Clinical and Experimental Health Sciences

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Evaluating the Clinical Leadership Levels of Nurse Managers

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Received: 03.11.2020 **Accepted:** 16.07.2022

ABSTRACT

Objective: This study aims to determine the clinical leadership levels of nurse managers and related factors

Methods: This descriptive study was conducted on 109 nurse managers working at six hospitals – including four public hospitals, one private hospital, and one university hospital. The data was collected using Clinical Leadership Scale (CLS) including their personal and demographic information, as well as their clinical leadership traits.

Results: The participants had a total mean score of 2.72±0.19 on clinical leadership scale. The mean scores of the improving services subscale were higher in female nurse managers than those of male counterparts. Participants who formally studied on management earned higher mean scores for the overall CLS and its setting direction subscale than those who did not. Likewise, the participants who formally studied on leadership earned higher mean scores for the overall CLS and its personal qualities subscale than those who did not. These findings were statistically significant ($p<0.05$).

Conclusion: Nurse managers, especially those who formally studied on management and leadership, had high levels of clinical leadership.

Keywords: Hospitals, leadership, nurse managers, nurses, nursing

1. INTRODUCTION

Clinical leadership means that a person is accessible, has clinical skills, is supportive, a role model, and visible in their practice, provides guidance and assistance, inspires others, is an effective communicator, and exhibits proper conduct (1). Clinical leadership also determines the practitioner, partner, and leadership roles of clinicians, (2) and affects patient care and practice standards (3) by supporting patient-centred communication, continuity of care and interdisciplinary cooperation (4). Clinical leadership enhances quality of care, security culture, and patient satisfaction and reduces costs (5). As cited by Brown, Crookes & Dewing (6), Millward and Bryan (7) define clinical leadership within the scope of nursing as "managing both oneself and others by combining leadership and management skills to make a real difference in care and also leading both oneself and others." Effective clinical leadership is key to creating healthy, functional, and supportive working environments for nurses (8). Similarly, it is a new role for patient-centered care. For nurse managers, it refers to the efforts made by a nurse – who gains expertise through undergraduate and/or graduate education, and has the knowledge and skills to carry out patient care, change complex systems, and improve patients' outcomes (9). In addition, one can address clinical leadership in the delivery of care as both the supportive and guiding behaviors of a nurse towards their patients and team (10). Therefore, it can be likened to a renaissance in nursing (11) because as a model it supports shared leadership, which also pushes nurses to enjoy high job satisfaction. This, in contrast to other leadership approaches, which place a leader and its followers in the center (12,13). Furthermore, it is crucial that a leader maintains clinical leadership behaviors. This requires the integration of traditional leadership and management skills, the formation of an infrastructure for organizational objectives, and the allocation of organizational resources among clinical leaders as well as support by nurse managers (14,15).

Among the studies conducted on this concept bringing a new perspective to leadership, Brown et al., (6) has discovered

DOI: 10.33808/clinexphealthsci.820506

that clinical leadership ensures the development of self-leadership and self-management skills. Ennis, Happell & Reid Searl (16) has stated that clinical leaders need to stay calm and confident during crisis/uncertainty. In the same study, the researchers emphasize that behaviors exhibited by clinical leaders during stressful/crisis situations and their ability to manage unpredictable/unexpected clinical situations positively affect the clinical practice. Chávez and Yoder (17), upon investigating clinical leadership in nursing staff has found that this nursing group was good at solving clinical issues for other team members even though they themselves lacked any formal authority. Connolly et al. (10) –investigating the correlation between the clinical leadership skills of emergency room nurses and structural and psychological empowerment – has revealed that nurses exhibit clinical leadership behaviors whilst on the job even if they lack psychological empowerment. Another study has put forth that service/clinic charge nurses use clinical leadership skills to maintain care (18). There are only a limited number of studies that examine clinical leadership here in Turkey. These studies have revealed that clinical leadership perceptions of the nurses and physicians are high (12,19,20). Conversely, no Turkish study as of yet has been published about nurse managers who both exhibit the leadership traits, behaviors, and skills and primarily manage clinics. However, both employees and patients have high expectations from nurse managers and the clinical leadership makes leadership and managerial skills essential. Both situations require to conduct further studies on this topic. In addition, it is important to determine what factors affect the clinical leadership skills of nurse managers for both active audiences and institutional outcomes, as well.

Research Questions:

What are the clinical leadership levels of nurse managers?

What are the factors affecting the clinical leadership levels of nurse managers?

Do the socio-demographic characteristics of nurse managers affect their clinical leadership levels?

2. METHODS

2.1. Purpose and Type of the Study

The aim of this descriptive study was to determine the clinical leadership levels of nurse managers and related factors.

2.2. Population and Sample of the Study

The population of the study included 136 nurse managers working in four public hospitals, one university hospital, and one private hospital in a city. Attempts were made to reach the whole population without using the sample selection. In the end, 109 (80.4%) nurse managers agreed to participate in the study.

2.3. Data Collection Tools

All of this study's data were collected using a Clinical Leadership Scale, which asked the participants about their personal and demographic information.

The Clinical Leadership Scale (CLS) consists of two parts. The first section includes questions regarding personal and demographic information of the participants. The second section focuses on their clinical leadership traits. The first section features 12 questions that ask the participants about gender, age, marital status, level of education, what their duty is, where they work (hospital/unit), the duration of working in the institution and profession, and whether or not they've received any formal education on management, leadership, and clinical leadership. The name of the original scale is "Clinical Leadership Competency Framework Self-Assessment Tool," which was developed by the National Health Services (NHS) Leadership Academy in 2012 (21). It was adapted into Turkish in 2016 by Budak, who shortened its name to "Clinical Leadership Scale." It evaluates the clinical leadership traits of the participants. It features 40 questions and 5 subscales: personal qualities (items 1 to 8), working with others (items 9 to 16), managing services (items 17 to 24), improving services (items 25 to 32), and setting direction (items 33 to 40). It is a 3-point Likert scale. The items are rated between 1 and 3 points: 1 =Almost never, 2=Sometimes, and 3=Almost always. Scoring has a range of 2 points. This range is equally divided into three categories: "low" (1.00-1.66), "medium" (1.67-2.33), and "high" (2.34-3.00). Higher scores signify that clinical leadership perception increases. Budak calculated the Cronbach's Alpha of CLS as 0.95. For this study, its Cronbach's alpha value was found as 0.90 (22).

2.4. Data Collection

The data were collected from self-reports that the participants (i.e. nurse managers) filled out by hand between October 15, 2018 and January 15, 2019.

2.5. Data Analysis

Frequency, percentage, and mean were used to analyze the participants' demographic traits. Mean was used to identify the clinical leadership levels of the nurse managers. Mann Whitney U and Kruskal Wallis tests were employed to compare their socio-demographic traits with their CLS scores. The results were assessed at confidence interval of 95% and significance level of 5%.

2.6. Ethical Considerations

In order to conduct this study, written permission were obtained from the Provincial Directorate of Health (public hospital) on May 10, 2018, from the university hospital on April 12, 2018, and from the private hospital on July 26, 2018. In addition, ethics committee approval (the number: 24237859-566) was obtained on September 24, 2018. Permission to use the CLS was obtained over e-mail on

March 09, 2018. The participants were also asked to give their written/informed consent indicating that they agreed to participate.

3. RESULTS

It was determined that 93.6% of the participants were female; 72.5% were between 36 and 45 years; 90.8% were married, and 70.6% had a Bachelor's degree. 77.1% of the participants had more than 16 years of professional experience; 91.7% were charge nurses. 62.4% of the participants worked in the public hospital; 37.6% worked in internal medicine units, and 41.3% were working in their respective institutions for more than 16 years. 58.7% of the participants did not receive any formal education on management; 71.6% did not receive any formal education on leadership, and 80.7% did not receive any formal education on clinical leadership (Table 1).

Table 1. *Socio-demographic characteristics of the nurse managers (n=109)*

Socio-demographic characteristics	n	%
Gender		
Female	102	93.6
Male	7	6.4
Age		
Between 26-35 years	14	12.8
Between 36-45 years	79	72.5
45 years and over	16	14.7
Marital status		
Married	99	90.8
Single	10	9.2
Level of Education		
High school	10	9.2
Associate degree	9	8.3
Bachelor's degree	77	70.6
Graduate degree	13	11.9
Position		
Director of healthcare services	3	2.8
Supervisor	6	5.5
Charge nurse of the service	100	91.7
Hospital		
University	31	28.4
Public	68	62.4
Private	10	9.2
Unit		
Internal medicine	41	37.6
Surgical	33	30.2
Emergency/Intensive care	26	23.9
Management	9	8.3
Duration of working in the Institution		
0-5 years	8	7.3
6-10 years	29	26.6
11-15 years	27	24.8
16 years and more	45	41.3
Duration of working in the profession		
6-10 years	11	10.1
11-15 years	14	12.8
16 years and more	84	77.1
Studying formally on management		
Yes	45	41.3
No	64	58.7
Studying formally on leadership		
Yes	31	28.4
No	78	71.6
Studying formally on clinical leadership		
Yes	21	19.3
No	88	80.7

Table 3. *Comparison of demographic characteristics of the nurse managers with their CLS total and subscale scores (n=109)*

Demographic characteristics	n	PQS		WOS		MSS		ISS		SDS		CLS	
Gender		Mean Rank	Med.	Mean Rank	Med.	Mean Rank	Med.	Mean Rank	Med.	Mean Rank	Med.	Mean Rank	Med.
Female	102	55.95	2.750	54.85	2.750	56.43	2.812	56.98	2.875	55.74	2.750	56.34	2.800
Male	7	41.14	2.625	57.21	2.750	34.21	2.625	26.21	2.500	44.29	2.500	35.50	2.625
MWU =		260.0		341.5		211.5		155.5		282.0		220.5	
p =		0.221		0.844		0.066		**0.010***		0.347		0.091	
Age													
Between 26-35 years	14	56.93	2.750	64.54	2.875	61.21	2.875	53.21	2.875	63.61	2.875	61.11	2.825
Between 36-45 years	79	56.34	2.750	52.47	2.750	54.29	2.750	56.89	2.875	54.04	2.750	54.86	2.750
45 years and over	16	46.72	2.748	59.13	2.750	53.06	2.812	47.22	2.750	52.22	2.562	50.34	2.725
X^2_{KW} values =		1.344		2.159		0.667		1.379		1.270		0.874	
p values =		0.511		0.340		0.716		0.502		0.530		0.646	
Marital status													
Married	99	55.38	2.750	55.07	2.750	54.57	2.750	56.33	2.875	55.74	2.750	55.36	2.775
Single	10	51.25	2.687	54.35	2.812	59.30	2.937	41.80	2.750	47.65	2.625	51.40	2.812
MWU =		457.5		488.5		452.0		363.0		421.5		459.0	
p =		0.688		0.944		0.645		0.153		0.434		0.705	
Level of education													
High school	10	59.90	2.750	65.90	2.812	69.30	2.875	51.35	2.812	62.65	2.812	62.75	2.812
Associate degree	9	44.33	2.625	46.33	2.750	50.17	2.750	45.33	2.625	38.67	2.375	41.11	2.625
Bachelor's degree	77	53.75	2.750	54.08	2.750	52.33	2.750	56.82	2.875	56.04	2.667	54.80	2.800
Graduate degree	13	66.00	2.750	58.04	2.750	63.15	2.875	53.73	2.875	54.27	2.625	59.85	2.800
X^2_{KW} values =		3.080		2.159		3.822		1.329		3.167		2.655	
p values =		0.379		0.540		0.281		0.722		0.367		0.448	
Position													
Director of healthcare services	3	60.67	2.750	79.00	2.875	95.00	3.000	90.00	3.000	74.67	2.875	88.83	2.900
Supervisor	6	46.92	2.687	49.83	2.750	46.67	2.750	57.50	2.875	50.75	2.750	49.67	2.775
Charge nurse of the service	100	55.32	2.750	54.59	2.750	54.30	2.750	53.80	2.875	54.67	2.750	54.31	2.775
X^2_{KW} values =		0.519		2.007		5.487		4.102		1.318		3.667	
p values =		0.771		0.367		0.064		0.129		0.517		0.160	
Hospital													
University	31	53.61	2.750	58.39	2.750	55.23	2.750	59.29	2.875	53.48	2.750	56.16	2.750
Public	68	55.80	2.750	52.18	2.750	53.58	2.750	53.78	2.875	55.54	2.750	54.15	2.800
Private	10	53.85	2.687	63.65	2.812	63.95	2.875	50.00	2.750	56.00	2.750	57.20	2.812
X^2_{KW} values =		0.121		1.732		0.979		0.981		0.104		0.140	
p values =		0.941		0.421		0.613		0.612		0.949		0.932	
Unit													
Internal medicine	41	53.72	2.750	52.60	2.750	52.06	2.750	50.99	2.750	53.04	2.750	50.74	2.775
Surgical	33	55.97	2.750	56.35	2.750	54.23	2.750	55.59	2.875	49.65	2.625	54.12	2.750
Emergency/Intensive care	26	57.00	2.750	55.50	2.750	57.92	2.875	55.96	2.875	63.60	2.812	60.15	2.812
Management	9	51.50	2.750	59.56	2.875	62.78	2.875	68.33	3.000	58.72	2.750	62.72	2.850
X^2_{KW} values =		0.326		0.516		1.188		2.442		3.241		2.003	
p values =		0.955		0.915		0.756		0.486		0.356		0.572	
Duration of working in the Institution													
0-5 years	8	60.94	2.750	54.63	2.750	52.88	2.812	48.88	2.812	58.44	2.750	55.75	2.762
6-10 years	29	56.76	2.750	55.48	2.750	62.16	2.875	55.43	2.875	57.71	2.750	59.19	2.800
11-15 years	27	51.78	2.750	52.46	2.750	51.72	2.750	55.70	2.875	52.37	2.750	51.37	2.750
16 years and more	45	54.74	2.750	56.28	2.750	52.73	2.750	55.39	2.875	54.22	2.750	54.34	2.725
X^2_{KW} values =		0.682		0.269		2.128		0.346		0.536		0.892	
p values =		0.877		0.966		0.546		0.951		0.911		0.827	
Duration of working in the profession													
6-10 years	11	60.50	2.750	71.05	2.875	62.23	2.875	52.36	2.875	65.82	2.875	64.32	2.825
11-15 years	14	42.82	2.625	42.11	2.750	54.46	2.812	55.96	2.875	52.21	2.750	49.64	2.775
16 years and more	84	56.31	2.750	55.05	2.750	54.14	2.750	55.18	2.875	54.05	2.687	54.67	2.762

X^2_{KW} values =		2.660		5.437		.667		.098		1.515		1.371
p values =		0.265		0.066		0.716		0.952		0.469		0.504
Studying formally on management												
Yes	45	59.16	2.750	60.46	2.875	61.14	2.875	61.24	2.875	63.70	2.875	63.68	2.825
No	64	52.08	2.750	51.16	2.750	50.68	2.750	50.61	2.750	48.88	2.625	48.90	2.700
MWU =		1253.0		1194.5		1163.5		1159.0		1048.5		1049.5	
p =		0.240		0.121		0.083		0.075		**0.015***		**0.016***	
Studying formally on leadership												
Yes	31	65.10	2.750	62.58	2.875	63.50	2.875	62.15	2.875	61.26	2.875	65.23	2.850
No	78	50.99	2.750	51.99	2.750	51.62	2.750	52.16	2.875	52.51	2.750	50.94	2.725
MWU =		896.0		974.0		945.5		987.5		1015.0		892.0	
p =		**0.032***		0.105		0.071		0.125		0.186		**0.033***	
Studying formally on clinical leadership												
Yes	21	62.86	2.750	62.33	2.875	56.95	2.875	53.74	2.875	59.36	2.875	60.33	2.850
No	88	53.13	2.750	53.25	2.750	54.53	2.750	55.30	2.875	53.96	2.750	53.73	2.750
MWU =		759.000		770.000		883.000		897.500		832.500		812.000	
p =		0.196		0.225		0.748		0.834		0.476		0.389	

*PQS: Personal qualities; WOS: Working with others; MSS: Managing services; ISS: Improving services; SDS: Setting direction; CLS: Clinical Leadership Scale Total; MWU: Mann–Whitney U test; Med.: Median; *p<0.05*

The participants earned total mean score of 2.72±0.19 in the CLS. Their mean score was 2.70±0.19 for the "personal qualities" subscale, 2.74±0.19 for the "working with others" subscale, 2.75±0.23 for the "managing services" subscale, 2.77±0.24 for the "improving services" subscale, and 2.66±0.31 for the "setting direction" subscale (Table 2).

Table 2. *Mean scores of the nurse managers for CLS and its subscales (n=109)*

Subscales	Mean	SD	Min.	Max.
Personal qualities (PQS)	2.70	0.19	2.13	3.00
Working with others (WOS)	2.74	0.19	2.00	3.00
Managing services (MSS)	2.75	0.23	1.75	3.00
Improving services (ISS)	2.77	0.24	2.00	3.00
Setting direction (SDS)	2.66	0.31	1.88	3.00
Clinical Leadership Scale Total (CLS)	2.72	0.19	1.98	3.00

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

The socio-demographic characteristics of the participants were compared with their CLS mean scores. The scores of "improving services" subscale was higher in the female participants than the male participants; this was statistically significant (MWU=155.500; p=0.010). In addition, total scores of CLS (MWU=1049.500; p=0.016) and scores of "setting direction" (MWU=1048.500; p=0.015) subscale were higher in nurse managers who formally studied on management than those who did not; this was statistically significant. The total scores of CLS (MWU=892.000; p=0.033) and scores of "personal qualities" (MWU=896.000; p=0.032) subscale were higher in nurse managers who formally studied on leadership than those who did not; this, too, was statistically significant (p<0.05) (Table 3).

No statistically significant differences were found between participants' CLS total and subscale scores and their age, marital status, level of education, position, where they worked (hospital/unit), the duration of working in the institution and profession, and whether they received any formal education on clinical leadership (p>0.05).

4. DISCUSSION

Clinical leadership helps nurses achieve individual and team goals, maintain team processes, have effective healthcare teams, and enhance the quality of their work life (17). According to other leadership approaches, clinical leadership focuses on the effective conducting of clinical care by managing personal and interpersonal skills, despite the quantitative shortage of health manpower and complex health system processes. In addition, a clinical leader is a professional health manager who centers health care, improves patient and employee safety, and has a refined sense of both self-awareness and self-management, is also able to maintain individual development, is open to innovation and change, and has good interpersonal relations and planning skills (23). Therefore, the benefits of clinical leadership enhance patient care, improve patient outcomes and patient satisfaction, and foster a healthy practice environment (24). In this context, it is important to evaluate clinical leadership because it can have such a positive outcome in health services, especially for nurse managers. Accordingly, upon studying the clinical leadership levels of the nurse managers, we discovered that most of the participants who were female, between 36 and 45 years old, married, had a Bachelor's degree, and had over 16 years of professional experience had high levels of clinical leadership. This proved to be desirable outcome for nursing because clinical leadership is an important and

challenging role that requires nurses to be experts at what they do, responsible, and accountable to enhance clinical results and improve nursing care for their patients (25). Some Turkish studies have revealed that physicians and nurses alike both have a high sense of clinical leadership, which supports the results of the present study (12,19,20). In addition, one international study reported that emergency room nurses had particularly high levels of clinical leadership (10). When the CLS subscales were examined in this study, it was found that participants received high scores on all of the subscales. Similarly, those studies conducted on physicians and nurses showed that they earned high clinical leadership subscale scores as well (12,19,20). In this study, they earned the highest score on the "improving services" subscale, and the lowest score on the "setting direction" subscale. This suggested that the participants were very open to provide higher quality services, improve those services, and enhance patient safety and innovation. However, they focused less on using information and evidence, and on decision making. Likewise, in a separate study conducted on physicians and nurses,the participants earned the lowest score on the "setting direction" subscale (12). Moreover, in this study, female nurse managers earned higher scores for the "improving services" subscale than their male counterparts. In other words, it appears that female nurse managers were much more adept at providing high quality healthcare service, enhancing that service, and ensuring patient safety. In their study, Budak and Özer (19) found that female physicians and nurses had higher clinical leadership levels in the same domain than men did.

Leadership is always required. Assessment and feedback on leadership development are necessary throughout training healthcare professionals, as well (26). Continuing education, as well as on-the-job and other forms of training initiatives can be used to teach nurses about care coordination, and to make them more knowledgeable, skilled, and capable clinical leaders (27). In this study, the participants who formally studied on management generally had higher levels of clinical leadership than those who didn't. They earned higher scores especially in "setting direction" subscale, determining the content for change, using information and evidences, decision-making, using new methods, and evaluating the effect. In Budak (12) and Budak and Özer (19)'s studies, physicians and nurses who formally studied on management earned higher scores in the setting direction subscale. It was seen that leadership education had a positive effect on the participants' leadership levels, the same held true for those had formal management education as well. The participants who formally studied on leadership generally had higher levels of clinical leadership than those who did not – having higher scores on the "personal qualities" subscale. This is effective for maintaining self-awareness, self-management, self-development and acquiring ethical and honest behaviors. Budak and Özer (19) also found in their study that physicians and nurses who formally studied on leadership earned higher scores in the "personal qualities" subscale. One international study revealed that leadership training programs given to nurse managers boosted their leadership skills over time (28). Cunningham and Kitson (29) found that leadership training program was effective in developing nurses' clinical leadership skills. They concluded that programs focusing on effective clinical leadership development were needed to teach nurses how to provide better patient-centered care. The researchers stated that courses developed in partnership with nursing leaders to focus on management, health policy, information, and nursing education could greatly improve nurses' leadership skills. They also noted that such courses could be integrated in continuing education programs aimed at improving the leadership of undergraduate/graduate nursing students (30). Another study reported that participative leadership education offered to nurses improved their leadership skills and autonomy in their professional practice, as well as offered positive outcomes for patients (31). Kelly, Wicker & Gerkin (32), upon examining the correlation between transformational leadership practices, nurses' traits and formal leadership education, have discovered that institutions need to devise and offer formal leadership education programs to train transformational nurse leaders. Duygulu and Kublay (33) found in their study that in Turkey, transformational leadership training programs designed for service charge nurses made them better leaders.

In the present study, the participants' age, marital status, level of educational, position, where they worked (hospital/unit), the duration of working in the institution, and whether they received any formal education on clinical leadership did not appear to have any impact on their leadership levels. Other studies have yielded similar results. Again, namely that age, marital status, level of education, total duration of working in the institution and profession, whether the hospital was a city or district hospital and the status of receiving a formal education on clinical leadership had no impact on clinical leadership levels of physicians and nurses (12,19). Yet another study involving physicians and nurses showed that participants who formally studied on clinical leadership had higher level of clinical leadership than those who did not. (34). In the present study, why leadership training did not appear to affect the participants' clinical leadership level may be one of two reasons: either too few nurse managers received the training, or they did not put what they learned to practice.

5. CONCLUSION

Today, nurse managers need to have the traits and skills of a leader in order to manage clinics with senses of quality, advanced technology and learning organization. Accordingly, in this study, the nurse managers were desirably found to have high levels of clinical leadership. In addition, those who received education on management and/or leadership had high levels of clinical leadership in setting direction and personal qualities subscales of the CLS. This indicates to us once more just how important and necessary education is. Despite this, in this study, majority of the nurse managers did not receive any education on management, leadership, or clinical leadership. Nevertheless, clinical leadership levels of

female nurse managers were higher especially in the subscale of improving services. Therefore, it would be effective and beneficial to organize training programs on management, leadership, and clinical leadership to teach nurses (especially prospective managers) how to better enhance and manage clinical services – with a focus on male nurse managers as well, as their numbers are expected to rise in the coming years. Such programs could be offered periodically or on a regular basis, offer vocational skill training, and be conducted in small groups for gaining the related skills using interactive training methods like simulation and drama. They should be turned into institutional policy and shared with nurses. The findings of this study could be used as a reference point for scouting out prospective nurse managers for positions in top management. This study could also open the doors to compare the results of studies on other leadership approaches, and help fill in a major gap in the literature when it comes to this subject.

Key points for policy, practice, and/or research

- Nurse managers – especially those have formally studied on management and leadership – generally had high levels of clinical leadership.
- The selection criteria used to employ nurse managers state that candidate managers should also be outstanding clinical leaders
- Data of this study could be used for comparative purposes. It also reveals that leadership and management training is necessary for grooming leaders.

The results of this study are limited to the opinions of only the nurse managers working at the hospitals where this study was conducted. Therefore, these findings should not be generalized for all nurses. Other studies should be done in different regions and hospitals to achieve that.

REFERENCES

[1] Stanley D. Clinical leadership characteristics confirmed. Journal of Research in Nursing 2014;19(2):118-128.

[2] Şahinoğlu AH. ABC of Clinical Leadership. Istanbul: Nobel Medical Bookstore; 2016 (In Turkish).

[3] Duffy K, Carlin A. Enhancing the clinical leadership of band 6 staff. Nursing Standard 2014;29(11):40-47.

[4] Dierckx de Casterlé B, Willemse AN, Verschueren M, Milisen K. Impact of clinical leadership development on the clinical leader, nursing team and care-giving process: A case study. Journal of Nursing Management 2008;16(6):753-763.

[5] Mountford J, Webb C. When clinicians lead. Health International 2009;9:18-25.

[6] Brown A, Crookes P, Dewing J. Clinical leadership development in a pre-registration nursing curriculum: What the profession has to say about it. Nurse Education Today 2016;36:105-111.

[7] Millward LJ, Bryan K. Clinical leadership in health care: A position statement. Leadership in Health Services 2005;18(2):13-25.

[8] Mannix J, Wilkes L, Daly J. Attributes of clinical leadership in contemporary nursing: An integrative review. Contemporary Nurse 2013;45(1):10-21.

[9] Baernholdt M, Cottingham S. The clinical nurse leader–new nursing role with global implications. International Nursing Review 2011;58(1):74-78.

[10] Connolly M, Jacobs S, Scott K. Clinical leadership, structural empowerment and psychological empowerment of registered nurses working in an emergency department. Journal of Nursing Management 2018;26(7):881-887.

[11] Cook MJ. The renaissance of clinical leadership. International Nursing Review 2001;48:38-46.

[12] Budak F. Clinical Leadership in Healthcare Management. Ankara: Siyasal Publishing; 2018 (In Turkish).

[13] Cummings GG, Tate K, Lee S, Wong CA, Paananen T, Micaroni SP. Leadership styles and outcome patterns for the nursing workforce and work environment: A systematic review. International Journal of Nursing Studies 2018;85:19-60.

[14] Bender M. Conceptualizing clinical nurse leader practice: an interpretive synthesis. Journal of Nursing Management 2016;24(1):E23-E31.

[15] McKee L, Charles K, Dixon-Woods M, Willars J, Martin G. 'New' and distributed leadership in quality and safety in health care, or 'old' and hierarchical? An interview study with strategic stakeholders. Journal of Health Services Research & Policy 2013;18(2 Suppl):11-19.

[16] Ennis G, Happell B, Reid-Searl K. Clinical leadership in mental health nursing: The importance of a calm and confident approach. Perspectives in Psychiatric Care 2015;51(1):57-62.

[17] Chávez EC, Yoder LH. Staff nurse clinical leadership: A concept analysis. Nursing Forum 2015;50(2):90-100.

[18] Eggenberger T. Exploring the charge nurse role: Holding the frontline. Journal of Nursing Administration 2012;42(11):502-506.

[19] Budak F, Özer Ö. Exploring the impacts of personal factors on clinical leadership in a university hospital. Journal of Research in Nursing 2018;23(8):711-724.

[20] Özer Ö, Budak F, Şentürk S, Gün Ç. The examination of the clinical leadership perceptions of physicians and nurses regarding socio-demographic variables. Journal of Educatıon and Research in Nursıng 2018;15(2):99-105 (Turkish).

[21] National Health Service (NHS). The leadership framework self-assessment tool. 2012. Available at: http://www.leadershipacademy.nhs.uk/wp-content/uploads/2012/11/NHSLeadershipFramework-LeadershipFrameworkSelfAssessmentTool.pdf (Accessed 27 February 2019).

[22] Budak F. Turkish validity and reliability study of clinical leadership scale: Public hospitals sample. Acıbadem University Health Sciences Journal 2017;2:85-91 (In Turkish).

[23] Özkan Ş. Clinical leadership and reflections to health institutions. International Journal of Leadership Studies: Theory and Practice 2021;4(1):40-50.

[24] Grindel CG. Clinical leadership: A call to action. Medsurg Nursing 2016;25(1);9-16.

[25] Davidson PM, Elliot D, Daly J. Clinical leadership in contemporary clinical practice: implications for nursing in Australia. Journal of Nursing Management 2006;14(3):180-187.

[26] Swanwick T, McKimm J. What is Clinical leadership and why is it Important?. The Clinical Teacher 2011;8(1):22-26.

[27] Joseph ML, Huber DL. Clinical leadership development and education for nurses: Prospects and opportunities. Journal of Healthcare Leadership 2015;7:55-64.

[28] Krugman M, Smith V. Charge nurse leadership development and evaluation. Journal of Nursing Administration 2003;33(5):284-292.

[29] Cunningham G, Kitson A. An evaluation of the RCN clinical leadership development program: Part 2. Nursing Standart 2000;15(13-15):34-40.

[30] Heller BR, Drenkard K, Esposito-Herr MB, Romano C, Tom S, Valentine N. Educating nurses for leadership roles. The Journal of Continuing Education in Nursing 2004;35(5):203-210.

[31] George V, Burke LJ, Rodgers B, Duthie N, Hoffmann ML, Koceja V. Developing staff nurses shared leadership behavior in professional nursing practice. Nursing Administration Quarterly 2002;26(3):44-59.

[32] Kelly LA, Wicker TL, Gerkin RD. The relationship of training and education to leadership practices in frontline nurse leaders. Journal of Nursing Administration 2014;44(3):158-163.

[33] Duygulu S, Kublay G. Transformational leadership training programme for charge nurses. Journal of Advanced Nursing 2011;67(3):633-642.

[34] Karakaya A, Daşlı Y. Effects of clinical leadership perceptions of healthcare workers on teamwork. Uluslararası Sağlık Yönetimi ve Stratejileri Araştırma Dergisi 2021;7(2):402-411.

