-1.2*	0.48	(0.31, 0.31-0.62)
Direct bilirubin (mg/dL)	*0-0.5*	0.23	(0.15, 0.15-0.30)
Amylase (U/L)	*28-100*	36	(31.2, 30-60)
Lipase (U/L)	*8-78*	25	(17.5, 19-36.5)
Electrolytes			
Sodium mmol/L	*135-145*	137	(5, 135-140)
Potassium mmol/L	*3.5-5.1*	4.25	(0.795, 3.89-4.69)
Calcium mg/dL	*8.4-10.6*	8.60	(0.6, 8.3-8.9)
Complete blood count			
White blood cells, WBC (10^3/uL)	*4.5-11*	7.10	(5.69, 5.22-10.9)
Lymphocytes %	*10-50*	16.3	(16.3, 10.3-26.6)
Neutrophiles %	*45-78*	74.3	(16.3, 61.4-82.4)
Monocytes %	*0-12*	6.59	(4.91. 4.52-9.43)
Hemoglobulin (g/dL)	*14.1-17.5*	12.3	(4.52, 10.9-13.7)
Hematocrit %	*40-52*	36.6	(7.44, 32.6-40.1)
Mean corpuscular volume, MCV (fL)	*80-97*	85.6	(7.22, 82.4-89.6)
Prothrombin time, PT (s)	*11.4-16.2*	14.5	(2.10, 13.7-158)
Activated partial thromboplastin time, aPTT (s)	*22-40*	35.1	(7.25, 31.9-39.2)
International normalized ratio, INR	*0.8-1.2*	1.07	(0.215, 0.98-1.1)9

Table 5. Statistical analysis of factors associated with the number of drug-related problems

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI for odds ratio	p value	OR	95% CI for odds ratio	p value
Age, years	1.023	(0.99-1.05)	0.124			
Gender						
Male	0.88	(0.46-2.46)	0.880			
Female	Reference					
Hospital stay (days)	1.051	(0.99-1.12)	0.113			
LDH U/L	1.00	(0.99-1.00)	0.889			
CRP mg/L	0.990	(099-1.00)	0.439			
Procalcitonin ng/mL	1.150	(0.71-1.77)	0.629			
Ferritin ng/mL	1.000	(1.00-1.00)	0.508			
D-Dimer ng/mL	1.000	(1.00-1.00)	0.881			
No of comorbidities	2.097	(1.43-3.08)	**<0.0001**	1.952	(1.07-3.54)	**0.028**
No of medication	1.32	(1.17-1.49)	**<0.0001**	1.344	(1.12-1.61)	**0.001**
Urea mg/dL	1.035	(1.01-1.06)	**0.006**	0.001	(0.00-10.34)	0.129
BUN mg/dL	1.000	(1.00-1.00)	**0.006**	1.000	(1.00-1.00)	0.128
Creatine mg/dL	2.225	(0.71-7.02)	0.172			
Sodium mmol/L	1.066	(0.96-1.18)	0.215			
Potassium mmol/L	2.409	(1.16-5.00)	**0.018**	5.252	(1.57-17.56)	**0.007**
WBC 10^3/µL	0.985	(0.945-1.028)	0.486			
Lymphocyte %	0.967	(0.94-1.00)	**0.050**	0.953	(0.91-0.99)	**0.041**
Quick COVID severity index	1.169	(1.01-1.35)	**0.037**	1.245	(0.98-1.58)	0.070
LACE index	1.233	(1.07-1.42)	**0.003**	0.798	(0.61-1.04)	0.093

OR: Odds ratio, CI: Confidence interval, LDH: Lactate dehydrogenase, CRP: C-reactive protein, BUN: Blood urea nitrogen, WBC: White blood cell, COVID: Coronavirus disease

DRPs. Hence, clinical pharmacists could provide pharmaceutical care for patients with COVID-19 in the hospital setting (7,23).

Previous studies held in either in community pharmacy settings or hospital setting detected different number of DRPs per patient (7,24,25). In our study, the mean number of DRP per patient was recorded as 1.93±1.91/patient. Our results were similar with the studies by Stafford et al. (24) and Rhalimi et al. (25) study. On the other hand, Wang et al. (7) recorded higher number of DRPs per patients than our findings. This difference could be explained by the settings of different studies. For instance, the study by Stafford et al. (24) and our data were obtained from hospital settings. However, the studies by Wang et al. (7) and Rahlimi et al. (25) were conducted in a community pharmacy settings.

Multivariate analysis showed that the number of medication used was associated as independent risk factor for the number of DRPs, which was consistent with previous studies (7,26-28). Polypharmacy is a strong risk factor for DRPs. As a result, the number of used medications increased number of DRPs increased simultaneously. The presented finding extended the understanding that a higher number of medications was also an important predictor of DRP in patients with COVID-19 (OR=1.344; 95% CI=1.12-1.61, p<0.001), that was not studied extensively in the literature before (27,28). Multiple medication use is inevitable with comorbidities. However, many of the DRPs may be prevented with well-planned pharmaceutical care services.

Another significant finding of our study was that serum potassium level was associated with DRPs. Compromised kidney functions are directly related with potassium levels. Many of our participants suffered from HT and DM both of which might compromise the kidney functions. Patient with kidney diseases is more prone to drug related problems (29,30). On the other hand, one of the most used antihypertensive drugs is diuretics which can either spare potassium or prevent potassium secretion in kidneys. Also, hypokalemia or hyperkalemia may be directly related with the pharmacokinetic parameters of drugs. As in our findings, the level of serum potassium was related with DRPs which was consistent with literature (29,30). Pharmacists should take into consideration that fluctuation of serum potassium level may result as adverse events.

Study Limitations

This study had some limitations such as the generalizability of the results was limited as the sample was taken from only one center. Future multicentered studies with larger sample size are required to investigate the pharmaceutical care needs of patients with COVID-19. The sample size was rather small. Finally, the

lack of a control group was a limitation of the present study, which was worth noting.

Conclusion

To the best of our knowledge, presented study was the first to investigate the incidence, type, and related factors of DRPs detected by clinical pharmacists in patients with COVID-19 in hospital settings. This study showed that a considerable proportion of patients had DRPs and the most common category was the potential drug-drug interactions. In the multivariate model, the number of medications, number of comorbidities, serum potassium levels and lymphocytes ratio were the significant related factors with the number of DRPs.

COVID-19 pandemic is still an important healthcare problem all around the world. Pharmacists and pharmacist-led cognitive services will detect, prevent, and decrease unfavorable drug-related events. To improve healthcare services, pharmacists should take responsibility and should become an indispensable component of the COVID-19 healthcare team.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Ministry of Health and the Local Ethics Committee of Bezmialem Vakıf University's clinical research ethics committee (approval number of 5/42, 06.05.2020).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: M.Y.B., M.S., F.V.İ., Design: M.Y.B., M.S., F.V.İ., Data Collection or Processing: M.Y.B., M.S., Analysis or Interpretation: M.Y.B., M.S., Literature Search: M.Y.B., F.K.O., B.D., Writing: M.Y.B., M.S., F.K.O., B.D., F.V.İ.

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Original Article

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Bond Strength of Different Composite Resin Materials and CAD/CAM Restorative Materials to Each Other and Dentin Tissue

Farklı Kompozit Rezin Materyaller ve CAD/CAM Restoratif Materyallerin Birbirine ve Dentin Dokusuna Bağlanma Dayanımı

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ABSTRACT

Objective: The aim of the current study is to investigate the strongest and weakest points of the three different structures of prosthodontic restorations constituting the coronal structure when considered as a whole: the remaining tooth, composite resin, and computer-aided design and computer-aided manufacturing (CAD/CAM) restorative materials.

Methods: Seventy extracted caries-free molars, CAD/CAM blocks [Lava Ultimate (LU), Vita Enamic (VE), IPS e.max CAD (IPS)], and composite resin materials Clearfil Majesty Posterior [CMP], Light Core [LC], Filtek Bulk Fill Posterior [FBP], EverX Posterior [EP] were used for this study. Dentin and CAD/CAM sections were embedded in acrylic. Clearfil SE Bond was used as adhesive material. Composite resin materials were applied to the dentin surface using a Teflon mold. LU and VE were sandblasted with 50-µm Al2O3 for 10-sec. IPS was etched with HF for 20-sec. Ceramic Primer-2 was applied to the surfaces. Composite bars (2.3x3 mm) were adhered to CAD/CAM blocks using RelyX-U200. In addition, CAD/CAM bars were also adhered to dentin. The shear bond strength test was performed. Failure modes were examined using a stereomicroscope. Differences were analysed using one-way ANOVA and Tukey Post Hoc test.

Results: The highest shear bond strength values of the composite resin materials to dentin tissue were observed in EP ($p<0.05$). Shear bond strength values of composite resin materials to IPS were found

ÖZ

Amaç: Bu çalışmanın amacı koronal yapıyı oluşturan üç farklı yapıyı bir bütün olarak ele alarak protetik restorasyonların en güçlü ve en zayıf noktalarını geriye kalan dental dokular, kompozit rezin ve CAD/CAM materyalleri yönünden incelemektir.

Yöntemler: Bu çalışmada 70 adet çürüksüz çekilmiş azı dişi, CAD/CAM bloklar [Lava Ultimate (LU), Vita Enamic (VE), IPS e.max CAD (IPS)] ve kompozit rezin materyaller Clearfil Majesty Posterior [CMP], Light Core [LC], Filtek Bulk Fill Posterior [FBP], EverX Posterior [EP] kullanıldı. Dentin ve CAD/CAM kesitleri akriliğe gömüldü. Adeziv materyal olarak Clearfil SE Bond kullanıldı. Kompozit rezin materyaller dentin yüzeyine Teflon kalıp kullanılarak uygulandı. LU ve VE 50 µm Al2O3 ile 10-sn kumlandı. IPS, 20-sn HF ile asitlendi. Yüzeylere Ceramic Primer-2 uygulandı. Kompozit çubuklar (2.3x3 mm) RelyX-U200 kullanılarak CAD/CAM bloklara yapıştırıldı. Ayrıca CAD/CAM çubuklar da dentine yapıştırıldı. Makaslama bağlanma dayanım testi yapıldı. Kırılma tipleri stereomikroskop kullanılarak incelendi. One-way ANOVA ve Tukey Post Hoc testi istatistiksel değerlendirmede kullanıldı.

Bulgular: Kompozit rezin materyallerin dentin dokusuna olan makaslama bağlanma dayanımı için en yüksek değerler EP'de gözlendi ($p<0,05$). Kompozit rezin materyallerin IPS'ye bağlanma dayanım değerleri LU ve VE'den daha yüksek bulundu. Üç farklı CAD/CAM restoratif materyalinin dentin dokusuna makaslama bağlanma dayanımları istatistiksel olarak benzerdi.

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higher than to LU and VE. The shear bond strengths of the three different CAD/CAM restorative materials to the dentin tissue were statistically similar.

Conclusion: The type of composite resin materials affects the shear bond strength to dentin tissue and CAD/CAM restorative materials. However, the type of CAD/CAM restorative material does not affect the shear bond strength to dentin tissue.

Keywords: CAD/CAM, shear bond strength, composite resin, dentin

Sonuç: Kompozit rezin materyalinin tipi, dentin dokusuna ve CAD/CAM restoratif materyallere olan makaslama bağlanma dayanımını etkilemektedir. Ancak CAD/CAM restoratif materyalinin tipi dentin dokusuna olan makaslama bağlanma dayanımını etkilememektedir.

Anahtar Sözcükler: CAD/CAM, makaslama bağlanma dayanımı, kompozit rezin, dentin

Introduction

At present, different restorative materials are available for the restoration of teeth with high coronal loss. The choice of restorative materials is critical in the long-term success of the coronal restoration. The restorative materials must provide resistance and retention to dental tissues and have sufficient mechanical resistance to occlusal forces (1). In previous years, amalgam, glass ionomer, and hybrid ionomer were used as restorative materials (2). Nowadays, resin-based restorative materials with different physical and mechanical properties that can be bonded to dental tissues are frequently used.

Materials that mimic different tooth structures such as enamel and dentin (biomimetic) have been developed, and products on a very wide scale have been introduced to the market. One of the main objectives of restorative dentistry is that restorative materials should be compatible with natural dental tissues and should have similar physical and mechanical properties (3). Despite the variety of materials available, long-term success in the restoration of teeth with excessive material loss depends on many factors. At this stage, each of the variables such as the amount of remaining dental tissues, the structural properties of the restorative material, and the bond strength of the ceramic or composite resin restorative material to dental tissue and each other are effective on the long-term clinical success of the restorations.

Restorative materials used in computer aided design/computer aided manufacturing (CAD/CAM) systems are frequently preferred for teeth with excessive coronal material loss. For long-lasting restorations or to obtain clinical success, adequate adhesion must be provided between the ceramic structure, composite resin material, and tooth. However, the literature is insufficient on which bonding agent or adhesive cement, or which ceramic material or composite resin material produces the greatest bond strength to tooth structures. Recently, there has been a significant increase in CAD/CAM restorative material diversity. As an alternative to CAD/CAM ceramic blocks, composite containing CAD/CAM blocks have been developed. Resin blocks have a softer structure compared with ceramic blocks, which facilitates the milling of the material. While all-ceramic systems have disadvantages such as repair difficulties, deterioration of polishing properties during the adaptation process, necessity to be repolished by a technician, and rapid crack formation in the material; the soft structure of composite materials allows them to be easily produced and these materials can be easily repaired by clinicians with direct composite resin materials. In addition, various studies have shown that resin-based CAD/CAM restorative materials are as successful as ceramic materials when the bond strength (4-6), flexural strength and modulus of resilience are evaluated (7,8).

In this study, we considered teeth with excessive material loss as a single structure and investigated the strongest and weakest connection points. The aim of the study was to investigate the connection between the three different structures constituting the coronal structure (tooth, composite resin materials, and CAD/CAM restorative materials). Therefore, this study had three different objectives: the first objective was to investigate the bond strength of composite resin materials (conventional posterior composite, fiber reinforced composite, bulk-fill composite, and light-cured core build-up composite) to dentin tissue. The second objective was to investigate the bond strength of different composite resin materials to the CAD/CAM restorative materials (resin nanoceramic, nanohybrid, or lithium disilicate ceramic). The third objective was to investigate the bond strength of different CAD/CAM restorative materials to dentin tissue.

The tested null-hypotheses were as follows: (1) the type of composite resin materials does not affect bond strength to dentin tissue; (2) the type of composite resin materials does not affect bond strength to different types of CAD/CAM restorative materials; and (3) the type of CAD/CAM restorative materials does not affect bond strength to dentin tissue.

Methods

The present study was approved by the Kocaeli University Non-Invasive Clinical Research Ethics Committee (no: 2019/264). Seventy extracted molar teeth, three different CAD/CAM restorative materials [Lava Ultimate (3M ESPE, St Paul, MN, USA), Vita Enamic (Vita Zahnfabrik, Bad Sackingen, Germany), IPS e.max CAD (Ivoclar Vivadent AG, Schaan, Liechtenstein)] and four different composite resin materials [Clearfil Majesty Posterior (Kuraray, Okayama, Japan), Light Core (Bisco Dental Products, Schaumburg, IL, USA), Filtek Bulk Fill Posterior (3M ESPE, St Paul, MN, USA), EverX Posterior (GC Corporation, Tokyo, Japan)] were used for the shear bond strength test in this study. The above-mentioned materials are presented in Tables 1, 2, and 3.

The enamel tissue of the teeth was removed with a low-speed diamond precision-cutting machine (Micracut 151/Metkon,

Table 1. CAD/CAM restorative materials used in the study

Materials	Type	Inorganic composition	Organic composition	Filler content (wt%)	Manufacturer and batch
Vita Enamic (VE)	Hybrid-ceramic (Polymer infiltrated feldspar ceramic)	SiO_2, Al_2O_3, Na_2O, P_2O_5, B_2O_3, ZrO_2, CaO	UDMA, TEGDMA	86 wt% feldspar ceramic, 14 wt% polymer	Vita Zahnfabrik, Bad Sackingen, Germany-51540
IPS e.max (IPS)	Lithium disilicate	SiO_2, Li_2O, K_2O, P_2O_5, ZrO_2, Al_2O_3, MgO, coloring oxides	-	-	Ivoclar Vivadent AG, Schaan, Liechtenstien-U16370
Lava Ultimate (LU)	Nano-ceramic	SiO_2, ZrO_2, Si/ZrO_2 cluster	Bis-GMA, UDMA, Bis-EMA, TEGDMA	80 wt% nanoceramic, 20 wt% resin	3M ESPE St Paul, MN- N664028

Bis-EMA: Bisphenol-A-polyethylene-glycol-diether dimethacrylate, Bis-GMA: Bisphenol-A-diglycidyl dimethacrylate, TEGDMA: Triethylene gly-col dimethacrylate, UDMA: Urethane dimethacrylate

Table 2. Composite resin restorative materials used in the study

Materials	Type	Inorganic composition	Organic composition	Filler content (wt%)	Manufacturer and batch
Clearfil Majesty Posterior (CMP)	Nano-hybrid	Alumina and glass ceramics	BisGMA AUDMA TEGDMA	92	Kuraray, Okayama, Japan-4D0060
Light Core (LC)	-	7.8% fiber and glass filler	Bis-GMA Bis-EMA	79	Bisco Dental Products, Schaumburg, IL, USA-1800006221
Filtek Bulk Fill Posterior (FBP)	Nano-hybrid	Based on silica, zirconia, and ytterbium trifluoride	AUDMA UDMA DDDMA	76.5	3M ESPE, St Paul, MN, USA-N721168
EverX Posterior (EP)	Short Fiber reinforced composite	E-glass short fibers and barium borosilicate glass particulate	Bis-GMA TEGDMA PMMA	74.2	GC Corporation, Tokyo, Japan-1506222

AUDM: Aromatic urethane dimethacrylate, Bis-EMA: Bisphenol-A-polyethylene-glycol-diether dimethacrylate, Bis-GMA: Bisphenol-A-diglycidyl dimethacrylate, DDDMA: 1,12-dodecanediol dimethacrylate, PMMA: Polymethyl methacrylate, TEGDMA: Triethylene gly-col dimethacrylate, UDMA: Urethane dimethacrylate

Table 3. Other materials used in the study

Materials	Chemical composition		Manufacturer and batch
Relyx U 200	Base paste: Silane-treated glass powder, 2-Propenoic acid, 2-methyl,1,10 -[1-(hydroxymethyl)-1,2-ethanodiyl] ester, TEGDMA, silane-treated silica, glass fiber, sodium persulfate, tert-butyl peroxy-3,5,5-trimethylhexanoate İnorganic Fillers %43	Catalyzer paste: Silane-treated glass powder, dimethacrylate substitute, silane-treated silica, sodium p-toluenesulfonate, 1-Benzyl-5-phenylbarbituric acid, calcium salts, 1,12-Dodecanediol dimethacrylate, calcium hydroxide, titanium dioxide.	3M/ESPE, St. Paul, MN, USA-665734
Clearfil SE Bond	Primer: MDP, HEMA, hydrophilic aliphatic dimethacrylate, di-camphorquinone, N, N-Diethanol-p-toluidine, water	Bond: MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, di-camphorquinone, N, N-Diethanol-p-toluidine, silanized colloidal silica	Kuraray Noritake Dental Inc., Okayama, Japan- Primer:AW0272 Bond:AX0436
Ceramic primer 2	Ethyl alcohol, phosphoric acid ester, silane, MDP, MDTP, DMA		GC Corporation, Tokyo, Japan-1605251

Bis-GMA: Bisphenol-A-diglycidyl dimethacrylate, DMA: Dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, MDTP: Methacryloyloxydecyl dihydrogen thiophosphate, TEGDMA: Triethylene glycol dimethacrylate

Turkey) with water cooling. Then, 2 mm thickness dentin sections were formed. The sections were embedded in acrylic molds. The upper surface of the specimen was abraded with 600 grit silicon carbide paper (LaboPol-1, Struers, Willich, Germany) for 60-sec with water. The obtained samples were randomly divided into four groups and each group was assigned a different type of composite resin material (n=10). Group 1A: Dentin-CMP, Group 2A: Dentin-LC, Group 3A: Dentin-FBP, and Group 4A: Dentin-EP. Clearfil SE Bond (Kuraray Noritake Dental Inc., Okayama, Japan) was used as adhesive agent. It was then polymerized using Elipar S10 (3M/ESPE, St. Paul, MN, USA). Composite resin materials were applied to the flat dentin surface using 2.3x3 mm size Teflon cylinder mold (Ultradent Product, Inc., Utah, USA) (Figure 1) and polymerized.

The IPS, LU, and VE blocks were cut using a low-speed precision cutting machine with a thickness of 2 mm and a total of 120 samples were created (n=10). IPS specimens were fully crystallized at 845 °C for 10-min in a ceramic furnace (Programat P300, Ivoclar Vivadent AG, Liechtenstein). The sections were embedded in acrylic molds. Then, all samples were ground with 600-grit SIC paper for surface standardization. LU and VE samples were sandblasted with 50-μm Al_2O_3 for 10-sec. IPS samples were treated with acid for 20-sec (9% Hydrofluoric acid; Ultradent-Porcelain-Etch), washed for 15-sec and air-dried. The ceramic sections were placed in an ultrasonic bath for 10-min and air-dried. The surface was treated with Ceramic primer 2 (GC Corporation Tokyo, Japan) and air-dried for 10-sec. Composite cylinder bars obtained from the composite resin materials using a Teflon cylinder mold were polymerized for 20-sec. The composite resin materials were adhered to the prepared adherend ceramic surface with self-adhesive resin-cement RelyX U200 (3M/ESPE, St. Paul, MN, USA). Each surface was polymerized using Elipar S10 for 20-sec. The obtained samples were divided into 12 groups. Group-1B: LU-CMP, Group 2B: LU-LC, Group 3B: LU-FBP, Group 4B: LU-EP, Group 5B: VE-CMP, Group 6B: VE-LC, Group 7B: VE-FBP, Group 8B: VE-EP, Group 9B: IPS-CMP, Group 10B: IPS-LC, Group 11B: IPS-FBP, and Group 12B: IPS-EP.

The cylinder bar samples of 2.3 mm diameter and 3 mm height were prepared from CAD/CAM materials (n=10). The obtained samples were divided into three groups. Group 1C: Dentin-LU, Group 2C: Dentin-VE, and Group 3C: Dentin-IPS. The surface of the cylinder bars obtained from LU and VE were sandblasted with 50-μm Al_2O_3 particles for 10-sec. The surface of the cylinder bars obtained from IPS was etched with 9% HF (Porcelain Etch; Ultradent Products, Inc., Köln, Germany) for 20-sec subsequently washed and dried for 15-sec. Ceramic primer 2 was applied to the surface and air-dried for 10-sec. The samples were adhered to the flat dentin surface using self-adhesive resin cement RelyX U200. Each surface was polymerized for 20-sec (Elipar S10). An overview of the groups can be seen illustrated in Figure 2, moreover the test specimen is shown in Figure 1. The polymerized specimens were stored in distilled water for 24-h.

The shear bond strength test was carried out following the guidelines of ISO 29022:2013 (9). A Teflon cylinder mold with a diameter of 2.3 mm was used to obtain the composite resin specimen. The ISO standard was mainly set to compare the adhesion of dental composite to teeth; some modifications were made, such as the use of flat dentin and ceramic surface with composite and ceramic specimens. The composite and ceramic cylinder bar were positioned perpendicular to the dentin and ceramic material surface. They were then subjected to shear bond strength test on a testing machine Shear Bond Tester (Bisco, Schaumburg IL Inc, USA) (Figure 1) at a crosshead speed of 0.5 mm/min and the values were recorded (MPa). Failure modes were analyzed using an M3B stereomicroscope (x30) (Wild, Heerbrugg, Switzerland) and evaluated as adhesive, cohesive, and mixed. The differences between the groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test ($p<0.05$).

Results

The mean shear bond strength and standard deviation values of the groups are presented in Tables 4-7 and Figure 3. According to the result of the bond strength of the composite resin materials to dentin tissue, the highest bond strength values to dentin tissue were observed in EP, and the lowest values were observed in CMP. The bond strength values of the groups in a descending order were Group-4A (20.58±6.55 MPa) > Group-3A (18.56±3.74 MPa) > Group-2A (16.79±2.52 MPa) > Group-1A (14.30±2.31 MPa). Consequently, a statistically significant difference was only found between Group 1-A and Group 4-A ($p<0.05$).

According to the result of the shear bond strength of composite resin materials to CAD/CAM restorative materials, the highest bond strength values were observed between IPS and FBP (34.64±4.65 MPa). The lowest bond strength values were between VE and CMP (22.31±2.84 MPa). There was a statistically significant difference between the groups ($p<0.05$). Generally, bonding of composite resin materials to IPS was found greater than to LU and VE.

The differences in the shear bond strength values of CAD/CAM restorative materials to dentin tissue were statistically insignificant ($p>0.05$).

When groups A, B, and C were compared, significantly lowest shear bond strength values were observed in Group C (dentin tissue-CAD/CAM restorative materials) ($p<0.05$).

Table 4. The shear bond strength values of composite resin materials to dentin tissue

Groups	Mean (MPa) ± Std. deviation
Group-1A: Dentin-Clearfil Majesty Posterior	$14.30±2.31^{A}$
Group-2A: Dentin-Light Core	$16.79±2.52^{AB}$
Group-3A: Dentin-Filtek Bulk Fill Posterior	$18.56±3.74^{AB}$
Group-4A: Dentin-EverX Posterior	$20.58±6.55^{B}$

Means followed by distinct superscript letters represent statistically significant differences in each column ($p<0.05$).

Table 5. The shear bond strength values of composite resin materials to CAD/CAM restorative materials (mean (MPa) ± std. deviation)

Materials	Clearfil Majesty Posterior	Light Core	Filtek Bulk Fill Posterior	EverX Posterior
Lava Ultimate	Group-1B	Group-2B	Group-3B	Group-4B
	26.10±5.30^{AB1}	26.49±6.93^{AB1}	27.87±7.78^{A1}	26.13±4.58^{A1}
Vita Enamic	Group-5B	Group-6B	Group-7B	Group-8B
	22.31±2.84^{A1}	25.77±5.26^{A1}	29.27±4.66^{A1}	29.09±5.16^{A1}
IPS e.max CAD	Group-9B	Group-10B	Group-11B	Group-12B
	30.59±5.02^{B1}	34.16±6.67^{B1}	34.64±4.65^{A1}	34.23±4.87^{A1}

Means followed by distinct superscript numbers represent statistically significant differences in each row ($p<0.05$). Means followed by distinct superscript letters represent statistically significant differences in each column ($p<0.05$).

Table 6. The shear bond strength values of CAD/CAM restorative materials to dentin tissue

Groups	Mean (MPa) ± std. deviation
Group-1C: Dentin-Lava Ultimate	10.15±1.79^{A}
Group-2C: Dentin-Vita Enamic	11.15±2.50^{A}
Group-3C: Dentin-IPS e.max CAD	12.87±4.02^{A}

Means followed by distinct superscript letters represent statistically significant differences in each column ($p<0.05$).

Table 7. The shear bond strength values of Groups A, B, and C

Groups	Mean (MPa) ± std. deviation
Group-A: Dentin-Composite resin	17.56±4.62^{A}
Group-B: CAD/CAM-Composite resin	28.89±6.41^{B}
Group-C: Dentin-CAD/CAM	11.39±3.05^{C}

Means followed by distinct superscript letters represent statistically significant differences in each column ($p<0.05$).

Distribution of the failure types corresponding to the groups is presented in Figure 4. The most common failure type was adhesive, and the least common failure type was mixed. The stereomicroscope photographs of failure types are shown in Figure 5.

Discussion

This study was designed to evaluate the bond strength of different CAD/CAM restorative materials and composite resin materials to dentin tissue and each other. The first and second tested null hypotheses were rejected. However, the third null hypothesis was not rejected since there was no influence of the type of CAD/CAM restorative materials on shear bond strength to dentin tissue.

Clinicians frequently face challenging situations while restoring teeth with excessive material loss, and at this stage, material choice is an important issue. The remaining tooth structures and selected composite and ceramic restorative materials must complement each other. A failure of bonding between one of these structures means clinical failure of the restoration. There is a great range of composite resin materials on the market such as conventional composites, fiber reinforced composites, and bulk-fill composites. All of these materials are produced to provide easy clinical stages for clinicians and restore the lost tooth structures.

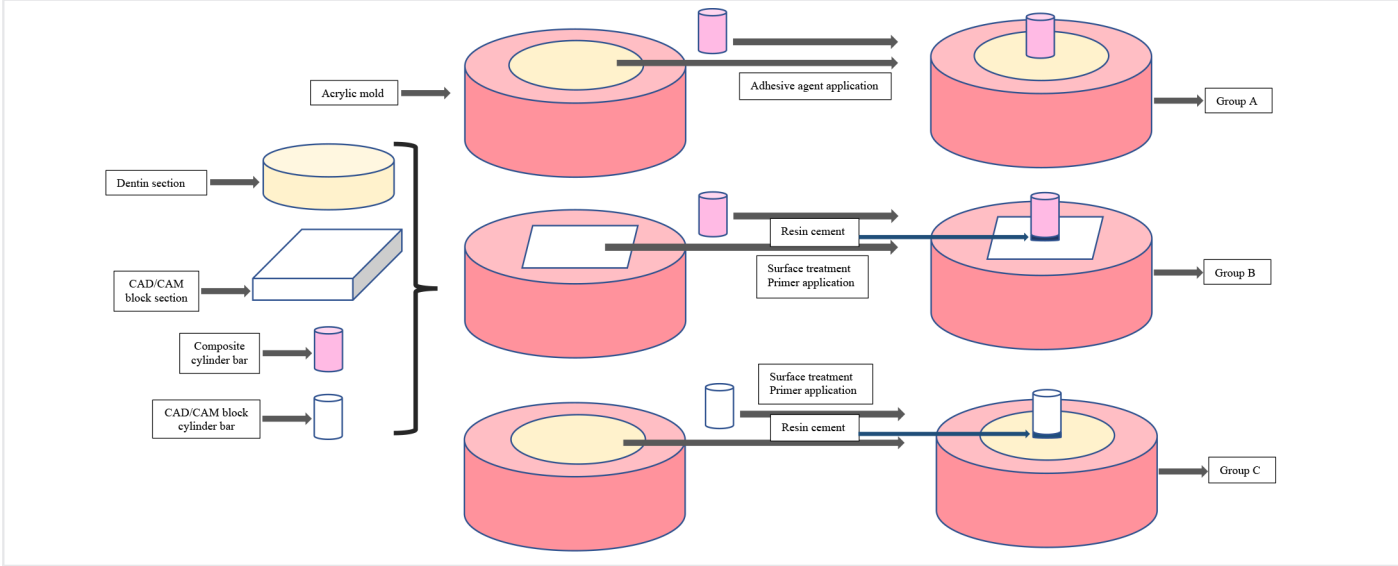

Figure 2. Schematic overview of Group A, B and C

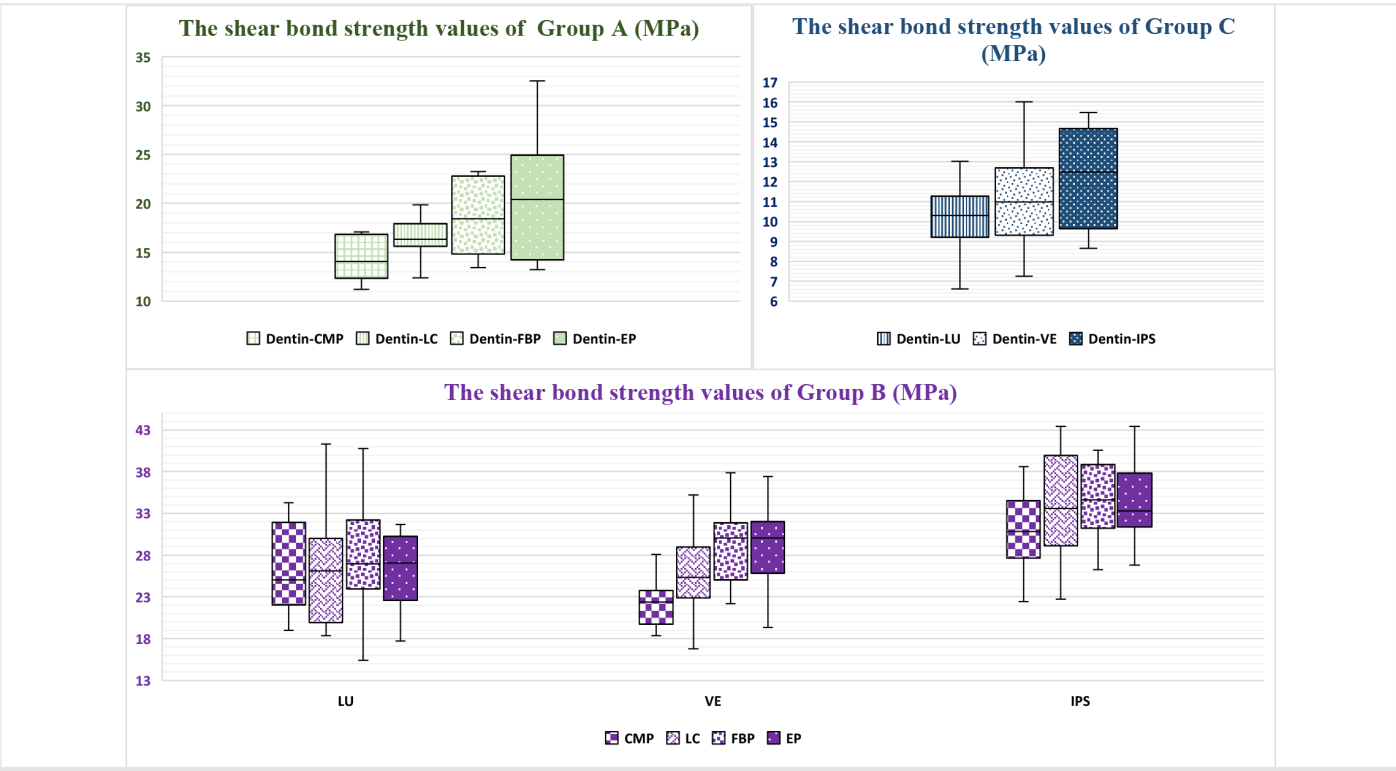

Figure 3. Shear bond strength values (MPa) obtained by the tested groups

Commonly preferred CAD/CAM resin blocks in the clinical practice are Vita Enamic (polymer infiltrated ceramic) and Lava Ultimate (resin nanoceramic). Nanoceramics have a polymeric matrix and contain about 80% by weight ceramic nanoparticle filler. Furthermore, the size of the fillers embedded in the polymer matrix is less than 100 nm. These fillers consist of silica nanoparticles and zirconia nanoparticles or a combination of both. One of the major advantages of this material is that it can be repaired directly in the event of fracture of the prosthetic component (10-12). Polymer-infiltrated ceramics combine ceramic and polymer properties. They have a hybrid structure with a permeable feldspathic ceramic and polymer network. The production of this material requires two stages. Initially, a porous pre-sintered ceramic network is produced and conditioned with a binder, then the polymer is infiltrated into this network. Polymer-infiltrated ceramics have dentin-like wear resistance, elasticity similar to dentin, and high flexural strength (10-13). IPS e.max CAD (Ivoclar Vivadent, Liechtenstein) is a lithium disilicate glass-ceramic structure that is frequently preferred in clinics because of its superior esthetic and mechanical properties. IPS e.max CAD blocks are manufactured in a pre-crystallized metasilicate phase. They are partially crystallized by heat treatment. After the restoration is milled, the lithium metasilicate crystals are modified to lithium disilicate crystals through vacuum heat treatment (11,14,15).

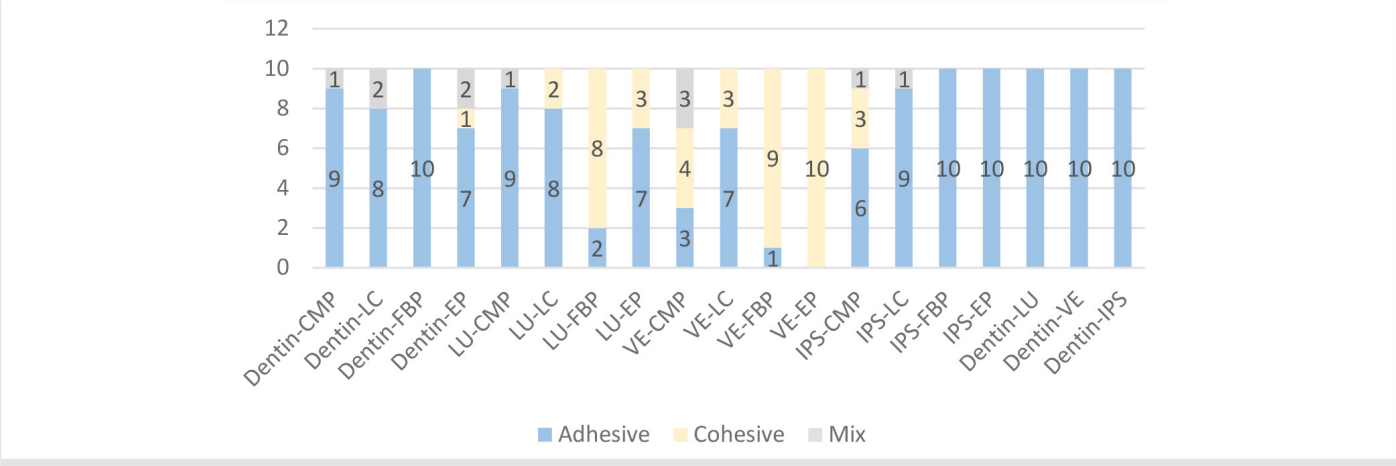

Figure 4. Distribution of fracture types according to experimental groups

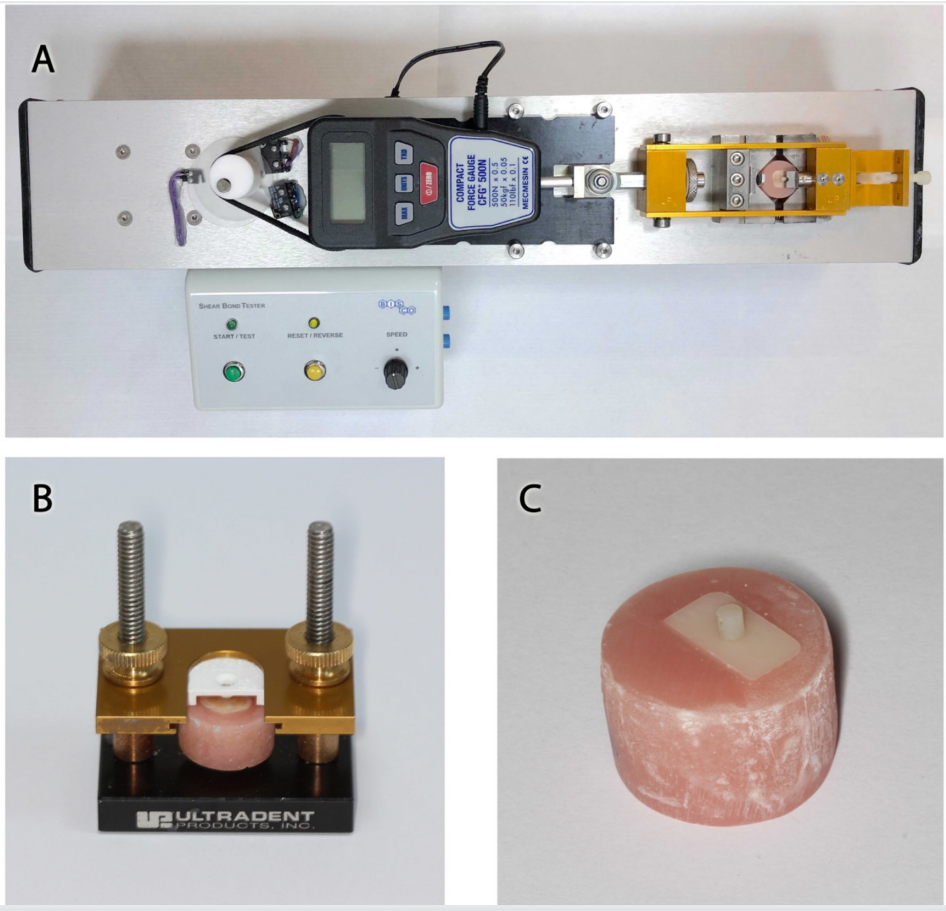

Figure 1. A) Shear Bond Tester B) Teflon cylinder mold C) Test specimen

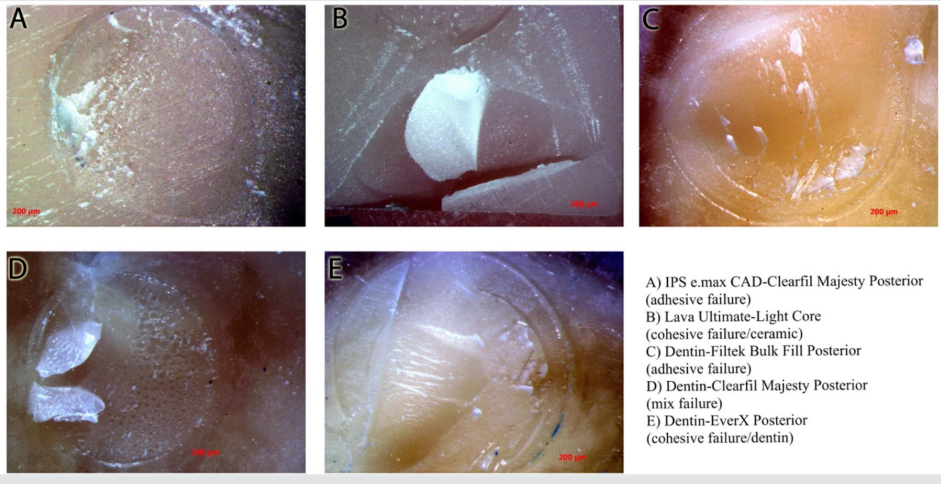

Figure 5. The stereomicroscope photographs of typical failure patterns (x30)