How to cite this article: Ozkol Kılınc K, Kurt S, Ozturk H. Evaluating the Clinical Leadership Levels of Nurse Managers. Clin Exp Health Sci 2022; 12: 556-563. DOI: 10.33808/clinexphealthsci.820506

Clinical and Experimental Health Sciences

Examining the Relationships Between Adolescents' Emotion Regulation Strategies and Social Media Addiction

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ABSTRACT

Objective: This study aims to examine the relationship between adolescents' emotional regulation strategies and social media addiction.

Methods: 1151 adolescents aged between 14 and 18 participated in the study (Mage = 14.44, SD =4.97). 63.8% of the participants in the study were girls and 36.2% were male. Researchers used the Emotion Regulation Scale and the Social Media Addiction Scale. In the study, we performed correlation analysis to determine the relationships between the variables. We used the structural equation model to test the predictive relationship between emotion regulation on social media addiction. We used SPSS 22, LISREL 9.1 program for statistical analysis.

Results: The results show that there are low-level, negative correlations between social media addiction and reappraisal of emotions. Similarly, there are low-level, positively significant relationships between suppression of emotions and social media addiction. Another result of the study reveals that reappraisal and suppression of emotions have a predictive effect on social media addiction.

Conclusion: The results show that reappraisal emotions reduce social media addiction, while suppression increases social media addiction.

Keywords: Emotion regulation, social media addiction, adolescents

1. INTRODUCTION

In the last 15 years, communication and socialization among adolescents have changed dramatically. Especially social networking sites such as Twitter, Instagram, and Facebook are among the social media tools that adolescents use to communicate with their friends. Adolescents can use technological tools to explore the world, express themselves and maintain friendships. Tzavela et al. (1) revealed that there are relationships between the use of new technology and adolescent needs. Despite the convenience provided by the use of new technology among adolescents, excessive use can cause psycho-social problems such as arguments with other people, decreased academic success and social withdrawal (2).

Social media addiction is a mental problem that negatively affects the cognitive, behavioral and emotional state of individuals, and causes problems in communication with others (3; 4). Recent studies show that social media addiction is an important problem in adolescents (5; 6). For example, Güney and Taştepe (7) determined that approximately 64% of adolescents experience moderate and high levels of social media addiction.

Emotion regulation is the expression, monitoring, and evaluation of emotions (8). Gross, Richards, and John (9) describe emotion regulation as a subtype of affect regulation, referring to attempts to consciously or unconsciously influence emotional experience. Gratz and Roemer (10) evaluate emotion regulation as an effort to regulate emotions in the face of events.

Cognitive reappraisal and suppression are two commonly used strategies to regulate emotions. Reappraisal involves changes in negative emotional contexts in the way a situation is interpreted in a way that reduces its emotional impact. Suppression prevents the external symptoms of emotions and behaviors expressing emotions (8).

Relationships Between Emotion Regulation and Social Media Addiction

Previous research has shown that difficulties in emotion regulation are associated with problematic internet use (11). Difficulties in emotion regulation can continue problematic Facebook use (12). Yu et al. (13) determined that adolescents'

DOI: 10.33808/clinexphealthsci.869465

unhealthy emotion regulation strategies lead to problematic internet use. Caselli and Spada (14) revealed links between emotion regulation and the use of social networking sites. Therefore, studies in the literature reveal that emotion regulation strategies are related to social media addiction.

Theoretical Theory

Emotion regulation refers to efforts to reduce the time it takes to experience negative emotions and to re-experience them in a positive way (15). Through emotion regulation, individuals can control, direct and understand their emotions (16; 17).

According to the Emotion Regulation Process Model (18; 9), the first step that constitutes the emotion regulation step begins with a trigger situation that will activate the emotions of the person. In the second stage, when individuals encounter a situation or event that they cannot change, they try to regulate their emotions by changing the direction of their attention instead of changing the situation. In the next stage, the individual organizes the emotions by reappraisal the event that led to the formation. In the last stage, individuals prevent the emergence of negative emotions by adding different meanings to the event.

According to the Emotion Regulation Process Model, individuals use cognitive reappraisal and suppression strategies for cognitive and response change of their emotions (8). Cognitive reappraisal is a way of changing the perception of the event that caused the emotion (19). Reappraisal emerges early as a leading strategy before emotional response tendencies. Individuals who cognitively reappraisal their emotions feel more positive emotions (20). Individuals who use a suppression strategy may feel inadequate, their negative emotions and depressive symptoms may increase (21). In this context, suppression is considered as maladaptive regulation strategy.

In summary, in terms of the emotion regulation process model approach, social media addiction may be associated with a lower level of social media addiction in individuals who reappraisal their emotions, and social media addiction at a higher level in individuals who suppress their emotions.

Purpose of the study

Emotion regulation is the process of adapting to the environment (8). The inability to deal with or regulate negative emotions can lead to impulsivity and the development of dysfunctional behaviors (22). Difficulties with emotion regulation can be a risk factor for addiction (23; 24). Previous studies have revealed significant relationships between emotion regulation and problematic internet use (25) and pathological use of social networking sites (26). However, it is noteworthy that studies investigating the relationship between emotion regulation and social media addiction are limited. Accordingly, current research suggests that a certain emotion regulation strategy (cognitive reappraisal and suppression) can predict social media addiction.

2. METHOD

2.1. Study Group

We created the following criteria to identify participants. (I) Residing in Erzurum, Turkey. (II) be between the ages of 14-19. (III) Reading and understanding Turkish. (IV) using at least one of the social networking sites in the last 6 months (Instagram, Twitter, Facebook, etc.). (V) Obtaining parent informed consent form approval to administer the scale to students.

The population of the research consists of 12.000 students studying in 46 secondary schools in Erzurum, Turkey in December of the 2020 academic year. We applied the random sampling method to determine which schools would participate in the study. We identified 11 schools using the random method. We used the power analysis method to calculate the sample size of the study. Results revealed that at least 955 sample sizes are required for models with an alpha level of .05 and power level of .80. This number obtained is sufficient for the sample size in the current study. Considering the possible data losses, we applied the scales to 1175 students. We did not use the data of 15 students who did not meet the normality criteria and 9 students who filled in the scales incompletely. As a result, we conducted the analysis with the dataset of 1151 students.

The age of the participants ranges from 14 to 18 (Mage = 14.44, SD =4.97). 63.8% of the students in the final sample were girls (n= 734), 36.2% were boys (n= 417). 08% of the students were in the preparatory class (n = 9), 13% in the 9th grade (n = 150), 26%, 6 in the 10th grade (n = 306), 27.5% in the 11th grade. in the 12th grade (n = 316) and 32.1% (n = 370) in the 12th grade.

2.2. Measures

2.2.1. Social Media Addiction Scale for Adolescents

We used the scale developed by Özgenel, Canpolat, and Ekşi (27) to determine the social media addiction levels of adolescents. The scale consists of a five-point Likert type, nine questions, and a single factor structure. The Cronbach's Alpha value of the scale is .90. In this research, Cronbach's Alpha value was found to be .88. The highest score that can be obtained from the scale is 45, and the lowest score is 9. The scale is evaluated by taking a total score.

2.2.2. Emotion Regulation Scale

We used the scale developed by Özgüle and Sümer (28) to determine the emotion regulation strategies of adolescents. The scale consists of two sub-dimensions as reappraisal, and suppression and ten questions. Cronbach's Alpha value of the scale was found as .78 and .64, respectively. In this study, Cronbach's Alpa value was found as .80 and .70, respectively.

The highest score that can be obtained from the scale is 50, and the lowest score is 10. The scale is evaluated according to the total scores obtained from the sub-dimensions.

2.3. Data Collection Process

Data was collected online as no face-to-face training in schools due to COVID-19. Therefore, online data collection scales prepared using Google Forms were sent to the participants. To apply the online scales, the researchers contacted the teachers working in the guidance services in 11 schools determined by the random method. The researchers explained the purpose of the study to the psychological counselors in the guidance service. Teachers were then asked to send the online scales to classroom messaging groups.

We also asked individuals who agreed to participate in the study to fill in informed consent forms. We ensured that only volunteers were included in the study. We also informed the individuals that they could stop completing the scales at any time and that the results would be kept confidential. The online data collection process was completed within 20 days. The data collected online were analyzed in computer. Since it is not possible to pass the next question without checking the answer in the online application, there is no missing data in the study.

2.4. Data Analysis

Before analyzing the data, we examined the outlier and normality values. In this dataset, we found that the data of 24 individuals violated the parametric conditions and we excluded these data from the dataset. In the final step, we examined Mardia's skewness and kurtosis values using Lisrel 9.1 software to test the multivariate normality of the dataset. As a result of this process, Mardia's assumptions of multivariate normality were confirmed (p>.05). After all these processes, we decided to analyzed process based on 1151 data. We performed a Pearson correlation analysis to determine whether there is a relationship between emotion regulation and social media addiction. We used the SPSS-22 program for descriptive and correlation analysis between variables. Then, next, we performed a structural equation model (SEM) analysis to test the predictive level of emotion regulation on social media addiction. Lisrel 9.1 program was used for this process.

2.5. Ethical Approval

The approval of the ethics committee of the study was obtained from the Ethics Committee of Atatürk University Educational Sciences Unit on 12.11.2020.

3. RESULTS

The Relationships Between Emotion Regulation and Social Media Addiction and Descriptive Results

Pearson correlation analysis was used to reveal the relationship between the reappraisal and suppression sub-dimensions of the students' emotion regulation scale and their social media addiction scores. In addition, descriptive statistical operations related to variables were made. Both descriptive and correlation results for variables are presented in Table 1.

Table 1. *Descriptive and Correlation Results Regarding the Variables*

	M	SD		Social Media
Degişkenler			Reappraisal	SupressionAddiction
Reappraisal	22.80	6.59	1	
Supression	15.25	4.95	-.31,4*	1
Social Media Addiction	17.75	5.85	-.08*	.21*

**p<.01*

When Table 1 is examined, among the reappraisal (r = – .08, p <.01) sub-dimension of the emotion regulation scale of social media addiction, there is a low level, negative direction; It was found that there were low level and positive significant relationships between suppression subscale (r = .21, p <.01).

Measurement Model

The measurement model was established with 3 latent variables (reappraisal, suppression and social media addiction) and 19 observed variables. The established measuring model was found to fit well [χ2/sd=4.20, GFI=.93, CFI=.92, NFI= .93, IFI= .95, SRMR= .051 ve RMSEA=.055]. Besides, it was found that all observed variables place a significant load on the relevant latent structures (p<.01 between .42 and .78).

Structural Model

Three models were established during the research process. In this context, the relationship between reappraisal and suppression of emotions and social media addiction was examined (Model I). In Model 1, the researchers determined that the predictive effect of re-evaluation (β = – .22. t = – 5.26, p < .01) and suppression of emotions (β = .32, t = 6.82, p < .01) on social media addiction was significant. It can be said that the goodness of fit indices of the structural model are at acceptable levels (Table 2). Reappraisal and suppression of emotions explain 10% of the variance of social media addiction. Model fit indices are shown in Table 2.

Table 2. *Model Fit Indexes*

Model	x2/df	GFI	CFI	NFI	IFI	SRMR	RMSEA
Model I	4.64	.94	.94	.92	.94	.052	.056

In Figure 1, the road diagram of the structural equation model is given.

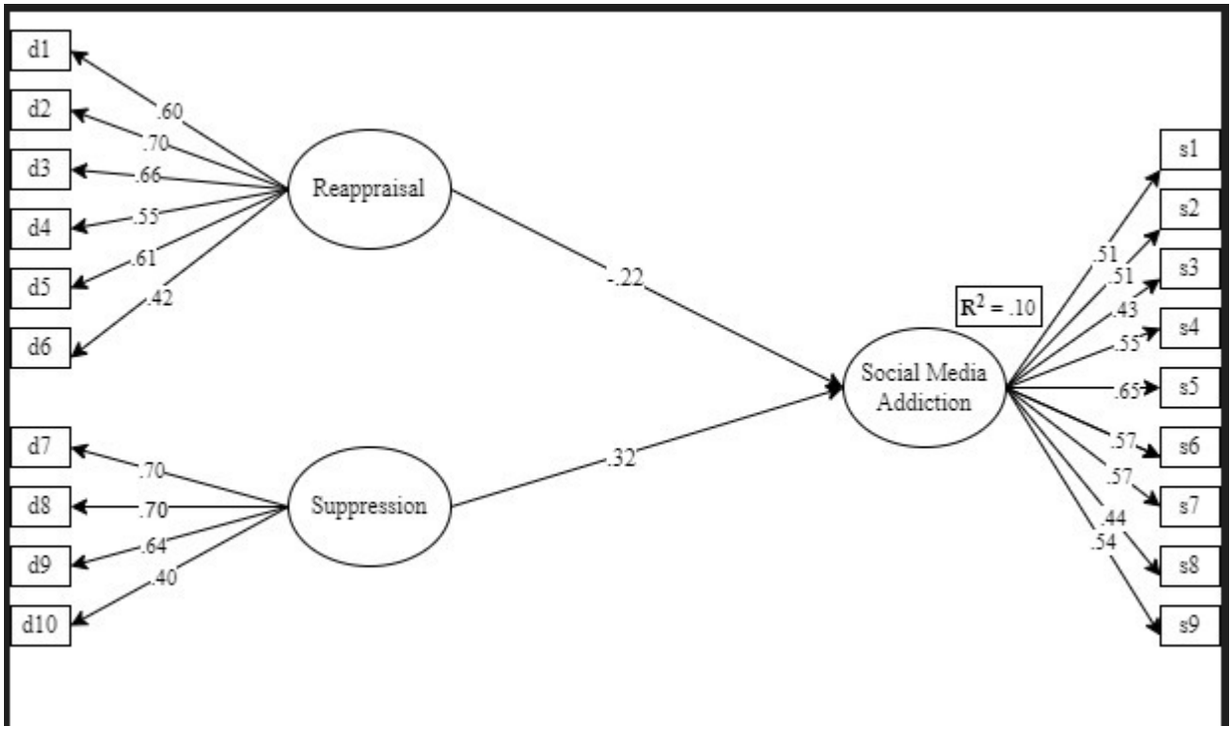

Figure 1. *Relationships Between Reappraisal and Suppression of Emotions and Social Media Addiction*
Standardized Values for the Level of Reappraisal and Repression of Emotions Predicting Social Media Addiction

4. DISCUSSION

In this study, we examined the predictive level of adolescents' emotion regulation on social media addiction. In this direction, we tested the relationship between emotion regulation and social media addiction with a structural model. The results show that there are low-level, negative correlations between social media addiction and reappraisal of emotions. Similarly, there are low-level, positively significant relationships between suppression of emotions and social media addiction. Another result of the study reveals that reappraisal and suppression of emotions have a predictive effect on social media addiction.

In the study, we found that the reappraisal dimension of the emotion regulation scale predicted social media addiction at a negative and significant level. These findings coincide with the findings obtained from studies examining the relationship between emotion regulation and problematic internet, Facebook and social media use, which are evaluated within the scope of social media use. For example, the negative prediction of positive emotion on internet addiction is similar to the result of this study (29). Reporting that reappraisal emotions reduce internet addiction in similar studies support the result of this study (30; 31). Satıcı and Deniz (32) reported that the emotion regulation reappraisal strategy negatively predicted smartphone addiction, which is consistent with this finding.

Social media addiction of individuals who reappraisal their emotions may be at a lower level. Individuals who experience and express their emotions more healthily by reappraisal the events they experience in daily life may use social media less. Emotional changes and emotional intensity can be experienced frequently during adolescence. At such times, individuals who reassess their emotions can display behaviors that can protect themselves against negative behaviors. In other words, adolescents who reevaluate their emotions may spend less time on social media.

The study found that the suppression dimension of the emotions regulation scale positively and significantly predicted social media addiction. The positive prediction of negative emotion on internet addiction is similar to the result of this study (29). In a similar study, individuals who suppress their emotions experience more internet addiction (31) and smartphone addiction (32) parallel to the results of the study. The positive prediction of dysfunctional emotion regulation on social media addiction is in line with the results of this study (33). Individuals who have problems in emotion regulation can use problematic Facebook (34).

Social media addiction of individuals who suppress their emotions may be at a higher level. Individuals who use Facebook experience emotional avoidance, lack of awareness and acceptance of their own emotional reactions, or inadequate impulse control, which can negatively affect their mood (35). Rozgonjuk and Elhai (36) emphasize that

suppressing emotions harms mental health and is not very effective against addictions. Suppressing emotions may not reduce negative emotional experiences. Therefore, suppression can be a factor that increases social media addiction.

5. CONCLUSIONS

The results of this study reveal that adolescents' use of social media increases when they suppress their emotions, and social media use decreases when they reappraisal their emotions.

Limitations and Recommendations

This study has some limitations that must be accepted. Firstly, the findings obtained from this study were collected with self-reported measurement tools based on the perceptions of the participants. Therefore, new studies using different data collection methods may be recommended. The second limitation is the cross-sectional collection of data. Therefore, experimental work can be done. In this context, the preparation of psycho-educational programs in which adolescents can express their feelings can reduce their social media addiction.

REFERENCES

[1] Tzavela EC, Karakitsou C, Dreier M. et al., Processes discriminating adaptive and maladaptive Internet use among European adolescents highly engaged online. Jour. Adol. 2015; 40: 34-47.

[2] Reiner I, Tibubos AN, Hardt J, M¨uller KW, Wölfling KW, Beutel ME. Peer attachment, specific patterns of internet use and problematic internet use in male and female adolescents. Eur. Ch. Adol. Psych. 2017; 26(10): 1257-1268.

[3] Griffiths MD, Szabo A. Is excessive online usage a function of medium or activity? An empirical pilot study. J. Behav. Addict, 2014; 3:74-77.

[4] Unlü F. Social media addiction and social isolation in middle age individuals. PESA Intern. Journ. Soc. Res. 2018; 4(1): 161-172.

[5] Deniz L, Gürültü E. High school students' social media addiction. Kast. Educ. Jour. 2018; 26(2): 355-467.

[6] Paul LL, Lee SN. Impact of internet literacy, internet addiction symptoms, and internet activities on academic performance. Soc. Scie. Comp. Rev. 2012; 30(4): 403-418.

[7] Güney M, Tastepe T. Social media usage and social media addiction in adolescent. Journ. Ank. Hea Sci. 2020; 9(2): 183-190. doi: https://www.doi.org/10.46971

[8] Gross JJ. Emotion regulation: Affective, cognitive, and social consequences. Psychoph. 2002; 39 (3): 281-291.

[9] Gross JJ, Richards JM, John OP. Emotion regulation in everyday life, in Emotion Regulation in Families: Pathways to Dysfunction and Health, D. K. Snyder, J. A. Simpson, and J. N. Hughes, Eds., pp. 13-35, American Psychological Association, 2006; Washington, DC, USA.

[10] Gratz KL, Roemer L. Multidimensional assessment of emotion regulation and dysregulation: Development, factor structure, and initial validation of the difficulties. J. Psych. Behav. Assess. 2004; 26: 41-54.

[11] Chong WH, Chye S, Huan VS, Ang RP. Computers in Human Behavior Generalized problematic Internet use and regulation of social emotional competence: The mediating role of maladaptive cognitions arising from academic expectation stress on adolescents. Comput. Hum. Behav. 2014; 38: 151-158.

[12] Marino C, Caselli G, Lenzi M, Monaci MG, Vieno A, Nikcnevic AV, Spada MM. Emotion regulation and desire thinking as predictors of problematic facebook use. Psych. Quart. 2019; 90: 405-411 https://doi.org/10.1007/s11126.019.09628-1

[13] Yu JJ, Kim H, Hay I. Understanding adolescents' problematic Internet use from a social/cognitive and addiction research framework. Comp. Hum. Behav. 2013; 29(6): 2682-2689.

[14] Caselli G, Spada MM. Metacognitions in desire thinking: A preliminary investigation. Behav. Cogn. Psych. 2010; 38(5): 629-637.

[15] McRae K, Hughes B, Chopra S, Gabrieli JD, Gross JJ, Ochsner KN. The neural bases of distraction and reappraisal. Jou. Cogn. Neuros. 2010; 22(2): 248-262.

[16] Ecirli H, Ogelman HG. Validity-reliability study of the scale of emotion regulation strategies for children aged five-six. Intern. Peer-Rev. Jour. Hum. Acad. Scie, 2015; 4(13): 85-100.

[17] Southam-Gerow MA. Emotion regulation in children and adolescents: A practitioner's guide. 2013; Guilford Press.

[18] Gross JJ. The emerging field of emotion regulation: An integrative review. Rev. Gen. Psych., 1998; 2(3): 271-299.

[19] John OP, Gross JJ. Healthy and unhealthy emotion regulation: Personality processes, individual differences, and life span development. Jour. Person. 2004; 72(6): 1301-1334.

[20] Gross JJ, John OP. Individual differences in two emotion regulation processes: Implications for affect, relationships, and well-being. Jou. Person. Soc. Psych. 2003; 85(2): 348-362 https://doi.org/10.1037/0022-3514.85.2.348

[21] Nyklicek I, Vingerhoets A, Zeelenberg M. Emotion regulation and well-being: A view from different angles. In Emotion regulation and well-being (1-9). 2011; Springer, New York, NY

[22] Rogier G, Velotti P. Conceptualizing gambling disorder with the process model of emotion regulation, Jour. Behav. Addict. 2018; 7(2): 239-251. https://doi.org/10.1556/2006.7.2018.52

[23] Aldao A, Nolen-Hoeksema S, Schweizer S. Emotion-regulation strategies across psychopathology: A meta analytic review. Clin. Psychol. Rev. 2010; 30(2): 217-237.

[24] Hayes SC, Strosahl KD, Wilson KG, Bissett RT, Pistorello J, Toarmino D. et al. Measuring experiential avoidance: A preliminary test of a working model. Psych. Rec., 2004; 54: 553-578

[25] Casale S, Caplan SE, Fioravanti G. Positive metacognitions about internet use: The mediating role in the relationship between emotional dysregulation and problematic use. Addict. Behav. 2016; 59: 84-88.

[26] Hormes JM, Kearns B, Timko CA. Craving Facebook? Behavioral addiction to online social networking and its association with emotion regulation deficits. Add. 2014; 109(12): 2079-2088.

[27] Ozgenel M, Canpolat O, Eksi H. Social media addiction scale for adolescents: Validity and reliability study. Addicta: Turk. Jour. Addict, 2019; 6: 629-662. doi.org/10.15805/addicta.2019.6.3.0086

[28] Ozgüle ETU, Sümer N. Emotion regulation and psychological adjustment in adolescence: Turkish adaptation of the emotion regulation scale. Turk. Psych. Articl., 2017; 20(40): 1-18.

[29] Sevinc H, Tas I. The relationship between internet addiction and the need for thinking and expressing emotions in university students. Jour. Educ. Live., 2020; 34(2): 523-541.

[30] Ercengiz M, Sar AH. The predictive role of Internet addiction in emotion regulation in adolescents. Sakar. Univ. Journ. Educ., 2017; 7(1): 183-194.

[31] Trumello C, Babore A, Candelori C, Morelli M, Bianchi D. Relationship with parents, emotion regulation, and callous-unemotional traits in adolescents' internet addiction. BioMed Res. Intern. 2018; 1-10. doi.org/10.1155/2018/7914261.

[32] Satıcı B, Deniz ME. Modeling emotion regulation and subjective happiness: Smartphone addiction as a mediator. Add. 2020;7(3): 146-152.

[33] Erdem G. Investigation of the relationship between social media use, peer relationships and emotion regulation among high school students. Unpublished master's thesis, 2019; Institute of Social Sciences, Near East University, Lefkoşa, Kıbrıs.

[34] Marino C, Gini G, Vieno A, Spada MM. The associations between problematic Facebook use, psychological distress and well-being among adolescents and young adults: A systematic review and meta-analysis. J. Aff. Disord. 2018; 226: 274-281.

[35] Hallion LS, Steinman SA, Tolin DF, Diefenbach GJ. Psychometric properties of the difficulties in emotion regulation scale (DERS) and its short forms in adults with emotional disorders. Fron. Psychol. 2018; 9:539-549. doi:10.3389/ fpsyg.2018.00539.

[36] Rozgonjuk D, Elhai JD. Emotion regulation in relation to smartphone use: Process smartphone use mediates the association between expressive suppression and problematic smartphone use. Curr. Psych. 2019; Advanced Publication Online. doi:10.1007/s12144.019.00271-4.

How to cite this article: Peker A, Nebioglu Yildiz M. Examining the Relationships Between Adolescents' Emotion Regulation Strategies and Social Media Addiction. Clin Exp Health Sci 2022; 12: 564-569. DOI: 10.33808/clinexphealthsci.869465

Clinical and Experimental Health Sciences

Evaluation of the Gender Perception of Students of the Faculty of Medicine; a Public University Faculty of Medicine in Western of Turkey

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Received: 20.03.2021 **Accepted:** 08.02.2022

ABSTRACT

Objective: The aim of the study was to determine the level of perception of gender in medical school students and to examine some variables that are thought to be related.

Methods: Study was carried at a public university faculty of medicine in 2018-2019 academic year and it consists of 1213 students without attendance problems and 1023 (84.3%) students were reached. The questionnaire form included some sociodemographic characteristics of the individuals, some variables thought to be related with gender perception and questions of the Perception of Gender Scale.

Results: In this study, medical students' gender perception levels and variables affecting it were evaluated and it was concluded that the students' gender perceptions were positive.

Conclusion: Medical faculty students should be role models in society's adoption of equality by looking at all practices from a gender equality perspective.

Keywords: Gender of perception, Faculty of medicine students, Türkiye

1. INTRODUCTION

Sex is the physiological and biological feature of the individual as a female or male (1). Gender, on the other hand, is defined as stereotyped roles, behaviors, activities, qualifications and opportunities that society sees suitable for men and women according to the World Health Organization (WHO) (2). While sex differences are inherent features of female and male, gender differences are learned, gained in the process of socialization and vary from individual to individual, intercultural and over time (3,4).

Gender, in other words, is the meaning and expectations of society and culture to be men and women; It is about how society sees male and female, thinks and how they expect them to act (3,5). Gender roles also include roles deemed appropriate for women and men in relation to these expectations, and these are transformed into stereotypes by society (3). Men are expected to be independent, objective, aggressive, strong, logical, brave, unable to express their feelings easily, show themselves in the public area and bring home the bacon. Women are expected to be docile, sensitive, dependent, subjective, obedient, able to express their feelings easily, do housework and service baby-sitting (1,3,6,7). When individuals or groups do not comply with established gender norms, roles, responsibilities or relationships, they often face stigma, discriminatory practices or social exclusion (8).

Gender-based values affect the basic determinants of health and equality such as nutrition, hygiene, awareness of health problems, health-seeking behavior and access to healthcare services as well as affecting people's daily lives (9). As a result of social norms, risky behaviors are more common in men. For example, deaths in traffic accidents are higher in males due to smoking, alcohol use and risky driving. In addition, the job areas where men work are more risky because work-related diseases are more common in men (1,9,10). However, the negative consequences of gender discrimination in women are observed more than men in every period of life. Violence against women in norms that value male authority and

DOI: 10.33808/clinexphealthsci.900405

privilege, deprivation of women from education and other social resources that improve and protect health, gender selective abortions significantly increase women's health risks (8,9).

Gender equality is the basic human right and increases economic and social development in all areas (11,12). United Nations ''5th Sustainable Development Goal'', is mentioned that women-girls should be empowered to achieve gender equality. It is aimed to accelerate the progress towards gender equality in all social areas (at home, workplace, health and educational attainment, political participation, leadership and economy) (12,13). It is reported by The European Observatory on Health Systems and Policies and the World Health Organization that gender inequality causes an important public health problem by depriving women of their economic, social, political rights and access to education, training and health services (14,15). Considering the fact that they are in constant contact with the society and the effects of gender on health, physicians also have a great role in achieving this goal. It is important to evaluate the gender perceptions of medical students, who will become future physicians, to make arrangements for adopting gender equality perceptions and attitudes among physicians. The aim of the study was to determine the level of gender perception in medical school students and to examine some variables that are thought to be related.

2. METHODS

The study is a cross-sectional study conducted between September and December 2018 on students of a public university faculty of medicine in the 2018-2019 academic year. The ethic permission was acquired from Eskisehir Osmangazi University Ethics Committee (Date: 09.10.2018, Protocol number: 25403353-050.99-E.110622). Additionally, necessary administrative permissions were obtained from the Diaconate of the Faculty of Medicine.

Study was carried at a public university faculty of medicine in 2018-2019 academic year and it consists of 1213 students without attendance problems and 1023 (84.3%) students were reached.

In this study, a questionnaire form was prepared using the literature for data collection (16-18). The questionnaire form included some sociodemographic characteristics of the individuals (age, gender, maternal education status, etc.), some variables thought to be related with gender perception and questions of the Gender Perception Scale.

In order to collect data in the study, verbal consent was obtained from those who accepted to participate in the study after the students were informed about the research subject and purpose by going to the classes at appropriate days and hours. The questionnaire forms were distributed to students and they were filled in by them under observation. This process took approximately 15-20 minutes.

The Perception of Gender Scale (PGS) is a self-report assessment tool designed to evaluate the gender roles and perceptions of individuals. Developed by Altınova and Duyan in 2013, the scale consists of 25 questions in 5-point Likert type. For each opinion stated in the items, individuals are asked to state their opinions as "I totally agree" "5", "I agree" "4", I am undecided "3", I do not agree "2", and I totally disagree "1". Items 2, 4, 6, 9, 10, 12, 15, 16, 17, 18, 19, 20, 21, 24 and 25 are negative and calculated in reverse. Accordingly, the score that can be obtained from the scale is between 25 and 125, and it is accepted that the gender perception changes positively as the score increases (16).

Family income levels of the students were evaluated as 'low', 'moderate' and 'high' according to their own statements.

The most played game as a child was categorized as "baby-house game", "car game" and "other". In the other category; There were outside games, football, puzzles and computer games.

Family attitude was categorized as "democratic" and "other". In the other category; authoritarian, indifferent, overly permissive, inconsistent, overprotective, perfectionist family attitudes were present.

The person who made the decisions in the family was categorized as "parents together" and "other". The other category included mother, father, family elders and other individuals.