In this study, the highest bond strength values of the composite resin materials to dentin tissue were observed with EP and the lowest values were observed with CMP, and there was a statistically significant difference between them. Garoushi et al. (16), investigated the effect of short fiber fillers on microleakage and shrinkage stress of composites. They reported that the glass fibers content of fiber reinforced composite resins kept polymerization shrinkage stress down and therefore might be effective in reducing microleakage in restorations. Fiber reinforced composites can control polymerization shrinkage stresses by fiber orientation. Thereby, it is assumed that reducing the polymerization shrinkage stresses of composites leads to the increased bonding performance of fiber reinforced composites to dentin tissue compared with conventional composite resins. Tsujimoto et al. (17) conducted a study with the hypothesis that fiber reinforced composites could improve the bonding

performance to dentin tissue. They investigated the bonding performance and interface characteristics of short fiber reinforced resin composites (EverX Posterior) in comparison with different composite resins (Clearfil AP-X, Filtek Suprema Ultra Universal Restorative). The authors reported that the bond strength values of short fiber reinforced resin composites to dentin tissue were statistically similar to the other composite resins. They also reported that the bonding performance of composites to dentin was basically influenced by the type of adhesive system. The researchers attributed the different bond strength values of composites to surface free energies of cured adhesives systems and reported that surface free energy had a strong influence on bond strength values. In the current study, unlike Tsujimoto et al. (17), it was observed that the shear bond strength values of fiber reinforced composite resin materials to dentin tissue were significantly higher than conventional composite resin materials. The different results obtained in these studies may be caused by the selection of different types of composite resin materials and the different inorganic content ratios these materials have.

Omran et al. (18), investigated the effect of increment thickness on light transmittance and bond strength of composite materials [G-aenial Anterior (control), Tetric Evo Ceram Bulk Fill, SDR, EverX Posterior] to the dentin tissue. According to the results of the study, bond strength and light irradiance decreased with increasing increment thickness of resin composites. EverX Posterior shows relatively higher bond strength values than bulk-fill composites. The researchers reported that the high values of EverX Posterior were due to micromechanical coupling between short fibers and dentin tissue. That connection may have increased the bond strength values. G-aenial Anterior (control) showed lower bond strength values. This material exhibits a lower translucency and more opacity, which means that the light transmittance is less, and this reduces the bond strength values (18-20). In addition, it has been reported in previous studies that a high filler ratio reduces the translucency of the composite material, which may lead to a decrease in conversion rates (21,22). In the current study, the lowest shear bond strength values to dentin tissue were observed in Clearfil Majesty Posterior; these low bond strength values might be related to the inorganic filler ratio of this material.

Recently developed bulk-fill composite resins aim to reduce polymerization shrinkage and simplify processes. Bulk-fill composites polymerize at a depth of 4 mm or more, which can be achieved without increasing the polymerization time (23,24). The cavity can be filled and polymerized simultaneously with bulk-fill composite resins, inter-layer contamination is avoided, and the time spent in the clinic is reduced (25,26). However, it is reported that as the layer thickness increases, less light reaches the bottom surface of the composite. To eliminate this effect, the filler amount was reduced, the translucency of the composites was increased, and more reactive photo activators, pre-polymerized particles, and glass fibers were added in bulk-filled composites (27-30). Pereira et al. (30), investigated the bond strength, nano-leakage, and marginal adaptation of bulk-fill composites. Filtek Z350 XT (conventional composite), Tetric N-Ceram Bulk Fill, Filtek Bulk Fill Posterior, and Sonic Fill were evaluated. As a result of that study, the Tetric N-Ceram Bulk Fill composite showed higher bond strength values than Sonic Fill. Researchers have reported that composite resin type is more effective on bond strength values than thermal and mechanical aging processes. In the current study, EP bulk-fill composite showed higher bond strength values than conventional posterior composite. The reason that EP exhibits higher bond strength to dentin tissue may be related to differences in the filler components (short fibers), filler ratio, and degree of conversion (22,26), which improve the polymerization rate of these composites.

The bond strength of CAD/CAM restorative materials to dentin tissue or composite resin materials is also effective in the clinical success of restoration with excessive material loss teeth. There are several ranges of CAD/CAM restorative materials on the market. It has been reported in different studies that the moduli of elasticity of ceramic materials are higher than in composites (31-35). The differences in the moduli of elasticity among the materials could have significant impact on the bond strength values. Ustun et al. (4), reported that when chairside CAD-CAM restorative materials were cemented with total etch, self-etch and self-adhesive systems to dentin tissue, differences in shear bond strength values were observed. However, in the present study, LU, VE, and IPS e.max exhibited similar bond strengths to dentin tissue. The reason for the similar bond strength values of the three CAD/CAM restorative materials to dentin may be related to the adhesive system used. The type of adhesive agent or surface treatment processes may affect bond strength values as well as the selected restorative materials.

Elsaka (12), reported that the bond strength values were significantly affected by the surface treatment protocol and the type of CAD/CAM restorative materials. In our study, IPS shows significantly higher bond strength than LU and VE with different composite resin material combinations. IPS was treated with different surface treatment protocols from LU and VE. Differences in surface treatment procedures may be effective in the bond strength values of CAD/CAM restorative materials. Straface et al. (36), investigated the influence of etching time and hydrofluoric acid concentration on the bond strength of CAD/CAM restorative materials. Etching time was found to significantly affect bond strength values. It was recommended to apply 5% and 9% HF acid to the surfaces of the materials from 15 to 60 seconds. Hou et al. (37), evaluated the bond strength of different CAD/CAM restorative materials. Four different CAD/CAM restorative materials (Vita Enamic, IPS Emax CAD, IPS Empress CAD, Vita Mark II) were used and divided into groups according to different surface treatments. Significantly, highest SBS values were obtained with HF acid etching for Vita Mark II and Vita Enamic and 400 mJ laser surface treatment for IPS e.max CAD. It was shown that SBS was significantly affected by different surface treatment protocols. In our investigation, three different CAD/CAM restorative materials were used. LU and VE samples were sandblasted with 50-μm Al_2O_3 particles, and HF and silane were used for IPS samples. The highest bond strength values between IPS and composite resin materials may be related

to the HF acid and silane application. The CAD/CAM material type and differences in surface treatment protocols applied to CAD/CAM restorative materials may have affected the bond strength values.

When failure modes were evaluated: for Group-A, mainly adhesive failures occurred; for Group-C, adhesive failures were observed for all samples. Bonding to dentin tissue is a sensitive and complex process. In this study, low values were determined for the shear bond strength of composite resin materials and CAD/CAM restorative materials to dentin tissue. Therefore, more adhesive failures may have been observed for Group-A and Group-C. For Group-B more cohesive failures were observed for Vita Enamic, while adhesive failures were mainly observed for Lava Ultimate and IPS e.max CAD materials. The polymer infiltrated ceramic nature of Vita Enamic may have caused these cohesive failures. As a result, it was observed that more cohesive failures occured with higher shear bond strength values (Group-B) and adhesive failures mainly occured with lower shear bond strength values (Group-A and Group-C).

Study Limitations

In this study, when the shear bond strength of composite resin materials to dentin, composite resin materials to CAD/CAM restorative materials, and CAD/CAM restorative materials to dentin tissue were compared, the lowest shear bond strength was observed in the bonding of CAD/CAM restorative materials to dentin tissue. The high organic content of dentin tissue, dentin tubule structure, intratubular moisture, and pressure affect the bond strength of dentin tissue. The sensitivity of bonding to dentin tissue and the use of self-adhesive resin for cementation may reduce the bond strength values of CAD/CAM restorative materials to dentin tissue. Therefore, care should be taken in the selection of composite resin and CAD/CAM restorative material type and adhesive protocol in clinical practice.

The limitations of the present study were that oral environmental conditions were not provided as in vivo and only one type of adhesive resin was used. In future studies, the shear bond strength of different types of adhesive resin and restorative materials could be examined by representing the oral environment. In this study, the effect of composite resin and CAD/CAM restorative material type on the shear bond strength values were investigated. The composite resin material type was effective in bonding to dentin and CAD/CAM restorative materials. However, CAD/CAM restorative material type did not affect the bond strength values to dentin tissue. The shear bond strength values increased when bulk-fill composites were used as composite resin material and IPS as CAD/CAM restorative material. With the use of these materials, the clinical success of the restorations can be increased.

Conclusion

Within the limitations of this study**,** the type of composite resin materials may affect the SBS to the dentin tissue and different CAD/CAM restorative materials. However, the type of CAD/CAM restorative materials does not affect the bond strength to dentin tissue. Composite resin materials, which can be used in a bulk-fill form provide clinicians convenience in many aspects. The choice of IPS as a restorative material in combination with bulk-fill composite resin materials for the restoration of teeth may provide an advantage in terms of restoration durability. However, we think that this issue should be further investigated by long-term clinical studies.

Ethics

Ethics Committee Approval: The present study was approved by the Kocaeli University Non-Invasive Clinical Research Ethics Committee (no: 2019/264).

Peer-review: Externally and internally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: S.B.A., B.D.İ., Concept: S.S., N.T., Design: S.S., N.T., Data Collection or Processing: S.B.A., B.D.İ., Analysis or Interpretation: N.T., S.B.A., Literature Search: N.T., Writing: N.T., S.B.A.

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Original Article

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Bioactive Components and Antioxidant and Antimicrobial Activities of *Rhus coriaria*, a Sumac Species found in Turkey

Türkiye'de Bulunan Sumak Türlerinden *Rhus coriaria* Türünün Biyoaktif Bileşenleri ile Antioksidan ve Antimikrobiyal Aktivitesi

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ABSTRACT

Objective: In our study, it was aimed to analyze the antioxidant and antimicrobial activities of the extracts of the fruits of *Rhus coriaria* L. (sumac) species collected from Gaziantep.

Methods: Ethanol extracts of 80% (R2) and 100% (R3) were prepared from Rhus coriaria fruits. Chemical analysis of the extracts were performed by liquid chromatography-high resolution mass spectrometry method, their antioxidant activities were investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, and their antimicrobial activity was investigated using broth microdilution method.

Results: In the chemical analysis of R2 and R3 extracts, fumaric acid, an organic acid with the highest concentration, was found at concentrations of 31076.55 and 23348.37 mg/kg, respectively. While the phenolic components with the highest concentration in R2 were observed as hyperoside (622.24 mg/kg), ellagic acid (343.63 mg/kg) and p-coumaric acid (182.91 mg/kg), the phenolic components with the highest concentration in R3 were observed as ellagic acid (607.30 mg/kg), hyperoside (440.41 mg/kg) and p-coumaric acid (178.61 mg/kg). In antioxidant activities of R2 and R3 extracts, DPPH free radical scavenging activities were found to

ÖZ

Amaç: Çalışmamızda Gaziantep şehrinden toplanan *Rhus coriaria* L. (sumak) türünün meyvelerine ait ekstrelerin kimyasal analizi yapılarak, antioksidan ve antimikrobiyal aktivitelerinin incelenmesi amaçlanmıştır.

Yöntemler: Rhus coriaria meyvelerinden %80 (R2) ve %100'lük (R3) etanol ekstraktları hazırlanmıştır. Ekstraktların kimyasal analizi sıvı kromatografi-yüksek çözünürlüklü kütle spektrometre yöntemi, antioksidan aktiviteleri 1,1-diphenyl-2-picrylhydrazyl (DPPH) serbest radikal giderim aktivitesi, antimikrobiyal etkinliği sıvı mikrodilüsyon yöntemi kullanılarak araştırılmıştır.

Bulgular: R2 ve R3 ekstraktlarının kimyasal analizinde en yoğun miktarda bir organik asit olan fumarik asit sırasıyla 31076,55 ve 23348,37 mg/kg olarak saptanmıştır. R2'de konsantrasyonu en yüksek olan fenolik bileşenler hyperoside (622,24 mg/kg), ellagic acid (343,63 mg/kg) ve p-coumaric acid (182,91 mg/kg) iken, R3'te konsantrasyonu en yüksek olan fenolik bileşenlerin ellagic acid (607,30 mg/kg), hyperoside (440,41 mg/kg) ve p-coumaric acid (178,61 mg/kg) olduğu gözlenmiştir. R2 ve R3 ekstraktlarının antioksidan aktivitelerinde DPPH serbest radikal giderim aktivitesinin sırasıyla %70,78±0,002 ve %11,19±0,001

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be 70.78%±0.002% and 11.19±0.001%, respectively. Antimicrobial activities of R2 and R3 extracts were found to be 125 and <3.9 µg/mL in S. aureus strain ATCC 25923, 15.625 and 31.25µg/mL in *A. baumannii* strain ATCC 19606, 62.5 µg/mL in H. pylori strain ATCC 43504, 62.5 µg/mL in *C. glabrata* strain ATCC 2001, <3.9 µg/mL in *C. albicans* strain ATCC 66027, respectively.

Conclusion: The higher antioxidant activity in the R2 extract obtained from R. coriaria fruits grown in our country may be due to the higher phenolic component content compared to the R3 extract. It is thought that the more effective antimicrobial activity detected in the R3 extract may be due to the higher amount of ellagic acid compared to the R2 extract.

Keywords: *Rhus coriaria*, phenolic component, antioxidant activity, antimicrobial activity

olduğu tespit edilmiştir. R2 ve R3 ekstraktlarının antimikrobiyal aktiviteleri; *S. aureus* ATCC 25923 kökeninde sırasıyla 125 ve <3,9 µg/mL, *A. baumannii* ATCC 19606 kökeninde sırasıyla 15,625 ve 31,25 µg/mL, *H. pylori* ATCC 43504 kökeninde 62,5 µg/mL, *C. glabrata* ATCC 2001 kökeninde 62,5 µg/mL ve *C. albican*s ATCC 66027 kökeninde <3,9 µg/mL olarak saptanmıştır.

Sonuç: Çalışmamızda ülkemizde yetişen *R. coriaria* meyvelerinden elde edilen R2 ekstresinde daha yüksek saptanan antioksidan aktivitenin, R3 ekstresine kıyasla fenolik bileşen içeriğinin daha fazla olmasından kaynaklanabilir. R3 ekstresinde saptanan daha etkin antimikrobiyal aktivitenin, R2 ekstresine kıyasla fazla miktarda ellagic acid içermesinden dolayı olabileceği düşünülmüştür.

Anahtar Sözcükler: *Rhus coriaria*, fenolik bileşen, antioksidan aktivite, antimikrobiyal aktivite

Introduction

Since ancient times, plants have been used for wellness and for the treatment of various diseases. One of these medicinal plants, the genus Rhus, which is called sumac in the regions where it grows, spreads in temperate and tropical regions, and includes more than 250 flowering plant species from the *Anacardiaceae* family. Rhus species have taken place in the treatment of many diseases in traditional treatment methods with medicinal plants in the culture of the societies in the regions where they are grown. Today, it has been reported that some Rhus species have important biological activities and nutritional values. Their important effects here are due to the large number of bioactive secondary metabolites they contain (1-3).

Rhus coriaria (sumac), which is found in many Mediterranean and Middle Eastern countries such as Lebanon, Syria, Jordan and Iran, grows naturally in Turkey, in the Mediterranean and Southeastern Anatolia. Since the dried and dark red powdered fruits of this plant have an acidic and sour taste, sumac is consumed as a flavor-enhancing spice in salads and meals (2). Besides the consumption of *R. coriaria* as a food, it has been used as a traditional medicine in the Middle East and South Asian countries for thousands of years in the treatment of various diseases, including cancer (2). While *R. coriaria* is used for wound healing, diarrhea, cold and ulcer treatment in traditional Turkish medicine, it has also been prescribed for the treatment of many illness such as liver diseases, urinary system diseases, dental diseases and high cholesterol in Arab countries (1,2). Similarly, *R. coriaria* was used in the treatment of respiratory diseases such as common cold in Cyprus and in the Ottoman Empire (1).

Many therapeutic effects of *R. coriaria* such as antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic activities can be attributed to its various biological properties (2). In fact, in many studies on the biological activity of *R. coriaria*, it has been reported that it has antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, antiulcer, hepatoprotective and neuroprotective effects depending on its bioactive components (1,2). For instance, its antitumor effect has been investigated in various studies on breast cancer, cervical cancer, and colorectal cancer (1,4-6). Particularly, phenolic components of *R. coriaria* can interfere with biological events in the cell by scavenging free radicals, inhibiting enzymes and modulating signal transmission with their strong antioxidant activity (3).

In addition to these reported biological activities of *R. coriaria*, the antimicrobial activity of its fruit extracts has been demonstrated against various microorganisms. Anti-bacterial effect against bacteria that cause severe disorders with their toxins and have intracellular life mechanisms, such as *Shigella dysenteriae*, *Salmonella typhimurium*, *Escherichia coli*, as well as *Bacillus cereus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, which are among the important intestinal pathogens, has been reported in various studies. Its effect against *Helicobacter pylori*, which has been proven to be associated with gastric cancer, is remarkable. In addition, its antimicrobial activity against potential pathogenic microorganisms, which are associated with various clinical pictures and may include some resistant strains, such as *Klebsiella pneumoniae*, *Stapyhlococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, has been observed in many studies (7-9).

In our study, it was aimed to analyze the chemical components of extracts obtained from *R. coriaria* fruits collected in Gaziantep, Turkey and to investigate their antioxidant and antimicrobial activities.

Methods

Supply of Herbal Material and Preparation of Extracts

In this study, extracts of dried fruits of *Rhus coraria*, which were collected from Gaziantep were used. Fruit samples were crushed into powder before being extracted. Solvent was added on it at a ratio of 1:10. As solvents, 80% and 100% ethanols were used. The extracts were prepared by maceration method in a shaking incubator at 35 °C for 24 hours. The solvents of the extracts obtained were completely removed with a rotavapor and lyophilizer, coded as R2 and R3, stored at +4°C until the experimental stage.

Chemical Profile by LC-HR/MS

Phenolic components in extracts of *R. coriaria* fruits were determined by LC-HR/MS method. LC-HR/MS experiments

were performed by a Thermo Orbitrap Q-Exactive ESI Mass Spectrometry system (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The samples were separated on a C18 (150x3 mm; 3 µm) column (Fortis Technologies, UK) at 25 °C. The chromatographic conditions, particularly the composition of mobile phase and its pH, were optimized through several trials to achieve good sensitivity and symmetric peak shapes of analytes. For that purpose, at various flow rates, different solvents of mixtures, such as methanol, acetonitrile, formic acid and acetic acid were tested. The best results were acquired using methanol: formic acid as the mobile phase and it was applied to the gradient program. The mobile phase was a mixture of mobile phase A (1% formic acid solution in water) and B (1% formic acid solution in methanol), the gradient program of which was 0-1.00 min 50% A and 50% B, 1.01-3.00 50% A and 50% B, 3.01-6.00 0% A and 100% B, 6.01-7.00 min 50% A and 50% B and finally 7.01-10.00 min 50% A and 50% B. The flow rate of the mobile phase was 0.35 mL/min. The injection volume was 10 µL. The dihydrocapsaicin was used as an internal standard.

Detection of Antioxidant Activity

In this study, the antioxidant effect of *R. coriaria* extracts was determined by using 1,1-diphenyl-2-picrylhydrazil free radical [1,1-Diphenyl-2-picrylhydrazine (DPPH)] (Sigma Aldrich, Germany) (10). The presence of antioxidant activity was evaluated with the decrease in the absorbance value of DPPH at 517 nm, proportionally. DPPH solution at a concentration of 40 µg/mL was added on the solutions of R2 and R3 extracts prepared with ethanol at concentrations of 10, 25, 50 and 100 µg/mL. Ethanol was used as a control. After 30 minutes of incubation at room temperature, in the dark, absorbance values were measured at 517 nm in a spectrophotometer (Synergy H1 Reader, BioTek, U.S.A). The absorbance values of the samples were evaluated against the control. Free radical scavenging activity was calculated using the following equation.

$$\text{DPPH Removal Activity (\% inhibition)} = \frac{\text{Acontrol-Asample}}{\text{Acontrol}} \times 100$$

(Acontrol: Absorbance of control, Example: Absorbance of sample)

Detection of Antimicrobial Activity

Standard Strains Used in the Study

In our study, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 49461, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 49461, ATCC 700CC, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063, *Acinetobacter baumannii* ATCC 19606, *Helicobacter pylori* ATCC 43504, *Candida albicans* ATCC 66027 and *Candida glabrata* ATCC 2001 strains were used in the investigation of antimicrobial activity.

The standard strains were cultured in Sabouraud Dextrose Agar, 5% Sheep Blood Agar, Mac Conkey agar and Columbia agar [(10% defibrinated horse blood and supplement with Vancomycin (10 mg/L), Cefsulodin (5 mg/L), Trimethoprim (5 mg/L) and Amphotericin B (5 mg/L)] for *Candida* species, Gram-positive strains, Gram-negative strains and *Helicobacter pylori*, respectively.

Detection of Antibacterial Activity

In our study, MIC values of standard bacterial strains were determined by using the resazurin microplate method to determine the antibacterial activities of *R. coriaria* extracts (11). All experiments were repeated twice and streptomycin (Sigma Aldrich, Germany) was used as a standard drug. Stock solutions of the studied samples at a concentration of 1000 µg/ml were prepared with dimethyl sulfoxide (DMSO) and passed through membrane filters with a diameter of 0.22 µm. 50 µl of Brucella broth (BD BBL, USA) for *H. pylori* and 50 µL of Mueller Hinton Broth (Merck, Germany) for other bacteria were dispensed into all wells of microplates. MIC range was set as 3.9-1,000 µg/mL by adding 1,000 µg/mL serial dilutions of the prepared solutions to the first wells of the microplates. The final concentration of streptomycin was adjusted to 83 µg/mL and serial dilutions were made by adding 50 µL to the first well. Serial dilutions were made by placing DMSO (Sigma Aldrich, Germany) as a negative control in one column of the microplate and 50 µL of each bacteria as a positive control on another column. 3 McFarland in Brucella broth containing 10% Fetal Bovine Serum (Lonza, USA) from colonies of *H. pylori* and 0.5 McFarland standard in Mueller Hinton Broth from other strains were prepared and diluted 1:100. Ten µLof the prepared suspension was added to the wells. The plates were covered with parafilm, the microplates of *H. pylori* were incubated in a microaerophilic environment (Thermo ScientificTM OxoidTM CampyGenTM, UK) for 72 hours at 37 °C, while the others were incubated at 37 °C in an aerobic environment for 24 hours. After incubation, 10 µl of 33.75 mg resazurin (Sigma Aldrich, Germany) and 20% Tween 80 (Merck, Germany) dissolved in 5 ml distilled water were added to all wells, plates were left to incubate for 2-4 hours and the results were evaluated visually. The lowest concentration that prevented the color change from purple to pink was determined as the MIC value.

Detection of Antifungal Activity

In our study, MIC values of standard strains were determined by using the resazurin microplate method to determine the antifungal activities of *R. coriaria* extracts (11). All experiments were repeated twice and fluconazole (Sigma Aldrich, Germany) was used as a standard drug. Stock solutions of the studied samples at a concentration of 1,000 µg/mL were prepared with DMSO and passed through membrane filters with a diameter of 0.22 µm. Fifty µL of Mueller Hinton Broth was distributed in each well, serial dilutions of the prepared solutions were made by adding 1,000 µg/mL to the first well and the MIC range was set as 3.9-1,000 µg/mL. The final concentration of fluconazole was adjusted to 30 µg/mL and serial dilutions were made by adding 50 µL to the first well. Serial dilutions were made by adding DMSO to one column of the microplate as a negative control and

50 µL of standard strains to another column as a positive control. Suspensions equivalent to 0.5 McFarland standard were prepared from fresh yeast colonies and diluted at a ratio of 1:100. Ten µL of the prepared suspensions was added to the wells. Plates were covered with parafilm and incubated in an aerobic environment at 37 °C for 48 hours. After the incubation, 10 µL of 33.75 mg of resazurin was dissolved in 5 mL of distilled water and 10 µL of 20% Tween 80 was added to all wells, the plates were left to incubate for 12-24 hours and the results were evaluated visually. The lowest concentration that prevented the color change from purple to pink was determined as the MIC value.

Results

LC-HR/MS Analysis Results

Chemical analysis of *R. coriaria* extracts was made by LC-HR/MS method and 21 components were determined. The components determined and their concentrations (mg/kg) are given in Table 1. In Figure 1, some LC-HR/MS chromatograms of R2 and R3 extracts are shown.

Fumaric acid, an organic acid with the highest concentration in R2 and R3 extracts, was found at concentrations of 31076.55 and 23348.37 mg/kg, respectively. Among the phenolic compounds, the highest amount of hyperoside, ellagic acid and p-coumaric acid was detected in the R2 extract, and their concentrations were 622.24 mg/kg, 343.63 mg/kg and 182.91 mg/kg, respectively. The phenolic components with highest amount in the R3 extract were ellagic acid, hyperoside and p-coumaric acid, and their concentrations were 607.30 mg/kg, 440.41 mg/kg and 178.61 mg/kg, respectively (Table 1).

Antioxidant Activity

The DPPH free radical scavenging activity was investigated at four different concentrations (10, 25, 50, 100 µg/mL). The effects were compared with BHA (Butylated hydroxy anisole), which was used as a standard. At the concentration of 100 µg/mL, inhibition was observed in R2 and R3 at rates of 70.78%±0.002% and 11.19±0.001%, respectively (Table 2). Inhibition values of standard substances and samples are shown in Figure 2.

Antimicrobial Activity

The antimicrobial activities of R2 and R3 extracts on Gram-positive, Gram-negative bacteria and yeasts were investigated by broth microdilution method. Antibacterial activity of R2 extract; MIC values of *A. baumannii* ATCC 19606, *H. pylori* ATCC 43504 and *S. aureus* ATCC 25923 strains were determined as 15.625, 62.5 and 125 µg/mL, respectively. Antibacterial activity of R3 extract; MIC values of *S. aureus* ATCC 25923, *A. baumannii* ATCC 19606 and *H. pylori* ATCC 43504 strains were <3.9, 31.25 and 62.5 µg/mL, respectively. Antifungal activity of R2 and R3 extracts; MIC values of *C. glabrata* ATCC 2001 and

Table 1. Chemical components of *Rhus coriaria* extracts (mg/kg)

Substance name	m/z	Ionization Mode	*Rhus coriaria*		U %
			R2	R3	
(-)-Epigallocatechin	307.0812	Positive	3.99	1.21	3.09
Chlorogenic acid	353.0878	Negative	90.43	75.16	3.58
Fumaric acid	115.0037	Negative	31076.55	23348.37	2.88
Caffeic acid	179.0350	Negative	7.41	6.15	3.74
(+)-t*rans* taxifolin	303.0510	Negative	0.39	0.39	3.35
Luteolin-7-rutinoside	593.1512	Negative	0.64	0.83	3.06
p-Coumaric acid	163.0401	Negative	182.91	178.61	3.31
Rutin	609.1461	Negative	25.17	17.55	3.07
Hyperoside	463.0882	Negative	622.24	440.41	3.46
Dihydrokaempferol	287.0561	Negative	0.51	0.50	2.86
Apigenin 7-glucoside	431.0984	Negative	9.02	6.68	3.59
Ellagic acid	300.9990	Negative	343.63	607.30	4.20
Quercitrin	447.0933	Negative	109.40	75.46	3.78
Myricetin	317.0303	Negative	56.73	8.24	4.18
Quercetin	301.0354	Negative	37.93	23.41	2.95
Salicylic acid	137.0244	Negative	7.67	4.93	1.89
Naringenin	271.0612	Negative	2.65	2.25	4.20
Kaempferol	285.0405	Negative	15.81	14.44	3.56
3'-O-methyl quercetin	315.0510	Negative	0.23	0.16	3.58
Apigenin	269.0456	Negative	0.86	0.75	2.87
Acacetin	283.0612	Negative	0.10	0.11	3.98

***m/z:** Mass to charge ratio, ****U:** *Measurement uncertainty*

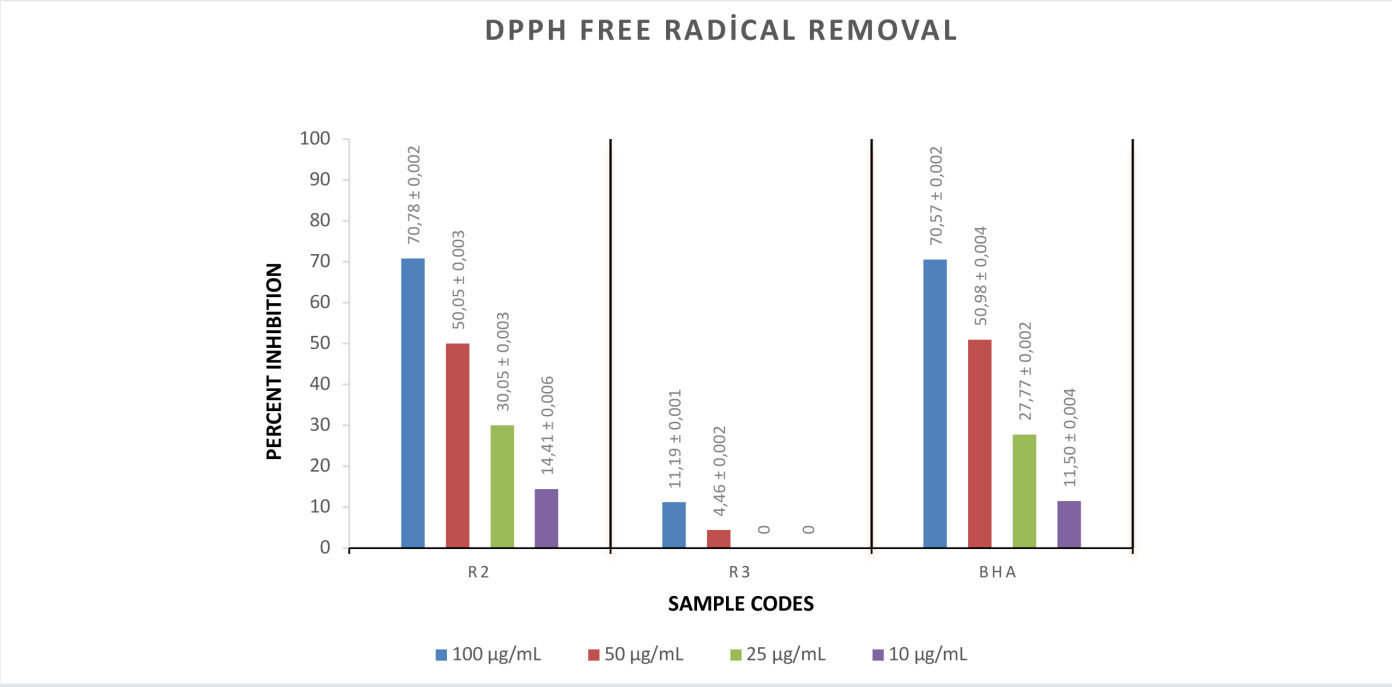

Figure 1. DPPH free radical scavenging activity of *Rhus coraria* extracts (%)
DPPH: 1,1-diphenyl-2-piciylhydrazine

C. albicans ATCC 66027 strains were determined as 62.5 and <3.9 µg/mL, respectively (Table 3).

Discussion

R. coriaria (sumac), which belongs to the *Anacardiaceae* family, is one of the important species of the Rhus genus that grows in the Mediterranean region. In the regions where *R. coriaria* grows, it is used as a flavoring spice and acidifier in appetizers and meals. Although it varies according to the region where *R. coriaria* fruits are grown, it is rich in minerals such as potassium, calcium, magnesium, phosphorus, aluminum, iron, sodium and zinc, it also contains vitamins such as thiamine, riboflavin, pyridoxine, cyanocobalamin, nicotinamide, biotin and ascorbic acid (12-14). It also has many phytochemical compounds, including tannins, flavonoids, terpenoids, anthocyanins (15). When *R. coriaria* was evaluated in terms of phenolic components, it was determined that it mostly contained gallic acid, and also contained flavonoids defined as quercetin, myricetin 3-rhamnoside and quercetin 3-glucoside (16,17). The information in the literature have shown that products with rich phenolic compounds reduce oxidative stress and the risk of chronic diseases. It has been reported that the antioxidant, antibacterial, antifungal, anti-inflammatory and anticarcinogenic activities of *R. coriaria*, which has a very rich content, resulting from the phenolic components and organic acids it contains, can have a protective effect against various diseases (1,2). The polarity and concentration of the solvent used in the extraction of phenolic compounds from plant materials are the most important parameters that reveal the bioactivity of the extract. In terms of human consumption, mostly hydroalcoholic (ethanol: water) extraction is more and widely preferred. Therefore, in this study, the bioactivity of the extracts prepared with 3 different concentrations of ethanol solution was evaluated (18,19).

Table 2. DPPH free radical scavenging activity of *Rhus coraria* extracts (%)

Concentration	R2	R3	BHA
100 µg/mL	70.78±0.002	11.19±0.001	70.57±0.002
50 µg/mL	50.05±0.003	4.46±0.002	50.98±0.004
25 µg/mL	30.05±0.003	0	27.77±0.002
10 µg/mL	14.41±0.006	0	11.50±0.004

DPPH: 1,1-diphenyl-2-piciylhydrazine

In our study, it was determined by LC-HR/MS that *R. coriaria* contained fumaric acid, which was one of the organic acids, in the highest amount. While the amount of fumaric acid was reported as 3.40 mg/kg in a study from China (14) and in *R. coriaria* species growing in Syria, in a study from our country, it was determined that methanol extracts contained 452.78 mg/kg and water extracts contained 180.72 mg/kg fumaric acid (20). In the study reported by Isik et al. (21) from our country with aqueous extracts, the fumaric acid concentration of *R. coriaria* was determined as 44.78 µg/L, while in another study, fumaric acid could not be detected in the samples collected from the city of Kahramanmaraş (12). In our study, the amounts of fumaric acid in 80% and 100% ethanol extracts, respectively, were found as 31,076 mg/kg in R2 and 23348 mg/kg in R3, which were quite high compared to other components. These differences in fumaric acid content reported in the literature may be caused by the genus of *R. coriaria*, the geographical region where it grows, the aqueous extract or the solvents such as methanol and

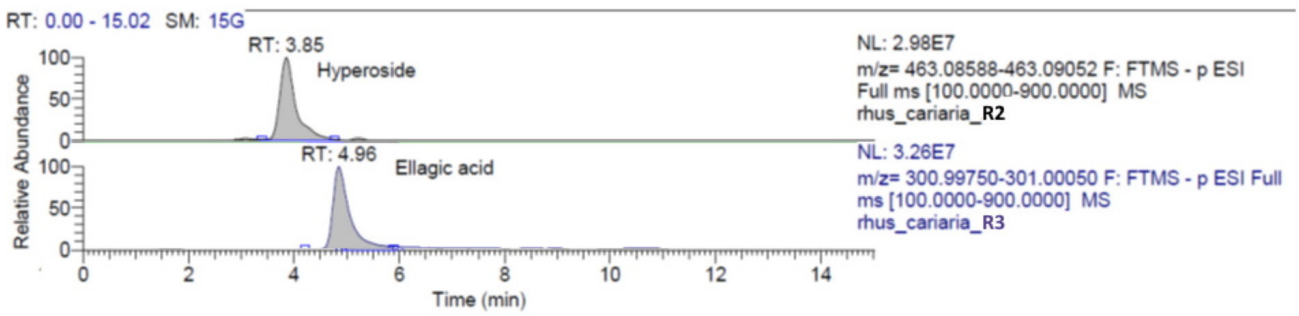

Figure 2. LC-MS/MS chromatograms

Table 3. Antimicrobial activity of *Rhus coraria* extracts (MIC)

	MIC (µg/mL)	
Microorganisms	***Rhus coraria* (80% ethanol) (R2)**	***Rhus coraria* (100% ethanol) (R3)**
E. faecalis ATCC 29212	250	250
S. aureus ATCC 25923	125	<3.9
S. epidermidis ATCC 49461	62.5	62.5
E. coli ATCC 25922	1000	250
P. aeruginosa ATCC 27853	125	125
K. pneumoniae ATCC 70063	125	125
H. pylori ATCC 43504	62.5	62.5
A. baumannii ATCC 19606	15.625	31.25
C. albicans ATCC 66027	62.5	62.5
C. glabrata ATCC 2001	<3.9	<3.9

MIC:

ethanol used in the extract. In a study, the anti-inflammatory and analgesic activities of *Fumaria indica*, which contained a large amount of fumaric acid, were associated with fumaric acid, while *Rhus coriaria*, in which we detected high levels of fumaric acid, might have a role in the similar effect (22). In addition, the antimicrobial activity of fumaric acid was known and it was thought that the fumaric acid concentrations of the extracts we used in our study might also contribute to the antimicrobial activity (23).

It has been reported that components such as quercetin, quercitrin, hyperoside, myricetin, kaempferol and rutin, which are in the flavonol class among flavonoids, have antioxidant, anticarcinogenic, antidiabetic, antiprotozoal, antidepressant, and hepatoprotective properties (3). The hyperoside (quercetin-3-D-galactoside) component, which was shown to have anti-inflammatory, anticancer, antiviral effects in many studies, was found at concentration of 622.24 mg/kg in the R2 extract and 440.41 mg/kg in the R3 extract (24). *R. coriaria* was shown to have an anti-cancer effect in several cancer types including breast and colorectal cancer, and it was one of the rare studies in which it was reported that *R. coriaria* contained isohyperoside (4-6). Investigation of the similar activity of this component, which we detected in large amounts in regional *R. coriaria* species, could generate important data.

Phenolic acids such as chlorogenic acid, caffeic acid, p-coumaric acid, ellagic acid and salicylic acid are considered to be powerful natural antioxidants with many biological activities such as anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic and hepatoprotective activities (3). Many activities of ellagic acid, which is an important component of fruits and vegetables, such as anti-inflammatory, antiulcerative, anticarcinogenic and antioxidant activities have been reported (25). Phytochemicals such as ellagic acid are thought to function either directly as an antioxidant against the negative effects of oxidative stress or by activating cellular antioxidant enzyme systems (26). While the amount of ellagic acid in *R. coriaria* species reported from our country was 12.29 mg/kg in the ethanol extract, this component could not be detected in the aqueous extract (20). However, in our study, ellagic acid was found at concentration of 343.63 mg/kg in R2 extract and 607.30 mg/kg in R3 extract, and their concentrations were high. It was thought that ellagic acid, which we detected in large amounts in our samples, might have a role in the antioxidant activity of regional *R. coriaria* species. While p-coumaric acid, which was another phenolic acid, was detected at concentration of 0.25 µg/g in *R. flexicaulis* extracts grown in Egypt, this component could not be detected in *R. coriaria* extracts in a study conducted in our country (27,28). In our study, p-coumaric acid was detected in 80% (R2) and 100% ethanol (R3) extracts of *R. coriaria* for the first time in our country. The concentrations of p-coumaric acid, caffeic acid, chlorogenic acid, salicylic acid were 182.91, 7.41, 90.43 and 7.67 mg/kg, respectively, in the R2 extract, and 178.61, 6.15, 75.16, and 4.93 mg/kg, respectively, in the R3 extract. Those were higher concentrations than the literature.