The data obtained in the study were computerized and evaluated using SPSS version 15.0. The scores from PGS was evaluated regarding normal distribution, and the Kolmogorov-Smirnov normal distribution test and graphs were used to show that the scores taken from both scales were normally distributed. Mann-Whitney U and Kruskal-Wallis tests, (Bonferroni correction) and multiple linear regression analyses were used. The logarithms of the scores were taken to apply linear regression. A multivariate linear regression model was performed to determine the variables affecting the PGS scores.

3. RESULTS

Of the 1023 students who constituted the study group, 52.8% (540) were female and 47.2% (483) were male. The ages of the study group ranged from 17-30, with a mean of 21.36 ± 2.06 years. The scores obtained from PGS ranged between 32.0 and 125.0, and mean is 108.06 ± 16.57. The scores obtained from the scale were higher in women, those aged 23 and over, and students without siblings, while it was lower in students whose mothers' education level was primary school and below. Students whose father's education level was primary school and below was found to be lower than those of university students. Comparison of students' PGS scores according to sociodemographic characteristics is given Table 1.

Table 1. *Comparison of students' PGS scores according to sociodemographic characteristics*

Sociodemographic characteristics	n (%)	PGS scores Median (min.-max.)	Kw/z ; p
Sex			
Female	540 (52.8)	117.0 (32.0-125.0)	**11.788 ; <0.001**
Male	483 (47.2)	105.0 (33.0-125.0)	
Age groups (year)			
17-19	232 (22.7)	113.5 (38.0-125.0)	**6.175 ; 0.046**
20-22	451 (44.1)	114.0 (32.0-125.0)	
23 <*	340 (33.2)	116.0 (33.0-125.0)	
Mothers' education level			
Primary education*	351 (34.3)	110.0 (56.0-125.0)	**29.983 ; <0.001**
High school	283 (27.7)	115.0 (49.0-125.0)	
University	389 (38.0)	116.0 (32.0-125.0)	
Fathers' education level			
Primary education*	185 (18.1)	109.0 (58.0-125.0)	**17.183 ; <0.001**
High school	246 (24.0)	114.0 (38.0-125.0)	
University *	592 (57.9)	116.0 (32.0-125.0)	
Siblings			
No	107 (10.5)	117.0 (32.0-125.0)	**2.843 ; 0.004**
Yes	916 (89.5)	114.0 (33.0-125.0)	
Family type			
Nucleer	928 (90.7)	115.0 (32.0-125.0)	**13.394 ; 0.001**
Expended *	67 (6.5)	108.0 (56.0-125.0)	
Broken	28 (2.7)	120.0 (49.0-125.0)	
Socioeconomic level			
High	332 (32.5)	116.0 (32.0-125.0)	**17.714 ; <0.001**
Moderate	659 (64.4)	114.0 (38.0-125.0)	
Low	32 (3.1)	103.0 (49.0-125.0)	

In the study group, those whose family attitude was democratic, those who spent most of their lives in the province, those who did not live with grandparents such as grandparents until the age of 18, those who played more baby-house when they were child, those who reported that the parents make decisions together in the family, and the students who took their mother as a role model, the gender perception was more positive. Comparison of students' PGS scores according to some variables thought to be related to the gender perception is given in Table 2.

Multiple linear regression analysis results showing the variables related to the scores of the students in the study group obtained from the PGS is given in Table 3.

Table 2. *Comparison of students' Perception of Gender Scale scores according to some variables thought to be related to the gender perception*

Variables	n (%)	PGS scores Median (min.-max.)	Kw/z ; p
Family attitude			
Democratic	473 (46.2)	116.0 (33.0-125.0)	**3.992 ; <0.001**
Other	550 (53.8)	113.0 (32.0-125.0)	
Region of the majority of life			
Province *	718 (70.2)	116.0 (32.0-125.0)	**21.446 ; <0.001**
District	257 (25.1)	111.0 (33.0-125.0)	
Town	48 (4.7)	107.5 (59.0-124.0)	
Living with grandparents until the age of 18			
No	760 (74.3)	115.0 (33.0-125.0)	**3.732 ;<0.001**
Yes	263 (25.7)	110.0 (32.0-125.0)	
Most played game when child			
Baby-house game *	391 (38.2)	117.0 (38.0-125.0)	**53.356 ; <0.001**
Car game	245 (23.9)	108.0 (32.0-125.0)	
Other	387 (37.8)	113.0 (33.0-125.0)	
How decisions are made in the family			
Parents together	612 (59.8)	116.0 (33.0-125.0)	**4.487 ; <0.001**
Other	411 (40.2)	112.0 (32.0-125.0)	
Role model person			
Mother*	341 (33.3)	117.0 (49.0-125.0)	**30.283 ; <0.001**
Father	325 (31.8)	111.0 (32.0-125.0)	
Other	357 (34.9)	114.0 (33.0-125.0)	

Table 3. *Multiple linear regression analysis model of the variables that affect students' Perception of Gender Scale scores*

	β (%95 CI)	β (%95 CI)
Sex	-0.055*** (-0.064 – -0.046)	-0.054*** (-0.065 – -0.044)
Age groups	0.0 (-0.007 – 0.006)	
Mothers' education level	0.006 (0.0-0.013)	
Fathers' education level	0.002 (-0.005 – 0.009)	
Sibling	-0.009 (-0.024 – 0.006)	
Family type	-0.008 (-0.019 – 0.004)	
Socioeconomic level	-0.007 (-0.017 – 0.002)	
Family attitude		0.018*** (0.009-0.028)
Where most of life is spent		-0.013** (-0.020 – -0.005)
Living with grandparents until the age of 18		0.017** (0.007-0.027)
Most played toy when child		0.001 (-0.005 – 0.007)
How decisions are made in the family		0.008 (-0.001 – 0.017)
Role model person		-0.002 (-0.007 – 0.004)
R^2:	0.144	0.170
F:	24.445***	29.602***

*CI: Confidence Interval, *:p≤0.05, **:p≤0.01, ***:p≤0.001*

4. DISCUSSION

The access to healthcare services and the quality of it is not received the same among genders in almost every part of the world due to gender perception (17,18). Therefore, medical doctors having high awareness and displaying positive attitude towards gender perception is considered to be one of the key factors in order for health service distribution to be equal. In this study, medical students' gender perception levels and variables affecting it were evaluated and it was concluded that the students' gender perceptions were positive. Female students, those who didn't live with their grandparents until they were 18, those who lived most of their lives in cities and those whose parents' attitude are democratic had a more positive gender perception.

The amount of studies assessing the gender perception of medical faculty students are very few. In our study group, the gender perception was more positive than the study conducted by Aydin et al. using the same scale on postgraduate individuals (19). Two different studies conducted on medical students in İzmir also reported that the gender perception was equitable and positive (18,20). There are studies suggesting that the gender perception of university students studying in the health field is more positive compared to the students in other fields (21,22).

Because of the societal role of a mother in a family being undertaking every household chores and bearing with every negative event that happens creates a traditional gender perception for the males of the family (23). In the study, female students' gender perception was more positive than males which was in line with the relevant literature (18,21,22,24).

Individuals' social relationships and environments increase with age, and they interact with more people and gain different perspectives. It is predicted that increasing social relations and changing environment will create a more egalitarian perception of gender apart from the gender perception settled in the society (25-27). In our study, the gender perception of the students over the age of 23 was found to be more positive than the students under the age of 23. In a study conducted on adolescents in the literature, it was reported that individuals' gender perceptions were more positive with increasing age (28). In the studies conducted by Zeyneloğlu and Arıcı on university students, it was reported that there was no difference between age and gender perception (29,30).

It is expected that the level of gender perception of their children will change positively with the increase in the education level of the parents due to reasons such as the increase in the education level of the families, the fact that people with higher education levels are open to new ideas and are not under the cultural influence of the society, and women enter the working life (30-32). In the study, while the gender perception level of those whose mother's education level is primary school or below is lower, the gender perception level of those whose father's education level is university was found to be more positive than those whose mother's education level is primary school and below. Similar result was reported in the study conducted by Arıcı (30). In some studies, it has been reported that as the educational status of the mother increases, the perception of gender changes positively, while there is no relationship between the educational status of the father (31,33). In another study, no relationship was found between the education level of the parents and the perception of gender (34). The different results here may be due to the different cultures of the community in which the study was conducted and the different schools where the students study.

Family is the first social environment individuals live in. Although every part of an individuals' life plays a role in their perception of gender, the family they live with since their birth has the biggest share in it (35). The attitude of the family, which is shaped by the culture and the societal norms in families is one of the major factors affecting the gender perception (17,36). In the study, the students who defined the attitude of their family as democratic was found to have a more positive perception on gender.

It is reported that the place where the individuals spend most of their life affects their predictions against life (37). It has been reported that as the time spent in the urban area increases, the gender perception becomes more egalitarian and positive (35). In the study, students' who spent most of their lives in city centers was found to be gender perception was more positive. Those who live in urban areas had a more positive gender perception in a study conducted in Namibia as well (38). In some other studies however no difference was reported regarding the living place (19,23).

In the study, those who did not live with their grandparents until they were 18 had a more positive gender perception. Living with the elders of the family may have resulted in having a more traditional gender perception due to their influence on children.

It has been determined that the toy preferences of children who adopt the roles assigned to men and women in the society and grow up in families with traditional role-sharing in this way differ in accordance with traditional roles (4). In the study; It was determined that the level of gender perception of the students whose most played games as children were baby-house game were more positive. Similar results are reported in the study of Hupp et al. (39).

In the model obtained as a result of multiple linear regression analysis, it was found that independent variables explain 17 % of the gender perception. Since gender perception is a multi-dimensional concept and highly influenced by the cultural traditions of individuals and the social structure of the environment they live in, it's hard to define and include every factors that possibly affects the gender perception in quantitative studies.

The research being a cross-sectional study resulting with inadequacy on explaining the cause-effect relationship and since it can't be generalized due to including only the medical faculty students, can be considered as the limitations of the study. It has been concluded that more advanced studies and qualitative studies that will provide access to in-depth information are needed to obtain more information on the subject.

5. CONCLUSION

Medical faculty students who will be the healthcare providers of the future should be role models in society's adoption of equality by looking at all practices from a gender equality perspective and shaping stereotypes and attitudes more equitably. The perception of gender of the faculty of medicine students was found to be positive. Sex and the characteristics of the social environment were related to perception of gender. Through the medical education curriculum and the student communities in the faculty, the awareness of students about the perspective of gender perception must be increased and this process should be sustained after the graduation with briefings during their educations. In addition to that, a cooperation should be created between faculty members and students.

Funding: None declared.

Conflicts of interest: The authors report no conflicts of interest

REFERENCES

[1] World Health Organization. Division of Family and Reproductive Health. Gender and health: technical paper. World Health Organization 1998.

[2] World Health Organization. Gender. Available from: https://www.who.int/health-topics/gender. Available date: 03.02.2020.

[3] Dokmen ZY. Gender: Social psychological explanations 5th Edition. İstanbul: Sistem Publishing; 2014.

[4] Lindsey LL. Gender roles A Sociological Perspective. Sixth Edition, USA: Routledge; 2016:1-5.

[5] Akın A. Toplumsal cinsiyet (gender) ayırımcılığı ve sağlık. Toplum Hekimliği Bülteni 2007;26(2):1-9. (Turkish)

[6] Temel A, Yakın M, Misci S. Örgütsel cinsiyetlerin örgütsel davranışa yansıması. Yönetim ve Ekonomi 2006;13(1):27-38. (Turkish)

[7] Koenig AM. Comparing prescriptive and descriptive gender stereotypes about children, adults, and the elderly. Frontiers in Psychology 2018;9:1086.

[8] World Health Organization. Gender mainstreaming for health managers: A practical approach. World Health Organization 2011.

[9] Sen G, Ostlin P, George A. Unequal, Unfair, Ineffective and Inefficient: Gender Inequity in Health: why it Exists and how We Can Change it: Final Report to the WHO Commission on Social Determinants of Health. World Health Organization 2007.

[10] Manandhar M, Hawkes S, Buse K, Nosrati E, Magar V. Gender, health and the 2030 agenda for sustainable development. Bulletin of the World Health Organization 2018;96(9):644.

[11] Global Health 5050. The Global Health 5050 Report (2018). Available from: https://globalhealth5050.org/report/ Available date: 03.02.2020.

[12] Dugarova E. United Nations Development Programme (2018). Gender Equality as an Accelerator for Achieving the Sustainable Development Goals. Available from: https://www.undp.org/content/undp/en/home/librarypage/womens-empowerment/gender-equality-as-an-accelerator-for-achieving-the-sdgs.html Available date: 03.02.2020

[13] Resolution A/RES/70/1. Transforming our world: the 2030 agenda for sustainable development. In: Seventieth United Nations General Assembly, New York, 25 September 2015. New York: United Nations; 2015. Available from: https://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang= Available date:03.02.2020

[14] Alvarez-Dardet C, Vives-Cases C. Three waves of gender and health. Eurohealth 2012;18(2):4-7.

[15] World Health Organization. Strategic action plan for the health of women in Europe-endorsed at a WHO meeting, Copenhagen 5-7 February 2001.

[16] Altınova HH, Duyan V. Toplumsal Cinsiyet Algısı Ölçeğinin Geçerlik Güvenirlik Çalışması. Journal Of Society & Social Work 2013;24(2). (Turkish)

[17] Connell R. Gender, health and theory: conceptualizing the issue, in local and world perspective. Social science & medicine 2012;74(11):1675-1683.

[18] Varol ZS, Ciceklioglu M, Taner S. Bir tıp fakültesi birinci sınıf öğrencilerinde toplumsal cinsiyet algı düzeyi ve ilişkili faktörlerin değerlendirilmesi. Ege Tıp Dergisi 2016;55(3):122-128. (Turkish)

[19] Aydin R, Demirci AD, Korukcu O, Kabukcuoglu K. Gender Perception of Academicians Using Social Media and the Influencing Factors. Clinical and Experimental Health Sciences 2019;9(4):310-315.

[20] Direk N, Irmak B. Dokuz Eylül Üniversitesi Tıp Fakültesi öğrencilerinde toplumsal cinsiyet rollerine yönelik tutumlar. DEÜ Tıp Fakültesi Dergisi 2017;31(3):121-128.

[21] Celik AS, Pasinlioglu T, Gonca TAN, Koyuncu H. Üniversite öğrencilerinin cinsiyet eşitliği tutumlarının belirlenmesi. Florence Nightingale Hemşirelik Dergisi 2013;21(3):181-186.

[22] Anderson SJ, Johnson JT. The who and when of "gender-blind" attitudes: Predictors of gender-role egalitarianism in two different domains. Sex Roles 2003;49(9-10):527-532.

[23] Pınar G, Taşkın L, Eroğlu K. Başkent Üniversitesi Öğrenci Yurdunda Kalan Gençlerin Toplumsal Cinsiyet Rol Kalıplarına İlişkin Tutumları. Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi 2008;15(1):47-57.

[24] Lee M, Coulehan JL. Medical students' perceptions of racial diversity and gender equality. Medical education 2006;40(7):691-696.

[25] Yılmaz DV, Zeyneloğlu S, Kısa S, Taşkın L, Eroğlu K. Üniversite öğrencilerinin toplumsal cinsiyet rollerine ilişkin görüşleri Uluslararası İnsan Bilimleri Dergisi 2009;6(1):775-792.

[26] Koksal SE. Uluslararası göç sürecinde kadının gündeme gelişi ve "getto'daki kadın. Kadın Araştırmaları Dergisi 1993;1(1):121-126.

[27] Demirel S, Kısa S, Kocaoz S, Vevfikulucay D, Eroğlu K, Taşkın L. Üniversite son öğrencilerinin toplumsal cinsiyet rollerine ilişkin görüşlerini etkileyen etmenler. 4. Uluslararası Üreme Sağlığı ve Aile Planlaması Kongresi Ankara:20-23 Nisan 2005. (Turkish)

[28] Kantoğlu, A. (2017). Bir grup ergenin toplumsal cinsiyet algılarının sosyodemografik özelliklere göre değerlendirilmesi. Yüksek Lisans Tezi, Işık Üniversitesi, Sosyal Bilimler Enstitüsü, İstanbul. (Turkish)

[29] Zeyneloğlu, S. (2008). Ankara'da hemşirelik öğrenimi gören üniversite öğrencilerinin toplumsal cinsiyet rollerine ilişkin tutumları. DoktoraTezi, Hacettepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara. (Turkish)

[30] Arıcı, F. (2011). Üniversite öğrencilerinde toplumsal cinsiyet rollerine ilişkin algılar ve psikolojik iyi oluş. Yayımlanmamış Yüksek lisans Tezi, Hacettepe Üniversitesi, Sosyal Bilimler Enstitüsü, Ankara. (Turkish)

[31] Kodan Çetinkaya, S. Üniversite öğrencilerinin şiddet eğilimlerinin ve toplumsal cinsiyet rollerine ilişkin tutumlarının incelenmesi. Nesne 2013;1(2):21-43. (Turkish)

[32] Karasu F, Göllüce A, Güvenç E, Çelik S. Üniversite öğrencilerinin toplumsal cinsiyet rollerine ilişkin tutumları. SDÜ Sağlık Bilimleri Enstitüsü Dergisi 2017;8:1-7. (Turkish)

[33] Aylaz R, Güneş G, Uzun Ö, Ünal S. Üniversite öğrencilerinin toplumsal cinsiyet rolüne yönelik görüşleri. Sürekli Tıp Eğitimi Dergisi 2014;23(5),:183-189. (Turkish)

[34] Çuhadaroğlu A, Akfırat ON. Üniversite öğrencilerinin benlik kurgularıyla toplumsal cinsiyet algıları arasındaki ilişkinin incelenmesi. Gaziantep Üniversitesi Sosyal Bilimler Dergisi 2017;16(4):1031-1046. (Turkish)

[35] Isiktekin Atalay B, Tozun M, Bugrul N, Ayhan E, Koyuncu T, Aygun SM, Arslantas D, Alaettin Unsal. Gender roles attitudes and their correlations with self-esteem in high school students: a cross-sectional study from West of Turkey. International Resarch Journal of Social Sciences 2016;5(11):8-16.

[36] Skarpenes O, Nilsen ACE. Regional gender inequality in the Norwegian culture of equality. Gender Issues 2015;32(1):39-56.

[37] Doyal L. Gender equity in health: debates and dilemmas. Social Science & Medicine 2000;51(6):931-939.

[38] LaFont, S. Beliefs and attitudes toward gender, sexuality, and traditions amongst Namibian youth. Gender Research & Advocacy Project, Legal Assistance Centre 2010.

[39] Hupp JM, Smith JL, Coleman JM, Brunel AM. That's a boy's toy: Gender-typed knowledge in toddlers as a function of mother's marital status. The Journal of Genetic Psychology 2010;171(4):389–401.

How to cite this article: Aygar H, Isiktekin Atalay B, Akbulut Zencirci S, Unsal A, Arslantas D. Evaluation of the Gender Perception of Students of the Faculty of Medicine; a Public University Faculty of Medicine in Western of Turkey. Clin Exp Health Sci 2022; 12: 570-575. DOI: 10.33808/clinexphealthsci.900405

Clinical and Experimental Health Sciences

Serum Amino Acid Profile in Chronic Sinusitis

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Received: 15.04.2021 **Accepted:** 17.05.2022

ABSTRACT

Objective: Serum amino acid profile is known to vary in many diseases. The changes in the serum amino acid profile provide important information about diseases and the effectiveness of treatment. The aim of this study is to investigate whether serum amino acids are effective in the development of CRS.

Methods: A total of 23 healthy volunteers between the ages of 20 and 40 years were allocated to the control group (Group 1) and 27 patients with chronic rhinosinusitis were allocated to the study group (Group 2). The patients whose history, symptom, and examination findings were consistent with chronic sinusitis and who also who had sinusitis findings on a paranasal sinus tomography and were diagnosed with chronic sinusitis for at least one year were included in Group 2. A total of 32 serum-free amino acid levels were measured in both group using the LC-MS/MS system. In the study, the JASEM amino acid kit was used for LC-MS/MS analysis.

Results: The serum taurine level was found to be statistically significantly lower in Group 2 compared to Group 1 (p:0.002). A significant alteration was not observed in the serum levels of remaining 31 amino acids.

Conclusion: According to the data we obtained from the study, decreased serum taurine level may be a factor in the etiopathogenesis of chronic rhinosinusitis and therefore taurine supplementation may be considered as a new therapeutic target in the treatment of chronic rhinosinusitis.

Keywords: Chronic sinusitis; Taurine; Human serum; Amino acids; LC-MS/MS

1. INTRODUCTION

Rhinosinusitis (RS) is the inflammation of nasal mucosa and paranasal sinus mucosa. It is associated with symptoms including nasal obstruction, post-nasal discharge, facial pain and a sensation of pressure, a smelling disorder, cough. and a sensation of fullness in the ear (1-3). RS develops primarily due to viral but also bacterial and fungal infections (4). The disease is defined as chronic rhinosinusitis (CRS) if symptoms and signs of the inflammation last for longer than three months. CRS is a chronic inflammatory disease which may develop due to both infectious and non-infectious factors (5). CRS is a very common disease and represents an important public health problem. In Europe, a high prevalence of up to 19.7% were reported (6). This ratio is between 14-16% in America (7).

CRS is a multi-factorial disease. The causes include anatomic, congenital, genetic, neoplastic, allergic and endocrinologic factors; sinonasal ciliary dysfunction; and smoking (8-9). The exact etiology and mechanism of CRS has not yet been revealed. The pathophysiology of sinusitis should be investigated to determine two important points in detail. One of them is that acute sinusitis is not recovered completely and the second one is that acute sinusitis tuns into chronic inflammatory disease

Many scientific studies have investigated regarding the relationship between diseases and biomolecules like amino acids, metabolites, enzymes and hormones for better understanding the pathophysiology of the diseases. Amino acids are required for the synthesis of many important molecules like protein, hormones, and neurotransmitters (10). Amino acids also regulate the immune response that defends the body against diseases through the activation of T and B lymphocytes, natural killers and macrophages; cellular redox including gene expression and lymphocyte proliferation; and producing antibodies, cytokines and other cytotoxic substances (11). For example, taurine is found in high concentrations in phagocytes and inflammatory lesions (12).

Taurine, a sulfur amino acid, is the most abundant amino acid in the body. Taurine plays a role in cyto-protection

DOI: 10.33808/clinexphealthsci.916590

and the regulation of inflammation through protecting the tissue against oxidative damage. It has been suggested that a deficiency in taurine influences immune cell functions since it is found in high levels in leukocytes (13,14). While taurine plays a role in many physiologic events, it is found in high concentrations in inflammatory cells where oxidative activity is high and in retina, kidney, heart tissues where oxidative products are at high levels (15-16).

In various studies in the literature, the association between different disease groups like oncologic diseases (hepatocellular cancer (17), breast cancer and lung cancer (18),colon cancer (19), renal cell cancer (20), cervical intra-epithelial neoplasia and cervical squamous cell cancer (21)), metabolic disorders including diabetes mellitus and metabolic syndrome (22), renal disorders (23), sepsis (24), nasal polyposis (25), and serum/plasma amino acid profiles was investigated with regard to biochemical parameters.

Based on the data mentioned above, it was suggested that patients with CRS could have an altered serum amino acid profile. For these analyses, the strongest technique, the LC-MS/MS method, was used for detection of the amino acid profile of the patients with CRS and discussed light of the literature.

2. METHODS

2.1. Materials

Ethics committee approval was obtained from the Erzurum Regional Research and Training Hospital (2020/14-157). Twenty-three healthy volunteers between the ages of 20 and 40 (Group 1) and 27 patients with CRS (Group 2) who were admitted to Erzurum Regional Research and Training Hospital in Turkey were included in the study. Healthy volunteers who were matched with the patient group for age, gender, and body mass index were included in Group 1. A detailed medical history was obtained from all participants and a basic rhinologic examination, a nasal endoscopic examination, and a routine ear-nose-throat examination were done. The patients whose history, symptom, and examination findings were consistent with chronic sinusitis and who also who had sinusitis findings on a paranasal sinus tomography and were diagnosed with chronic sinusitis for at least one year were included in Group 2.

Exclusion criteria were as follows: smoking and/or alcohol use, atopic dermatitis, asthma, nasal polyposis, acute sinusitis, chronic drug use, a history of systemic or topical steroid use for any reasons during the last three months, pregnancy, lactation, the presence of an upper respiratory tract infection during the study, and hepatic, renal, hematologic, cardiovascular, metabolic, neurologic, of psychiatric disorders or malignity (25).

In this study, JASEM quantitative amino acid LC-MS/MS kits were used. These kits include amino acid standards, internal standard, analytical column and mobile phase (JASEM Laboratory Systems and Solutions A.S).

2.2. Analytic Chemical Analysis

2.2.1. Measurement of Free Amino Acids

Blood samples were taken into gel tubes from the antecubital vein following one night of fasting. Biochemistry tubes were centrifuged at 3500 rpm for 10 min to separate the serum, and serum samples were stored at – 80 °C until the day of examination and tested after they reached room temperature.

For both patient and control groups, 50 µL of serum samples were transferred into Eppendorf tubes, 50 µL internal standard was added to them, and they were vortexed for 10 sec. 700 µm of amino acid solvent solution (Mobile Phase A: Mobile Phase B, V:V:1:4) was added to each tube. Each sample was vortexed for one min and centrifuged at 3000 rpm and at 4 °C for eight min thereafter. The superior phase was separated and filtered using 0.45 µm filters. Samples were measured in triplicate with the LC-MS/MS system (Agilent 6460 Triple Quadropol, USA). Chromatography and mass spectrometry conditions of the LC-MS/MS method that was used for the separation and definition of amino acids are presented in Tables 1 and 2. Thirty-two amino acids defined as the result of the analysis are listed in table 4. Table 5 gives m/z values for amino acids. MRM chromatograms of the control and patient groups of amino acids obtained from the study are given in figure 2. MRM chromatogram and mass spectrum of the control and patient groups of taurine are also given in figure 3 and figure 4, respectively.

Table 1. *Solvent Composition Schedule during the gradient elution for LC-MS/MS*

Time Flow: 0.7 mL/min Column	Change Solvent Composition A: MPA (Jasem AA Kit) AA Kit) Jasem AA Column	B: MPB (Jasem
1.00 min	78.00 %	22.00 %
4.00 min	70.00 %	30.00 %
5.00 min	70.00 %	30.00 %
5.10 min	22.00 %	78.00 %
9.00 min	22.00 %	78.00 %

Table 2. *Mass conditions*

Parameters	Value (+)	Value(-)
Gas Temp (°C)	150.00	150.00
Gas Flow (L/min)	11.00	11.00
Nebulizer (psi)	40.00	40.00
SheathGasHeater	375.00	375.00
SheathGasFlow	11.00	11.00
Capillary (V)	2000.00	0.00
VCharging	0.00	0.00
Injection Volume (µl)	1.00	
Ion source	AJS ESI	
Ion Mode	Positive	

2.3. Statistical Analysis

An independent samples t test was used for the comparison of the mean differences between groups. Analyses were done using the IBM SPSS 20.0 package program. A p level of<0.05 was accepted as statistically significant.