Oxidative stress results from an imbalance between production and elimination of reactive oxygen species (ROS) (2). Phytochemicals known as secondary metabolites and especially phenolic compounds have strong antioxidant activity. In recent years, it has been reported that some plant species with antioxidant activity can reduce the risk of various diseases with the effect of phenolic compounds. Due to their strong antioxidant capacity, *R. coriaria* species have been suggested for various pathological conditions (29,30). For instance, *R. coriaria* extract has been reported to reduce UV-A-induced ROS production in HMEC-1 cells, and to block DNA damage significantly (29). Moreover, in

another study, it was observed that *R. coriaria* extract inhibited the progression of skeletal muscle atrophy and created a very strong antioxidative effect in human myoblasts exposed to oxidative stress with hydrogen peroxide (30). In terms of the relationship between ROS levels and liver damage; the protective effect of aqueous *R. coriaria* extract against hydroperoxide (CHP)-induced oxidative stress was demonstrated in rat hepatocytes (31).

Due to the antioxidant activity of *Rhus coriaria,* it is known to have a protective role in various health problems such as cancer, cardiovascular and neurodegenerative diseases caused by oxidative damage (2,13). Studies have shown that antioxidant activity is proportional to the amount of phenolic component. In a study reported in 2013, DPPH free radical scavenging activities of methanol, ethanol and aqueous extracts obtained from Iraqi sumac (*R. coriaria*) were determined as 87%, 72% and 58%, respectively. In the study, it was observed that the total phenolic component concentrations were higher in methanol extracts in parallel with the antioxidant activity (32). In a study reported from Iran, the total amount of phenolic compounds in aqueous extracts of *R. coriaria* and antioxidant activity were highly correlated, and it was reported that DPPH free radical scavenging activities were found 37%, 90%, and 96%, at 1, 2 and 4 mg/mL concentrations, respectively (33). In a study from the Kahramanmaraş region in our country, it was reported that the antioxidant activity of the samples was 73%, and the total phenolic composition was 36.38-58.66 mg GAE/g dw (12). In another study reported from our country; the antioxidant activities of aqueous and methanol extracts of sumac fruits collected from the same region with *R. coriaria* in our study were found 42% and 56%, respectively, at 100 µg/ml concentration (34). In our study, DPPH free radical scavenging activities were found 70.78%±0.002% and 11.19±0.001%, respectively, in R2 and R3 samples at a concentration of 100 µg/mL. Higher activity might be observed in the R2 (80% ethanol) extract due to its higher content of phenolic compounds (Table 1). It is thought that the differences in the results reported in the literature may be due to the phenolic component content and amount of the *R. coriaria*, and the solvents used in the extraction.

Increasing antibiotic resistance is a major problem in the treatment of infections. The problem of resistance is increasing rapidly due to the fact that bacteria develop new resistance mechanisms and transfer resistance genes to other bacteria. One of the important problems in the development of resistance is to trigger the resistance with the use of antibiotics and thus, to activate the resistance genes. Activated resistance genes can also be transferred from one bacterium to another by different mechanisms (35). If there is no need for antibiotic use, this will partially contribute to the problem of resistance development. Studies have shown the antibacterial activity of essential oils, aqueous, methanol and ethanol extracts of *R. coriaria* on some species are known to be pathogenic. Consumption of R. coriaria may contribute to the prevention of foodborne infections in particular. For example, antibacterial activity of *R. coriaria* has been demonstrated on bacteria such as *E. coli*, *S. aureus*, *S. enterica*, *B. cereus*, *S. dysentariae*, *Y. enterocolitica*, which can cause foodborne infections (36-38).

In a study investigating the antibacterial activity of R. coriaria, the MIC value was reported as 0.025% for a multi-drug resistant *S. aureus* strain. This research is particularly important as it shows the effect on a resistant strain. In another study with aqueous extracts of *R. coriaria*, the MIC value for *S. aureus* was found as 0.49%. In a study by Gezici (34) from our country, it was observed that the methanol extract of *R. coriaria* was more effective, and its antimicrobial activity against *S. aureus* ATCC 6538 strain was reported as 15.62 µg/mL. On the other hand, in the study of Ceylan et al. (39) with methanol extracts of *R. coriaria* collected in Şırnak, the MIC values of *S. aureus* ATCC 6538 were determined as 500 µg/mL and 1,000 µg/mL. In our study, the MIC value of *S. aureus* ATCC 25923 strain was determined as 125 µg/mL with R2 80% ethanol extract, the MIC value was determined as <3.9 µg/mL with R3 100% ethanol extract, and more effective antibacterial activity with R3 was observed, compared to the MIC values of other bacteria used in the study (Table 3).

H. pylori, an important pathogen that can colonize the gastric mucosa, is known to cause gastritis, ulcers and gastric cancer. Urease activity of *H. pylori* has primary importance in the colonization to the gastric mucosa (40). Anti-urease activity has been shown in studies investigating the enzyme inhibition activity of *R. coriaria* (41). This activity can have a negative role in the colonization of *H. pylori*. In addition, studies on the antimicrobial activity of *R. coriaria* have also been reported to be effective against *H. pylori*. In a study reported in Iran, the mean MIC value of *R. coriaria* ethanol extracts in *H. pylori* strains isolated from patients with gastritis and peptide ulcers was found to be 214.28 µg/mL (42). In another study reported from Iran, the MIC value of *H. pylori* strain produced from gastric biopsy samples was determined as 80 mg/mL and its inhibitory effect on *H. pylori* was shown (43). Similarly, in the study of Kossah et al. (9), it was reported that *R. coriaria* extracts had an inhibitory effect against *H. pylori*, the MIC value was found to be 1,000 µg/mL in *H. pylori*. In our study, it was determined that both R2 and R3 *R. coriaria* extracts showed an inhibitory effect (MIC: 62.5 µg/mL) in the *H. pylori* ATCC 43504 strain, and it was thought that it could contribute to the reduction of *H. pylori*-related disorders with the effect of this activity.

In our study, the MIC value of *A. baumannii* ATCC 19606 strain, which was another important pathogen, was determined as 15.625 µg/mL with R2 80% ethanol extract. The MIC value of *A. baumannii* was determined as 15.625 µg/mL with R3 100% ethanol extract. In the study reported by Ashoori et al. (44) with *R. coriaria* hydroalcoholic extracts, the MIC value was reported as 1,024 µg/mL.

In addition to its antibacterial activity, *R. coriaria* has also been reported to have antifungal activity in various studies. It has also been shown that *R. coriaria* inhibits the adhesion of *C. albicans* to HEp-2 epithelial cells (45). In a study, the MIC value of alcoholic extracts of *R. coriaria* was reported as 1 mg/mL in *C. albicans* ATCC 60192 strain (7). In the study of Gezici et

al., the antimicrobial activity of methanol extract of *R. coriaria* in *C. albicans* ATCC 10231 strain was 62.25 µg/mL (34). In our study, the MIC values of both R2 and R3 extracts in *C. albicans* ATCC 66027 and *C. glabrata* ATCC 2001 strains were found to be 62.5 and <3.9 µg/mL, respectively (Table 3). When we evaluated the antimicrobial activity that we detected in our study, it could be said that the R3 extract showed more effective antibacterial activity compared to R2, and the antifungal activity was also similar. This activity of the R3 extract may be due to the fact that it contains almost 2 times more ellagic acid, which has antimicrobial activity, than R2 (Table 1).

Study Limitations

Due to economic limitations in our study, clinical bacterial strains and different solvent could not be included. New studies can be planned with these bacteria and different solvent.

Conclusion

As a result; in our study, it was observed that 80% (R2) and 100% (R3) ethanol extracts obtained from *R. coriaria* fruits collected from Gaziantep city in the southeast region of our country, contained high amounts of phenolic compounds. In our study, when the phenolic compound content and antioxidant activities of the extracts were examined; it could be said that the R2 extract showed higher antioxidant activity proportionally to the phenolic compound content compared to the R3 extract. However, in terms of antimicrobial activity, it was observed that the effect of the R3 extract was stronger than R2. This activity of R3 may be due to the fact that it contains more ellagic acid, which has antimicrobial activity, together with other components it contains, compared to R2 extract. In order to determine which bioactive component plays more effective role among the biological activities of *R. coriaria* and to fully understand its mechanism of action, detailed studies at the cell and protein level are needed. Evaluation of these components, which can be determined in future studies, in terms of therapeutic potential may be possible with comprehensive clinical studies.

Ethics

Ethics Committee Approval: Kocaeli Health and Technology University Non-Interventional Research Ethics Committee (number: 2022-03).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: R.Ç., G.I., P.Y.M., Design: R.Ç., B.B.A., P.Y.M., Data Collection or Processing: R.Ç., S.P.S., B.B.A., H.Ö.D., A.B., G.I., P.Y.M., Analysis or Interpretation: R.Ç., S.P.S., B.B.A., H.Ö.D., A.B., G.I., P.Y.M., Literature Search: R.Ç., S.P.S., B.B.A., H.Ö.D., A.B., G.I., P.Y.M., Writing: R.Ç., P.Y.M.

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

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Original Article

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Validity and Reliability of the Symptom-Management Self-Efficacy Scale for Breast Cancer Related to Chemotherapy

Meme Kanserinde Semptom Yönetimi-Öz Etkililik Ölçeğinin Geçerlik ve Güvenirliği

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ABSTRACT

Objective: Since breast cancer (BC) is one of the most common cancer types among women, it is very important for nurses to assess symptom-management and self-efficacy of patients during chemotherapy treatment. This study was carried out to examine the validity and reliability of the symptom-management self-efficacy scale for BC related to chemotherapy.

Methods: The study sample of this methodological research consisted of 248 women receiving chemotherapy treatment due to breast cancer in a public hospital in İstanbul between November 2017 ans March 2018. Translation-back translation method was used to assess the language validity of the scale. Kaiser-Mayer-Olkin and Bartlett's tests were applied to evaluate the sampling adequacy and the suitability of the data for factor analysis.

Results: The content validity of the Turkish form was 0.912; Cronbach alpha coefficient of the scale was 0.905. The factor loads of all the items belonging to the scale were above 0.40, and explained variance for the scale was as follows; 16,284 for the problem-solving sub-dimension; 13,517 for the sub-dimension of managing problems in emotional and interpersonal relationships, and total explained variance of the scale was found to be 46,944. For this reason, no items were removed from the scale and the scale was accepted as having 3 sub-dimensions as it was in the original.

Conclusion: Findings obtained from this study showed that the Turkish version of the scale was valid and reliable and could be used in research and clinical practice in Turkey.

ÖZ

Amaç: Meme kanseri (MK) kadınlarda en sık görülen kanser türlerinden biri olduğundan, hemşirelerin meme kanseri nedeniyle kemoterapi tedavisi alan hastaların semptom yönetimini ve öz-etkililiğini değerlendirmeleri oldukça önemlidir. Bu doğrultuda bu çalışma, MK'de semptom yönetimi-öz etkililik ölçeğinin Türk dilinde geçerlik ve güvenirliğini değerlendirmek amacıyla yapılmıştır.

Yöntemler: Metodolojik tipte yürütülen bu araştırmanın örneklemini Kasım 2017-Mart 2018 tarihleri arasında İstanbul'da bir devlet hastanesinde meme kanseri nedeniyle kemoterapi tedavisi gören 248 kadın oluşturmuştur. Ölçeğin dil geçerliliğinin değerlendirmesinde çeviri-geri çeviri yöntemi kullanılmıştır. Ölçeğin güvenirliğini değerlendirmek için kapsam geçerliliği, faktör analizi, Cronbach α katsayısı ve madde-toplam korelasyonu incelenmiştir. Verilerin faktör analizine uygunluğunu ve örneklem yeterliliğini değerlendirmek için Kaiser-Mayer-Olkin ve Bartlett testleri uygulanmıştır.

Bulgular: Ölçeğin Türkçe formunun kapsam geçerliliği 0,912; Cronbach alfa katsayısı ise 0,905 olarak bulunmuştur. Ölçeğe ait tüm maddelerin faktör yükleri 0,40'ın üzerinde olup, öl.eğin alt boyutlarına ait açıklanan varyansı; problem çözme alt boyutu için 16,284; kemoterapi semptomlarının yönetimi alt boyutu için 16,603; duygusal ve kişilerarası ilişkilerde sorunları yönetme alt boyutu için 13,517'dir ve ölçeğin açıklanan toplam varyansı 46,944'tür. Bu nedenle ölçekten hiçbir madde çıkarılmamış ve ölçeğin orijinaline uygun olarak 27 madde ve 3 alt boyuttan oluştuğu belirlenmiştir.

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Keywords: Validity-reliability, breast cancer, self-efficacy, symptom management

Sonuç: Bu çalışmadan elde edilen bulgular, ölçeğin Türkçe versiyonunun geçerli ve güvenilir olduğunu ve Türkiye'de araştırma ve klinik uygulamalarda kullanılabileceğini göstermiştir.

Anahtar Sözcükler: Geçerlik-güvenirlik, meme kanseri, öz-etkililik, semptom yönetimi

Introduction

Cancer is one of the leading causes of mortality and morbidity in the world.It was reported by the World Health Organization that in 2015, 571,000 of the 8.8 million cancer-related deaths were caused by breast cancer (BC) (1). According to the data of the American Cancer Association, it was stated that 231,840 women were diagnosed as having BC in 2015 in the United States (2). It was declared that 570,000 women lost their lives from BC in the world, which accounted for approximately 15% of cancer-related deaths in women in 2015 (3). Similarly, BC is the most common type of cancer in women in Turkey, and it is reported that more than 1.5 million women are diagnosed as having BC every year (4).

Surgical treatment, radiotherapy, hormonotherapy and chemotherapy are used in the treatment of BC (5). Chemotherapy is a form of treatment using chemotherapeutic drugs to prevent the destruction of cancer cells or the uncontrolled growth of these cells (6). Chemotherapy drugs used in cancer treatment extend the life span of the patients but cause a series of side effects in them (7,8). In chemotherapy, besides cancerous cells, healthy cells are also damaged, especially after chemotherapy, sexual functions are negatively affected. Besides, the following symptoms are observed; infection, bleeding tendency, anemia, weakness/fatigue, alopecia, nausea/vomiting, constipation, diarrhea, changes in the mouth, gums, and throat (8). In addition, it is stated that chemotherapy causes many symptoms related to bone marrow depression, shortness of breath, insomnia, skin, and eye (9). Since chemotherapy treatment has been given at the outpatient clinics, it is becoming more crucial for patients to manage symptoms related to chemotherapy at home (10). In the literature, it is pointed out that patients face many difficulties when performing self-management of side effects caused by chemotherapy treatment (10,11). These side effects and symptoms observed during and after chemotherapy treatment negatively affect the patient's lifestyle, self-care, daily activities, quality of life, symptom management, and self-efficacy (11). Therefore, it is important to evaluate the symptom management and self-efficacy of patients receiving chemotherapy with a valid and reliable instrument that can make more clear to understand the potential role of symptoms and self-efficacy during the treatment process (10).

Self-efficacy level has an important place in the behaviors of individuals in coping with illness, adaptation, and maintaining health, and it has been emphasized that belief is important in health behaviors (11,12). Self-efficacy is defined as the perception of an individual to successfully perform a certain action and to control events (13). Self-efficacy has an important role in guiding the individual's behaviors, feelings, and thoughts. Most studies show that self-efficacy positively affects the treatment process in patients receiving chemotherapy and shortens this process (14,15). It is also believed that patients with cancer with high self-beliefs are more likely to participate in effective symptom management strategies, which make it easier for them to adapt to the disease and treatment (16). Practices to increase self-efficacy reduce emotional responses such as anxiety and stress. Strong self-efficacy increases the probability of initiating and maintaining recommended health behaviors (17). In this process, determining the level of self-efficacy is reported to be important in determining the needs of individuals (12). Liang et al. (9) revealed in their study that when patients' self-efficacy was high, they could also provide symptom management. In another study, it was reported that patients who could fulfill their self-care responsibilities had higher self-efficacy against possible symptoms (11,18).

In addition to being the basis of care given to patients with cancer, symptom management has an important role in preventing or controlling symptoms that develop due to treatment, and symptom management can only be done by evaluating symptoms (19). Techniques used in chemotherapy change depending on the developments in chemotherapy and biotherapy methods, and therefore, new approaches in symptom management are expected to keep up with these changes. Chemotherapy treatment is carried out on an outpatient basis without the need for hospitalization. For this reason, it is very important to be able to manage chemotherapy-related symptoms at home. It has been pointed out in the literature that patients experience various difficulties related to the symptoms associated with chemotherapy (11,19).

In order to ensure symptom management in patients receiving chemotherapy and to evaluate patients' self-efficacy, nurses should understand the symptoms and causes associated with chemotherapy, and the frequency and severity of symptoms. It has been pointed out in the literature that self-efficacy is an important component of well-being and successful symptom management (16). At this point, it is obvious that nurses should determine patients' self-efficacy in order to achieve effective symptom management during the chemotherapy treatment. However, in the literature, there is no valid and reliable measurement tool in Turkish language that evaluates the symptoms related to chemotherapy in BC and determines the self-efficacy level of the patients. For this reason, it is believed that the adaptation of the symptom-management self-efficacy scale for BC (SMSES-BC)

related to chemotherapy to the Turkish society will be a guide for the nurses who care for this patient group and will contribute to the literature.

Methods

Settings and Participants

This study was conducted in a methodological type to examine the validity and reliability of the SMSES-BC related to chemotherapy. The population of the study consisted of women who received chemotherapy for BC in the chemotherapy unit of a public hospital in Istanbul between November 2017 and March 2018. The sample of the study was determined using the purposeful sampling method. Women who agreed to participate in the study, over the age of 18, diagnosed as having BC, completed the third course of chemotherapy treatment, and agreed to participate in the study were included in the study in line with the voluntary principle. By including a total of 248 women in the study, the condition of reaching a size of 5-10 times greater than the number of items in the calculation of the sample size in methodological studies was met.

Instruments

The data of the study were collected using the patient information form and the SMSES-BC related to chemotherapy via face-to-face interview technique from 248 women who were treated in the chemotherapy unit of a public hospital in Istanbul between November 2017 and May 2018 and agreed to participate in the study.

Patient Information Form consisted of a total of 12 questions; 6 questions about the patient's demographic characteristics (age, gender, marital status, educational status, number of children, social security) and 6 questions for the information about the disease (previous hospitalization experience, chronic disease, BC diagnosis year, smoking/alcohol use).

Symptom-Management Self-Efficacy Scale for BC related to chemotherapy was developed by Liang et al. (9) to evaluate symptom management and self-efficacy in patients with BC receiving chemotherapy. The scale was developed in Likert type (0= not sure at all, 10= very sure). It is a scale consisting of 27 items and three sub-dimensions. The total score of the scale is obtained by adding up the numerical values corresponding to the answers. The total score that can be obtained from the scale is between 0-270. The high score indicates that the person's perceived self-efficacy in managing their symptoms is high. SMSES-BC related to chemotherapy includes 3 sub-dimensions: problem-solving skill (7 items), management of chemotherapy symptoms (15 items), and management of emotional and interpersonal problems (5 items). In the scale, there are no items scored by inverting. In the study carried out by Liang et al. (9), the Cronbach alpha value for the original scale was calculated as 0.96, and the Cronbach alpha value of all subscales ranged from 0.88 to 0.95. Content validity of the scale was between 0.75 and 1.00 (11).

Methodology of Translation and Procedures

First of all, the language validity of the SMSES-BC related to chemotherapy was provided. In the adaptation of the English form of the scale into Turkish, the translation-back translation technique which was recommended in the literature and widely accepted for the translation and adaptation of tools in different languages was used (20). For this purpose, the scale, which was originally in English, was translated into Turkish by two professionals, one of whom was a professional translator and the other one had a good command of English and was an expert in the field. The most appropriate translation was adopted for each item by examining the form translated into Turkish by two faculty members who were experts in their field and had a good command of English. The Turkish translation of the scale was re-translated into English by two people (one was a nursing lecturer and the other was a professional translator) who had a good command of English and Turkish and did not see the original of the scale. After the translation and back-translation of the scale was completed, both forms were compared and necessary arrangements were made. After the arrangements were made, a pilot application was carried out in 20 patients who met the inclusion criteria. After the pilot application, the comprehensibility of the scale items was reviewed again and the scale was finalized. The items in the original scale and the back-translated scale were compared and semantic equivalence was achieved. The final version of the scale was sent to Liang et al. (9) of the suitability of the translation was obtained. In this way, the language validity phase of the scale was completed.

Content Validity

Content validity is to take expert opinions in order to determine whether the items in the measurement tool are suitable for the purpose of measurement and whether they represent the area to be measured (21). Along with the Turkish version of the language adapted scale, the English form was submitted to the opinions of 12 experts (two nurse lecturers, six specialist nurses, two oncologists, one psychologist, and one linguist) to determine its suitability in terms of language and content validity. The experts, whose opinions were received via e-mail, examined the items of the scale in terms of intelligibility and cultural compatibility. In order to obtain content validity index (CVI) value, expert opinions were evaluated using the Davis technique. According to the Davis technique, in which four-point grading is used, experts consider the items of the scale as follows; 1. "Not suitable", 2. "The item needs to be brought into an appropriate form", 3. "Appropriate but needs minor changes", 4. "Very suitable". In this research, CVI value was obtained as follows: the number of experts who chose option (a) and (b) was divided by the total number of experts, and the CVI for the item was obtained, and instead of comparing this value with a statistical criterion; a value of 0.80 was accepted as a criterion (22). The scale, of which language and content validities were made, was applied to 20 patients who were diagnosed as having BC in a public hospital in Istanbul and who were excluded from the chemotherapy sample in terms of applicability and understandability. Further, the expressions in the scale items were found to be understandable

by the patients. No adjustment was made in the scale items after the pilot study.

Ethical Consideration

Liang et al. (9), who developed the scale, was contacted by e-mail in order to carry out the validity and reliability study of the SMSES-BC related to chemotherapy in Turkish language and the necessary permission was obtained. Ethics committee approval (EKK/2017/101) and approval from the public hospital where the study was conducted were obtained to conduct the study (71211201-773.99). The study was conducted in accordance with the Declaration of Helsinki and based on the voluntary principle. Detailed information was given to the participants about the study, in addition, individuals were informed that participation in the study was not compulsory and the information obtained from the research would be kept confidential. Individuals who accepted to participate in the study were asked to read and sign the Informed Consent Form.

Data Analysis

The data were analyzed with SPSS for Windows 17.0 package program. Numbers, percentages, minimum and maximum values, mean and standard deviations were used in the analysis of the data. The CVI for content validity, varimax rotation, and principal component analysis for construct validity were applied. The suitability of the data for factor analysis was examined using Kaiser Meyer-Olkin (KMO) value and Bartlett's test. Cronbach alpha coefficient was calculated for internal consistency. Pearson correlation analysis was performed for item-total score correlation and time invariance.

Results

The mean age of 248 women in the study sample was 51.66±12.69. It was determined that 35.9% of the women participating in the study were primary school graduate, 52.8% had comorbidity and 22.2% of them were hypertensive. It was also found that 56.4% of women with BC also had BC in their mothers (Table 1).

Table 1. Participant characteristics (n=248)

Category		n	%
Education	Literate	35	14.1
	Primary school	89	35.9
	High school	62	25.0
	University	62	25.0
Employment	Employed	85	34.3
	Unemployed	163	65.7
Previous hospitalization	Yes	133	53.6
	No	115	46.4
Comorbidity	No	117	47.2
	Hypertension	55	22.2
	Diabetes + hypertension	24	9.7
	Diabetes	23	9.3
	Asthma	14	5.6
	Other*	15	6.0
First degree relative previously diagnosed with breast cancer	No	154	62.1
	Mother	53	56.4
	Sister	41	43.6
Duration of breast cancer diagnosis	1 year	60	24.2
	1-3 years	146	58.9
	3-5 years	30	12.1
	5 years and above	12	4.8
Smoking	Yes	92	37.1
	No	156	62.9
Mean age $\bar{X}$**± SD** 51.66±12.69			

*thyroid, vertigo, heart disease, hepatitis B

Validity

It was found that most of the items of the SMSES-BC related to chemotherapy were scored as "very appropriate" according to expert opinions and the CVI value was 0.912 (Table 2). KMO value of the scale was found as 0.895, and Barlett's test was found as 29621.730 (p=0.000). In the factor analysis, a 3-factor structure with an eigenvalue above 1.00 was observed. Further, factor loadings were found to vary between 0.410 and 0.855. It was determined that the 3-factor structure of the scale was the same as the original. The factor loads of all the items of the scale were above 0.40 and the explained variance was as follows; it was 16,824 for the problem-solving sub-dimension, 16,603 for the management of chemotherapy symptoms sub-dimension, and 13,517 for the management of problems in emotional and interpersonal relationships sub-dimension. The total explained variance of the SMSES-BC related to chemotherapy was 46.944 (Table 4). Therefore, no item was removed from the scale at this stage, and the scale was accepted as having 3 sub-dimensions.

Reliability

In the analysis performed to test the internal consistency of the SMSES-BC related to chemotherapy, the Cronbach alpha reliability coefficient was found to be 0.905. Further, item-total correlations for all items of the scale were positive, and deletion of any item did not cause a significant increase in the Cronbach's α coefficient. Therefore, no item was removed from the scale at this stage (Table 3).

Discussion

This research was carried out with the aim of bringing a measurement tool developed to determine the perceived self-efficacy and the management of symptoms related to chemotherapy in BC to the nursing literature of our country. To determine whether the SMSES-BC related to chemotherapy was valid and reliable in Turkish Language; content validity,

Table 2. Content validity index scores of the symptom-management self-efficacy scale for breast cancer related to chemotherapy items

Items		4	3	2	1	CVI score
1.	Coping with problems in social activities (e.g. stop meeting with friends, stop gossiping)	10	2	-	-	1.0
2.	Coping with emotional stress (e.g. feeling weak, anxious, afraid)	9	3	-	-	1.0
3.	Coping with palpitations (e.g. tachycardia)	8	4	-	-	1.0
4.	Managing fatigue (e.g. tiredness, weakness)	8	4	-	-	1.0
5.	Coping with interpersonal stress (e.g. stress from people who show interest in you)	6	6	-	-	1.0
6.	Coping with vomiting and nausea	12	-	-	-	1.0
7.	Coping with hormonal problems (such as night sweats, facial flushing)	9	3	-	-	1.0
8.	Seeking a place where you can express your feelings (e.g. religious practices, painting, reading books)	3	6	3	-	0.75
9.	Talking actively to healthcare professionals about the side effects of chemotherapy before treatment	8	3	1	-	0.91
10.	Coping with problems related to the oral mucosa (such as inflammation of the mucosa, cracked lips)	9	3	-	-	1.0
11.	Talking actively to healthcare professionals about the side effects of chemotherapy after treatment	4	4	2	2	0.67
12.	Coping with sleep problems (such as insomnia, sensitivity to stimuli while sleeping/waking up quickly)	10	2	-	-	1.0
13.	Coping with eating problems (such as difficulty swallowing, decreased appetite, change in taste)	7	5	-	-	1.0
14.	Coping with skin problems (such as darkening of the skin, redness, and itching)	6	5	1	-	0.91
15.	Prevention of infection (such as prevention of anemia, prevention of decrease in blood cells)	9	3	-	-	1.0
16.	Coping with pain (such as bone pain, muscle pain, spasm)	9	3	-	-	1.0
17.	Coping with nail problems (e.g. darkening of the nails, deterioration of the nail structure)	7	5	-	-	1.0
18.	Being able to get support from social groups (e.g. peer groups, religious officials)	2	6	4	-	0.67
19.	Coping with problems related to arms and legs (e.g. numbness, contraction)	2	6	4	-	0.67
20.	Talking actively with healthcare professionals to cope with the side effects of chemotherapy	4	7	1	-	0.91
21.	Coping with memory problems (such as forgetfulness)	7	5	-	-	1.0
22.	Access to internet resources to cope with chemotherapy-related problems	2	7	3	-	0.75
23.	Coping with hair loss	9	3	-	-	1.0
24.	Coping with social isolation	6	4	2	-	0.83
25.	Coping with work problems caused by chemotherapy (such as demanding rest due to illness)	3	6	1	2	0.75
26.	Getting support from people around her (e.g. healthcare staff, family, friend support)	9	3	-	-	1.0
27.	Coping with problems related to the digestive system (such as feeling bloated, constipation, diarrhea)	6	4	2	-	0.83
Content validity index		**0.912**				

explanatory factor analysis, and internal consistency were examined from 3 different aspects.

The first step in adapting a scale to a different language and culture is to provide language validity by translating the scale (20). Language validity of the scale was provided at the first stage in the translation of the SMSES-BC related to chemotherapy into Turkish language. Originally in English, the scale was translated into Turkish by two people who were fluent in English and Turkish. The most appropriate expressions were selected from the translations and the scale was finalized. The Turkish translation of the scale was re-translated into English by two people (one was a nursing lecturer and the other was a professional translator) who had a good command of English and Turkish and did not see the original of the scale. After the scale was translated into Turkish and the back translation was completed, both forms were compared and it was decided that the language equivalence of the scale was achieved by making the necessary arrangements.

In scale validity and reliability studies, evaluating the content validity is one of the primary stages (21). Content validity means evaluating the scale and the extent to which each item in the scale serves the purpose when examined as a whole. The method of obtaining expert opinion is used to evaluate the content validity (22).

Table 3. Item-total correlations and cronbach α coefficients of the symptom-management self-efficacy scale for breast cancer related to chemotherapy (n=248)

			Avg.	SD	Item-total correlations	If the item is removed cronbach α
9.	Talking actively to healthcare professionals about the side effects of chemotherapy before treatment		8.22	1.43	0.476	0.902
11.	Talking actively to healthcare professionals about the side effects of chemotherapy after treatment		8.20	1.38	0.496	0.902
18.	Being able to get support from social groups (e.g. peer groups, religious officials)		7.59	1.57	0.635	0.900
20.	Talking actively with healthcare professionals to cope with the side effects of chemotherapy		7.75	1.49	0.606	0.900
22.	Access to internet resources to cope with chemotherapy-related problems		6.37	2.98	0.505	0.903
25.	Coping with work problems caused by chemotherapy (such as demanding rest due to illness)		8.61	1.89	0.100	0.909
26.	Getting support from people around her (e.g. healthcare staff, family, friend support)		7.65	1.44	0.623	0.900
3.	Coping with palpitations (e.g. tachycardia)		6.92	2.06	0.633	0.899
4.	Managing fatigue (e.g. tiredness, weakness)		5.68	1.86	0.650	0.899
6.	Coping with vomiting and nausea		6.63	1.77	0.214	0.907
7.	Coping with hormonal problems (such as night sweats, facial flushing)		5.50	1.88	0.316	0.905
10.	Coping with problems related to the oral mucosa (such as inflammation of the mucosa, cracked lips)		6.37	2.21	0.432	0.903
12.	Coping with sleep problems (such as insomnia, sensitivity to stimuli while sleeping/waking up quickly)		5.90	2.09	0.555	0.901
13.	Coping with eating problems (such as difficulty swallowing, decreased appetite, change in taste)		6.44	1.70	0.448	0.903
14.	Coping with skin problems (such as darkening of the skin, redness, and itching)		5.94	1.88	0.415	0.903
15.	Prevention of infection (such as prevention of anemia, prevention of decrease in blood cells)		6.67	1.68	0.482	0.902
16.	Coping with pain (such as bone pain, muscle pain, spasm)		5.99	1.77	0.555	0.901
17.	Coping with nail problems (e.g. darkening of the nails, deterioration of the nail structure)		6.67	1.61	0.535	0.901
19.	Coping with problems related to arms and legs (e.g. numbness, contraction)		6.32	1.69	0.637	0.899
21.	Coping with memory problems (such as forgetfulness)		7.40	1.78	0.290	0.905
23.	Coping with hair loss		6.43	2.54	0.681	0.898
27.	Coping with problems related to the digestive system (such as feeling bloated, constipation, diarrhea)		5.56	2.05	0.392	0.904
1.	Coping with problems in social activities (e.g. stop meeting with friends, stop gossiping)		6.92	1.77	0.589	0.900
2.	Coping with emotional stress (e.g. feeling weak, anxious, afraid)		5.68	2.07	0.545	0.901
5.	Coping with interpersonal stress (e.g. stress from people who show interest in you)		6.88	1.49	0.559	0.901
8.	Seeking a place where you can express your feelings (e.g. religious practices, painting, reading books)		7.16	2.20	0.445	0.903
24.	Coping with social isolation		6.67	1.57	0.629	0.900
Problem Solving Cronbach α					0.804	
Management of chemotherapy symptoms Cronbach α					0.858	
Managing problems in emotional and interpersonal relationships Cronbach α					0.831	
Total the symptom management-self efficacy scale for breast cancer Cronbach α					**0.905**	

Table 4. Factor analysis findings for the symptom management-self efficacy scale for breast cancer (27 items)

Items		Factor/sub-dimension		
		1	2	3
9.	Talking actively to healthcare professionals about the side effects of chemotherapy before treatment	0.034	0.184	**0.855**
11.	Talking actively to healthcare professionals about the side effects of chemotherapy after treatment	0.047	0.217	**0.833**
18.	Being able to get support from social groups (e.g. peer groups, religious officials)	0.454	0.244	**0.545**
20.	Talking actively with healthcare professionals to cope with the side effects of chemotherapy	0.286	0.264	**0.694**
22.	Access to internet resources to cope with chemotherapy-related problems	0.270	0.238	**0.544**
25.	Coping with work problems caused by chemotherapy (such as demanding rest due to illness)	-0.022	-0.128	**0.499**
26.	Getting support from people around her (e.g. healthcare staff, family, friend support)	0.453	0.196	**0.595**
3.	Coping with palpitations (e.g. tachycardia)	0.448	**0.526**	0.159
4.	Managing fatigue (e.g. tiredness, weakness)	0.521	**0.589**	-0.011
6.	Coping with vomiting and nausea	0.007	**0.385**	-0.015
7.	Coping with hormonal problems (such as night sweats, facial flushing)	0.037	**0.520**	-0.004
10.	Coping with problems related to the oral mucosa (such as inflammation of the mucosa, cracked lips)	0.068	**0.542**	0.203
12.	Coping with sleep problems (such as insomnia, sensitivity to stimuli while sleeping/waking up quickly)	0.417	**0.506**	0.043
13.	Coping with eating problems (such as difficulty swallowing, decreased appetite, change in taste)	0.086	**0.522**	0.209
14.	Coping with skin problems (such as darkening of the skin, redness, and itching)	0.281	**0.410**	0.045
15.	Prevention of infection (such as prevention of anemia, prevention of decrease in blood cells)	0.251	**0.437**	0.210
16.	Coping with pain (such as bone pain, muscle pain, spasm)	0.196	**0.703**	0.076
17.	Coping with nail problems (e.g. darkening of the nails, deterioration of the nail structure)	0.234	**0.464**	0.310
19.	Coping with problems related to arms and legs (e.g. numbness, contraction)	0.403	**0.632**	0.058
21.	Coping with memory problems (such as forgetfulness)	-0.034	**0.428**	0.178
23.	Coping with hair loss	0.517	**0.586**	0.076
27.	Coping with problems related to the digestive system (such as feeling bloated, constipation, diarrhea)	0.125	**0.434**	0.170
1.	Coping with problems in social activities (e.g. stop meeting with friends, stop gossiping)	**0.784**	0.062	0,251
2.	Coping with emotional stress (e.g. feeling weak, anxious, afraid)	**0.723**	0.254	-0,003
5.	Coping with interpersonal stress (e.g. stress from people who show interest in you)	**0.640**	0.138	0,282
8.	Seeking a place where you can express your feelings (e.g. religious practices, painting, reading books)	**0.731**	0.075	0,010
24.	Coping with social isolation	**0.742**	0.147	0.273
Explained variance (%)		**16.824**	16.603	13.517
Total explained variance (%)		**46.944**		

In this study, the Davis technique was used to evaluate the content validity and expert opinions were obtained by evaluating the opinions of 12 experts on the items. In the Davis technique, the "CVI" for the item is obtained by dividing the number of experts who mark the "appropriate" and "appropriate but requires minor changes" options to the total number of experts. The fact that this value is 0.67 in studies in which 12 experts give opinions means that the content validity is at an acceptable level (21). Considering the recommended reference values for CVI, the CVI value found as 0.912 in this study showed that the content validity of the Turkish form of the scale was appropriate. According to the result, there was a consensus among the experts about the applicability

and understandability of the items of the scale. In other words, the content validity of the scale was provided.

Multiple methods are used by different researchers to evaluate the construct validity in scale development and validity-reliability studies. One of the most common of these is factor analysis. Factor analysis, one of the multivariate statistical techniques, makes many variables that are related to each other fewer, more meaningful, easily understood, and independent from each other and is widely used (24). In this study, the KMO coefficient was 0.895, and Bartlett test results were x^2=29621.730, p=0.000, and these results revealed the adequacy of the sample consisting of 248 participants for factor analysis (23). It is reported that if the number of samples included in the study is not sufficient, the results cannot be generalized to the society, the reliability of the obtained results should be supported by different applications and more comprehensive studies should be carried out by increasing the number of samples (25). In this study, factor analysis was performed to determine the construct validity of the scale in order to obtain clearer findings from the study after the content validity. In the factor analysis, a 3-factor structure with an eigenvalue above 1.00 was observed. Further, factor loads varied between 0.410 and 0.855. The 3-factor structure of the scale was determined to be the same as the original. Since all factor loads were above 0.30, no item was removed from the scale at this stage (23).

Internal consistency is calculated by using the Cronbach alpha coefficient and takes a value between 0.00 and 1.00. A high value means that the reliability is also high, and the Cronbach alpha coefficient is required to be at least 0.70 in order for a measurement tool to be reliable (26). The Cronbach alpha coefficient calculated to determine the internal consistency of the SMSES-BC related to chemotherapy was 0.905. In addition, the Cronbach alpha coefficients of the sub-dimensions of the scale were as follows; 0.804 for the problem-solving sub-dimension, 0.858 for the management of chemotherapy symptoms sub-dimension, and 0.831 for the management of problems in emotional and interpersonal relationships sub-dimension. In the study carried out by Liang et al. (9), the Cronbach alpha coefficient of the original form of the scale was found to be 0.96, and this result was similar to our study. Item-total correlations for all items of the scale were positive, and in line with this information, it could be said that the scale was a valid and reliable measurement tool.

Study Limitations

Despite the significant and satisfied results, the fact that this research was conducted in a single city of Turkey was the limitation of this study since there could be some cultural differences between different regions of our country and the validity and reliability could change depending on this fact.

Conclusion

The findings obtained from this study showed that the Turkish form of the SMSES-BC related to chemotherapy was valid and reliable for Turkish language and society. This adapted scale contains the same number of items and sub-dimensions as in its original language. As a result of this study, the dissemination of the scale in different regions by repeating it in a larger sample group in Turkey, testing its reliability, and planning different studies by considering other factors that may affect self-efficacy in patients can be recommended.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the ethics committee (EKK/2017/101) and approval from the public hospital where the study was conducted was obtained to conduct the study (71211201-773.99).

Informed Consent: The participants were informed about the aim of the study and confidentiality of their personal information, and their consent was obtained.

Peer-review: Externally peer reviewed.

Authorship Contributions

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

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Original Article

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The Effect of Social Intelligence Levels on Decision-making Styles: A Research in Turkish Healthcare Managers

Sosyal Zekanın Karar Verme Tarzı Üzerindeki Etkisi: Türk Sağlık Yöneticileri Üzerinde Bir Araştırma

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ABSTRACT

Objective: There is little information about the influence of social intelligence on decision making. The nature of decision-making is complex. Many factors influence it. Identifying these factors is important for making effective decisions. Social intelligence is an important factor which can influence decision-making style. This study was conducted to determine the effect of the social intelligence of healthcare managers on their decision-making style.