Table 3. *Demographic data of groups 1 and 2*

Variable	Grup 1 (n=23)	Grup 2 (n=27)	p-value
Gender(Male/Female)	11/12	14/13	p:0,654
Age(years)	33,50±5,30	34,26± 4,72	p:0,780
BMI (kg/m2)	25,1±1,20	24,8±1,16	p:0,802

Table 4. *Serum free amino acid values (nmol/mL) in patients with CRS compared with controls.*

Amino Acids	Group 1 (n:23) Mean±SD	Group 2 (n:27) Mean±SD	p-values
1-Methyl-L-Histidine	5,86±5,74	3,18±1,54	0,172
3-Amino Isobutyric acid	1,93±1,26	1,95±1,27	0,977
3-Methyl-L-Histidine	7,58±1,79	7,27±2,96	0,777
Beta-Alanine	1,28±0,69	1,12±0,51	0,569
DL-5-Hydroxy Lysine	1,48±0,34	1,30±0,48	0,341
Ethanolamine	8,46±4,22	9,10±7,32	0,575
Gamma-aminobutyric acid	0,35±0,13	0,40±0,19	0,569
L-2-Aminobutyric acid	2,97 ±1,89	3,41±2,96	0,699
L-Alanine	596±198	653±391	0,691
L-Anserine	23,48±5,90	25,84±16,25	0,672
L-Arginine	86,26±31,34	81,39±47,78	0,790
L-Asparagine	62,28±17,57	62,75±33,13	0,969
L-Carnosine	10,51±4,46	11,78±6,94	0,632
L-Citrulline	34,31±10,70	27,37±13,18	0,212
L-Cystine	53,55±24,76	50,08±32,03	0,789
L-Glutamine	547,13±131,71	557,79±286,24	0,916
L-Glycin	478,58±200,78	495,83±269,25	0,873
L-Histidine	92,46±20,72	94,65±43,57	0,887
L-Isoleucine	83,67±30,52	79,50±48,17	0,819
L-Leucine	167,60±43,62	174,05±107,04	0,862
L-Lysine	232,72±68,92	250,16±141,81	0,731
L-Methionine	33,21±11,81	30,49±16,28	0,674
L-ornithine	148,23±62,09	148,10±78,20	0,981
L-Phenylalanine	110,14±31,53	124,76±76,50	0,583
L-Proline	330,71±163,51	346,13±225,04	0,863
L-Serine	195,20±55,47	203,60±104,80	0,825
L-Threonine	150,56±57,25	158,35±82,51	0,809
L-Tryptophan	72,04±18,47	65,54±38,29	0,635
L-Tyrosine	76,13±24,15	80,42±52,32	0,816
L-Valine	251,90±57,57	258,66±144,30	0,892
*Taurine	104,15±20,01	59,69±34,28	0,002
Trans-4-hydroxy L-proline	8,08±3,14	6,55±3,06	0,286

**: p<0.05*

Table 5. *m/z values for amino acids*

Amino Acids	m/z
1-Methyl-L-Histidine	170.1→126.2
3-Amino Isobutyric acid	104.1→86.2
3-Methyl-L-Histidine	170.1→124.1
Beta-Alanine	90.1→72.1
DL-5-Hydroxy Lysine	163.1→128.1
Ethanolamine	62.1→44.2
Gamma-aminobutyric acid	104.0→87.1
L-2-Aminobutyric acid	104.2→58.3
L-Alanine	90.2→44.2
L-Anserine	241.1→109.1
L-Arginine	175.2→70.2
L-Asparagine	133.1→74.2
L-Carnosine	227.1→110.1
L-Citrulline	176.4→159.3
L-Cystine	241.0→74.2
L-Glutamine	147.1→84.2
L-Glycin	76.2→30.1
L-Histidine	156.1→110.1
L-Isoleucine	132.2→69.2
L-Leucine	132.2→43.3
L-Lysine	147.1→84.2
L-Methionine	150.1→104.1
L-ornithine	133.2→70.3
L-Phenylalanine	166.1→120.1
L-Proline	116.2→70.2
L-Serine	106.2→60.2
L-Threonine	182.1→165.0
L-Tryptophan	205.1→188.1
L-Tyrosine	182.1→165.0
L-Valine	118.2→72.2
Taurine	126.1 →44.3
Trans-4-hydroxy L-proline	133.2→68.2

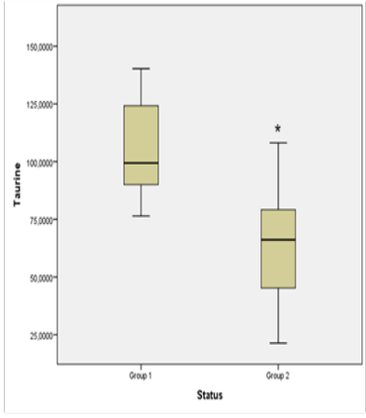

Figure 1. *Box-plot distribution of serum Taurine levels in Group 1(control) and Group 2 (patient).*Significant at p < 0.05 when compared to control*

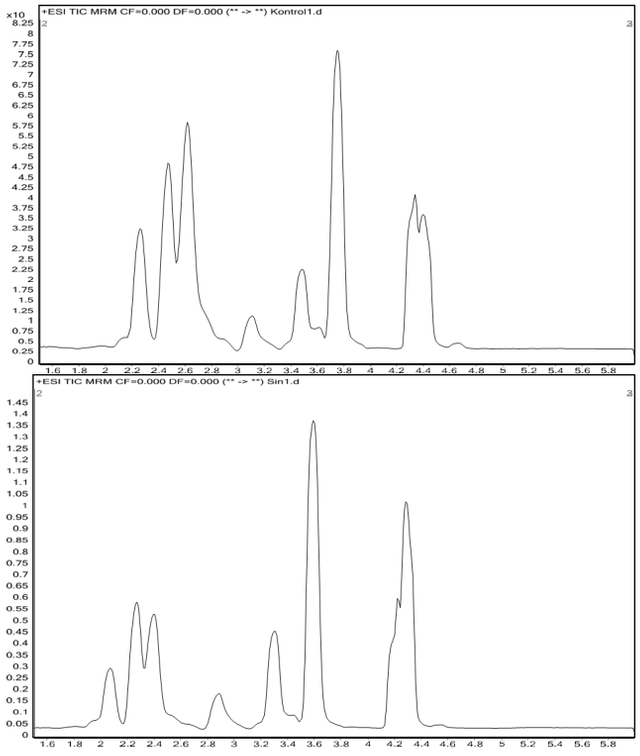

Figure 2. *Typical MRM chromatogram for amino acids obtained from the control group (A) Typical MRM chromatogram for amino acids obtained from the control group (B).*

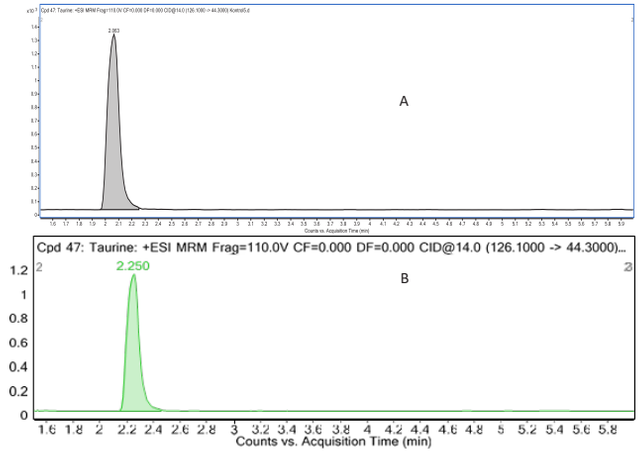

Figure 3. *Typical MRM chromatogram for Tauirine MRM choromatogram: A Control Group, B Patient Group*

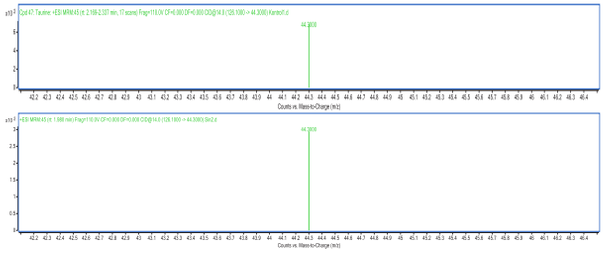

Figure 4. *Product ion mass spectra for Taurine: A Control Group, B Patient Group*

3. RESULTS

The average age of the individuals is 33,50±5,30 in group 1 and 34,26± 4,72 in group 2, and the average age between the two groups is similar (table 3).In Group 1, 11 patients were male and 12 were female while Group 2 included 14 males and 13 females. The female/male ratio was similar between groups and the mean BMI values were similar between groups (table 3). The serum-free taurine level was found to be statistically significantly lower in Group 2 compared to Group 1 (table 4, figure 1). A significant difference was not found between groups with regard to the remaining 31 free amino acid levels (table 4).

4. DISCUSSION

In CRS, long term neutrophil infiltration in paranasal sinuses and ROS are proposed to lead to the infection becoming chronic (26). Neutrophil granulocytes release proteolytic enzymes, and the protease amount exceeds antioxidant capacity and leads to tissue damage in the nasal and paranasal mucosa (27). Oxidative and immune-active products may continue inflammation even after the infection has been treated (28).

The main function of immune reactions is to fight micro-organisms and other foreign bodies (26). When the natural immune response is activated, it leads to the degranulation of inflammatory cells, the rapid release of reactive oxygen species (ROS) including the superoxide anion radical and the hydrogen peroxide radical. A balance exists between ROS production and elimination in most cellular processes of the organisms (29,30). This balance is preserved by endogenous antioxidant mechanisms (31). Hemostasis is impaired in the ROS over-expression resulting in oxidative stress, and tissue damage occurs through triggered inflammation (29,30). Additional protective mechanisms that restrict the severity of tissue damage like antioxidant defense mechanisms contribute to the control of the disease (31).

In various studies, it has been reported that taurine shows a cyto-protective effect due to its antioxidant activity (32,33). Taurine, whose antioxidant, antimicrobial, and anti-inflammatory activity is well known, was found to be lower in the serum of the patients with CRS in our study compared to the healthy control group. This suggests that taurine may be effective in the physiopathology and/or treatment of CRS, one of the chronic inflammatory diseases.

When neutrophil granulocytes, which are the dominant immune cells in inflammation, are activated, the myeloperoxidase enzyme is released into the extracellular environment. Taurine reacts with the enzyme products hypohalous acids (HOCl, HOBr) to form TauCl and TauBr products (34,35). Since TauCl is a less potent oxidant than HOCl, neutralization of HOCl represents one of the important antioxidant mechanisms of taurine. This reaction, which is catalyzed by myeloperoxidase, is also responsible for the anti-inflammatory activity of taurine since TauCl inhibits

the production of pro-inflammatory cytokines, inhibits the increase of nitric oxide and prostaglandin E2, reduces the activity of matrix metalloproteinases, and initiates leukocyte apoptosis to terminate acute inflammation (16,35).

The antimicrobial, antioxidant, and anti-inflammatory effects of TauCl and TauBr have enabled these agents to be used clinically, especially in the local treatment of infectious and inflammatory diseases (35). Nagl et al. reported that a 1-2% Lyc topical solution of N-chlorotaurine (NCT), a N-chloral derivative of taurine, was well tolerated by patients with bacterial conjunctivitis and was effective within 3-5 days (36). In the study by Neher et al., it was reported that NCT is a very well tolerated and highly effective drug for external otitis (37). In another study conducted by Neher A. et al., irrigation of nasal and paranasal sinuses was performed three times a week for a month in patients with chronic sinusitis, and NCT was applied. No toxic side effects were observed in the patients, and positive results including an increase in nasal breathing and ability to smell were obtained. It is not clear whether the positive results were due to NCT or irrigation (38). In the study by Nagl M. et al., the use of 1% NCT for five days was sufficient to eliminate the clinical signs of infection in infected leg ulcers, and NCT has also been reported to significantly suppress pain and granulation tissue formation and to accelerate re-epithelization (39). In addition, studies are available reporting that it is effective against the HIV27 virus (40).

Marcinkiewicz et al reported that the use of topical TauBr in acne vulgaris patients resulted in clinical improvement and could be a new treatment option for inflammatory acne (41).

In addition to successful topical treatments for various diseases, the rapid degradation of TauCl and TauBr in the systemic circulation limits the systemic use of these agents (35). The use of 5-aminosalicyltaurine (5-ASA-Tau), a colon-specific pro-drug administered orally in previous experimentally induced colitis (42) and dietary taurine supplementation in experimentally induced colitis mice in another study (43) yielded successful treatment outcomes. Based on these data, taurine supplementation can be considered a systemic treatment option when necessary in the treatment of diseases.

5. CONCLUSION

The result of this study showed that decreased serum taurine levels are associated with CRS. Identification of this relationship has shown that serum taurine levels may be another factor in the aetiology of CRS. In addition, both in vitro and clinical studies have shown that the use of taurine and taurine derivatives in the treatment of many infections and inflammatory diseases showed positive results. Based on this, we consider that taurine supplementation and/or nasal topical administration in CRS can be a supportive treatment option.

REFERENCES

[1] Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps group. Chinese journal of Otorhinolaryngology Head and Neck Surgery 2008;43(4):317–320.

[2] Hirsch AG, Stewart WF, Sundaresan AS, Young AJ, Kennedy TL, Scott Greene J. Nasal and sinus symptoms and chronic rhinosinusitis in a population-based sample. Allergy: European Journal of Allergy and Clinical Immunology 2017;72(2):274–281.

[3] Kucuksezer UC, Ozdemir C, Akdis M, Akdis CA. Chronic rhinosinusitis: Pathogenesis, therapy options, and more. Expert Opinion on Pharmacotherapy 2018;19:1805–1815.

[4] Murtaza M, Patawari P, Iftikhar HM, Shimmi SC, Hussain MS. Acute and chronic rhinosinusitis, pathophysiology and treatment. International Journal of Pharmaceutical Science Invention 2015;4(2):30–36.

[5] Bose S, Grammer LC, Peters AT. Infectious chronic rhinosinusitis. Journal of Allergy and Clinical Immunology: In Practice 2016 4(10):584–589.

[6] Fokkens W, Kok J, Bachert C. The high prevalence of chronic rhinosinusitis in Europe: Findings from the GALEN questionnaire. Allergy 2009;64(suppl. 90):597–598.

[7] Shashy RG, Moore EJ, Weaver A. Prevalence of the chronic sinusitis diagnosis in Olmsted County, Minnesota. Archives of Otolaryngology – Head and Neck Surgery 2004; 130:320–323.

[8] Lam K, Schleimer R, Kern RC. The etiology and pathogenesis of chronic rhinosinusitis: A Review of current hypotheses. Current Allergy and Asthma Reports 2015;15: 41.

[9] Gudis D, Zhao KQ, Cohen NA. Acquired cilia dysfunction in chronic rhinosinusitis. American Journal of Rhinology and Allergy 2012;26: 1–6.

[10] Wu G. Amino acids: Metabolism, functions, and nutrition. Amino Acids. 2009;37: 1–17.

[11] Calder PC, Yaqoob P. Amino acids and immune function. Metabolic and Therapeutic Aspects of Amino Acids in Clinical Nutrition 2003; 305–320.

[12] Schuller-Levis GB, Park E. Taurine and Its Chloramine: Modulators of Immunity. Vol. 29, Neurochemical Research 2004;117–126.

[13] Learn DB, Fried VA, Thomas EL. Taurine and hypotaurine content of human leukocytes. Journal of Leukocyte Biology 1990;48(2):174–182.

[14] Green TR, Fellman JH, Eicher AL, Pratt KL. Antioxidant role and subcellular location of hypotaurine and taurine in human neutrophils. BBA – General Subjects1991;1073(1):91–97.

[15] Warskulat U, Heller-Stilb B, Oermann E, Zilles K, Haas H, Lang F, et al. Phenotype of the Taurine Transporter Knockout Mouse. In: Methods in Enzymology 2007;439–458.

[16] Janusz Marcinkiewicz, Kontny E. Taurine, taurine derivatives and the immune system. In: Transworld Research Network 2012;101–129.

[17] Watanabe A, Higashi T, Sakata T, Nagashima H. Serum amino acid levels in patients with hepatocellular carcinoma. Cancer 1984;54(9):1875–1882.

[18] Cascino A, Muscaritoli M, Cangiano C, Conversano L, Laviano A, Ariemma S. Plasma amino acid imbalance in patients with lung and breast cancer. Anticancer Research 1995;15(2):507–510.

[19] Miyagi Y, Higashiyama M, Gochi A, Akaike M, Ishikawa T, Miura T. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. PLoS One 2011;6:9.

[20] Mustafa A, Gupta S, Hudes GR, Egleston BL, Uzzo RG, Kruger WD. Serum amino acid levels as a biomarker for renal cell carcinoma. Journal of Urology 2011;186(4):1206–1212.

[21] Hasim A, Aili A, Maimaiti A, Mamtimin B, Abudula A, Upur H. Plasma-free amino acid profiling of cervical cancer and cervical intraepithelial neoplasia patients and its application for early detection. Molecular Biology Reports 2013;40(10):5853–5859.

[22] Tochikubo O, Nakamura H, Jinzu H, Nagao K, Yoshida H, Kageyama N. Weight loss is associated with plasma free amino acid alterations in subjects with metabolic syndrome. Nutrition & Diabetes 2016;6:e197.

[23] Hong S-Y, Yang D-H, Chang S-K. The relationship between plasma homocysteine and amino acid concentrations in patients with end-stage renal disease. Journal of Renal Nutrition 1998;8(1):34–39.

[24] Vente JP, Von Meyenfeldt MF, Van Eijk HMH, Van Berlo CLH, Gouma DJ, Van Der Linden CJ. Plasma-amino acid profiles in sepsis and stress. Annals of Surgery 1989;209(1):57–62.

[25] Celik M, Şen A, Koyuncu İ, Gönel A. Plasma-free amino acid profiling of nasal polyposis patients. Combinatorial Chemistry & High Throughput Screening 2019;22(9):657–662.

[26] Melén I. Chronic sinusitis: Clinical and pathophysiological aspects. Acta Oto-Laryngologica 1994;114(S515):45–48.

[27] Ohlsson K, Odsson I. The neutral proteases of human granulocytes: Isolation and partial characterization of granulocyte elastases. European Journal of Biochemistry 1974;42(2):519–527.

[28] Itoh K, Katahira S, Matsuzaki T, Ohyama M, Fukuda K. Retention fluids of chronic sinusitis induce neutrophil adherence to microvascular endothelial cells. Acta Oto-Laryngologica 1992;112(2):882–889.

[29] Lugrin J, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. Biological Chemistry 2014;395:203–230.

[30] Paulis G. Inflammatory mechanisms and oxidative stress in prostatitis: The possible role of antioxidant therapy. Research and Reports in Urology 2018;10:75–87.

[31] Harijith A, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroads of inflammasome and inflammation. Frontiers in Physiology 2014;29:5.

[32] Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochemical Journal 1988;256(1):251–255.

[33] Schaffer SW, Azuma J, Mozaffari M. Role of antioxidant activity of taurine in diabetes. In: Canadian Journal of Physiology and Pharmacology 2009;91–99.

[34] Zgliczyński JM, Stelmaszyńska T, Domański J, Ostrowski W. Chloramines as intermediates of oxidation reaction of amino acids by myeloperoxidase. BBA – Enzymology1971;235(3):419–424.

[35] Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. 2014;46:7–20.

[36] Nagl M, Teuchner B, Pöttinger E, Ulmer H, Gottardi W. Tolerance of N-chlorotaurine, a new antimicrobial agent, in infectious conjunctivitis – A phase II pilot study. Ophthalmologica 2000;214(2):111–114.

[37] Neher A, Gstöttner M, Nagl M, Scholtz A, Gunkel AR. N-chlorotaurine-A new safe substance for postoperative ear care. Auris Nasus Larynx 2007;34(1):19–22.

[38] Neher A, Fischer H, Appenroth E, Lass-Flörl C, Mayr A, Gschwendtner A. Tolerability of N-chlorotaurine in chronic rhinosinusitis applied via yamik catheter. Auris Nasus Larynx 2005;32(4):359–364.

[39] Nagl M, Nguyen VA, Gottardi W, Ulmer H, HöPfl R. Tolerability and efficacy of N-chlorotaurine in comparison with chloramine T for the treatment of chronic leg ulcers with a purulent coating: A randomized phase II study. British Journal of Dermatology. 2003;149(3):590–597.

[40] Gottardi W, Nagl M. N-chlorotaurine, a natural antiseptic with outstanding tolerability [Internet]. Vol. 65, Journal of Antimicrobial Chemotherapy 2010:399–409.

[41] Marcinkiewicz J, Wojas-Pelc A, Walczewska M, Lipko-Godlewska S, Jachowicz R, Maciejewska A. Topical taurine bromamine, a new candidate in the treatment of moderate inflammatory acne vulgaris – A pilot study. European Journal of Dermatology 2008;18(4):433–439.

[42] Joo K, Lee Y, Choi D, Han J, Hong S, Kim YM. An anti-inflammatory mechanism of taurine conjugated 5-aminosalicylic acid against experimental colitis: Taurine chloramine potentiates inhibitory effect of 5-aminosalicylic acid on IL-1β-mediated NFκB activation. European Journal of Pharmacology 2009;(1–3):91–97.

[43] Shimizu M, Zhao Z, Ishimoto Y, Satsu H. Dietary taurine attenuates dextran sulfate sodium (DSS)-induced experimental colitis in mice. In: Advances in Experimental Medicine and Biology. Springer, New York, NY; 2009:265–271.

How to cite this article: Efe Atila N, Atila A. Serum Amino Acid Profile in Chronic Sinusitis. Clin Exp Health Sci 2022; 12: 576-581. DOI: 10.33808/clinexphealthsci.916590

Clinical and Experimental Health Sciences

University Students' Attitudes Toward Homophobia and Related Factors

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Received: 20.04.2021 **Accepted:** 22.12.2021

ABSTRACT

Objective: The aim of this study was to determine the levels of homophobic attitudes and related factors among senior students at a university in Turkey.

Methods: The descriptive study sample consisted of 317 senior undergraduate students who met the inclusion criteria and accepted to participate in the study between March 20 and April 24, 2017. The study data were collected using the "Personal Information Form" to determine the socio-demographic characteristics of the students and "Hudson-Ricketts Index of Homophobia" to determine their homophobic attitudes. Descriptive statistics, the independent samples t-test (Student's t-test) for the comparison of two groups and the one-way ANOVA test for the comparison of the means of more than two groups were used in the analysis of the data.

Results: In the study, it was determined that 156 (49.2%) students had low levels homophobic attitudes and 148 (46.7%) students had high levels homophobic attitudes. In addition, it was found that 13 (4.1%) students got 87 points and had neutral level homophobic attitudes. Among the students who participated in the study. it was found that the homophobic attitude levels of the students who were women and who had individuals with different sexual orientation in their social environment were found to be lower ($p<0.05$).

Conclusion: In this study, it was determined that university students have homophobic attitudes and the factors affecting homophobic attitudes of university students are gender and having different sexual orientation in the social environment.

Keywords: Homophobia, homophobic attitudes, homosexuality, university students, factors

1. INTRODUCTION

Sexual orientation is a phenomenon related to who an individual is attracted to in emotional romantic and sexual terms and is different from sexual identity. While sexual identity is about who the individual is (female, male, transgender), sexual orientation is related to the individual that the individual is interested in (1). Sexual orientation is not a feature chosen by the individual's own will, it is a condition that exists outside the will of the individual (2). Sexual orientation is not related to biological sex. Homosexuality, which emerges when the individual is directed to her/his own gender is not a disease but an orientation difference (2,3). The World Health Organization (WHO) states that homosexuality is a form of identity and existence that encompasses the private and public spheres of life (4).

Widely accepted opinion in the society is that each individual should be heterosexual, in other words, there should be no orientations other than heterosexuality. Homophobia resulting from this thought advocates the view that the identities of non-heterosexual individuals should be destroyed or kept secret (2). The Sexual Health Institute Association defines homophobia as behaviours and attitudes involving unfair judgments against homosexuals and homosexuality such as irrational hatred hate and humiliation (7). Since homophobia is a situation that is shaped and learned in the society, attitudes and behaviours towards sexual orientation may change over time (8). Today attitudes and behaviours of the society towards individuals with a different sexual orientation are usually negative and therefore these individuals have problems about being a part of the society. These problems are seen as discrimination and exclusion at the level of interpersonal relations and as ignorance and marginalization at the level of the entire society (5,6). In male-dominated societies such as Turkish society, attitudes towards sexual orientation differences may often be negative. Children raised in patriarchal societies can more easily internalize the gender roles identified by the society. This causes development of gender inequality and homophobia indirectly (9).

The educational experience of individuals in the society is one of the important tools in the development of prejudices and attitudes towards individuals with a different sexual orientation. Studies with university students showed that the

DOI: 10.33808/clinexphealthsci.923740

majority of students had negative attitudes towards individuals with a different sexual orientation (10-21). Şah, for example found that university students' and graduates' knowledge of sexual orientations was neither scientific nor accurate and that this knowledge often emerged as daily information learned through social practices (22). The study conducted by Sadıç and Beydağ shows that nursing students' attitudes towards lesbians and gays are at a medium level (23). Strong and Folse's study with nursing students revealed that the participants' attitudes towards lesbian, gay, bisexual, transgender and intersex (LGBTI) individuals were more positive after their education than their pre-education period (24).

As can be seen university education process is very important in adopting positive attitudes towards homosexuals. Also, exclusion and discrimination faced by individuals with a different sexual orientation can be prevented during this period. This process can become an effective tool for students to gain awareness of gender roles and to exhibit more tolerant attitudes towards sexual differences. Thus, it is thought that the exclusion and discrimination faced by homosexual individuals can be prevented. In this context, this study aimed to determine the levels of homophobic attitude of university students and related factors.

2. METHODS

2.1. Study Design and Participants

The population of this descriptive study consisted of 4505 senior students studying at the faculties and vocational schools in the central campuses of a state university in Turkey during 2016-2017 academic year. Prior to the data collection stage, necessary approval was obtained from this university Social Sciences Research Ethics Committee (Approval Date and Number: 15/03/2017, 2017/19). Before the data collection forms were administered, the students were informed about the purpose of the research and their verbal and written permission was obtained.

The study sample was selected using stratified sampling. The sampling formula with a known universe used in this study is shown below. The t value in this formula is the value for the selected alpha level 1.96 for a 95% confidence level. Based on the calculations, it was determined that a sample size of 354 students (171 female, 183 male students) would be large enough to represent the population. However, 37 students who could not be reached due to various reasons (e.g. student absenteeism, refusal to participate in the research, etc.) were not included in the sample and, therefore, the sample consisted of 317 (166 female, 151 male) students.

2.2. Data Collection and Instruments

Data were collected using a personal information form and the Hudson-Ricketts Index of Homophobia (HRIH). The data collection forms were filled in by the students between March 20 and April 24, 2017. It normally took 15 minutes on average to answer all the questions of the data collection forms.

The Personal Information Form

This form prepared by the researchers based on the relevant literature contains 12 questions about the students' descriptive information and the characteristics that are thought to affect their perspective on homosexuality (12-21,23-27).

The Hudson-Ricketts Index of Homophobia (HRIH)

The Hudson-Ricketts Index of Homophobia (HRIH) was developed as a 25-item scale by Hudson and Ricketts (1980) to measure attitudes towards homosexual individuals. The HRIH was adapted into Turkish by Sakallı and Uğurlu by omitting one of the 25 items in the original version. Each of the items is scored on a six-point Likert scale (1=Strongly Disagree to 6=Strongly Agree). In the scale, the response "Strongly Disagree" is considered as a positive cultural value and the response "Strongly Agree" is considered as a negative cultural value. The scores range from 24 to 144 with higher scores indicating higher levels of homophobia. After reversing the polarity of several questions (i.e. Items 5, 6, 8, 10, 11, 13, 17, 18, 23 and 24) and summing the individual items, the total score on the scale measures self-reported amount of homophobia. The median value of the total score was calculated and the participants were divided into two categories according these scores: low and high homophobia levels. The total Cronbach's alpha coefficient of the Turkish version of the HRIH was determined to be .94 (28). In our study, the Cronbach's Alpha coefficient of internal consistency was found to be .95.

2.3. Statistical Analysis

Data were analysed using SPSS 22.0 (Statistical Program for the Social Sciences) for Windows. Descriptive statistics were given as percentage, mean standard deviation,n minimum and maximum values. As a result of the statistical analyses, it was found that the data showed normal distribution and the variances were homogeneous. An independent samples t test was used to compare two groups and a one-way ANOVA was used to compare three and more groups. The significance level of the tests was set at $p<0.05$.

3. RESULTS

The sample comprised of 317 undergraduate university students, of which 166 (52.4%) were female and 151 (47.6%) were male, while 94.6% were single. The mean age of the students was 23.24 years (±1.56). The students came from various departments of the university but majority were from faculty of science and literature (26.2%), faculty of engineering (19%) and faculty of economics and administrative sciences (14.6%). Other sociodemographic characteristics of the students are shown in Table 1. Descriptive analysis was carried out to evaluate the students' level of homophobic attitudes. The results showed that in total, almost half of the sample and namely 156 (49.2%) of the students had low levels homophobic attitudes, 148 (46.7%) had high levels homophobic attitudes and only 13 (4.1%) of the students had neutral level homophobic attitudes (Table 2).

The mean HRIH scores of the male students (91.82±26.57) were higher than the mean HRIH scores of the female students (81.04±24.80) (p<0.05). The mean HRIH scores of the students who had individuals around them with a different sexual orientation (73.61±22.65) were lower than the HRIH mean scores of the students who did not have such individuals around them (90.49±25.95) (p<0.05). It was determined that there was no statistically significant relationship between the students' mean age and their mean HRIH scores (r=-0.004, p>0.001) (Table 3).

Table 1. *Distribution of the students' socio-demographic characteristics (n=317)*

Socio-Demographic Characteristics	X̄±SS	
Age	23.2±1.56	
	Frequency (n)	**Percentage (%)**
Sex		
Female	166	52.4
Male	151	47.6
Marital status		
Single	300	94.6
Married	17	5.4
Departments		
Faculty of science and literature	83	26.2
Faculty of engineering	60	19.0
Faculty of economics and administrative sciences	46	14.6
Faculty of education	38	11.7
Faculty of nursing	20	6.3
Faculty of communication	14	4.5
Faculty of medicine	11	3.5
Faculty of fine arts	11	3.5
Faculty of architecture	8	2.5
Faculty of tourism	8	2.5
Faculty of pharmacy	8	2.5
Faculty of sports science	5	1.6
Faculty of aquaculture	5	1.6
Type of high school graduated		
Anatolian high school	154	48.6
Regular high school	126	39.7
Vocational high school	37	11.7
Family income status		
Income equals to spending	203	64.0
Income more than spending	71	22.4
Income less than spending	43	13.6
Type of family		
Nuclear	237	74.8
Extended	80	25.2
Longest living place		
City	191	60.3
District	92	29.0
Village	34	10.7
Presence of individuals with a different sexual orientation in social environment		
No	236	74.4
Yes	81	25.6

Table 2. *Distribution of the students' Hudson-Ricketts Index of Homophobia score means (n=317)*

	Hudson-Ricketts Index of Homophobia Median Values	Frequency (n)	Percentage (%)	Total Score X̄±SS
Low Level of Homophobia	**<87**	156	49.2	64.86±15.67
High Level of Homophobia	**>87**	148	46.7	108.58±15.07
Neutral Homophobia	**87**	13	4.1	87.00

Table 3. *Distribution of the students' Hudson-Ricketts Index of Homophobia score means according to their characteristics (n=317)*

Socio-Demographic Characteristics	Frequency (n)	Total Score X̄±SS	P
Age	317	23.2±1.56	0.947* r – 0.004
Sex			
Female	166	81.04±24.80	**<0.001****
Male	151	91.82±26.57	
Marital status			
Single	300	85.92±26.14	0.465**
Married	17	90.70±27.18	
Type of high school graduated			
Anatolian high school	154	87.67±25.97	0.365***
Regular high school	126	83.63±26.36	
Vocational high school	37	88.64±26.42	
Family income status			
Income equals to spending	203	87.56±26.00	0.458***
Income more than spending	71	83.64±26.44	
Income less than spending	43	83.86±26.71	
Type of family			
Nuclear	237	85.08±26.81	0.197**
Extended	80	89.45±24.08	
Longest living place			
City	191	85.82±26.75	0.365***
District	92	84.75±24.03	
Village	34	92.05±28.44	
Presence of individuals with a different sexual orientation in social environment			
No	236	90.49±25.95	**<0.001****
Yes	81	73.61±22.65	

* Pearson Correlation Coefficient

** Student's t-test

***ANOVA Test

4. DISCUSSION

Negative attitudes towards homosexuality in the society lead to many social problems such as the exclusion and discrimination of homosexual individuals and their inability to express their sexual preferences. Therefore, homophobia appears as an important research topic to be investigated.

In this study, the majority of the senior students (49.2%) were found to have low levels of homophobic attitudes. On the

other hand, in our study, it was striking that there were also a considerable number of students (46.7%) with high levels of homophobic attitudes. The study of Sadıç and Beydağ shows that nursing students' attitudes towards lesbians and gays are at a moderate level (23). In the study of Gromer et al., it was found that students displayed a moderate amount of sexual prejudice toward lesbian and gay people (27). The fact that the homophobic attitudes of senior students were low in our study made us think that the students gained awareness about homophobia during the university education process.