Methods: A cross-sectional study was conducted with 170 healthcare managers (physician, nurse, and administrative manager) from three public hospitals in Turkey. The analysis of the data was done by using SPSS 26 package program. In order to determine the relationships and effects between the variables, independent t-test, analysis of variance and simple linear regression analysis were performed.

Results: The findings showed that the social intelligence of healthcare managers had a significant positive effect on rational decision-making style and a significant negative effect on dependent and avoidant decision-making style. Whereas healthcare managers with high social intelligence adopted rational style more in decision-making, they adopted dependent and avoidant style less.

Conclusion: Rational decision-making is the most promising, functional and effective decision-making style for physician, nurse and administrative managers in the healthcare industry which includes intensive complexity. Social intelligence is an important concept for healthcare managers considering effective decision-making in the quality of patient care, outcomes, and managerial

ÖZ

Amaç: Literatürde sosyal zekanın karar verme tarzı üzerindeki etkisi hakkında çok az bilgi vardır. Karar verme doğası gereği karmaşık bir süreci ifade eder ve üzerinde birçok faktör etkilidir. Etkili kararlar almak için bu faktörlerin belirlenmesi önemlidir. Sosyal zeka da, karar verme tarzını etkileyebilecek önemli bir faktördür. Bu araştırma, sağlık yöneticilerinin sosyal zekalarının karar verme tarzları üzerindeki etkisini belirlemek amacıyla yapılmıştır.

Yöntemler: Araştırma kapsamında, üç kamu hastanesinden 170 sağlık yöneticisi (hekim, hemşire ve idari yönetici) ile kesitsel bir çalışma yürütülmüştür. Verilerin analizi SPSS 26 paket programı ile yapılmıştır. Değişkenler arasındaki ilişkileri ve etkileri belirlemek için varyans analizi ve bağımsız t-testi ve çoklu doğrusal regresyon analizi kullanılmıştır.

Bulgular: Bulgular, sağlık yöneticilerinin sosyal zekasının rasyonel karar verme stili üzerinde anlamlı pozitif etkiye, bağımlı ve kaçıngan karar verme stili üzerinde ise anlamlı negatif etkiye sahip olduğunu göstermiştir. Sosyal zekası yüksek sağlık yöneticilerinin, rasyonel karar verme tarzı yüksek iken, bağımlı ve kaçınma karar verme tarzı düşüktür.

Sonuç: Rasyonel karar verme, yoğun ve karmaşıklık içeren sağlık sektöründe hekim, hemşire ve idari yöneticiler için en umut verici, etkili ve işlevsel karar verme tarzı olarak görülmektedir. Sosyal zeka, hasta bakımının kalitesi, sonuçları ve yönetsel kararlarda etkili kararlar vermeyi düşünen sağlık yöneticileri için önemli bir kavramdır. Sosyal zekası yüksek sağlık yöneticilerinin rasyonel karar verme eğilimleri de yüksektir.

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decisions. Healthcare managers with high social intelligence also tend to make rational decisions.

Keywords: Social intelligence, decision-making, physician executives, nurse administrators, health facility administrators

Anahtar Sözcükler: Sosyal zeka, karar verme, hekim yöneticiler, hemşire yöneticiler, sağlık tesisi yöneticileri

Introduction

A manager is a person who is given humane and physical resources and expected to use these resources to achieve certain goals. Works will be done by using resources and the objectives of the organization will be accomplished. The manager has to choose among a variety of works (alternatives). The chosen alternative reflects the decision of the manager (1, p.133-134). Decision-making is so important for management work that it is called the heart of management. Therefore, decision-making is the primary duty of the management (2).

Patient care and operation management require the interaction of multiple shareholders. For example, physicians, nurses, lower-middle-senior managers give clinical (e.g. treatment, diagnosis and drug prescription) and non-clinical (e.g. resource allocation, budget, technology achievement, service arrangement, strategic planning) decisions (3). The decisions taken by healthcare managers directly affect the quality, productivity, and effectiveness of the service. Correct decisions must be taken to provide quality and safe health services. However, the nature of decision-making is complex. Decision-making is a cognitive process on which many factors have effects (4). Social intelligence is one of these factors. Social intelligence skills which help the individual communicate and have social interaction with others also affect the quality of decision-making (5). Individuals with improved social intelligence are aware of their competencies and they can understand their environment at the same time. This enables them to control their emotions and make more effective decisions about their objectives (6). Managers make limited rational decisions by using their cognitive abilities and social relationships in healthcare services where environmental uncertainty and complexity are high (7).

Social intelligence has been the subject of a substantial amount of literature especially in the last two decades. However, little contribution has been made to the effect of social intelligence on decision-making behaviours. In studies conducted with healthcare professionals and managers, no study examining the effect of social intelligence on decision-making styles has been found. The aim of this article is to examine the relationship between the healthcare managers' social intelligence levels and decision-making styles. Moreover, it also aims to determine the relationship between the socio-demographic characteristics of healthcare managers and their social intelligence levels and decision-making styles. Social intelligence emphasizes using social skills and working in a team. Considering that healthcare service is a team service, social intelligence is important especially in terms of managers. Decision-making is one of the most important roles of managers. Therefore, this study will be the first in evaluating these two concepts together on healthcare managers.

Background

Social Intelligence

Some basic individual differences which cause people to have different degrees of achievement in social situations are called social intelligence in psychological literature (8). Social intelligence has a very wide field of study in psychology. Researchers have defined it differently over the years (9). It was first defined by Thorndike (10) as the ability to understand and manage people and act intelligently in human relationships. It was later explained by Guilford (11) in the behavioural intelligence model and popularized by Goleman (12) and Albrecht (13). According to Goleman (12), social intelligence includes both interpersonal and social skills. On the other hand, Albrecht (13) defined social intelligence as the ability to get along and cooperate with others.

Social intelligence is a concept that is difficult to define and measure because of its nature. Many researchers have put forward various opinions regarding the dimensions of social intelligence. Two basic dimensions are generally mentioned in the literature as cognitive (14) and behavioral dimensions (15). In this study, social intelligence was examined in three sub-factors stated by Silvera et al. (9). These factors are social skills, social knowledge process and social awareness. Social skill is known as sociality transformed into behaviour. This factor indicates that the individual behaves wisely in his/her social relationships. Social knowledge process is the ability of an individual to understand and predict the feelings and behaviours of other individuals in his/her relationship with them. Social awareness is the individual's awareness of his/her social environment and his/her ability to act in accordance with this environment (9).

It is suggested that social intelligence is one of the most important factors affecting the success of individuals, improves social interaction and can be a precursor of success especially in the administrative domain (16). Social intelligence generally depends on effective social functionality, effective management, and leadership (8). Whether social intelligence will be used to achieve common objectives rather than personal objectives depends on the emotional maturity and social power motivation of the manager or leader (17, p.153). Riggio and Reichard (8) emphasized the importance of social and emotional skills for effective management and leadership. It is important for managers to use especially social intelligence to be effective. Social intelligence is the sensitivity to social problems and the ability to manage them effectively in terms of management (18, p.56). Social intelligence is the ability to determine the requirement of leadership and management in a given situation and to choose the appropriate reaction (17, p.152). Understanding the three-factor structure of social intelligence, which is also used in the

research, and benefiting from them will increase the effectiveness of managers.

Decision-making

According to Van Wart (1998), a decision is a judgement or a result which is reached or made. This definition highlights the choice of a single option among the alternatives. There are two main trends related to the purpose of this research at the heart of a set of decision models: decision-making styles and decision-making as a process. John Dewey argued that individual decision-making process consisted of three separate stages as "(1) What is the problem? (2) What are the alternatives? (3) Which alternative is the best?" (19). Decision-making style is based on the studies of a number of scientists. During the decision-making process, individuals acquire habits that can be affected by many factors they have previously developed. Decision-making style consists of a learnt set of habits (19-21). Decision-making styles were explained by Scotte and Bruce (21) through five factors:

"Rational decision-making style": It is the style expressed by extensive research and rational evaluation for alternatives. Information entry is of the highest order.

"Intuitive decision-making style": It is the opposite of rational decision-making. It is a style expresssed by relying on intuition and feeling rather than knowledge in decision-making.

*"Dependent decision-making style"***:** It is the style in which seeking advice and guidance from others comes forward.

*"Avoidant decision-making style"***:** It is the style expressed by attempts to avoid decision-making completely.

*"Spontaneous decision-making style"***:** It is the style in situations that require immediate decision-making.

Intuitive, dependent and avoidant decision-making styles can be viewed as fundamental decision-making styles as they are used more frequently on a daily basis. However, rational decision-making is a more improved decision-making style. People use information and facts and analysis and step-by-step procedures to make decisions. Intuitive decision-making mostly does not require reasoning or logic. Most people do not do extensive research on what route to take to work in a crowded city or which of the various options to choose for dinner; they figure it out through intuition (22).

One of the most fundamental processes on healthcare management is decision-making. The decision-making style of the managers directly affects the quality, productivity and effectiveness of the services provided (4). Managers should know the decision-making styles and make use of the appropriate decision-making style when necessary, in order to make effective decisions and improve decisions (23).

Social Intelligence and Decision-making

In the literature, theoretical aspects of social intelligence are focused mostly. However, there is an important gap in both theory and practice in terms of the effect of social intelligence skills on decision-making models. Decision makers generally face problems that cannot be easily solved and sometimes there might be negative effects on others even in the issues they believe that they have solved. Therefore, evaluating the effect of decisions on others should be an important factor of the decision-making process. The issue of how decisions will have an effect on others and how they will be interpreted requires social intelligence skills (24). Although there are more studies in the literature on the relationship between emotional intelligence and decision-making, the effect of social intelligence on decision-making cannot be denied. Hence, Goleman (12) defined social awareness related to social intelligence, the individual's relationship and interaction with others and relationship management skills as well as self-management and self-awareness while defining emotional intelligence skills that are effective on decision-making. The conducted studies have shown that social skills have a positive statistically significant effect on decision-making (5). Again, while social intelligence skills such as communication, influencing and persuasion were emphasized among the skill requirements that purchasing managers needed (25), among the basic skills of nurse managers skills which required social intelligence such as communication skills and relationship management were mentioned (26).

Methods

Aim

The aim of this study was to examine the relationship between social intelligence and decision-making styles of healthcare managers. In particular, the aim of the study was to analyse the effect of social intelligence levels of managers on decision-making styles.

Based on previous discussion, the following hypotheses were established.

H1: There is a significant difference between socio-demographic characteristics and decision-making styles dimensions.

H2: There is a significant difference between socio-demographic characteristics and social intelligence

H3: There is a significant correlation between social intelligence as well as its sub-dimensions and decision-making styles dimensions.

H4: Social intelligence has a positive impact on decision making styles dimensions.

The relationships proposed in the hypotheses in this study are presented in Figure 1.

Sample and Procedure

A cross-sectional study was conducted in three public hospitals. The invitation to participate in this study was sent to 260 healthcare managers working in these hospitals. It was sent to all the managers without sampling selection. The data were collected using a questionnaire in person. A questionnaire was distributed to the managers who agreed to participate in the

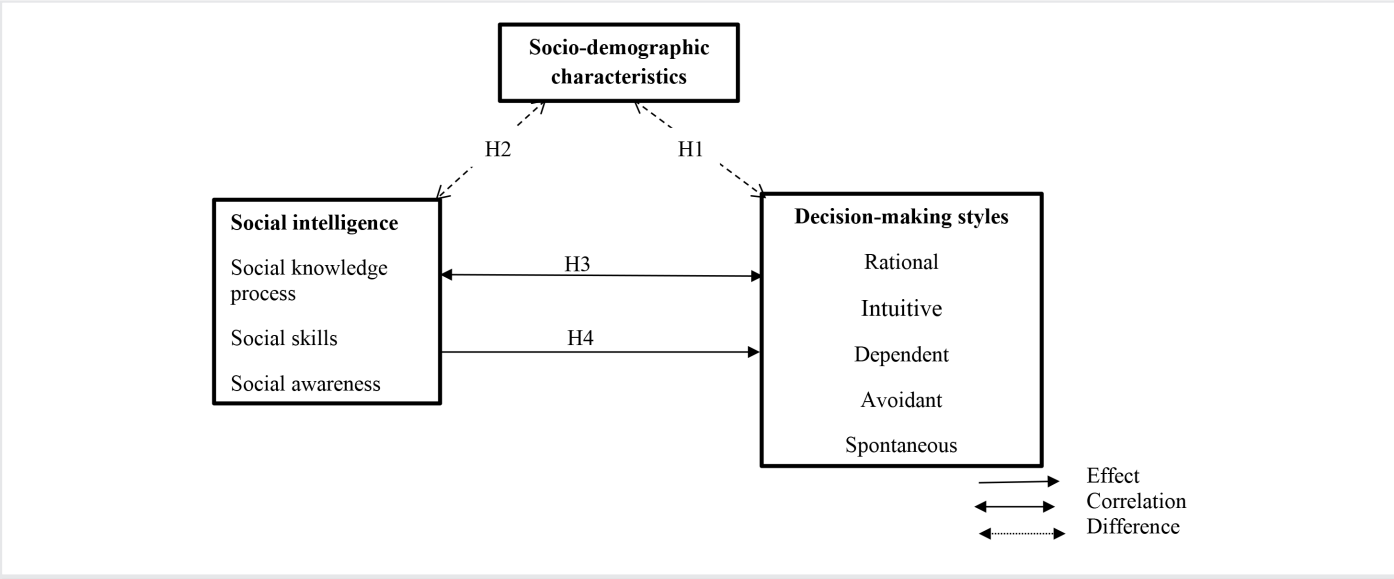

Figure 1. The proposed research model

study, brief information was given about the purpose of the study and the confidentiality of the answers was ensured. A total of 170 valid questionnaires were obtained from three public hospitals. General response rate was 65%. As a result, the sample size of the present study was suitable for testing the model.

Ethical Consideration

The research protocol was approved by the ethics committee of a public university (date: 09.30.2019; number: 31900080-600-E.15719). Written permission was obtained from the hospitals where the study was conducted. Potential participants were informed about the scope, aim, content and method of the study and privacy and anonymity of the data. Participation was based on voluntarism. Informed consents of the participants who agreed to participate in the study were taken. This study was carried out in accordance with 1964 Helsinki Declaration.

Measures

Multi-item scales were used to measure the structures in this study and the scales available in the literature were adopted. The questionnaire form consisted of three parts: social intelligence, decision-making styles and socio-demographic characteristics. All item expressions were measured in 5-point Likert type (1= strongly disagree, 5= strongly agree). Scale factor scores were evaluated in the range of 1-5.

"Tromso social intelligence scale" developed by Silvera et al. (9) is used to measure the social intelligence levels of managers. The scale is consisted of a 3-factor structure as "social knowledge process, social skills and social awareness" and 21 items. In this study, the Cronbach's α values of the factors were determined as 0.84, 0.83 and 0.71, respectively and the Cronbach's α value of the general scale was determined as 0.60.

The Decision-making styles scale was developed by Scotte and Bruce (21). The scale is consisted of a 5-factor structure as "rational, intuitive, dependent, avoidant, and spontaneous decision-making" and 25 items. In this study, the Cronbach's α value of the scale was determined as 0.78. In this study, Cronbach's α values of the factors were determined as 0.84, 0.68, 0.70, 0.90 and 0.72, respectively.

Analysis

The analysis of the data was done by using SPSS 26 package program. The descriptive statistics used were percentages, means, and standard deviations. Anova and independent t test were used to determine the relationships between the healthcare managers' socio-demographic characteristics, social intelligence, and decision-making styles. Simple linear regression analysis was used to measure the effect of the managers' social intelligence levels on decision-making styles. The model developed by Baron and Kenny (27) was used in the regression analysis. Before starting the regression analysis, whether there was multicollinearity between variables and correlation relations were determined. The variance inflation factor (VIF) and tolerance values were examined to determine the multicollinearity. It means that there is a multicollinearity problem when VIF is greater than 10.0 and the tolerance is less than 0.10 (28). As a result of the analysis, it was determined that VIF values (2.04; 2.63 and 1.81) and tolerance values (0.48, 0.37 and 0.55) were suitable.

Results

A total of 170 healthcare managers were included in this study. The average age of the participants was 40.11. Slightly more than half of the participants were women (64.7%) and had bachelor's degree (54.1%) and the majority were married (83.5%). Approximately half of them were nurse managers (49.4%). The participants' average working time in management positions was 6.77 years (SD =5.35) and slightly more than half of them were low level managers (60.6%). A few of them (20%) were working in management positions for more than 10 years. All details of the characteristics of healthcare managers are shown in Table 1.

As a priority in the study, standard deviation and general averages were evaluated to perform error control in the data. The mean scores of the managers in the social intelligence scale were determined as (3.42±0.32) and the mean scores on the decision-making styles scale were determined as (3.13±0.39). Social knowledge process which was one of the social intelligence sub-factors of the managers had the highest average (3.97±0.71). In addition, the managers had the highest average (4.35±0.52) in the rational decision-making sub-factor in the decision-making style scale. As a result of the normality analysis, it was determined that the skewness and kurtosis values of the scales and their sub-factors were between -2 and +2 values (29). According to the results obtained, it was found appropriate to use parametric tests in the analysis of the data (Table 2).

The independent t test showed that males had a significantly higher mean scale score than the females on the dependent (3.44±0.81) and spontaneous (2.61±0.84) decision-making scales. This result revealed that male managers are more dependent and spontaneous than female managers while making decisions. According to the Anova test, those with high school educational level had a significantly higher mean scale score on the intuitive (4.08±0.78), dependent (3.78±0.75) and avoidant (2.87±0.97) scales than those with graduate and master's degrees. In addition, managers with high school educational level had a significantly higher mean scale score in the spontaneous decision-making scale (2.80±0.8) than only the ones with graduate degrees. Again, only in the spontaneous decision-making scale, administrative manager (2.72±0.80) had significantly higher mean scale score than the nurse managers (2.24±0.68). According to the results of the test conducted to determine whether there was a significant difference between managers' decision-making styles in terms of management experience variable, it was determined that there was a significant difference between the groups in rational decision-making (p=0.000), dependent decision-making (p=0.000), and avoidant decision-making (p=0.000) scales. In the rational decision-making scale, it was observed that the difference was between the managers with less than 5 years and more than 11 years of management experience and that the managers with more management experience showed more rational decision-making behaviour. It was observed that there was a significant difference in terms of the dependent and avoidant decision-making scale between the managers with less than 5 years and 6-10 years and more than 11 years of management experience. Those with less management experience showed more dependent and avoidant decision-making behaviour (Table 3). Accordingly, H1 hypothesis was accepted except for the marital status and management position variables.