Considering other studies conducted with university students, it was seen that contrary to the results of our study, students mostly exhibited homophobic attitudes (10,19-21) and had negative attitudes towards lesbians and gays (11-14,16,18,22). In the study conducted by Bakır Ayğar et al. (2015) with students studying at Mersin University Faculty of Education, it was determined that students' homophobic attitudes were high (15). Kara's study with 97 university students (2018) found that 55 students had lower levels of homophobic attitudes while 42 students had higher (29). In a study by Varol et al. (2016), more than half of the students (53.6%) agreed with the statement, "I hate it when I see a man acting like a woman" (30). To sum up, we found that the homophobic attitudes were found to be low in the majority of the senior students in contrast with other similar studies in the literature. This result could have been caused by education given to the students until the senior year. University education and the different social interactions that might occur during this period may be effective in reducing homophobic attitudes.

In our study, the levels of homophobic attitudes of male students were found to be higher than female students. This result is also confirmed by other studies in the literature (11,12,14-17,20-23,27,29,31). In the studies, it is striking that male students have more negative attitudes towards male homosexuals (gays). In some of these studies, it is also emphasized that male individuals see gays as a "threat to the ideal male perception in society" (27,32). Additionally it is stated in these studies that men adopt the attributed gender roles especially by the patriarchal society more than women (10,31,33). This finding we obtained in the study may be due to the adoption of gender roles by male students who grew up in patriarchal societies.

In our study, homophobic attitude levels of students who have communication with individuals with different sexual orientations in their social environment were found to be lower than other students (Table 3). Consistent with our findings, similar studies found that participants who were acquainted with homosexual individuals and who had social relations with such individuals had more positive attitudes towards homosexuality than others (11,14,19-21,23,31). A study conducted by Rowniak found that students who stated that there were no individuals in their social environment with a different sexual orientation had higher levels of homophobic attitudes (34).

In the study of Gromer et al., it was found that, contrary to our findings, the levels of homophobic attitudes of university students did not change with personal acquaintance with a gay or lesbian person (27). As shown by the findings from our study and other comparable studies, prejudice and negative attitudes towards individuals with a different sexual orientation may change positively with the presence of social connections with these individuals. These social ties can help students to understand the difficulties experienced and felt by individuals with a different sexual orientation, to enable students to empathize with them, and to decrease their homophobic attitudes gradually.

5. CONCLUSION

In this study, university students were found to have homophobic attitudes. In addition, the homophobia level of male students and students who are not gay or lesbian in their social environment were determined to be higher.

In many courses and field practices during the education process, university students should be informed about the psychosocial and cultural characteristics of groups such as children, youth, women, elderly, refugees who are exposed to prejudice, discrimination, marginalization, exclusion and even stigmatization. In the courses included in the curriculum, efforts should be made to increase awareness and acceptance of human and professional values, especially social justice, human rights, respect for differences and the right to self-determination. Different and various educational or drama-type practices should be carried out that positively change homophobic and heterosexist perceptions and attitudes towards students. The fact that individuals have social relations and connections with is homosexual individuals effective in reducing homophobic attitudes should be taken into consideration. The inclusion of homosexual individuals as participants in educational studies and lessons can have positive effects on the perception and attitude change of students. In addition, seminars and panels focusing on workshops aimed at reducing homophobic attitudes and behaviors can be organized. In all these activities, the participation of especially male students can be increased.

Limitation of the Study

The research is limited to university students. Since this research was conducted with students of only one universitythe results cannot be generalized to students studying at different universities.

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

Financial Disclosure: None.

REFERENCES

[1] Levay S. Gay, straight and the reason why the science of sexual orientation. Second edition. New York: Oxford University Press; 2011.

[2] Kaptan S, Yüksel S. Homosexuals, social exclusion and mental health. Community and Physician 2014;29(4):259-265.

[3] Basar K. Different facets of sexual identity: sex, gender identity, gender role and sexual orientation. Community and Physician 2014;29(4):245-251

[4] World Health Organization. Developing sexual health programmes. Available from: URL: http://apps.who.int/iris/bitstream/10665/70501/1/WHO_RHR_HRP_10.22_eng.pdf

[5] The Sexual Health Institute Association Homophobia and Heterophobia. Available from: URL: http://www.cised.org.tr/sayfa218.html.

[6] Keleher A, Smith ER. Growing support for gay and lesbian equality since 1990. J Homosex 2012;59(9):1307-1326.

[7] Buz S. Social work with lesbian gay bisexual transsexuals and transvestites. SSW 2011;22(1):137-148.

[8] Evans R, Nagoshi JL, Nagoshi C, Wheeler J, Henderson J. Voices from the stories untold: Lesbian, gay, bisexual, trans, and queer college students' experiences with campus climate J. Gay Lesbian Soc Serv 2017;29(4):426-444.

[9] Çelik DB, Şahin NH. Sexual orientations: approach of health care professional. Literature Symposium 2012;(1):15-23.

[10] Feng Y, Lou C, Gao E, Tu X, Cheng Y, Emerson MR, Zabin LS. Adolescents' and young adults' perception of homosexuality and related factors in three Asian cities. J Adolesc Health 2012;50(30):52–60.

[11] Saraç L. Attitudes of future physical education teachers in Turkey toward lesbians and gay men. Psychol Rep 2012;111(3):765-775.

[12] Flórez-Salamanca L, Herazo E, Oviedo HC, Campo-Arias A. Prevalence and predictors of high sexual prejudice among medical students from two Colombian cities. SAGE Open 2014;4(2):1–8.

[13] Costa PA, Almeida R, Anselmo C, Ferreira A, Pereira H, Leal I. University students' attitudes toward same-sex parenting and gay and lesbian rights in Portugal. J Homosex 2014;61(12):1667-1686.

[14] Papadaki V, Plotnikof K, Gioumidou M, Zisimou V, Papadaki E. A comparison of attitudes toward lesbians and gay men among students of helping professions in Crete, Greece: the cases of social work, psychology, medicine, and nursing. J Homosex 2015;62(6):735-762.

[15] Bakır-Ayğar B, Gündoğdu M, Ayğar H. The attitudes of students at Faculty of Education Mersin University towards homosexuals. J Int Soc Res 2015;8(41):769-777.

[16] Saraç L. Relationships between religiosity level and attitudes toward lesbians and gay men among Turkish university students. J Homosex 2015;62(4):481-494.

[17] Sanberk İ, Çelik M, Gök M. An investigation of university students' homophobia levels regarding gender and gender roles. J Hum Sci 2016;13(3):4011-4019.

[18] Unlu H, Bedük T, Duyan V. The attitudes of the undergraduate nursing students towards lesbian. J Clin Nurs 2016;25(23-24):3697–3706.

[19] Gonenc IM, Senturk Erenel A. Determining homophobic attitudes of nursing students in Turkey and the factors affecting them. Clin Exp Health Sci 2019;9(1):21-28.

[20] Kwak HW, Kim MY, Kim MY. Severity and influencing factors of homophobia in Korean nursing students. Int J Environ Res Public Health 2019;25;16(23):4692.

[21] Bozkurt F, Korkut-Owen F. University students' attitudes toward homosexuals. TPCGJ 2020;10(58):495-513.

[22] Şah U. The social representations of sexual orientation among Turkish youths. Turkish Psychology Articles 2011;14(27):88-99.

[23] Sadıç E, Beydağ KD. Nursing students' attitudes toward lesbians and gay and affecting factors. Journal of Nursing Science 2018;1(2):5-13.

[24] Strong KL, Folse VN. Assessing undergraduate nursing students' knowledge, attitudes and cultural competence in caring for lesbian, gay, bisexual, and transgender patients. J Nurs Educ. 2015;54(1):45-49.

[25] Osmanaga F. Student's attitudes toward homosexuality. ESJ 2015;11(23):170-183.

[26] Collier KL, Horn SS, Bos HMW. Sandfort TGM. Attitudes toward lesbians and gays among American and Dutch adolescents. Sex Roles 2015;52(2):140-150.

[27] Gromer J, Campbell M, Gomori T, Maynard D. Sexual prejudice among Barbadian university students. J Gay Lesbian Soc Serv 2013;25(4):399-419.

[28] Sakallı N, Uğurlu O. Effects of social contact with homosexuals on heterosexual Turkish university students attitudes towards homosexuality. J Homosex 2001;42(1):53-62.

[29] Yunus K. Determination of homophobic attitudes' of social work students. Turkish Journal of Social Work 2018;2(1):16-27.

[30] Varol ZS, Çiçeklioğlu M, Taner Ş. Evaluation of levels of gender perception and related factors among first-year students in a medical faculty. Ege Journal of Medicine 2016;55(3):122-128

[31] Woodford MR, Silverschanz P, Swank E, Scherrer KS, Raiz L. Predictors of heterosexual college students' attitudes toward LGBT people. J LGBT Youth 2012;9(4):297-320.

[32] Şah U. The relationship of the descriptions of homosexuality, bisexuality and transsexuality with levels of homophobia and acquaintanceship with LGBT people. Studies in Psychology 2012;32(2):23-48.

[33] Higa D, Hoppe MJ, Lindhorst T, Mincer S, Beadnell B, Morrison DM, Wells EA, Todd A, Mountz S. Negative and positive factors associated with the well-being of lesbian, gay, bisexual, transgender, queer, and questioning (LGBTQ) youth. Youth Soc 2014;46(5):663-687.

[34] Rowniak SR. Factors related to homophobia among nursing students. J Homosex 2015;62(9):1228-1240.

How to cite this article: Vefikuluçay Yilmaz D, Degirmenci F, Aksoy A, Koptur A, Aksu A. University Students' Attitudes Toward Homophobia and Related Factors. Clin Exp Health Sci 2022; 12: 582-586. DOI: 10.33808/clinexphealthsci.923740

Clinical and Experimental Health Sciences

The Protective Effect of Rheum Ribes L., and Quercetin on Protein Carbonyl Levels Against Carbon Tetrachloride-Induced Liver and Kidney Damage in the Rats

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Received: 26.05.2021 **Accepted:** 23.07.2022

ABSTRACT

Objective: This study was designed to examine the potential protective effects of Rheum ribes L., and quercetin on protein carbonyl (PCO) in kidney and liver tissue, trace elements (Fe, Cu, Zn) and mineral (P) in serum samples in Wistar rats of carbon tetrachloride (CCl4)-induced oxidative damage.

Methods: The 2, 4-dinitrophenylhydrazine (DNPH) method is the most reliable method widely used to measure carbonyl levels in proteins. In this study, the effect of Rheum ribes L. (Rr) and quercetin on protein carbonyl, trace elements (Fe, Cu, Zn) and mineral (P) levels against carbon tetrachloride (CCl4) mediated liver and kidney damage was investigated. For this purpose, 56 Wistar albino female rats weighing 200 ± 220 g were used. Groups were designed as: controls, 0.3 ml DMSO, 1 ml/kg olive oil, 1 ml/kg CCl4, 100 mg/kg Rr, 100 mg/kg quercetin, 100 mg/kg Rr+1 ml/kg CCl4 and 100 mg/kg quercetin+1 ml/kg CCl4 groups.

Results: The results showed that the CCl4 group had significantly higher level of protein carbonyl (PCO) than the control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.01$, and $p<0.01$, respectively). A significant elevation in the group of CCl4 + quercetin, compare to control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.01$, and $p<0.05$, respectively) in the liver tissue. Additionally, the CCl4 group had significantly higher level of PCO than the control, DMSO, olive oil, Rr and quercetin groups ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). Similarly, the CCl4 + Quercetin group had increased level of PCO compared to the control and Rr.groups ($p<0.05$ and $p<0.05$) in the kidney tissue.

Conclusion: In the study, it was seen that the bioactive substances in Rheum ribes L. (root) and quercetin, a standard antioxidant, could be an alternative against the toxic effect of CCl4.

Keywords: Carbon tetrachloride, protein carbonyl, quercetin, rheum ribes L., trace elements.

1. INTRODUCTION

Proteins, which are among the most common organic molecules in living things, are molecules with much different functionality, since they participate in the structure of enzymes and hormones in living systems. Proteins constitute the main “working force” for all biological processes (1). Although the folding pattern of proteins and their precise three-dimensional structure, their tight dependence on their activities and functions (1) make research difficult, they are at an important and critical point in the diagnosis of diseases. Almost all vital function depends on this macromolecule class (2). Because of all these functions of proteins and their abundance in biological systems and high rate constants for reactions, they are the main targets for oxidants (3). For all these reasons, the living organism is exposed to a system that produces reactive oxygen species (ROS) that can damage nucleic acids, lipids and proteins (4).

Although some signs of oxidative stress such as DNA damage and lipid peroxidation have been extensively evaluated and reliable biomarkers have been found to determine the degree of damage associated with it, the attack of ROS on proteins and protein carbonyl formation is a newly investigated topic (5,6).

Carbonyl groups are introduced into proteins by various oxidative means, particularly by metal catalyzed oxidation (MCO) of specific protein amino acid side chains or the addition of carbonyl-containing oxidized lipids (4-hydroxynonenal, malondialdehyde) or sugars (7). Protein oxidation is expressed as covalent change of proteins induced directly by free radicals such as hydrogen peroxide (H_2O_2) and hydroxyl (OH^-) and/or indirectly induced by the reaction with secondary products of oxidative stress (8). Protein carbonyl (PCO) products are formed as a result of damage to the amino acid residue or peptide backbone such

DOI: 10.33808/clinexphealthsci.943255

as histidine (His), proline (Pro), arginine (Arg) and lysine (Lys) as a result of the interaction of reactive oxygen species (ROT) mentioned above with proteins. This damage is irreversible and responsible for cell death (8).

It is a fact that protein oxidation that occurs in certain situations will be protected by the existing environmental, genetic and dietary factors, as well as affect the balance between antioxidant and peroxidant activities (4).

The antioxidant activities of natural products are generally observed in phenolic compounds. Quercetin is a common ingredient found in many fruits and vegetables. Quercetin, a plant-derived flavonoid, is thought to have a multifunctional molecular structure and may act partially through a mitochondrial mechanism, although its biological properties and underlying mechanisms of action are not fully known (9). Recent studies have shown that quercetin exhibits antioxidative, anti-inflammatory and anti-apoptosis properties, and also protects the liver from damage caused by hepatoxins (10). *Rheum ribes L.,* belonging to the Polygonacea family, is a perennial herbaceous plant that grows in the Eastern Anatolia Region in Turkey. It has been reported that the leaves and body of the plant are sour and strengthens the stomach (11). It has been shown in studies that the content of the Rheum ribes L. plant contains high phenolic content and is rich in Fe, Zn and vitamin C (12, 13). Chemical components of anthraquinone and stilbene have been identified in the roots of *Rheum ribes L.* (14). Anthraquinones stimulate fluid balance, electrolyte exchange and colon smooth muscle movement.

The aim of this study is to determine the effects of quercetin and *Rheum ribes L*., which have important antioxidant effects, on protein carbonyl in liver and kidney tissues damaged with carbon tetrachloride (CCl_4).

2. METHODS

2.1. Chemical Reagents

All chemicals and reagents used were of analytical grade. Quercetin (sigma, USA), 10% DMSO, (sigma, USA), CCl_4 (sigma-Aldrich, USA), Guanidin-HCl (sigma, USA), Olive oil (Turkiye)

2.2. Plant Material and Extraction

Rheum ribes L. plant used in the study was collected from the Van region in May – June 2017. Since the ingredients of the product in the spring are at the highest level, the products grown in the spring were selected. The sample material was recorded by performing the necessary identification procedures in the herbarium of *Rheum ribes L*. (root), Van YYU Faculty of Science, Department of Biology.

The water extraction of *Rheum ribes* L. roots was performed by modifying the decoction method used by Eddouks et al. (15). 10 grams of ground *Rheum ribes L.* roots were boiled in 100 ml distilled water at 250 rpm for 10 minutes and then allowed to cool for 15 minutes. Then, the water of the extract, which was filtered through Watman filter paper, was placed in 50 mm falcon tubes (not exceeding 1/3 when tilted) and kept at – 80 °C for a week, and then lyophilized in a – 80 °C lyophilizer for 48 hours. The yield of the extract obtained was calculated to be 5.184%. After determining the yield, dose calculations were made in the rats according to their weights and the amount of plant to be given daily was determined. The plant extracts prepared daily by decoction method for 7 days were dissolved in 15 ml of distilled water and vortexed sufficiently and then kept in closed glass bottles until the time of application. It was given to rats by gavage at 10.00 every day throughout the study. *Rheum ribes L.,* plant was administered to the fifth and seventh groups throughout the study at a dose of 100 mg/kg.

2.3. Preparation of Quercetin

Quercetin was administered daily at a dose of 100 mg / kg to the sixth and eighth groups by oral gavage.

2.4. Preparation of Dimethyl Sulfoxide (DMSO)

DMSO (% 10) was added daily to 5 ml of distilled water and 25 µl was administered to the second group as 0.3 ml / kg by oral gavage.

2.5. Preparation of CCl_4

CCl4 was mixed with olive oil at a ratio of 1:1 and administered intraperitoneally as a single dose to the fourth, seventh and eighth groups on the 7th day as 1ml / kg.

2.6. Animals

In this study, 56 female Wistar albino rats were used. Rats at Van Yüzüncü Yıl University Experimental Medicine Application and Research Center; it was fed with standard pellet at room temperature set to 22 ± 2 °C, illuminated at a rhythm of 12 hours light – 12 hours dark. The rats were kept in standard plastic cages with free feed and water intake. Before starting the study, the study approval (decision dated 27.02.2020 and numbered 2020/02) was obtained from Van Yüzüncü Yıl University, Animal Experiments Local Ethics Committee.

2.7. Establishment of Experimental Groups and Experiment Plan

In the study, 8 groups were formed with 7 rats in each group.

1. Group (n= 7): It was determined as the control group and fed with normal water and standard pellet feed for 7 days.

2. Group (n=7): DMSO group (0.3 ml intragastrically) was determined and administered for 7 days.

3. Group (n=7): Olive oil group (1 ml/kg intraperitoneal) was determined and a single dose was administered on the 7th day.

4. Group (n=7): Carbon tetrachloride group (1 ml/kg intraperitoneal, dissolved in olive oil 1:1 ratio) was determined and a single dose was administered on the 7th day.

5. Group (n=7): *Rheum ribes L.* (root) water extract group (100 mg/kg intragastrically) was determined and administered for 7 days.

6. Group (n=7): The quercetin group was determined (100 mg/kg intragastrically) and administered for 7 days.

7. Group (n=7): *Rheum ribes L.* (root) water extract (100 mg/kg intragastrically) was administered for 7 days and carbon tetrachloride (1 ml/kg in intraperitoneal olive oil at a ratio of 1:1) as a single dose on the 7th day.

8. Group (n=7): Quercetin was administered (100 mg/kg intragastrically) for 7 days and carbon tetrachloride (1 ml/kg intraperitoneal, 1:1 in olive oil) as a single dose on the 7th day.

2.8. *Performing Tissue Homogenate Processes*

Liver and kidney tissues taken from rats were kept at – 65 ºC and gradually dissolved until they reached room temperature. Tissue extraction process for protein carbonyl determination method in tissues was performed as follows. For the extraction, 1 mL of buffer containing 6.8 g of KH_2PO_4 and 2.92 g of EDTA was prepared. 0.5 g of kidney and liver tissue was weighed in a precision balance (Denver instrument SI-234) and put into tubes and 2 mL of buffer was added. Tissues were homogenized on ice until disintegrated and a further 3 mL buffer was added again. Then it was centrifuged at +4 ºC in a cooled centrifuge (EBA 20 Hettich) device at 9500 rpm for 10 minutes. Clear supernatants obtained from liver and kidney tissue were made ready for analysis (16).

2.9. *Protein Carbonyl Determination Method*

PCO content in liver and kidney tissues were determined according to the method described by Reznick and Packer (17). The basic principle protein carbonyl derivatives are one of the most used markers in the detection of oxidative protein damage. The formation of derivatives of carbonyl groups with 2-4 dinitrophenylhydrazine (DNPH) is followed by the formation of dinitrophenylhydrozane (DNP) products. Finally, the DNP formation was determined at 360 nm at UV. The value found was expressed as nanomole protein carbonyl per mg.

2.10. *Trace Element and Mineral Determination*

Trace element (Cu, Zn, Fe) and mineral (P) analyzes measured in serum were performed using inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.11. *Statistical Analysis*

The results are presented as means ± the standard error of the mean (X±SEM). Variance analysis (ANOVA) was applied. Tukey's test was applied for post hoc comparison. Statistical significance was considered as p<0.05. The statistical analysis was carried out using SPSS®, version 23.0 statistical software (SPSS Inc. Chicago Ill, USA).

3. RESULTS

Within the scope of our study, the protein carbonyl (PCO) data in the liver and kidney tissues of the rats and the comparison of the liver PCO levels between the groups are given in table 1, and the comparison of the PCO levels in the kidney tissue is given in the Table 2.

Statistical analysis showed that in the CCl_4 group was significantly higher than the control, DMSO, Olive oil, *Rheum ribes L.*, and Quercetin groups in the PCO levels (p<0.00, p<0.05, p<0.001, p<0.01, and p<0.01, respectively). However, CCl_4 + Quercetin group was also significantly higher than control, DMSO, Olive oil, *Rheum ribes L.*, and Quercetin groups regarding PCO levels (p<0.001, p<0.05, p<0.001, p<0.01, and p<0.05, respectively) in the liver. (Table 1 and Figure 1).

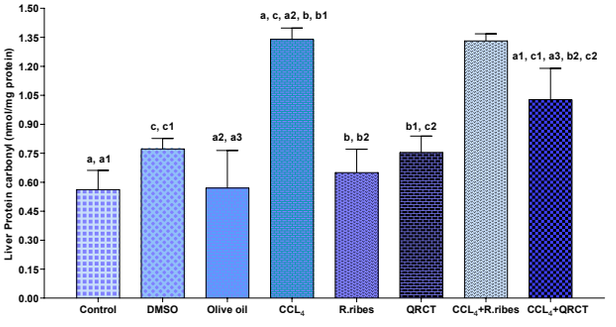

Fig 1. *Comparison of protein carbonyl (PCO) levels between liver tissue control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 1 demonstrates Protein carbonyl (PCO) values in control, DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin groups in the liver and kidney tissues.

Table 1. *Protein carbonyl (PCO) findings of control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups in the liver and kidney tissues.*

Groups/Parameters	Liver PCO (nmol/mg prot.)	Kidney PCO (nmol/mg prot.)
Control	$0,56 \pm 0,10^{a,a1}$	$1,05 \pm 0,06^{a,c}$
DMSO	$0,77 \pm 0,05^{c,c1}$	$1,15 \pm 0,11^{b}$
Olive oil	$0,57 \pm 0,19^{a2,a3}$	$1,09 \pm 0.09^{a1}$
CCl_4	$1,34 \pm 0,06^{a,c,a2,b,b1}$	$1,74 \pm 0,08^{a,b,a1,a2,a3}$
Rheum ribes L.	$0,65 \pm 0,12^{b,b2}$	$1,08 \pm 0,10^{a2,c1}$
Quercetin	$0,75 \pm 0,08^{b1,c2}$	$1,07 \pm 0,09^{a3}$
CCl_4 + *Rheum ribes L.*	$1,03 \pm 0,16$	$1,46 \pm 0,16$
CCl_4 + Quercetin	$1,33 \pm 0,04^{a1,c1,a3,b2,c2}$	$1,53 \pm 0,04^{c,c1}$

a,a_1,a_2,a_3:p<0.001, b,b_1,b_2: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

According to the statistical analysis in kidney tissue, PCO levels were significantly higher in the CCl_4 group than the control, DMSO, Olive oil, *Rheum ribes L.,* Quercetin groups (p<0.001, p<0.01, p<0.001, p<0.001 and p<0.001, respectively). Moreover, PCO were increased in CCl_4 + Quercetin group compared to control and *Rheum ribes L.* groups (p<0.05, and, p<0.05) (Table 1, Figure 2).

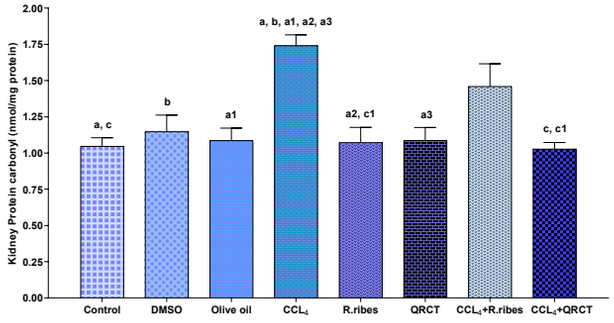

Fig 2. *Comparison of protein carbonyl (PCO) levels between kidney tissue control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 2 shows the trace element (Cu, Zn, Fe) and mineral (P) levels between control**,** DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin group.

Table 2. *Trace element (Cu, Zn, Fe) and mineral (P) levels between control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin group*

Groups/ Parameters	Cu (µmol/L)	Zn (µmol/L)	Fe (mmol/L)	P (mmol/L)
Control	0,95 ± 0,11	3,15 ± 0,06^{a}	0,118 ± 0,0001	6,34± 0,43^{c,b}
DMSO	1,16 ± 0,16	3,58 ± 0,07^{a,a1,c,b,c1,b1}	0,116 ± 0,0007	4,47± 0,25^{c,b1}
Olive oil	1,04 ± 0,10	3,15 ± 0,05^{a1}	0,118 ± 0,0004	6,32± 0,33^{b1,b2}
CCl_4	1,32 ± 0,13	3,39 ± 0,06	0,117 ± 0,0009	4,29± 0,30^{b,b2,c1}
Rheum ribes L.	0,99 ± 0,09	3,26 ± 0,07^{c}	0,118 ± 0,0004	6,07± 0,26^{c1}
Quercetin	1,03 ± 0,11	3,22 ± 0,06^{b}	0,117 ± 0,0003	5,55 ± 0,38
CCl_4 + *Rheum ribes L.*	1,11 ± 0,14	3,25 ± 0,04^{c1}	0,118 ± 0,0006	5,44 ± 0,42
CCl_4 + Quercetin	1,17 ± 0,17	3,22 ± 0,08^{b1}	0,117 ± 0,0009	5,50 ± 0,42

a,a_1:p<0.001, b,b_1,b_2: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

The trace element (Cu, Zn, Fe) and mineral (P) levels were measured in serum in our study and the proportional findings between these elements are shown in table 2. Regarding the zinc (Zn) trace element findings, between the group treated with 0.3 ml DMSO and the control group and the group treated with 1 ml kg^{-1} olive oil (p<0.001), between the group administered 100 mg kg^{-1} quercetin and the group treated with CCl_4 + quercetin (p<0.01) and between the group administered 100 mg kg^{-1} *Rheum ribes L.* and the group treated with CCl_4 + quercetin (p <0.05), a significant relationship was found (Table 2). No significant relationship was found between the control group and other groups in terms of Cu and Fe trace element levels (Table 2).

The CCl_4 group was also significantly lower than control, Olive oil and *Rheum ribes L.* groups regarding P level (p<0.01, p<0.01 and p<0.05, respectively), whereas the DMSO group had decreased level of P comparing with control and Olive oil groups (p<0.05 and p<0.01).

The mean ratio trace element and mineral Zn/Cu, P/Zn, P/Fe and Zn/Fe in the control**,** DMSO, olive oil, CCl_4, *Rheum ribes L.*, quercetin, CCl_4 + *Rheum Ribes L.*ve CCl_4 + quercetin groups are shown in Table 3 (Figure 3).

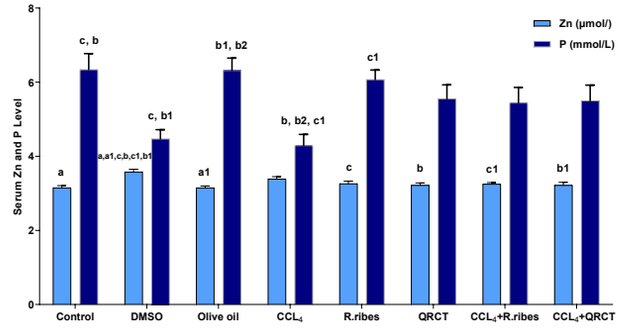

Fig 3. *Comparison of zinc (Zn) element and phosphorus (P) mineral levels in serum control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups. (Different letters on the error bars represent mean significant difference).*

Table 3. *Findings of the ratio trace element and mineral Zn/Cu, P/Zn, P/Fe and Zn/Fe in the control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups*

Groups/ Parameters	Zn/Cu	P/Zn	P/Fe	Zn/Fe
Control	3,55 ± 0,38	2,02 ± 0,13^{a,b}	54,01 ± 3,93^{b,c}	26,80±0,73^{b}
DMSO	3,81 ± 1,02	1,25 ± 0,06^{a,a1,c}	38,75 ± 2,22^{c,c1}	30,98 ± 0,58^{a,b,b1,c,c1,c2}
Olive oil	3,25 ± 0,36	2,01 ± 0,13^{a1,b1}	53,67 ± 2,71^{b1,c1}	26,8 ± 0,49^{a}
CCl_4	2,75 ± 0,35	1,27 ± 0,11^{b,b1,c1}	36,76 ± 2,43^{b,b1,c2}	28,93 ± 0,73
Rheum ribes L.	3,47 ± 0,36	1,86 ± 0,10^{c,c1}	51,77 ± 2,22^{c2}	27,71 ± 0,70^{c}
Quercetin	3,38 ± 0,39	1,73 ± 0,12	47,49 ± 3,30	27,53 ± 0,56^{c1}
CCl_4+*Rheum ribes L.*	3,25 ± 0,44	1,65 ± 0,11	46,17 ± 3,66	27,6 ± 0,44^{c2}
CCl_4+Quercetin	3,23 ± 0,54	1,72 ± 0,14	46,86 ± 3,68	27,43 ± 0,90^{b1}

a,a_1:p<0.001, b,b_1: p<0,01, c,c_1,c_2: p<0.05 (different letters in superscripts represent statistically significant differences between groups).

The results showed that the CCl_4 group had significantly lower levels of P/Zn ratio than the control, Olive oil and *Rheum ribes L.*groups (p<0.01, p<0.01, and, p<0.05, respectively). Additionally, the DMSO group had significantly lower levels of P/Zn than the control, Olive oil and *Rheum ribes L.*groups (p<0.001, p<0.001, and p<0.05, respectively). The CCl_4 group had increased levels of P/Fe compared to the control, Olive oil and *Rheum ribes L.* groups (p<0.01, p<0.01 and p<0.05, respectively). Similarly, DMSO group had significantly lower levels of P/Fe than the control and Olive oil group (p<0.05 and p<0.05). However, the DMSO group had a significantly higher level of Zn/Fe ratio than the control, Olive oil, *Rheum ribes L.,* Quercetin, CCl_4 + *Rheum ribes L.* and CCl_4 + Quercetin groups (p<0.01, p<0.001, p<0.05, p<0.05, p<0.05, and p<0.01, respectively). (Table 3, Figure 4).

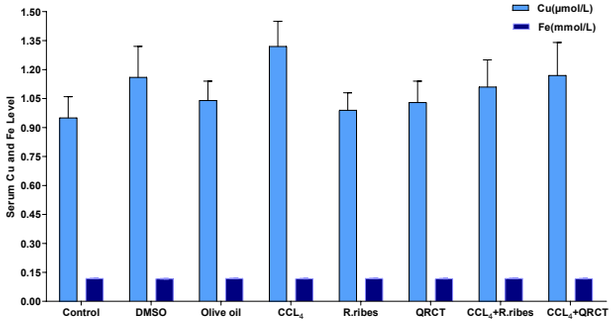

Fig 4. *Comparison of copper (Cu) and iron (Fe) element levels in serum control, DMSO, olive oil, CCl4, Rheum ribes L., quercetin, CCl4 + Rheum Ribes L.ve CCl4 + quercetin groups*

4. DISCUSSION

CCl_4 is known as hepatotoxin and nephrotoxin (18, 19). In experimental studies, the mechanism of CCl_4 induced damage is that cytochrome P450 transforms into free radicals trichloromethyl (CCl_3^-) and trichloromethyl peroxy ($CCl_3O_2^-$) and increases lipid peroxidation and protein oxidation in the liver, as well as the heart, kidney, brain, lung and testis. It has been reported to cause damage in many organs (19, 20, 21, 22). CCl_3 – radical undergoes both oxidative and reductive biotransformation and initiates biochemical events that lead to liver cell necrosis (18, 23). On the other hand, oxidative damage of proteins is also caused by the fenton-type reaction of free radicals and trace elements such as Fe^{+2}, Cu^{+2}. Conditions leading to the mobilization of Fe or Cu to redox active forms lead to high levels of protein carbonyls (24). As the carbonyl groups of proteins increase, they become more susceptible to oxidative damage (25). Symptoms such as increases in carbonyl levels, rheumatoid arthritis, ischemia-reperfusion damage to the heart muscles, and muscle damage caused by strenuous exercise are also examined (17).

In our study, a non-lethal dose of CCl_4 (1 ml/kg i.p) was used and the rats were decapitated 24 hours after CCl_4 treatment. This period has been reported in the literature review to be the most appropriate time for inducing liver damage (26, 27). In this study, the effect of these antioxidant substances on protein carbonyl in the presence of *Rheum ribes L.* and quercetin in CCl_4-induced liver and kidney damage was investigated. When table. 1 was examined, it was observed that the PCO levels of 1 ml kg^{-1} CCl_4 administered in both liver and kidney tissue groups increased compared to the control group (p<0.001, p<0.05). It was determined that the group given CCl_4 with 100 mg kg^{-1} quercetin also decreased the PCO levels compared to the CCl_4 group (p<0.01; p<0.05), but increased compared to the 100 mg kg^{-1} *Rheum ribes L.* group. These results show that CCl_4 causes oxidative damage on liver and kidney tissues, *Rheum ribes L.* and quercetin have been found to reduce this damage. In CCl_4 application, it can be thought that the presence of PCO, which is a high level of oxidative biomarker in kidney tissue parallel to the liver tissue, is most likely caused by the application of CCl_4 oxidative stress.

Cu, Fe and Zn are trace elements that have important roles in fulfilling the functions of many enzymes and protein metabolism in the organism (2, 28, 29). Cu is an essential trace mineral essential for many biological processes. This requirement is thought to be due to the Cu element acting as a cofactor for proteins involved in various biological reactions such as photosynthesis, respiration, free radical reactions, connective tissue formation, Fe metabolism, and neurological function (30). Iron is an essential element required as a cofactor for proteins that govern oxygen transport, such as hemoglobin and myoglobin (31). The antioxidant effect of Zn is due to its role in preventing the formation of free radicals and protecting against oxidative stress (32). Phosphates are the central building blocks in nucleic acids. P is an important mineral that regulates the fat content in the blood by taking part in carbohydrate and fat metabolism, apart from being in a critical position in important physiological functions including energy production, cellular replication and bone mineral metabolism (2, 29, 33). In our study, when trace elements (Cu, Fe, Zn) and mineral (P) levels were evaluated, the values of P, Zn/Cu, P/Zn and P/Fe were statistically significant between the control group and the group administered 1 ml kg^{-1} CCl_4 (p<0.05; p<0.001; p<0.001) a decrease was detected. Significant decrease in P level (p<0.01) between the 1 ml kg^{-1} CCl_4 applied group and the control group indicates that there is a sign of muscle damage. Because it has been understood that deficiency of P mineral causes muscle weakness (2), and one of the side effects of CCl_4 to the organism is muscle weakness (34). Therefore, it can be interpreted that the increases in protein carbonyl levels in the group administered 1 ml kg^{-1} CCl_4 may cause muscle damage in the organism.

The hepatoprotective herbal preparations recommended as an alternative treatment method for the elimination and prevention of hepatic disorders have been shown to be antioxidant in clinical and experimental studies (35) and black seed (36), nettle seeds studies have been included in the literature. In addition, there are studies in the literature in which CCl_4 and various antioxidants are used both single and multiple times. It has been shown that antioxidants such as ascorbic acid (37), selenium (38), a-tocopherol (39) combined

with CCl_4 prevented or partially protected hepatotoxicity in rats. In the literature review, no study was found on the effects of *Rheum ribes L.* and quercetin on protein carbonyl on liver and kidney tissue damage by causing oxidative stress with CCl_4 in rats. However, when looking at similar studies obtained in our study, Sundari et al. (40) found that oxidative protein accumulation in liver damage by causing oxidative stress with CCl_4 in rats is an early finding in CCl_4-mediated liver damage. Kılıçgün and Altıner (41) investigated the inhibition effect of Rosa canina (rosehip) protein oxidation in rats with liver damage with CCl_4 and found that the plant in question reduced liver protein oxidation.

5. CONCLUSION

Throughout our investigations and analyses, it was observed that oxidative stress increased in rats exposed to CCl_4 and the associated protein carbonyl levels in liver and kidney tissues increased. Considering the liver and kidney tissues between the groups, it was observed that the protective effect of the group administered with 100 mg kg-1 Rheum ribes L on PCO is higher than the group administered with100 mg kg-1 quercetin. These findings show that the protective effect of Rheum ribes L plant on CCl4-induced liver and kidney toxicities. In addition, it can be considered that in further studies, *Rheum ribes L.,* active ingredient dosage and method will be a preliminary study in terms of determining protein oxidation.

REFERENCES

[1] Chevion M, Berenshtein E, Stadtman ER. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. Free Radical Research 2000;33:99-108.

[2] Ferrier DR. Mikrobesinler: Minareller. Lippincott Görsel Anlatımlı Çalışma Kitapları Biyokimya. 2019.p.399 (Turkish)

[3] Davies MJ. Reactive species formed on proteins exposed to singlet oxygen. Photochemical Photobiological Sciences 2004; 3(1): 17-25.

[4] Stadtman ER, Levine RL. Protein oxidation. Annals of the New York Academy of Sciences 2000; 899 (1); 191-208.

[5] Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clinica Chimica Acta 2003; 329(1-2); 23-38.

[6] Purdel NC, Margina D, Ilie M. Current methods used in the protein carbonyl assay. Annual Research Review in Biology 2014;2015-2026.

[7] Requena JR, Levine RL, Stadtman ER. Recent advances in the analysis of oxidized proteins. Amino Acids 2003;25(3-4): 221-226.

[8] Büyükgüzel E. Protein oksidasyonun biyokimyasal ve moleküler mekanizması. Karaelmas Fen ve Mühendislik Dergisi, 2013;3(1):40-51 (Turkish)

[9] Zhang M, Swarts SG, Yin L, Liu C, Tian Y, Cao Y, Ju S. Antioxidant properties of quercetin. Adv Exp Med Biol. 2011;701:283-289.

[10] Alrawaiq NS, Abdullah A. A review of flavonoid quercetin: metabolism, bioactivity and antioxidant properties. International Journal of PharmTech Research 2014;6(3): 933-941.

[11] Baytop T. Türkiye'de Bitkiler ile Tedavi, İstanbul Üniv. Yay. No. 3637, Ecz. Fak. No.40, İstabul, 1984;240-376**. (Turkish)**

[12] Oktay M, Yildirim A, Bilaloglu V, Gülçin I. Antioxidant activity of different parts of isgin (*Rheum ribes L.*). Asian Journal of Chemistry 2007;19(4): 3047.

[13] Andiç S, Tunçtürk Y, Ocak E, Köse S. Some chemical characteristics of edible wild Rhubarb species (Rheum ribes L.). Res J Agric Biol Sci. 2009;5: 973-977.

[14] Naqishbandi AM, Josefsen K, Pedersen ME, Jäger AK. 2009. Hypoglycemic activity of Iraqi Rheum ribes root extract. Pharmaceutical Biology 2009;47(5): 380-383.

[15] Eddouks M, Maghrani M, Zeggwagh NA, Michel JB. Study of the hypoglycaemic activity of Lepidium sativum L. aqueous extract in normal and diabetic rats, J Ethnopharmacology 2005;97: 391–395.

[16] Xia E, Rao G, Van Remmen H, Heydari AR, Richardson A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. The Journal of Nutrition 1994;125(2): 195-201.

[17] Reznick AZ, Packer L. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Methods in Enzymology 1994;233: 357-363.

[18] Slater TF. Activation of carbon tetrachloride: Chemical principle and biological significance. Free radicals, Lipid Peroxidation and Cancer 1982;243-270.

[19] Abraham P, Wilfred G. Oxidative damage to the lipids and proteins pf the lungs, testis and kidney of rats during carbon tetrachloride intoxication. Clinica Chimica Acta; International Journal of Clinical Chemistry 1999;289(1-2): 177-179.

[20] Ruprah M, Mant TGK, Flanagan RJ. Acute carbon tetrachloride poisoning in 19 patients: implications for diagnosis and treatment. The Lancet 1985;325(8436):1027-1029.

[21] Chen H, Tappel AL. Protection of multiple antioxidants against heme protein oxidation and lipid peroxidation induced by $CBrCl_3$ in liver, lung, kidney, heart, and spleen. Journal of Agricultural and Food Chemistry 1996;44(3): 854-858.

[22] Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. BMC Pharmacology 2005;5(1): 2.

[23] Mehmetçik G, Özdemirler G, Koçak-Toker N, Çevikbaş U, Uysal M. Effect of pretreatment with artichoke extract on carbon tetrachloride-induced liver injury and oxidative stress. Experimental and Toxicologic Pathology 2008;60(6): 475-480.

[24] Stadtman ER, Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metabolism Reviews 1998;30(2): 225-243.

[25] Levine RL. Carbonyl assay for determination of oxidatively modified proteins. Methods. Enzymol. 1994;233: 246-257.

[26] Bhadauria M, Nirala SK, Shukla S. Propolis protects CYP 2E1 enzymatic activity and oxidative stress induced by carbon tetrachloride. Molecular and Cellular Biochemistry 2007;302(1-2): 215-224.

[27] Lee CP, Shih PH, Hsu CL, Yen GC. Hepatoprotection of tea seed oil (Camellia oleifera Abel.) against CCl4-induced oxidative damage in rats. Food and hemical Toxicology 2007;45(6): 888-895.

[28] Gooneratne SR, Christensen DA. Effect of chelating agents on the excretion of copper, zinc and iron in the bile and urine of sheep. The Veterinary Journal 1997;153(2): 171-178.

[29] Andersen SM. Vitamins and Minerals in The Traditional Greenland Diet. NERI Technical Report 2005;528.

[30] Kuo YM, Gybina AA, Pyatskowit JW, Gitschier J, Prohaska JR. Copper transport protein (Ctr1) levels in mice are tissue specific and dependent on copper status. The Journal of Nutrition 2006;136 (1): 21-26.

[31] Robach P, Cairo G, Gelfi C, Bernuzzi F, Pilegaard H, Vigano A, Lundby C. Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. Blood 2007;109(11): 4724-4731.

[32] Kasnak C, Palamutoğlu R. Doğal antioksidanların sınıflandırılması ve insan sağlığına etkileri. Turkish Journal of Agriculture-Food Science and Technology 2015;3(5): 226-234. (Turkish)

[33] Tran L, Batech M, Rhee CM, Streja E, Kalantar-Zadeh K, Jacobsen SJ, Sim JJ. Serum phosphorus and association with anemia among a large diverse population with and without chronic kidney disease. Nephrology Dialysis Transplantation 2015;31(4): 636-645.

[34] Pope AM, Rall DP. Environmental medicine*:* İntegrating a missing element into medical education.1995

[35] Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research 2010;3(1): 91-100.

[36] İlhan N, Seçkin D. Protective effect of Nigella sativa seeds on CCl_4-induced hepatotoxicity. FÜ Sağlık Bil Dergisi, 2005;19(3): 175-179.

[37] Bayram I, Özbek H, Uğraş S, Tuncer I, Reçber D. Askorbik asit ve alfatokoferol'ün karbon tetraklorürle oluşturulmuş akut karaciğer toksisitesi modelinde karaciğeri koruyucu etkisi. Van Tıp Dergisi 2004; 11: 32-38 (Turkish).

[38] Şahin A, Yener Z, Dağoğlu G. Karbon tetraklorür (CCl_4) ile deneysel olarak karaciğer nekrozu oluşturulan ratlarda Vitamin E + Selenyum ve Nigella sativa'nın karaciğer yıkımını engelleyici etkileri. Turkish Journal of Veterinary and Animal Sciences 2003; 27: 141-152. (Turkish).

[39] Klebanoff SJ. Myeloperoxidase: Friend and foe. J Leukoc Biol 2005; 77(5): 598 – 625.

[40] Sundari PN, Wilfred G, Ramakrishna B. Does oxidative protein damage play a role in the pathogenesis of carbon tetrachloride-induced liver injury in the rat? Biochim Biopyis Acta 1997; 1362: 169-176.

[41] Kılıçgün H, Altıner D. Karbontetraklorür ile karaciğer hasarı oluşturulmuş sıçanlarda Rosa canina'nın (kuşburnu) in vivo antioksidan Etkisi. Fen Bilimleri Dergisi, 2009;30(2): 10-16 (Turkish)

How to cite this article: Bakir A, Ekin S, Oztas S, Oto G. The Protective Effect of Rheum Ribes L., and Quercetin on Protein Carbonyl Levels Against Carbon Tetrachloride-Induced Liver and Kidney Damage in the Rats. Clin Exp Health Sci 2022; 12: 587-593. DOI: 10.33808/clinexphealthsci.943255

Clinical and Experimental Health Sciences

Investigation of Therapeutic Ultrasound Dose on Muscle Phantom: An Experimental Study Investigation of Therapeutic Ultrasound Dose

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Received: 13.06.2021 **Accepted:** 02.11.2021

ABSTRACT

Objective: The study aims to investigate the standardized, traceable dose amounts that will create the desired therapeutic physiological change in the tissue and prevent the risk of tissue damage in the light of metrology principles

Methods: In the study carried out in TÜBİTAK National Metrology Institute Medical Metrology Laboratory, a muscle phantom simulating the acoustic properties of muscle tissue was created and thermocouples were placed in it. Ultrasound at different intensities and durations at 1 MHz frequency was applied to the phantom. The temperature changes measured by the thermocouples were recorded. Each measurement was repeated three times and averaged

Results: 14 minutes application at 1 W/cm^2 density, 10 minutes application at 1.5 W/cm^2 density, ~7 minutes application at 2 W/cm^2 density, and ~4 minutes application at 2.5 W/cm^2 density have been achieved for the temperature range needed to produce therapeutic effect.

Conclusions: In order to achieve the therapeutic effect, the ultrasound doses used in the procedure should be checked. Measurements in multi-layer phantoms would be useful in future research.

Keywords: Therapeutic ultrasound, muscle phantom, dose, metrology.

1. INTRODUCTION

Therapeutic ultrasound is an accepted therapeutic agent in reducing pain and spasm in tissues. It is a high-frequency current that is preferred because of its thermal and mechanical effects on deep tissues, and it is a frequently used, easy-to-apply, and economical electrotherapy modality (1–3). As a result of the absorption of ultrasound in the tissues, mechanical vibration and sonic energy are transformed into heat energy in proportion to the intensity of ultrasound. Due to the increase in temperature in the tissue, circulation increases, the inflammatory process improves and an analgesic effect occurs (4). To achieve the beneficial therapeutic effect, the tissue temperature must be kept between 40-45°C for at least 5 minutes. Destructive consequences occur when the local tissue temperature exceeds 45°C (5)

Despite its widespread use, there are conflicting results in the literature regarding the therapeutic effect of therapeutic ultrasound. In previous studies to investigate the effectiveness of ultrasound, the duration of application differed, a standard dose data was not found, and quantitative information supporting the effectiveness of application protocols recommended for different clinical conditions was not presented.(6–10) Treatment plan is based on the clinician's individual experience. As a result, therapeutic ultrasound applications do not include standardized, comparable dose concepts supported by traceable measurements. The lack of accurate dose information makes it difficult to determine dose-response curves and to create effective treatment plans (11). This can lead to excessive or inadequate treatment of the tissue and even to the patient's harm.

Test materials that can describe the special characteristics of human tissues in ultrasound applications are called phantoms (12). It is used to test US systems or to investigate the interaction of sound waves with tissue. There are many phantoms in the literature that mimic human tissue. To develop measurement techniques and validate theoretical models, tissue-mimicking phantoms should have acoustic and thermal properties equivalent to human tissues Speed

DOI: 10.33808/clinexphealthsci.950896

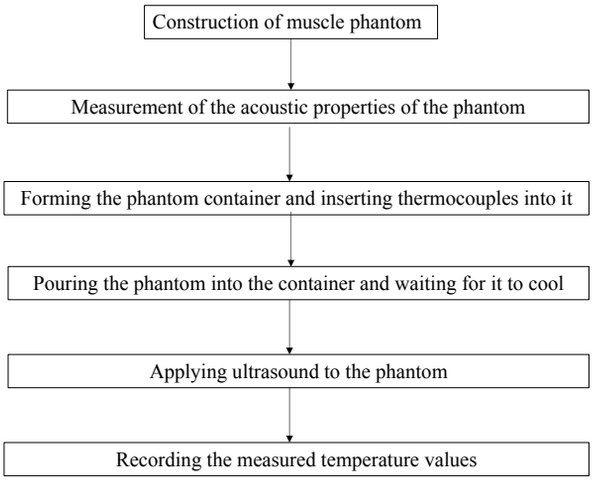
of sound, attenuation coefficient, and acoustic impedance of phantom and the tissues must be similiar. (11,13,14)

This study was planned due to the lack of a clear framework for therapeutic US applications in the literature. The aim of the study is to investigate the standardized, traceable dose amounts of therapeutic ultrasound that will create the desired therapeutic physiological change in the tissue and prevent the risk of tissue damage with reference to the temperature increase on the muscle phantom in the light of metrology principles.

2. METHODS

This research was conducted in the TÜBİTAK Metrology Institute Medical Metrology Laboratory from April 8, 2018 to December 31, 2020. The study was conducted under the conditions required for the competence of the test and calibration laboratories. All instruments to be used in measurements were calibrated at first. The algorithm with the study progress steps is given in Table 1.

Table 1. *Study algoritm*



2.1. Construction of Muscle Phantom and Measurement of the Acoustic Properties

Materials to be used in phantom construction were prepared and their masses were measured. Agarose powder (A9539-500G Sigma-Aldrich, USA), degassed water, aluminum powder Al2O3 (Nanokar, Turkey), and glycerin (Cleanmaster, Turkey) were used to prepare the muscle phantom.

Speed of sound, attenuation coefficient, and acoustic impedance are the most essential acoustic properties of soft tissue phantoms. The attenuation coefficient and speed of sound of the phantom was then measured ten times and averaged. In the speed of sound and attenuation coefficient measurements, a digital storage oscilloscope (Tektronix TDS 2002C, USA) and a pulser receiver (Panametrics Model 5052 PR, USA) were used, as well as 1 MHz probes (M639 SMN2M5, Meccasonics LTD, UK). Calculations of density and acoustic impedance were carried out. Uncertainty calculations are important in determining how much information is missing from a measurement result JCGM 100: 2008 GUM was used to guide the uncertainty measurements (15). The factors affecting the speed of sound and attenuation coefficient measurements results were identified. The components of the uncertainty budget were determined. Measurement uncertainty was calculated. It was possible to calculate expanded uncertainty, U, by taking the square root of the number of the variances and applying a coverage factor k=2, which corresponds to a coverage probability of approximately 95% for normal distribution.

2.2. Preparation of Phantom Container and Insertion of Thermocouples

A phantom container of 58x108x74 mm was drawn with the SolidWorks application. The center of the area where the ultrasound will be applied was determined. The spaces where the thermocouples will be placed were drawn at a depth of 3 cm from the phantom surface, to the center of the application area and 1cm and 2cm from this point. Then the phantom was removed from the 3D printer (Zaxe X1-Plus ZAXE) and 3 connectable 10 kOHM NTC type thermocouples were placed in the spaces. Thermocouples are directly connected to the temperature measurement device

2.2.1. Pouring the Phantom Into the Container

The level where the muscle phantom will be pour was marked on the container. The prepared muscle phantom was poured into the container where the thermocouples were placed and left to cool.

2.3. Dose Calculations

Acoustic dose is defined as the energy deposited per unit mass of a medium supporting an acoustic wave (16). The minimal amount of energy that was determined to be beneficial for therapeutic ultrasound was 2250 Joules each session. For the calculation of the energy required during the ultrasound application, given formula is used (17).

Total energy per session (Joules) = Intensity (W/cm^2) x time (seconds) x US head size (cm)

2.4. Applying Ultrasound to the Phantom

Before the measurements, it was checked whether the device to be used in the study applies the power that appears on the it's screen.. The device used was PUG, a portable ultrasonic power meter created by TÜBTAK UME Medical Metrology Laboratory. Then phantom container placed in the experimental setup.

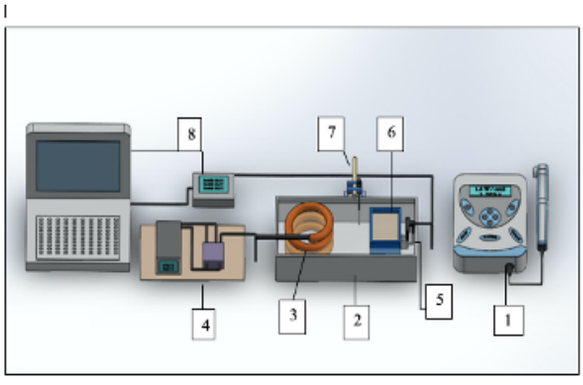
Multi-Channel Temperature Measurement Device (Model 1, 2019) which provides its energy via USB, was connected to the computer. Data can be read and commands can be entered

on the screen. The software of the Temperature Measurement Device was developed in TÜBİTAK UME Medical Metrology Laboratory, and the data can be transferred to Excel as raw data from this software interface (https://www.ume.tubitak.gov.tr/en/laboratuvarlarimiz/projects-18, accessed: 25.09.2021). They can be read instantly directly from the screen display.

To mimic human muscle tissue, the phantom must be held at 37°C (18) . A temperature system was created to bring the phantom's temperature to 37°C. The thermocouple measuring the temperature of the system working with electricity was prepared using the heating resistor that provides heating and prevents the temperature from dropping below 37°C and the screen showing the set temperature

In the measuring setup, a phantom cup containing thermocouples was mounted. The phantom was fitted with the apparatus (with the size of two times of the transducer head) prepared in accordance with the effective radiating area (ERA). With the temperature control mechanism, the phantom's temperature was raised to 37 °C. The 5 cm ultrasound head of the ultrasound device with an ERA of 4 was used (Chattanooga Intelect Mobile Ultrasound).

In line with the times calculated by the dose measurements, ultrasound was applied at a frequency of 1 MHz and in continuous mode. The application started when the value measured by the thermocouples showed 37°C. The ultrasound head was moved at a speed of about 4 centimeters per second. Degassed water was used as coupling agent. The value measured by the thermocouples was recorded by applying at a density of 1, 1.5, 2, 2.5 watt / cm^2 for different durations. Each measurement was taken three times and the average was calculated. The temperature of the phantom was expected to decrease to 37 ° C after each measurement. The therapeutic dose was described as the intensity and duration that enabled the temperature to rise above 40 ° C for 5 minutes. The parameters that will affect the results of the temperature measurements were determined and the uncertainty value of the temperature measurement results was calculated.the experimental set up is given in figure 1



1. Ultrasound Device
2. Termal Equiliubrium Pool
3. Heater
4. Temperature Control System
5. Thermocouples
6. Muscle phantom
7. Thermometer
8. Temperature Record System

Figure 1. *Temperature measurement set up by the ultrasound application*

3. RESULTS

3.1. Results of Measurement of Acoustic Parameters of Muscle Phantom

The mass ratios of the materials used in phantom construction are given in Table 2. The acoustic parameters of the phantom we constructed are reported in Table 3. The acoustic properties of muscle tissue are included in the Table 3 too (19).

Table 2 *Materials and mass ratios used in phantom construction*

Material	Composition ratios (%) – by mass
Distilled Water	81.5
Agarose	1.5
Aluminum Powder (Al_2O_3 0,3µm)	7
Glycerin	10

Table 3. *Acoustic parameter of muscle phantom and muscle tissue*

Acoustic parameter	Result of measurement (mean± standard deviation)	Muscle tissue (19)
Speed of sound (m/s)	1549.8±3.89	1547
Attenuation coefficient (dB/cm MHz)	1.14 ±0.08	1.09
Acoustic impedance (MRayl)	1.632	1.62
Density (kg/m^3)	1053.5	1050

3.2. Results of Temperature Measurement with Ultrasound Application

Temperature data depending on different time and intensity after ultrasound applications are given below. In al figures T1, T2 and T3 show the temperatures measured by the thermocouples.

T1. Thermocouple in the center of the application area

T2. Thermocouple placed 2 cm away from T1

T3. Thermocouple placed 3 cm away from T1

The temperature changes caused by the application of ultrasound at 1Mhz frequency and 1 W/cm^2 intensity in the phantom depending on time are given in Figure 2.

In the 10-minute and 12-minute applications, the temperature increased to 40 °C but in only 14 minutes of application, it remained above 40 °C for 5 minutes.

Figure 3 shows the temperature changes in the phantom generated by ultrasound at 1Mhz frequency and 1.5 W/cm2 intensity as a function of time.

In 7 minutes of application, the temperature rise is approximately 2 °C. The temperature did not rise above 40 °C. In the 8-minute application, the phantom temperature rose above 40 °C after approximately 7 minutes of the application and remained above 40 °C until the 11th minute. In the

10-minute application, the phantom temperature remained above 40 °C for more than ten minutes.

Figure 4 depicts the temperature fluctuations in the phantom as a function of time caused by ultrasound at 1Mhz frequency and 2 W/cm^2 intensity.

According to the graphic data in Figure 4, the peak temperature value of the 6-minute application is 39.2 °C. With the 7-minute ultrasound application, the phantom temperature reached 40 °C in the 6th minute. When it drops below 40°C again, it is ten minutes. After 8 minutes of application, the time it stays above 40°C is more than ten minutes.

The temperature changes caused by the application of ultrasound at 1Mhz frequency and 2.5 W/cm^2 intensity in the phantom depending on time are given in Figure 5.

As seen in Figure 5, an increase of 2.45°C was achieved with a 3-minute application. In the 4-minute application, the phantom temperature remained above 40 °C for approximately four and a half minutes. In the 5-minute application, the temperature remained above 40 °C for five minutes.

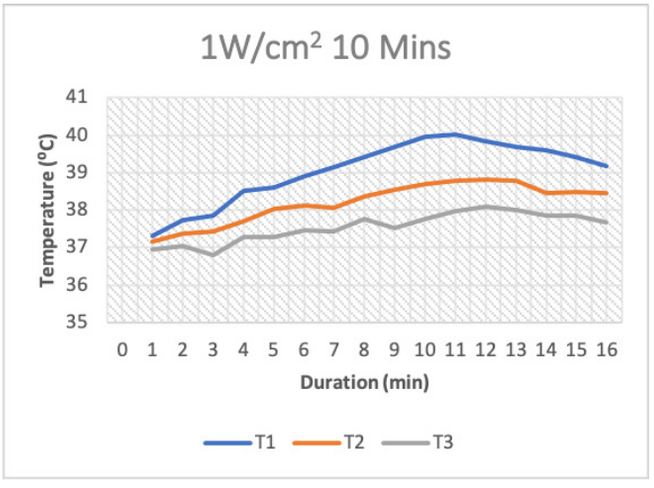


Figure 2A

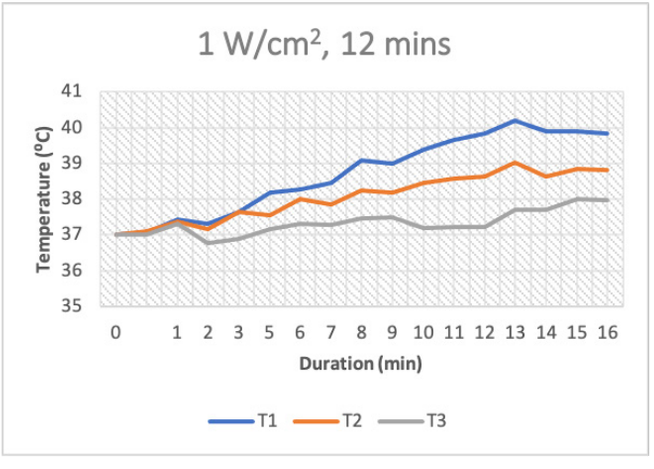


Figure 2B

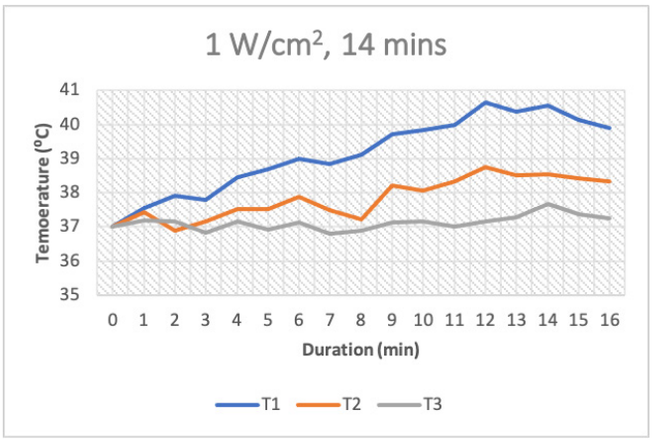


Figure 2C.