Table 1. Demographic characteristics of the participants (n=170)

Characteristics		Mean	SD
Age		40.11	7.14
Year in profession		17.23	8.04
Year in present organisation		9.29	6.83
Year in present management position		4.96	4.62
Years in management position		6.77	5.35
Categories		N	%
Gender	Female	60	35. 3
	Male	110	64.7
Marital status	Married	142	83.5
	Un married	28	16.5
Education	High school	22	12.9
	Bachelor	92	54.1
	MS/PhD	56	32.9
Job role	Physician manager	37	21.8
	Nurse manager	84	49.4
	Administrative manager	49	28.8
Management position	Senior	15	8.8
	Middle	52	30.6
	Lower	103	60.6
Unit	Clinic and polyclinic	51	30.0
	Administration	74	43.5
	Laboratory-X-ray, operating room, emergency, ıntensive care	45	26.5
Management experience	<5 years	67	39.4
	6-10 years	69	40.6
	>11 years	34	20.0

SD: Standard deviation

Table 2. Descriptive statistics of the key study variables

Scale and its subdimesions	N	Min	Max	Mean	SD	Skewness	Kurtosis
Social intelligence scale	170	1.00	5.00	3.42	0.32	-0.549	0.944
Social knowledge process	170	1.00	5.00	3.97	0.71	-0.387	-0.082
Social skill	170	1.00	5.00	2.42	0.61	-0.601	0.233
Social awareness	170	1.00	5.00	3.88	0.62	0.463	0.010
Decision-making styles scale	170	1.00	5.00	3.13	0.396	0.765	2.086
Rational decision-making	170	1.00	5.00	4.35	0.52	-0.572	0.305
Intuitive decision-making	170	1.00	5.00	3.58	0.68	-0.236	0.303
Dependent decision-making	170	1.00	5.00	3.29	0.71	0.256	-0.490
Avoidant decision-making	170	1.00	5.00	1.98	0.84	1.030	0.828
Spontaneous decision-making	170	1.00	5.00	2.45	0.77	0.490	-0.257

Table 3. Relationships between sosyo-demografic characteristics, decision-making styles and social intelligence

Charecteristics	Categories	N	Mean	SD	F	P	Difference
Dependent DMS[a]	Male	60	3.44	0.81	6.230	0.044	
	Female	110	3.21	0.64			
Spontaneous DMS[a]	Male	60	2.61	0.84	4.601	0.052	
	Female	110	2.36	0.71			
Rational DMS[a]	Male	60	4.32	0.57	1.593	0.209	
	Female	110	4.36	0.49			
Intuitive DMS[a]	Male	60	3.71	0.68	0.051	0.822	
	Female	110	3.52	0.68			
Avoidant DMS[a]	Male	60	3.71	0.68	3.623	0.060	
	Female	110	3.52	0.68			
Social intelligence[a]	Male	60	3.40	0.37	3.115	0.079	
	Female	110	3.43	0.28			
Independent variable: education							
Intuitive DMS[b]	High school (1)	22	4.08	0.78	6.398	0.003	1-2 p=0.010; 1-3 p=0.014
	Bachelor (2)	92	3.50	0.69			
	MS/PhD (3)	56	3.52	0.54			
Dependent DMS[b]	High school (1)	22	3.78	0.75	6.212	0.003	1-2 p=0.002; 1-3 p=0.003
	Bachelor (2)	92	3.22	0.67			
	MS/PhD (3)	56	3.20	0.70			
Avoidant DMS[b]	High school (1)	22	2.87	0.97	17.355	0.000	1-2 p=0.000; 1-3 p=0.000
	Bachelor (2)	92	1.90	0.69			
	MS/PhD (3)	56	1.75	0.81			
Spontaneous DMS[b]	High school (1)	22	2.80	0.81	3.922	0.022	1-2 p=0.018
	Bachelor (2)	92	2.32	0.71			
	MS/PhD (3)	56	2.53	0.80			
Rational DMS[b]	High school (1)	22	4.18	0.54	1.408	0.248	
	Bachelor (2)	92	4.36	0.53			
	MS/PhD (3)	56	4.40	0.50			
Social intelligence	High school (1)	22	3.2	0.46	3.444	0.034	1-3 p=0.027
	Bachelor (2)	92	3.43	0.29			
	MS/PhD (3)	56	3.47	0.27			
Independent variable: job role							
Spontaneous DMS[b]	Physician manager (1)	37	2.58	0.81	7.166	0.001	2-3, p=0.001
	Administrative manager (2)	49	2.72	0.80			
	Nurse manager (3)	84	2.24	0.68			
Rational DMS[b]	Physician manager (1)	37	4.36	0.49	0.319	0.727	
	Administrative manager (2)	49	4.39	0.59			
	Nurse manager (3)	84	4.31	0.49			
Dependent DMS[b]	Physician manager (1)	37	3.37	0.72	0.454	0.636	
	Administrative manager (2)	49	3.22	0.82			
	Nurse manager (3)	84	3.29	0.65			
Avoidant DMS[b]	Physician manager (1)	37	1.89	0.85	0.275	0.760	
	Administrative manager (2)	49	1.97	0.99			
	Nurse manager (3)	84	2.02	0.75			

Table 3. Continued

Charecteristics	Categories	N	Mean	SD	F	P	Difference
Inutiative DMS[b]	Physician manager (1)	37	3.50	0.67	2.716	0.069	
	Administrative manager (2)	49	3.77	0.67			
	Nurse manager (3)	84	3.51	0.69			
Social intelligence	Physician manager (1)	37	3.44	0.30	0.099	0.906	
	Administrative manager (2)	49	3.42	0.37			
	Nurse manager (3)	84	3.41	0.29			
Independent variable: management experience							
Rational DMS[b]	< 5 years (1)	67	4.17	0.55	9.082	.000	1-3, p=0.000
	6-10 years (2)	69	4.38	0.45			
	>11 years (3)	34	4.62	0.47			
Dependent DMS[b]	<5 years (1)	67	3.58	0.72	10.703	.000	1-2, p=0.001 1-3, p=0.000
	6-10 years (2)	69	3.13	0.69			
	>11 years (3)	34	3.02	0.54			
Avoidant DMS[b]	<5 years (1)	67	2.36	0.97	14.989	0.000	1-2, p=0.000 1-3, p=0.000
	6-10 years (2)	69	1.80	0.64			
	>11 years (3)	34	1.58	0.62			
Intuitive DMS[b]	<5 years (1)	55	3.57	0.75	0.777	0.461	
	6-10 years (2)	51	3.68	0.61			
	>11 years (3)	64	3.52	0.68			
Spontaneous DMS[b]	<5 years (1)	55	2.41	0.92	0.592	0.554	
	6-10 years (2)	51	2.55	0.76			
	>11 years (3)	64	2.41	0.63			
Social intelligence[b]	<5 years (1)	67	3.33	0.33	6.586	0.002	1-3 p=0.001
	6-10 years (2)	69	3.44	0.31			
	>11 years (3)	34	3.56	0.23			
Independent variable: management position							
Decision making sytle	Senior	15	3.04	0.27	1.332	.267	
	Middle	52	3.08	0.38			
	Lower	103	3.17	0.41			
Social intelligence	Senior	15	3.59	0.258	2.369	.097	
	Middle	52	3.39	0.26			
	Lower	103	3.41	0.34			
Independent variable: gender							

[a]t-test for independent group, [b]Analysis of variance

Managers with more than 11 years of management experience had a significantly higher mean scale score than managers with less than 5 years of management experience (p=0.002) in the social intelligence scale (Table 3). Accordingly, H2 hypothesis was rejected except for the management experience variable.

The correlation relationships between variables are shown in Table 4. While social intelligence was significantly positively related with rational decision-making style (r=0.513, p<0.01), it was significantly negatively related with dependent (r=-0.267, p<0.01) and avoidant (r=0.463, p<0.01) decision-making styles. While social knowledge process was significantly positively related with rational decision-making style (r=0.551, p<0.01), it was significantly negatively related with dependent (r=-0.438, p<0.01) and avoidant (r=0.595, p<0.01) decision-making styles. While social skills were significantly positively related with rational decision-making style (r=0.523, p<0.01), it was significantly negatively related with dependent (r =-0.463, p<0.01), avoidant (r=-0.671, p<0.01) and spontaneous (r=-.0163, p<0.01) decision-making styles. While social awareness was significantly negatively related to rational decision-making style (r=-0.362, p<0.01), it was significantly positively related with dependent (r=0.557, p<0.01) and avoidant (r=0.640, p<0.01) decision-making styles. Accordingly, H3 hypothesis was accepted but not in the intuitive and spontaneous decision-making styles dimensions.

A simple linear regression analysis was performed to determine

Table 4. Correlations between social intelligence and its sub-dimensions and decision-making styles

Factors	Social knowledge process	Social skills	Social awareness	Rational	Intuitive	Dependent	Avoidant	Spontaneous
Social knowledge process	1	0.711**	-0.529**	0.551**	0.012	-0.438**	-0.595**	-0.037
Social skills	0.711**	1	-0.664**	0.523**	-0.089	-0.463**	-0.671**	-0.163*
Social awareness	-0.529**	-0.664**	1	-0.362**	0.089	0.557**	0.640**	0.117
Overall social intelligence	0.861**	0.743**	-0.180**	0.513**	0.009	-0.267**	-0.463**	-0.057

the effect of general social intelligence levels of healthcare managers on their decision-making styles (Table 5). Social intelligence levels predicted rational, dependent, and avoidant decision-making styles. It was observed that increased social intelligence was related with increased rational decision-making (β=0.513, p=0.000), decreased dependent (β =-0.267, p=0.000), and avoidant (β=-0.463, p=0.000) decision-making styles. General social intelligence clarified 25% of the change in rational decision-making style (adjusted R^2=0.259), 6.6% of the change in dependent decision-making style (adjusted R^2=0.066), 20% of the change in avoidant decision-making style (adjusted R^2=0.209) (Table 5). Based on the results H4 hypothesis was accepted but not in the intuitive and spontaneous decision-making styles dimensions.

Discussion

This study examined the relationship between socio-demographic characteristics, social intelligence and decision-making styles in a sample of healthcare managers. The discussion part of this study was presented in three parts in order to achieve the purpose of the study.

I. Evaluating the social intelligence and decision-making styles of healthcare managers.

First of all, the findings of the study showed that the managers adopted avoidant decision-making style the least while they adopted rational decision-making style the most. This finding was similar to Şen et al. (30) and Küçükkendirci et al. (4) who showed that public health managers at the provincial level adopted rational decision-making style more. In addition, another study similarly revealed that nurse managers adopted rational decision-making behaviour (31). However, the findings did not agree with Aliakbari et al. (32) who reported that clinical nurses mostly adopted the intuitive decision-making style. In particular, the importance of intuitive decision-making in nursing services was emphasized (33). Because, unlike the studies which consider intuition and rationality as alternative decision-making approaches, there are also studies which argue that intuition is not an independent process from analysis and that intuition and analysis are complementary to each other in decision-making (34).

The findings regarding the social intelligence of healthcare managers showed that the managers had a high level of social intelligence, especially in terms of their social information processing skills. This may be related to the managers' constant interaction with those around them and it demonstrates their ability to recognize and anticipate the feelings and behaviours of others. This finding was similar to Korauš et al. (35) who reported that the managers had higher empathy levels than the ones who were not managers.

II. Evaluating the relationship between healthcare managers' social intelligence, decision-making styles and socio-demographic characteristics.

Second, the relationships between social intelligence and decision-making styles were examined according to socio-demographic characteristics. Regarding the socio-demographic characteristics and decision-making styles of healthcare managers, the study revealed that the managers' decision-making styles were significantly related with gender, education level, job role, and management experience. When the relationship between gender and decision-making style was analyzed, it was found that male managers were more dependent and spontaneous than female managers while making decisions. The findings were similar to previous studies. Küçükendirci et al. (4) showed that male managers in public health institutions adopted a more dependent and intuitive decision-making style. However, there was no significant difference between gender and rational, intuitive, and avoidant decision-making styles. There are different research results in the literature on this subject. For example, Şen et al. (30) did not find a significant difference between the gender of public health administrators and the five decision-making styles. However, Acar et al. (36) revealed in his study on education managers that male managers made more intuitive decisions than female managers, they referred to more opinions of others when making decisions, and they adopted more avoidant decision-making behavior. When the relationship between education level and decision-making style was analyzed, no significant relationship was found only between rational decision-making style and education level. A significant relationship was found between education level and intuitive, avoidant, dependent and

Table 5. The influence of overall social intelligence on decision-making styles

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constant	1.468	0.373		3.933	0.000
Social intelligence	0.841	0.109	0.513	7.754	0.000
Summary statistics of the research model regarding the effect of social intelligence on dependent decision-making styles R=0.267, adj.R^2=0.066, F=12.846, p=0.000					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constant	5.342	0.574		9.303	0.000
Social intelligence	-0.598	0.167	-0.267	-3.584	0.000
Summary statistics of the research model regarding the effect of social intelligence on avoidant decision-making styles R=0.463, adj.R^2=0.209, F=45.718, p=0.000					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constant	6.172	0.623		9.915	0.000
Social intelligence	-1.223	0.181	-0.463	-6.762	0.000
Summary statistics of the research model regarding the effect of social intelligence on intuitive decision-making styles R=0.009, adj.R^2=-0.006, F=0.015, p=0.904					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constant	3.520	0.570		6.172	0.000
Social intelligence	0.020	0.166	0.009	0.121	0.904
Summary statistics of the research model regarding the effect of social intelligence on spontaneous decision making styles R=0.057, adj.R^2=-0.003, F=0.015, p=0.463					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Constant	2.923	0.639		4.571	0.000
Social intelligence	-0.137	0.186	-0.057	-0.736	0.463
Summary statistics of the research model regarding the effect of social intelligence on rational decision-making styles R=0.513, adj.R^2=0.259, F=60.120, p=0.000					

spontaneous decision-making styles. It was found that healthcare managers with high school education level adopted a more intuitive, avoidant, dependent and spontaneous decision-making style. Based on this finding, it can be said that the managers with a lower education level tend to avoid responsibility, consider their feelings and intuitions as the main source of knowledge, and make sudden decisions without thinking. There are different research results related to this finding in the literature. There are studies reporting that education level has no effect on decision-making styles (4,30,36). In addition, Demir et al. (37) found that proportion of athletes with high school graduate rational and dependent decision-making style was significantly higher than athletes with bachelor's degree and primary school graduate.

When the relationship between job role and decision-making style was analyzed, a significant relationship was found only between the spontaneous decision-making style and job role. It was revealed that administrative managers adopted spontaneous decision-making style more than nurse managers. Spontaneous decision-making behaviour is described as a tendency to make rapid, impulsive, and immediate action (21). The nature of the decisions taken by the administrative managers makes their taking quick decisions understandable. In addition, spontaneous decision-making may be insufficient in making decisions about patient care. This finding was similar to the study finding of Krasniqi et al. (38) which showed that directors of corporations adopted more intuitive and spontaneous decision-making behaviour. According to the authors, the managers who use intuitive and spontaneous decision-making styles make brave and quick decisions and tend to be more entrepreneurial. There are also studies that do not find a significant relationship between job role and decision-making styles (30).

When the relationship between managers' decision-making style and their management experience was analyzed, no significant relationship was found between management experience and intuitive and spontaneous decision-making styles, while a significant relationship was found between management experience and rational, dependent, and avoidant decision-making style. It was seen that the managers with more management experience adopted rational decision-making style while the managers with less management experience adopted dependent decision-making style the most and then adopted avoidant decision-making style. Rational decision-making is defined as the most promising, functional, and effective decision-making process for managers, leaders, and individuals (22). These results were similar to the findings of other studies (4,22). However, there are studies which do not report the work experience as a manager in a meaningful way as well (31).

Regarding the socio-demographic characteristics and social intelligence of healthcare managers, a significant relationship was found between the education level and management experience variable and social intelligence. No significant relationship was found with gender, job role, and management position. Being male or female did not change the social intelligence level in this study. Many studies in the literature reported that social intelligence levels do not differ according to gender (6,39). But there are also studies that report significant differences in the social intelligence levels in terms of gender (40). Regarding the job role variable, it was found that being a physician, nurse and administrative manager did not significantly differentiate the social intelligence level. Similarly, Kul and Yüksekbilgili (41) found no significant difference in the social intelligence level according to the working position (doctor, nurse-midwife, health officer, technician). Finally, regarding the management position; there was no significant difference in the social intelligence levels of senior, middle and lower managers. There are studies with different results in the literature regarding this finding. For example, Korauš et al. (35) showed that senior managers had higher social intelligence than low-level managers.

Healthcare managers with more than 11 years of management experience had a significantly higher level of social intelligence than the managers with less than 5 years of management experience. Managers should have a high level of social competence as well as a high level of knowledge about the management profession (35). Therefore, managers with more management experience may have improved their social competencies. Similarly, Kul and Yüksekbilgili (41) found a significant relationship between professional experience and social skill. The authors revealed that the social skill level was higher in healthcare profession with 5-10 years of professional experience than in other groups (below 5 years and over 10 years). In terms of the education level variable, managers with a master's degree or higher had a significantly higher the social intelligence level than managers with a high school education level. Similarly, Özdemir and Adıgüzel (39) found that the social intelligence levels of healthcare profession with a master's degree were significantly higher. However, there are also results showing that education level is not effective on social intelligence (40, 41).

III. Relating healthcare managers' social intelligence and decision-making styles.

Third, there was no significant relationship between healthcare managers' social intelligence and their intuitive and spontaneous decision-making style. There was a statistically significant negative relationship between dependent and avoidant decision-making styles, while there was statistically significant positive relationship between healthcare managers'social intelligence and their rational decision-making styles. To our knowledge, there is no study examining the relationship between social intelligence and decision-making styles in the literature. However, as emphasized by Albrecht et al. (13), emotional intelligence may be associated with social intelligence and well-known and accepted leadership and management approaches. In this respect, these findings are consistent with the study of Ibrahim and Elsabahy (31). The authors showed that there was a statistically significant positive relationship between nurse managers' emotional intelligence and their rational, intuitive, and spontaneous decision-making styles, and a statistically significant negative relationship between emotional intelligence and avoidant decision-making style.

When the effect of social intelligence on decision-making styles was analyzed, it was determined that the social intelligence of healthcare managers had a significant positive effect on rational decision-making styles. The social intelligence abilities of healthcare managers can be explained by their understanding and anticipation of the behaviour of others and their behaving accordingly. In this sense, it can be said that managers with high social intelligence tend to make rational decisions that enables determining and evaluating the alternatives in a comprehensive way regarding the decision criteria. Al-Mehsin (5) showed that social skill level had an effect on the quality of decision-making.

It was determined that the social intelligence of healthcare managers had a significant negative effect on dependent and avoidant decision-making style. Healthcare managers with high social intelligence tend to be less prone to avoidant and dependent decision-making behaviour. This situation can be explained by the fact that individuals, who are aware of the social environment, understand the emotions and behaviours of others and act accordingly, prefer social support and avoidant strategy less (35). Because, perceiving the social environment and behaving accordingly do not cause people to seek advice from others or avoid any situation. This finding was consistent with the study findings of Korauš et al. (35) in which they examined the effect of managers' social intelligence on performance motivation.

Study Limitations

The primary limitation of this study was that it analyzed the role of social intelligence in explaining the decision-making style of healthcare managers in only three public healthcare facilities. Because it is not known whether similar findings have emerged when analyzing other healthcare facilities and other countries, this questionnaire should be repeated in future

research in other contexts. Subsequently, the research design used might be a limitation; a cross-sectional study design could not establish a causal relation between the variables that were investigated. Longitudinal or qualitative studies will provide further theoretical detail underlying the findings of this study. In addition, there are many factors (e.g. organizational factors such as perception, personality, size, ownership, technology) that can affect the decision-making style of managers (30).

It is suggested that future studies should investigate other variables that may have an effect on the decision-making style of healthcare managers as well as social intelligence.

Implications

Various theoretical and practical implications emerged from the research findings. The theoretical implication of this research is that it provides a better understanding of the decision-making behaviour of healthcare managers by evaluating the effect of social intelligence on the decision-making style. Decision making is an important function in management. However, to our knowledge, there is no previous study examining the influence of social intelligence on decision-making styles in the sample of healthcare managers. The study offered empirical evidence as to influence of social intelligence on decision making style in the sample of physicians, nurses, and administrative managers. These findings showed that the social intelligence of healthcare managers had a significant positive effect on rational decision-making style and a significant negative effect on dependent and avoidant decision-making style. Considering how important rational decision-making style is for the management work, it can be said that social intelligence is an important driving force in rational decision-making style.

There are also some practical implications of this research. Regardless of the level, all managers (physician-nurse-administrative) in health institutions make certain types of decision. Their decisions affect patients, employees, and others. One of the factors that predict effective decision-making style of healthcare managers is social intelligence. Social intelligence is an important skill. Healthcare managers have high social intelligence which will lead the organization to success. Another practical implication is that this study raises awareness in terms of social intelligence and decision-making styles for healthcare managers at all levels when selecting and evaluating managers for health institutions.

Conclusion

The aim of this study was to examine the relationship between healthcare managers' social intelligence and their decision-making styles. Previous research has shown that emotional intelligence has an impact on decision making in managers. Yet, we still know relatively little about the relationship between social intelligence and decision-making style in the context of healthcare managers. This research provided evidence regarding that the social intelligence of healthcare managers could predict decision-making style. Research findings showed that healthcare managers with high social intelligence adopted more rational decision-making behaviour and less dependent and avoidant decision-making behaviour.

Ethics

Ethics Committee Approval: The research protocol was approved by the ethics committee of a public university (date: 09.30.2019; number: 31900080-600-E.15719).

Informed Consent: Written permission was obtained from the hospitals where the study was conducted.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: A.Y., Design: A.Y., Data Collection or Processing: S.A.K., Analysis or Interpretation: S.A.K., Literature Search: M.Ö., Writing: A.Y.

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