Figure 2. *Time-dependent temperature changes at a density of 1 W/cm2. A. At 10 mins application B. At 12 mins application C. At 14 mins application*

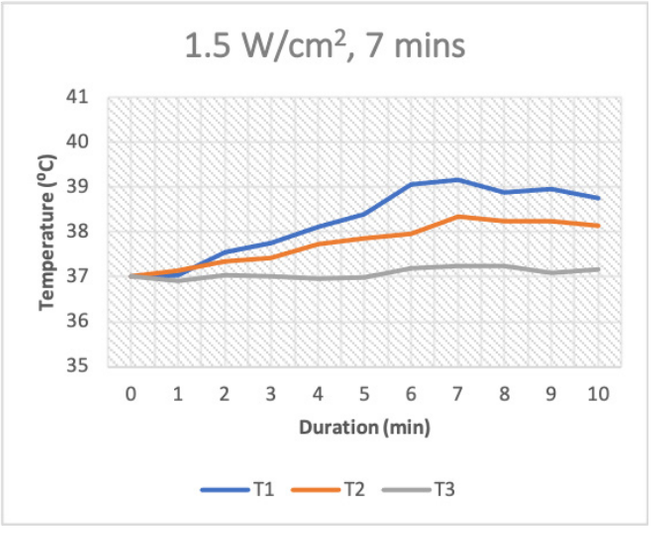


Figure 3A

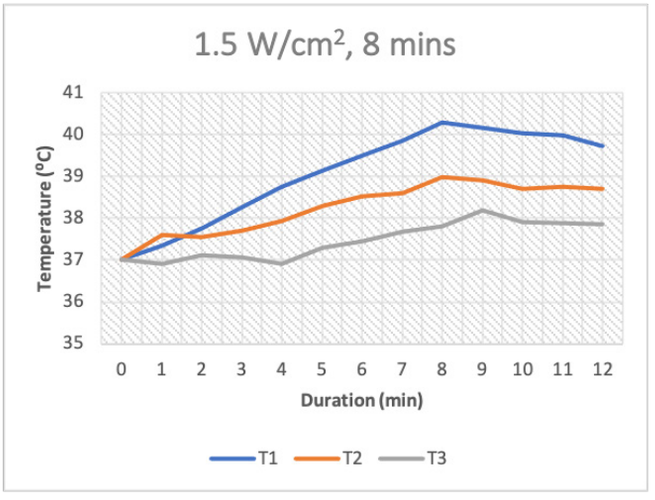


Figure 3B.

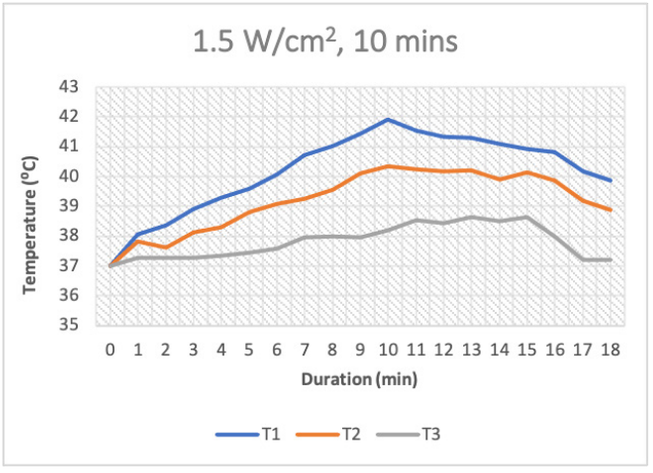


Figure 3C.

Figure 3. *Time-dependent temperature changes at a density of 1,5 W/cm2 A. At 7 mins application B. At 8 mins application C. At 10 mins application*

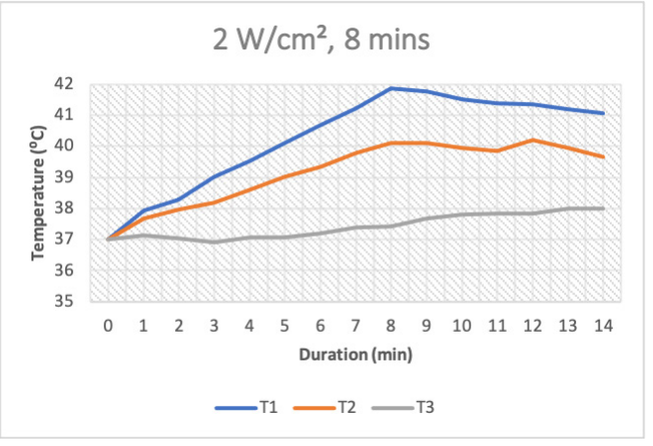


Figure 4C.

Figure 4. *Time-dependent temperature changes at a density of 2 W/cm2 A. At 6 mins application B. At 7 mins application C. At 7 mins application*

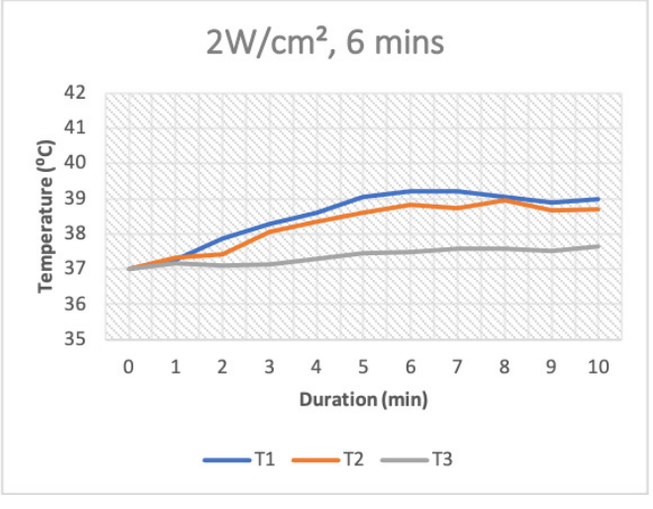


Figure 4A.

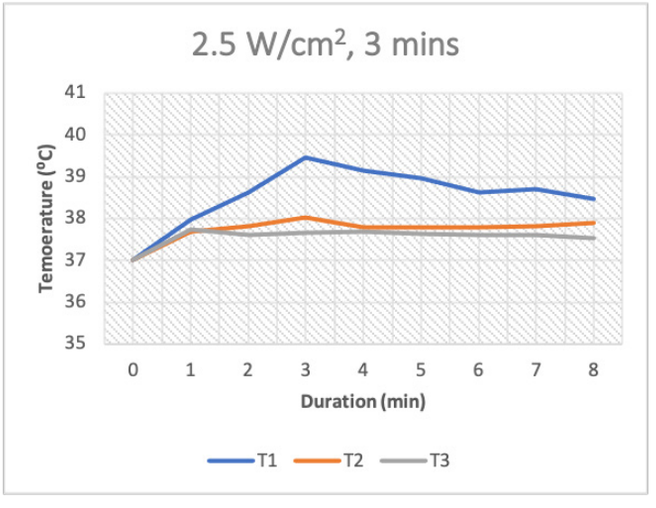


Figure 5A.

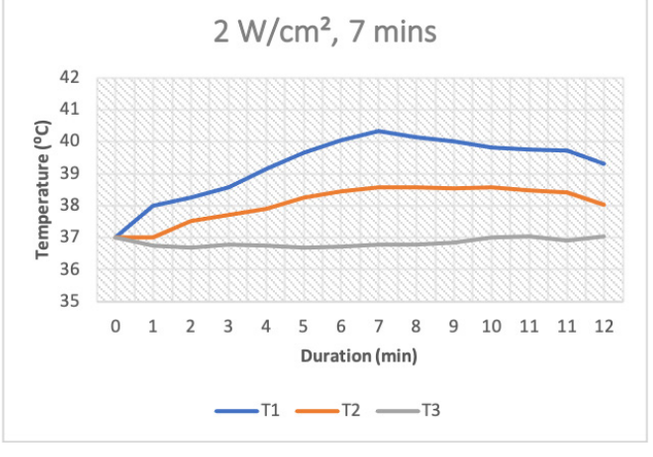


Figure 4B.

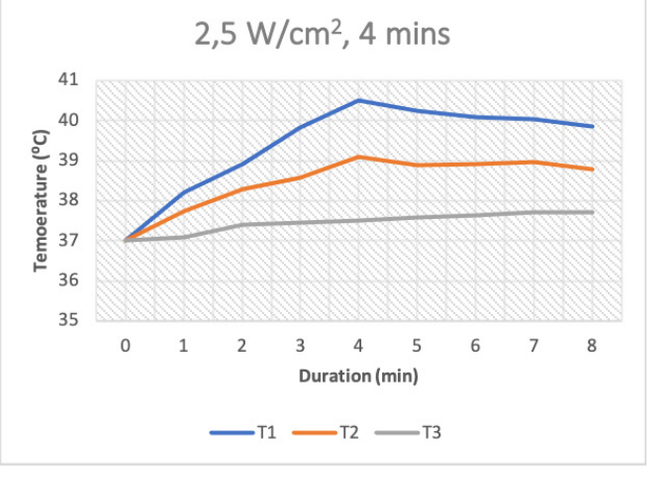


Figure 5B.

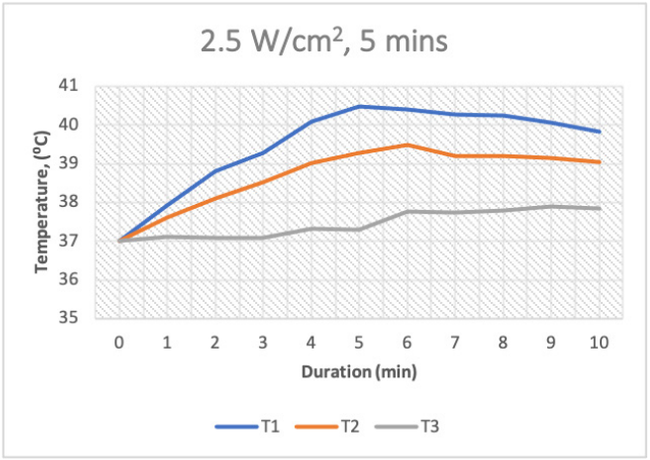


Figure 5C.

Figure 5. *Time-dependent temperature changes at a density of 2,5 W/cm2 A. At 3 mins application B. At 4 mins application C. At 5 mins application*

Depending on the applied density, the times required for the therapeutic effect at 1 MHz frequency are given in the Table 4

Table 4. *Intensity and time at which the therapeutic effect occurs*

Intensity (W/cm²)	Time (min)
1	14
1,5	8
2	6-7
2,5	4-5

3.4. Temperature Measurements' Uncertainty

The sources of standard uncertainty and the resulting combined standard uncertainty of temperature measurements during ultrasound application are listed in Table 5.

Table 5. *Uncertainty Budget for Temperature Measurements*

Sources of uncertainty	Error%	Probability Distribution	Divisor	Uncertainty%
Error of measurement system (Thermocouple system)	0.02	Normal	2	0.01
Environment temperature change and fluctation	0.05	Rectangular	1.73	0,03
Probe positionning error	0.08	Rectangular	1.73	0.05
Water temperature change	0.02	Rectangular	1.73	0.01
Thermocouple positioning	0.09	Rectangular	1.73	0.05
Distance change between probe-thermocouple	0.08	Rectangular	1.73	0.05
Repeatibility	0.09	Rectangular	1.73	0.05
Reproducebility	0.10	Rectangular	1.73	0.06
Loss of phantom's properperties	0.10	Rectangular	1.73	0.06
Combined Uncertainty				**0.13**
Expanded Uncertainty= U (k=2)				**0.26**

4. DISCUSSION

In light of the principles of metrology, the study intends to investigate the standardized, traceable dose quantities that will produce the required therapeutic physiological change in the tissue while avoiding the danger of tissue harm. For this purpose, phantom simulating muscle tissue was created and its acoustic properties were measured. Then, 1, 1.5, 2, 2.5 Watt/cm² intensity ultrasound was applied to this phantom at a frequency of 1 MHz and the time to create a therapeutic effect was determined.

Phantom construction is guided by Gutierez et al. (20). They have used graphite powder as a scatter agent in their work but we used aluminum powder. The scattering ratio used was changed until the phantom best reflected the acoustic properties of the muscle tissue. Phantom's speed of sound, attenuation coefficient was measured ten times and averaged. Mast's data were used as a basis for our research (19). The speed of sound was 1549.8±3.8 m/s (U=6.7), which was consistent with the Mast's data, 1547 m/s. According to Mast , the attenuation coefficient of muscle tissue is 1.09 dB / cm MHz, while various values such as 1.1 dB /cm MHz have been reported in the literature (21). These figures are similar to the attenuation coefficient we measured at the end of the investigation, 1.14±0.8 dB / cm MHz (U =0.55). The acoustic impedance and density of muscle tissue, according to Mast, are 1630 MRayl and 1050 kg/m3, respectively. The muscle phantom's acoustic impedance (1.632 MRayl) and density (1053,5 kg/m³) values were computed extremely near to the reference values. The table does not include the standard deviation in acoustic impedance and density calculations because it is so small. this phantom is appropriate for use in ultrasonic measurements.

1 MHz and 3 MHz frequencies are used in therapeutic ultrasound applications. Since the muscle tissue is located deeper, 1 MHz frequency application, which penetrates deeper, is preferred. There are different data on the amount of depth 1MHz penetrates. Watson states that 1 Mhz penetrates to an average of 4 cm depth, since tissue densities are different in humans (22). Cisowska-Adamiak et al showed that 1 MHz, has a half-depth penetration in the muscle of approximately 1.2 cm (23) As a result of the experiments in which we evaluated the temperature increase at different depths before starting the this study, we noted that the maximum temperature increase was 3 cm. Therefore tissue temperatures were measured at 3 thermocouple locations at 3 cm depths from the phantom surface.

One of the reasons for the use of continuous ultrasound is to increase the temperature that will create a biological effect in the tissue. Contrary to its name, thermal dose is time dependent (16) Therefore, the formula suggested by Hougton was used. The application time to meet the amount of energy required for effective treatment was calculated by placing the density and area of the device head into the formula. Ultrasound was applied to the phantom at times below and above the value found as a result of the calculations and temperature changes were observed.

The temperature measurement system has a measurement error of ±0.2 °C. The system takes a measurement every two seconds. The graphs show changes on a minute basis. The deviations seen in the graphs are thought to be caused by these factors. The application head movement was made to be approximately 4 cm/sec. No timer was used since the head movement speed had no effect on the temperature increase and all applications were made by the same person (24). Again, since the coupling agent used during the application did not affect the temperature change, water, which did not cause deterioration on the phantom surface, was used instead of gel (25).

It has been observed that the time required to exceed 40 °C at the end of 1W/cm^2 application should be 10 minutes and the application time should be 14 minutes for the therapeutic effect to ocur, in this study. Myrer et al. placed a thermocouple in the posterior calf and applied 1 W/cm^2 ultrasound for 10 minutes and showed that the temperature increased by about 6 °C (26). Draper et al. recorded a temperature increase of 3.5 °C in the medial triceps surae muscle at the same dose (27). In our study, an increase of 3.03 °C (U=0.26) was achieved after 10 minutes of application. This difference is thought to be caused by skin and adipose tissue in vivo studies.

As a result of our experiments, the time required for a therapeutic effect in 1.5 W/cm^2 applications was found to be approximately 8 minutes. It has been shown in previous studies that ultrasound is not effective in lower duration applications (28) Ebadi et al in which they investigated the effectiveness of ultrasound in patients with frozen shoulder, they applıed to the patients ultrasound for 6 minutes at an intensity of 1.5 W/cm^2. They showed that ultrasound did not have any effect on pain, range of motion and function (7). Yıldırım et al. compared the results of 4 and 8 minutes of application and concluded that 8 minutes of application was more effective in reducing pain and increasing the ability to carry out activities of daily living in patient with knee osteoarthritis. (29). These data support the results of our study, although it shows that the application times in the previous studies should be reviewed.

Test results from this study show that the time required for the application of 2 W/cm^2 is approximately 7 minutes. In the application of 2 W/cm^2, the rate of temperature increase in the tissue is 0.38 per minute (30). When we look at the graphic data given in the figure, it is seen that this speed is 0.43 in our study. This difference is considered by our study made from a single-layer pantom. But measurement uncertainty must also be taken into account.

Although it was stated that therapeutic ultrasound applications were performed at an intensity of 0.2-3 W/cm^2, it was seen that the intensity of 2.5 was not preferred in the study. If this application is to be preferred, according to the results of our data, it is thought that the application of 4 minutes at this intensity is more reliable and the applications performed over a period of time may cause tissue damage.

Since ultrasound applications are used in musculoskeletal problems, muscle phantom was preferred. Although the acoustic values of the muscle phantom meet the values given in the literature for soft tissue (including skin, fat, fascia, muscle), it would be more appropriate to use multi-layered phantoms in order to be more precise and simulate the human body in the best way (19). In this respect, the use of a single-layered phantom is seen as a limitation of our study. In this respect, the use of a single-coil phantom is seen as a limitation of our study.

5. CONCLUSION

In this study, the doses required to reach the temperatures that will reveal the therapeutic effect in ultrasound applications were investigated. This study is experimental study and it was made in accordance with metrology principles in terms of determining the factors affecting the measurement results and giving the uncertainty value. Therefore, the results can be considered as reliable. In order to achieve the therapeutic effect, the ultrasound doses used in studies should be checked.

Phantoms are more used in high intensity focused ultrasound applications. In this regard, they can be used also in measuring the efficıency of the physiotherapy devices as well as for dosing in physiotherapy**.** Measurements in multi-layer phantoms would be useful in future research.

Conflict of interest: The research is not financed, and the authors have no conflicts of interest to report.

REFERENCES

[1] Baker KG, Robertson VJ, Duck FA. A Review of Therapeutic Ultrasound : Phys Ther [Internet]. 2001 Jul 1 [cited 2020 Jan 6];81(7):1351–8. Available from: https://academic.oup.com/ptj/article-abstract/81/7/1339/2857679

[2] Lioce EEAN, Novello M, Durando G, Bistolfi A, Actis MV, Massazza G, et al. Therapeutic Ultrasound in Physical Medicine and Rehabilitation: Characterization and Assessment of Its Physical Effects on Joint-Mimicking Phantoms. Ultrasound Med Biol. 2014;40(11):2743–8.

[3] Rodriguez E, Ramírez Ramírez C. Effects of therapeutic ultrasound on pain intensity and quality of life in persons with knee osteoarthritis. Physiotherapy. 2015;101.

[4] Paliwal S, Mitragotri S. Therapeutic opportunities in biological responses of ultrasound. Vol. 48, Ultrasonics. 2008. p. 271–8.

[5] Watson T. Ultrasound in contemporary physiotherapy practice. Ultrasonics. 2008;48(4):321–9.

[6] Balci TO, Turk AC, Sahin F, Kotevoglu N, Kuran B. Efficacy of therapeutic ultrasound in treatment of adhesive capsulitis: A prospective double blind placebo-controlled randomized trial. J Back Musculoskelet Rehabil. 2018;31(5):955–61.

[7] Ebadi S, Forogh B, Fallah E, Babaei Ghazani A. Does ultrasound therapy add to the effects of exercise and mobilization in frozen shoulder? A pilot randomized double-blind clinical trial. J Bodyw Mov Ther [Internet]. 2017;21(4):781–7. Available from: https://doi.org/10.1016/j.jbmt.2016.11.013

[8] Rehman U, Rehman S. Effectiveness of Mobilization with Movement with and without Therapeutic Ultrasound in Rehabilitation of Idiopathic Adhesive Capsulitis. J Riphah Coll. 2013;1(1).

[9] Cakir S, Hepguler S, Ozturk C, Korkmaz M, Isleten B, Atamaz FC. Efficacy of Therapeutic Ultrasound for the Management of Knee Osteoarthritis. Am J Phys Med Rehabil. 2014;93(5).

[10] Zhang C, Xie Y, Luo X, Ji Q, Lu C, He C, et al. Effects of therapeutic ultrasound on pain, physical functions and safety outcomes in patients with knee osteoarthritis: A systematic review and meta-analysis. Clin Rehabil. 2016;30(10).

[11] Status R. Publishable JRP Summary Report for JRP HLT03 DUTy Dosimetry for ultrasound therapy. 2012;(March):2–3.

[12] Vardaki MZ, Kourkoumelis N. Tissue Phantoms for Biomedical Applications in Raman Spectroscopy: A Review. Biomed Eng Comput Biol. 2020 Jan;11:117.959.722094810.

[13] Zeqiri B, Scholl W, Robinson SP. Measurement and testing of the acoustic properties of materials: A review. Metrologia. 2010;47(2).

[14] Culjat MO, Goldenberg D, Tewari P, Singh RS. A review of tissue substitutes for ultrasound imaging. Ultrasound Med Biol. 2010;36(6):861–73.

[15] Joint Committee For Guides In Metrology. Evaluation of measurement data — Guide to the expression of uncertainty in measurement. Int Organ Stand Geneva ISBN [Internet]. 2008;50(September):134. Available from: http://www.bipm.org/en/publications/guides/gum.html

[16] Hpa. Health Effects of Exposure to Ultrasound and Infrasound Report of the independent Advisory Group on Non-ionising Radiation [Internet]. Environmental Hazards. 2010. Available from: http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/126.502.8759717

[17] Alexander LD, Gilman DRD, Brown DR, Brown JL, Houghton PE. Exposure to Low Amounts of Ultrasound Energy Does Not Improve Soft Tissue Shoulder Pathology: A Systematic Review. Phys Ther. 2010;90(1):14–25.

[18] González-Alonso J, Quistorff B, Krustrup P, Bangsbo J, Saltin B. Heat production in human skeletal muscle at the onset of intense dynamic exercise. J Physiol. 2000;524(2):603-15.

[19] Mast TD. Empirical relationships between acoustic parameters in human soft tissues. Acoust Res Lett Online. 2000;1(2000):37–42.

[20] Gutierrez MI, Lopez-Haro SA, Vera A, Leija L. Experimental Verification of Modeled Thermal Distribution Produced by a Piston Source in Physiotherapy Ultrasound. Biomed Res Int. 2016;2016.

[21] Singh RS, Culjat MO, Grundfest WS, Brown ER, White SN. Tissue mimicking materials for dental ultrasound. J Acoust Soc Am. 2008;123(4):EL39–44.

[22] Watson T. Therapeutic Ultrasound. http://www.electrotherpy.org [Internet]. 2015;(2012):1–18. Available from: http://www.electrotherapy.org/assets/Downloads/Therapeutic_Ultrasound_2015.pdf%0Ahttp://www.electrotherapy.org

[23] Cisowska-Adamiak M, MacKiewicz-Milewska M, Szymkuć-Bukowska I, Hagner W, Beuth W. Ultrasound therapy: Dose-dependent effects in LBP treatment. J Back Musculoskelet Rehabil. 2019;32(2):339–43.

[24] Weaver SL, Demchak TJ, Stone MB, Brucker JB, Burr PO. Effect of Transducer Velocity on Intramuscular Temperature During a 1-MHz Ultrasound Treatment. J Orthop Sport Phys Ther [Internet]. 2006;36(5):320–5. Available from: http://www.jospt.org/doi/10.2519/jospt.2006.2157

[25] Gulick DT, Ingram N, Krammes T, Wilds C. Comparison of tissue heating using 3 MHz ultrasound with T-Prep® versus Aquasonic® gel. Phys Ther Sport. 2005;6(3).

[26] Myrer JW, Measom GJ, Fellingham GW. Intramuscular Temperature Rises with Topical Analgesics Used as Coupling Agents during Therapeutic Ultrasound. J Athl Train. 2001;36(1):20–6.

[27] Gallo JA, Draper DO, Brody LT, Fellingham GW. A comparison of human muscle temperature increases during 3-MHz continuous and pulsed ultrasound with equivalent temporal average intensities. J Orthop Sports Phys Ther. 2004;

[28] Analan PD, Leblebici B, Adam M. Effects of therapeutic ultrasound and exercise on pain, function, and isokinetic shoulder rotator strength of patients with rotator cuff disease. J Phys Ther Sci. 2015;27(10).

[29] Yildiriim MA, Uçar D, Öneş K. Comparison of therapeutic duration of therapeutic ultrasound in patients with knee osteoarthritis. J Phys Ther Sci. 2015;27(12).

[30] Draper DO, Ricard MD. Rate of Temperature Decay in Human Muscle Following 3 MHz Ultrasound: The Stretching Window Revealed. J Athl Train. 1995;

How to cite this article: Gun N, Karaboce B, Yurdalan SU. Investigation of Therapeutic Ultrasound Dose on Muscle Phantom: An Experimental Study Investigation of Therapeutic Ultrasound Dose. Clin Exp Health Sci 2022; 12: 594-601. DOI: 10.33808/clinexphealthsci.950896

Clinical and Experimental Health Sciences

VDBP and VDR Mutations May Cause In-Stent Restenosis

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Received: 17.06.2021 **Accepted:** 06.03.2022

ABSTRACT

Objective: In-stent restenosis (ISR) is the narrowing of a stented coronary artery lesion. A considerable number of patients undergoing percutaneous coronary intervention (PCI) are affected by ISR. The predominant mechanism in the development of ISR is an inflammatory response to vessel wall injury during PCI. Vitamin D is reported to have anti-inflammatory properties, so it may also be related with ISR. Therefore, in this study the relationship between vitamin D receptor *(VDR)*, vitamin D binding protein *(VDBP)* gene variations and ISR were investigated.

Methods: Fifty-eight ISR patients who have chest pain, underwent angiography and were found to have restenosis in the previously inserted stent were included in the patient group and thirty-five patients who have chest pain and were not found to have restenosis in their previous stent in coronary angiography were included in the control group. rs7041 and rs4588 variations in *VDBP*; rs1544410 and rs2228570 variations in *VDR* were investigated by real-time polymerase chain reaction (RT-PCR). Results were evaluated statistically.

Results: The CC genotype of rs2228570 variation of *VDR* and the CA genotype of rs4588 variation of *VDBP* were found statistically high in patient group. rs7041 variation was found statistically high in patients who had myocardial infarction history before stent implantation. Additionally, it was demonstrated that vitamin D deficiency (vitamin D level<20 ng/ml) was found statistically high in patient group.

Conclusion: It was considered that rs2228570, rs4588 variations and the presence of vitamin D deficiency may play role in the formation of ISR.

Keywords: ISR, *VDR, VDBP*, RT-PCR

1. INTRODUCTION

Coronary artery disease (CAD) is a complex cardiovascular disorder which is related to pathophysiologic conditions, some environmental and genetic factors (1). Percutaneous coronary intervention (PCI) by balloon angioplasty and stenting have participated in the treatment of CAD, and have provided improvement in acute myocardial infarction (MI) (2). Unfortunately, a considerable number of patients undergoing PCI are affected by ISR (3-5). In-stent restenosis (ISR) was defined angiographically as the presence of >50% diameter stenosis at the stent site or within 10 mm proximal or distal to the stent. It is a complex disease considered to derive from several causative mechanisms, which have yet to be fully defined. The predominant mechanism in the development of ISR is an inflammatory response to vessel wall injury during PCI (6). Vitamin D is also reported to have anti-inflammatory properties, so it may also be related with ISR. Until now, vitamin D deficiency has been shown as a common risk factor for cardiovascular disease in several studies (7-9). 1,25-dihydroxyvitamin D (1,25$[OH]_2$D), the active form of vitamin D, binds to vitamin D binding protein (VDBP) in order to transport vitamin D metabolites to target tissues. VDBP binds to vitamin D receptor (VDR) to produce a biological effect (10). 1,25$[OH]_2$D acts through a specific VDR, which is a hormone structure that communicates with the vitamin D responsive element of several target genes and regulates the transcription of more than 200 heterogeneous genes. Therefore, vitamin D have crucial roles in the regulation of vascular smooth muscle cell proliferation, cell differentiation, vascular calcification, and angiogenesis (4, 7, 8, 11). It has been suggested that some candidate gene polymorphisms involved in the process of ISR are also associated with vitamin D metabolism (12).

DOI: 10.33808/clinexphealthsci.953893

The gene encoding human *VDR* is located on chromosome 12q12-q14, and has many common allelic variants (13). Two of the most common polymorphisms are named as *VDR FokI* (rs2228570) and *BsmI* (rs1544410) (14). Until now, only some studies have investigated the relationship between rs2228570, rs1544410 SNPs, vitamin D levels and the risk of CAD (15). To our knowledge these mutations were not investigated before for ISR. *FokI* polymorphism, which is located in exon 2 of a gene, identifies 2 translation initiation start sites (ATG) in the *VDR*. T>C polymorphism leads to shortening of the protein by three amino acids (16). Studies also show that *FokI* is a potential genetic marker for CAD. *Bsm*I (A>G) is located in intron 8 of *VDR*. It affects the mRNA stability and causes a reduction in VDR levels (17).

VDBP, also known as a human group specific component (GC globulin), has three common phenotypic alleles in chromosome 4. These alleles differ from one another by integrating two variants of *VDBP*: rs4588 and rs7041, which are both located in exon 11. rs7041 (G>T) encodes Asp432Glu and rs4588 (C>A) encodes Thr436Lys (18). It has been found that lower 1,25[OH]2D levels are associated with rs7041 and rs4588 (AA, AC) mutations (19).

In vivo vitamin D level is evaluated as normal (>30 ng/mL), insufficient (20–30 ng/mL) and deficient (<20 ng/mL) (10, 20). In this study vitamin D leves were also measured to investigate the relationship between vitamin D levels and ISR.

Until today, genetic factors that may be related with ISR could not be completely defined. Thus, in this study the effects of *VDR* and *VDBP* variations to ISR were investigated.

2. METHODS

2.1. Study Population

In this prospective study, 58 consecutive patients who applied to the cardiology outpatient clinic of Dr. Siyami Ersek Training and Research Hospital with chest pain, underwent angiography and were found to have restenosis in the previously inserted stent were included in the patient group. All coronary angiography procedures were performed via the femoral route of patients (Siemens Axiom Artis Zee, Germany). The diagnosis of ISR was performed by expert invasive cardiologists. Thirty-five consecutive patients who applied to the cardiology outpatient clinic with chest pain and were not found to have restenosis in their previous stent in coronary angiography were included in the control group. Patients who have kidney or liver disease, acute coronary syndrome, hyperparathyroidism, history of malignancy of use of drugs including calcium (Ca) and vitamin D were excluded from the study.

The present study protocol was approved by the Institutional Ethics Committee of Yeditepe University (Approval Date:22.06.2015, Approval Number:495). Written informed consent from participants was obtained following a detailed explanation of the experimental procedures.

2.2. Biochemical Analysis:

The blood samples were obtained at the time of angiographic procedures and biochemical parameters such as vitamin D, lipid and hemoglobin levels were measured (Coulter LH780, Beckman Coulter Ireland Inc., Mervue, Galway, Ireland). Vitamin D deficiency is defined as Vitamin D levels smaller than 20 ng/mL (10,20). All patients were prescribed statins, beta-blockers and acetylsalicylic acid, according to their electronic prescriptions.

2.3. Molecular Analysis

After blood samples were obtained from all patients, genomic DNA was isolated from 200 µl blood by using commercially available kits according to manufacturers' instructions (Roche, Basel, Switzerland). DNA concentrations were determined with a NanoDrop spectrophotometer (Thermo Scientific, Foster City, CA, USA). DNA samples with a DNA concentration greater than 30 ng/µL and OD260/280 ratio near to 1.8 were used for molecular analysis. rs7041 and rs4588 variations in *VDBP;* rs1544410 and rs2228570 variations in *VDR*; were analyzed by using Real-time PCR (RT-PCR) (Applied Biosystems, Foster City, CA, USA). RT-PCR Assay ID's and primer sequnces of variants which were attached with VIC and FAM fluorescent material are shown in Table 1. The RT-PCR cycle parameters for all variants were 60^{0}C for 1 min., 95^{0}C for 10 min. followed by forty cycles of 95^{0}C for 15 sec. and 60^{0}C for 1 min., then 60^{0}C for 1 min.

Table 1. *Primer sequnces of variants which are attached with VIC and FAM fluorescent material*

SNP ID (Assay ID)	Gene	Primer sequences of variants which are attached with VIC and FAM fluorescent material
rs7041 (C___3133594_30)	VDBP	GCTTTGCCAGTTCCGTGGGTGTGGC**[A/C]** TCAGGCAATTTTGCTTTTAGTCGCT
r4588 (C___8278879_10)	VDBP	CTTGTTAACCAGCTTTGCCAGTTCC**[G/T]** TGGGTGTGGCATCAGGCAATTTTGC
rs1544410 (C___8716062_10)	VDR	GAGCAGAGCCTGAGTATTGGGAATG**[C/T]** GCAGGCCTGTCTGTGGCCCCAGGAA
rs2228570 (C__12060045_20)	VDR	GGAAGTGCTGGCCGCCATTGCCTCC**[A/G]** TCCCTGTAAGAACAGCAAGCAGGCC

2.4. Statistical Analysis

Statistical Package for the Social Science (SPSS) 23.0 was used to analyze the results. Assumption of normal distribution was checked with the Kolmogorov-Smirnov test. Two independent samples t test was used to compare continuous variables' means between two groups which were normally distributed. Kruskal Wallis tests were performed to investigate the difference between genotypes and risk factors (which are not normally distributed) of ISR. If there were statistically significant differences for pairwise comparison, Mann-Whitney U test was performed and Bonferroni correction

was applied to p values. p values smaller than 0.05 (p<0.05) were considered as statistically significant.

3. RESULTS

3.1. Study Population

The baseline characteristics of the study population are shown in Table 2. When groups were compared according to the baseline characteristics; vitamin D, white blood cell (WBC) and hemoglobin levels were found statistically low whereas the presence of vitamin D deficiency (vitamin D level<20 ng/mL), family history of CAD, hypertension and C reactive protein (CRP) levels were found statistically high in patient group (p<0.05). Other parameters, which are shown in table 2 were not found statistically significant (p>0.05).

Table 2. *Baseline characteristics of the study population.*

Baseline Characteristics	Groups (number of participants)		p values
	Control group (n=35)	Patient group (n=58)	
Vitamin D level (ng/mL)	18.07 ± 4.97	12.10 ± 4.44	**<0.001****
Age (years)	59.17 ± 10.36	59.52 ± 8.80	0.86
Hyperlipidemia (%)	12 (34.3%)	29 (50%)	0.14
Total cholesterol (mg/dl)	180.63 ± 47.10	184.90 ± 42.54	0.65
Triglyceride (mg/dl)	166.17 ± 101.86	179.64 ± 123.20	0.59
WBC (x1000/ml)	9.12 ± 2.77	7.84 ± 2.59	**0.027***
HbA1c (%)	6.13 ± 1.22	6.65 ± 1.79	0.13
Hypertension (%)	14 (40%)	38 (65.6%)	**0.016***
Current smoking (%)	11 (31.4%)	20 (34.5%)	0.76
Diabetes mellitus (%)	11 (46.6%)	27 (31.4%)	0.15
Familial history of CAD (%)	19 (54.3%)	55 (94.8%)	**<0.001****
CRP (mg/ml)	0.92 ± 0.53	1.40 ± 0.9	**0.005***
Hemoglobin	14.02 ± 1.27	13.04 ± 2.46	**0.013***
Serum creatinine (mg/dl)	0.95 ± 0.21	1.02 ± 0.65	0.54
Total thrombocytes (ml)	239.80 ± 78.85	231.81 ± 58.04	0.58
Vitamin D deficiency	18 (51.4%)	54 (93.1%)	**<0.001****
COPD	7 (20%)	7 (12.1%)	0.30

**p<0.05, **p<0.001, HbA1c: Hemoglobin A1c, WBC: White blood cell, CAD: Coronary artery disease,*
CRP: Creactive protein, COPD: Chronic obstructive pulmonary disease

3.2. VDBP and VDR genotyping

The genotype distributions of study groups are shown in table 3. The CC genotype of variation of *VDR* and the CA genotype of rs4588 variation of *VDBP* were found statistically high in patient group (p<0.05). However rs7041 of *VDBP* and rs1544410 of *VDR* were mostly detected in patient group, they were not found statistically significant (p>0.05).

Table 3. *Genotype distributions of study groups*

Gene names and genotype distributions	Groups		p values
	Control group (n=35)	Patient group (n=58)	
VDBP			
rs4588			
CC	29 (82.9%)	34 (58.6%)	
CA	6 (17.1%)	20*(34.5%)	**0.036***
AA	0 (0%)	4 (6.9%)	
rs7041			
GG	10 (28.6%)	9 (15.5%)	
GT	10 (28.6%)	30 (51.7%)	0.076
TT	15 (42.9%)	19 (32.8%)	
VDR			
rs2228570			
TT	7 (20%)	1 (1.7%)	
TC	10 (28.6%)	22 (37.9%)	**0.009***
CC	18 (51.4%)	35* (60.3%)	
rs1544410			
GG	8 (22.9%)	26 (44.8%)	
GA	21 (60%)	25 (43.1%)	0.103
AA	6 (17.1%)	7 (12.1%)	

**p<0.05*

3.3. Statistically Significant Assocations Between Risk Factors and Variations

All of the risk factors which are shown in table 2, were also analyzed for investigating the association between these factors with variations. Of these, rs7041 variation was found statistically high in patients who had myocardial infarction history before stent implantation (p=0.048). (Figure 1). The other factors were not found statistically significant (p<0.05).

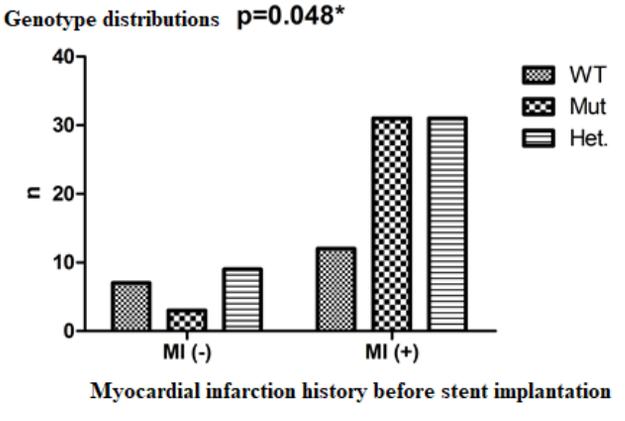

Figure 1. *Association between myocardial infaction (MI) history before stent implantation with rs7041 variation*

4. DISCUSSION

Mortality due to cardiovascular disease has risen day by day, and it is estimated that it may increase to 23.4 million deaths by the year 2030 (21). Although PCI is a reliable application to treat CAD, development of restenosis is a major problem after angioplasty (22). CAD can occur due to platelet activation, thrombus formation, endothelial dysfunction, activation in vascular smooth cell presentation or inflammation. It is demonstrated that vitamin D affects the renin-angiotensin system (RAS), and that vitamin D deficiency causes inflammation (23). Functional abnormality in endothelial cells takes place before and throughout the development of atherosclerosis, and especially during plaque fracture. Oxidized LDL appears to induce this cellular dysfunction. High levels of angiotensin II facilitates oxidation of LDL and its uptake by receptors on monocytes, macrophages and endothelial cells (24). Also RAS contributes to the development of arterial hypertension. In a brief study, it was shown that vitamin D could reduce high blood pressure in hypertension patients (25). This might be explained by the observation of endothelial dysfunction involved in both hypertension and restenosis. A recent study also showed that lower vitamin D level (<30 ng/ml) is related with CAD (21). It is suggested that vitamin D intake limits neointimal formation following coronary intervention, and provides protection against the development of coronary restenosis (8, 22). Vitamin D deficiency may also induce the production of C reactive protein (CRP), which is known to be directly related with the inflammation process (23, 24). According to these findings, it was considered that there might be an association between vitamin D deficiency and restenosis progression related with inflammation. At the end of the study, vitamin D deficiency (vitamin D level<20 ng/mL) was found statistically high in in ISR patients ($p<0.001$). Additionally hypertension and CRP level were also found statistically high in patient group ($p<0.05$).

ISR is the main obstacle of percutaneous coronary intervention. Heritable factors also may have a role in ISR. Many studies showed that investigating the effect of related genes and other risk factors on the development of restenosis has critical importance for new therapeutic approaches. *VDR* and *VDBP* polymorphisms have important impact on the regulation of vitamin D metabolism (19). Excluding vitamin D deficiency, *VDR* and *VDBP* genes are also the main regulators in vitamin D metabolism (10). Therefore, these main factors should be investigated together in order to evaluate the relationship between ISR and genes related with vitamin D metabolism. In our study, the CC genotype of rs2228570 variation of *VDR* and the CA genotype of rs4588 variation of *VDBP* were found statistically high in patient group ($p<0.05$). Contrary to these findings, in a separate study no significant association was found between these polymorphisms with ISR (25,26).

In the present study, the relationship between ISR and other risk factors were also investigated. Recent studies have shown that there is a relationship between vitamin D level and MI (27). Similarly, according to our results rs7041 variation was found statistically high in patients who had myocardial infarction history before stent implantation ($p=0.048$). Contrary to this finding, in another study no significant associations were found between MI, type 2 diabetes mellitus, death, BMI, lipid levels, blood pressure and HbA1c (28).

Multiple studies have shown that vitamin D deficiency is a risk factor for cardiovascular diseases (7, 9). Growing evidence has supported that vitamin D plays a critical role in modulating the anti-inflammation in other inflammatory diseases such as ulcerative colitis (UC). Recent studies suggest that UC is characterized by relapsing inflammatory process in the gastrointestinal tract; it is highly related with vitamin D metabolism and its regulator protein VDR due to its anti-inflammation effect (29, 30). Similarly, in the present study it was found that ISR, which is related with inflammation, has an association with vitamin D deficiency

5. CONCLUSION

According to these findings, it was considered that gene variations which are related with vitamin D metabolism and vitamin D deficiency may increase the risk of ISR. In conclusion, genetic screening of patients for VDR and VDBP variations before stent implantation may provide information about the possibility of ISR. Possible tracking of gene variations and risk factors with some other studies with high number of patients may help to clarify the mechanism of ISR.

Acknowledgements: We are grateful to Dr. Seval Turkmen for her valuable contribution.

Funding: None

Conflict of Interest: There is no conflict of interest to declare

REFERENCES

[1] Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. World J Cardiol. 2016;8:1-23.

[2] Schaffer A, Verdoia M, Barbieri L, Cassetti E, Suryapranata H, De Luca G. Impact of diabetes on homocysteine levels and ıts relationship with coronary artery disease: A single-centre cohort study. Ann Nutr Metab. 2016;68:180-188.

[3] Huang NF, Kurpinski K, Fang Q, Lee RJ, Li S. Proteomic identification of biomarkers of vascular injury. Am J Transl Res. 2011;3:139-418.

[4] Hamid H, Coltart J. 'Miracle stents'—a future without restenosis. Mcgill J Med. 2007;10:105-111.

[5] Looser PM, Kim LK, Feldman DN. In-Stent Restenosis: Pathophysiology and treatment. Curr Treat Options Cardiovasc Med. 2016;18:10.

[6] Ferrante G, Niccoli G, Biasucci LM, Liuzzo G, Burzotta F, Galiuto L, Trani C, Rebuzzi AG, Crea F. Association between C-reactive protein and angiographic restenosis after bare metal stents: An updated and comprehensive meta-analysis of 2747 patients. Cardiovasc Revasc Med. 2008;9:156-165.

[7] Al Mheid I, Patel R, Murrow J, Pires NM, Pols HA, Zwinderman AH, de Maat MP, Doevendans PA, DeWinter RJ, Tio RA, Waltenberger J, Frants RR, Quax PH, van der Laarse A, van der Wall EE, Uitterlinden AG, Jukema JW. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol. 2011;58:186-192.

[8] Monraats PS, Fang Y, Pons D, Pires NM, Pols HA, Zwinderman AH, de Maat MP, Doevendans PA, DeWinter RJ, Tio RA, Waltenberger J, Frants RR, Quax PH, van der Laarse A, van der Wall EE, Uitterlinden AG, Jukema JW. Vitamin D receptor: a new risk marker for clinical restenosis after percutaneous coronary intervention. Expert Opin Ther Targets. 2010;14:243-251.

[9] Sen F, Yilmaz S, Balci KG, Sen Ö, Gül M, Çay S, Topaloglu S, Aydogdu S. The relationship between vitamin D levels and saphenous vein graft patency. Coron Artery Dis. 2015;26:328-332.

[10] Young KA, Snell-Bergeon JK, Naik RG, Hokanson JE, Tarullo D, Gottlieb PA, Garg SK, Rewers M. Vitamin D deficiency and coronary artery calcification in subjects with type 1 diabetes. Diabetes Care. 2011;34:454-458.

[11] Gupta GK, Agrawal T, Del Core MG, Hunter WJ 3rd, Agrawal DK. Decreased expression of vitamin D receptors in neointimal lesions following coronary artery angioplasty in atherosclerotic swine. PLoS One. 2012;7:e42789.

[12] Verschuren JJ, Trompet S, Sampietro ML, Heijmans BT, Koch W, Kastrati A, Houwing-Duistermaat JJ, Slagboom PE, Quax PH, Jukema JW. Pathway analysis using genome-wide association study data for coronary restenosis—a potential role for the PARVB gene. PLoS One. 2013;8:e70676.

[13] Pan XM, Li DR, Yang L, Wang EY, Chen TY, Liu YJ, Liu M, Liao ZG. No association between vitamin D receptor polymorphisms and coronary artery disease in a Chinese population. DNA Cell Biol. 2009;28:521-525.

[14] He L, Wang M. Association of vitamin D receptor-a gene polymorphisms with coronary heart disease in Han Chinese. Int J Clin Exp Med. 2015;8:6224-6229.

[15] Jorde R, Schirmer H, Wilsgaard T, Joakimsen RM, Mathiesen EB, Njølstad I, Løchen ML, Figenschau Y, Berg JP, Svartberg J, Grimnes G. Polymorphisms related to the serum 25-hydroxyvitamin D level and risk of myocardial infarction, diabetes, cancer and mortality. The Tromso Study. PLoS One. 2012;7:e37295.

[16] Abu El Maaty MA, Hassanein SI, Gad MZ. Genetic variation in vitamin D receptor gene (Fok1:rs2228570) is associated with risk of coronary artery disease. Biomarkers. 2016;21:68-72.

[17] Laczmanski L, Milewicz A, Lwow F, Puzianowska-Kuznicka M, Pawlak M, Kolackov K, Jedrzejuk D, Krzyzanowska-Swiniarska B, Bar-Andziak E, Chudek J, Mossakowska M. Vitamin D receptor gene polymorphism and cardiovascular risk variables in elderly polish subjects. Gynecol Endocrinol. 2013;29:268-272.

[18] Stakisaitis D, Lesauskaite V, Girdauskaite M, Janulionis E, Ulys A, Benetis R. Investigation of vitamin D-binding protein polymorphism impact on coronary artery disease and relationship with longevity: own data and a review. Int J Endocrinol. 2016;8347379:1-7.

[19] Lafi ZM, Irshaid YM, El-Khateeb M, Ajlouni KM, Hyassat D. Association of rs7041 and rs4588 polymorphisms of the vitamin D binding protein and the rs10741657 polymorphism of CYP2R1 with vitamin D status among jordanian patients. Genet Test Mol Biomarkers. 2015;19:629-636.

[20] Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: A systematic review. Nat Rev Cardiol. 2009;6:621-630.

[21] Dhibar DP, Sharma YP, Bhadada SK, Sachdeva N, Sahu KK. Association of vitamin D deficiency with coronary artery disease. J Clin Diagn Res. 2016;10:OC24-OC28.

[22] Gupta GK, Agrawal T, Rai V, Del Core MG, Hunter WJ, Agrawal DK. Vitamin D supplementation reduces intimal hyperplasia and restenosis following coronary intervention in atherosclerotic swine. PLoS One. 2016;11:e0156857.

[23] Chen WR, Qian YA, Chen YD, Shi Y, Yin DW, Wang H, Zhu P, Liu HW, Sha Y. The effects of low vitamin D on coronary artery disease. Heart Lung Circ. 2014;23:314-319.

[24] Li DY, Zhang YC, Philips MI, Sawamura T, Mehta JL. Upregulation of endothelial receptor for oxidized low-density lipoprotein (LOX-1) in cultured human coronary artery endothelial cells by angiotensin II type 1 receptor activation. Circ Res. 1999;84:1043-1049.

[25] Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet. 1998;352:709-710.

[26] Pleva L, Kovarova P, Faldynova L, Plevova P, Hilscherova S, Zapletalova J, Kusnierova P, Kukla P. The rs1803274 polymorphism of the BCHE gene is associated with an increased risk of coronary in-stent restenosis. BMC Cardiovasc Disord. 2015;15:135.

[27] Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med 2008;168:1174-1180.

[28] Jorde R, Schirmer H, Wilsgaard T, Bøgeberg Mathiesen E, Njølstad I, Løchen ML, Joakimsen RM, Grimnes G. The DBP Phenotype Gc-1f/Gc-1f is associated with reduced risk of cancer. The tromso study. PLoS One. 2015;10:e0126359.

[29] Zheng SZ, Zhang DG, Wu H, Jiang LJ, Jin J, Lin XQ, Ding R, Jiang Y. The association between vitamin D receptor polymorphisms and serum 25-hydroxyvitamin D levels with ulcerative colitis in chinese han population. Clin Res Hepatol Gastroenterol. 2017;41:110-117.

[30] Xia SL, Yu LQ, Chen H, Hu DY, Shao XX, Guo MD, Jiang LJ, Lin XX, Lin XQ, Jiang Y. Association of vitamin D receptor gene polymorphisms with the susceptibility to ulcerative colitis in patients from southeast china. J Recept Signal Transduct Res. 2015;35:530-535.

How to cite this article: Kirac D, Erdem Yaman A, Gezmis H, Yesilcimen K, Avcilar T, Guney AI, Keles EC, Koc G, Akkanat R, Isbir T. VDBP and VDR Mutations May Cause In-Stent Restenosis. Clin Exp Health Sci 2022; 12: 602-606. DOI: 10.33808/clinexphealthsci.953893

Clinical and Experimental Health Sciences

A Comparison of Actual Cardiovascular Disease Risks to the Perceptions of Middle-aged Men: A Cross-Sectional Study

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Received: 17.08.2021 **Accepted:** 06.06.2022

ABSTRACT

Objective: To determine the actual cardiovascular diseases risk and to compare it with the perceived risk in 40-65 years old men.

Methods: We conducted a cross-sectional study in a population determined to be 21.039 men and sample consisted of 400 men. The actual cardiovascular diseases risks of these respondents were calculated using HeartScore, classified as low, moderate, high, or very high. The respondents' perception of their cardiovascular disease's risks was categorized as wrong optimists/pessimists and realists. We used multivariate logistic regression models to determine the relationships between perceived cardiovascular diseases risk and independent variables (age, diabetes, hypertension, etc.). Required ethics committee and institutional permissions were obtained for the duly conduct of the study.

Results: It was determined in our study that while 8.3% (n=33) of the men had a high-risk level for cardiovascular disease, 52.5% (n=210) had a very high level. Forty-eight percent of the participants perceive their CVD risks to be lower than they are and 23.8% to perceive it higher than they are. Correct estimation rate of CVD risk was 28.2%. The variables affecting the actual cardiovascular diseases risk were diastolic blood pressure, body mass index, and level of physical activity. 13.3% (n=53) of respondents perceived their cardiovascular diseases risks as high and 8% (n=32) as very high. The variables affecting an incorrect perceived cardiovascular diseases risk are being 61-65 years of age (odds ratio=0.34, 95% confidence interval: 0.16-0.73) and a diagnosis of diabetes mellitus (odds ratio=0.45, 95% confidence interval: 0.20-0.99).

Conclusion: We observed that more than half of the residents were at a very high level of risk for cardiovascular disease, and approximately one out of every two respondents perceived their risk of cardiovascular disease to be lower than the actual risk. It is recommended risk reducing behaviors be developed and awareness of risk be raised.

Keywords: Middle age, men, cardiovascular diseases, perceived risk.

1. INTRODUCTION

Of the 56 million deaths worldwide in 2019, approximately 18 million (32%) were caused by cardiovascular disease (CVD) (1). This rate is estimated to rise to 22.2 million by 2030. CVD is reported as the most common fatal disease (2,3). Turkey has the highest rates of mortality in Europe from coronary issues (2,4), and in 2016 the province in Turkey with the highest mortality rates due to circulatory system problems was Amasya with 50.8% (5).

The incidence of CVD disease is known to be higher in men (6). Habits related to an unhealthy lifestyle increase the risk of CVD (7,8,9,10). According to the data of the Ministry of Health, rates of tobacco use, systolic blood pressure (SBP) with fasting plasma glucose (FBG), total cholesterol (TC), alcohol and substance use, occupational risks, and insufficient physical activity along with other risk behaviors are generally higher in men (11). The same data shows that noncommunicable disease attributed to Disability Adjusted Life Years (DALY) is higher in men (19%) than in women (18%). Moreover, the CVD risk was found to be higher in men (13.3 %) than women (7.8%) in 2017 (11,12). Worldwide, more than one-third (35%) of men smoke while just over 6% of women do so according to data from the years 2000-2016 (13). According to the 2019 National Survey on Drug Use and Health (NSDUH) in Turkey, 85.6 percent of people ages 18 and older reported that 59.1 percent of men in this age group and 51.0 percent of women in this age group smoke (14). Regarding the cardiovascular risk factors in Turkey, daily tobacco use in women aged between 45-64 was 14.3% while the rate was 41.5% in men of the same age range. Similarly, in Turkey, alcohol consumption in men is higher (42.5%) than in in women (3.75%) (12). Nevertheless, according to the Turkey Health Survey Data (2016), men generally receive preventive health services at lower rates than women (blood pressure, cholesterol etc.) (5).

DOI: 10.33808/clinexphealthsci.984039

There is no routine screening program in Turkey for actual CVD risk (15,16). In terms of ease of use and accessibility (17), the HeartScore program, a risk assessment and management program aimed at supporting clinicians in optimizing individual cardiovascular risk reduction intended to be used by health professionals only and not by the general public (18,19,20), is suitable for screening men in the CVD risk group (19). The Ministry of Health suggests that the HeartScore program be used to evaluate the CVD risks of 40-65 aged individuals in Turkey. It has been emphasized that there is increased cardiovascular risk among this age group individuals (21). HeartScore contains broader groups and yields more reliable results (18,22). The program also free (18).

Risk perception is important for individuals to adopt healthy lifestyle behaviors in order to reduce CVD risks. Individuals with the awareness of an increased CVD risk may adapt more easily to attitude changes such as being more careful about their nutrition, quitting smoking and receiving regular medical check-up (23,24). Research indicates that men fail to perceive CVD risks (25,26,27), and commonly men perceive CVD risks at a lower level than what is actual (27,28,29).

The aim of this study was to determine the actual CVD risk level for the next ten years in the high-risk group of 40-65 age men and to compare it to the CVD risk level their perceived.

1.1. Study Questions

What are the actual CVD risk levels for men aged 40-65?

What are the perceived CVD risk levels for men aged 40-65?

Is there a difference between the actual CVD risks for men aged 40-65 years and the perceived CVD risks?

What are the factors affecting the perceived risk levels for men aged 40-65 regarding CVD?

2. METHODS

2.1. Study Design and Population

We conducted a cross-sectional study in a population determined to be 21,039 men in the 40-65 age range living in a city center of Turkey, in 2018 (5). Individuals over the age of 65 were not included in this study because the Turkish Ministry of Health considers individuals aged 40-65 years to be at risk for cardiovascular diseases (21). The sample was calculated with a 95% confidence interval (CI) and a 5% margin of error in case the number of population is known along with the sum of CVD mid-level 22% (n=72) and high-level 11.7% (n=37) determined by Akgöz & Gözüm (2019), with the HeartScore system used; and found as the minimum n=338 (20). The study aimed to reach 20% more of the specified value. 400 individuals were included in the scope of the study using the cluster sampling method. Each of the 40 neighborhoods in the central area of Amasya province was considered as a cluster in the first stage. Neighborhood names were put in alphabetical order, and 10 neighborhoods were included in the study as the field of research using the systematic sampling method (40/10=4) in the second stage. The number of participants from each neighborhood was determined using the percentage of males in the population in the third stage. In the fourth stage, volunteers in this age group who met at a specified place after an announcement by the local authorities. Volunteers were accepted until researchers reached the pre-specified number of participants.

2.2. Data Collection Tools

Research data was collected using the Demographic and Medical Information Data Collection Form, the HeartScore database (actual) and Perceived CVD Risk Defining Form, and the International Physical Activity Questionnaire (IPAQ Short Form) was used to determine the participants' physical activity levels (30).

2.2.1. Demographic Characteristics

The age of the participants was determined by their year of birth on their IDs., and their use of medication, their medical history (increased risk of CVD) (4), and their family history (first degree relatives with CVD) was evaluated by questions.

2.2.2. HeartScore Database (actual) and Perceived CVD Risk

We used HeartScore's non-HDL measurement Turkey form that was used to evaluate the actual CVD risks of individuals (17,31). It was adapted to a high-risk model. At the beginning, the researcher signed up on the system with his/her first name, last name, and email information in order to save data on the HeartScore Turkey form (31). After signing up, first-last names, birth dates (month/year), and gender information was defined on the system under the "Create New Patient Record" tab. After defining the patients on the system, participants were displayed in the "Patient List" tab. Individuals were picked in this tab to measure risks, and the "Create New Examination" tab was clicked. Researcher then recorded measured SBP and TC value along with the data about smoking/non-smoking and calculated the percentages of CVD risks of participants for the next 10 years (<1% low, 1≤-<5% moderate, 5≤-<10% high and ≥10% very high) (17,18). SBP indicates how much pressure your blood is exerting against your artery walls when the heart beats. The TC is a measure of the total amount of LDL-HDL cholesterol, and other lipid components (32). The perceived CVD risk level was ascertained when the participants were asked an open-ended question and graded their own risk as low, moderate, high, and very high. If they perceived their risk as lower than their actual risk, they were classified as wrong optimists; if they perceived their actual risks as higher that their actual risk, they were classified as wrong pessimists; and if they perceived their risk as the same as their actual risk, they were classified as realistic (24,27,33,34).

2.3. Measurements

2.3.1. Physical Activity

The Turkish version of the International Physical Activity Questionnaire/IPAQ developed by Booth was used to determine the physical activity levels of participants in terms of the risk factors for CVDs (30). It is a reliable and valid in the Turkish population (35). Physical activity was rated as the metabolic equivalent (MET) to minutes a week with the data evaluation of the IPAQ. Then, activity level was determined and classified as inactive (<600 MET-min.day/wk), low level active (600-3000 MET – min.day/wk), and sufficiently active (>3000 MET – min.day/wk) (30).

2.3.2. Height and Weight

To measure height, participants stood upright after taking their shoes off. Subsequently, a stadiometer was used for their height measurements, and the obtained value that appeared on the screen was saved. The stadiometer is a digital measurement device that is connected to the acrylic head piece and interfaces directly (36). To measure weight, individuals took their shoes off and stepped onto the digital weight scale; the number displayed on the scale was recorded (20,36). Body mass index (BMI) was calculated with a weight/height [kg m^{-2}] formula after the researcher measured weight and height and then classified (37).

2.3.3. Lipids

Lipid measurements were performed with the blood sample taken from the capillaries of the individuals and the data were obtained in this way (38). Participants' TC levels were determined after evaluation with the Accutrend Plus GCT device in which the result appears after approximately three minutes (19, 39). The measurements were carried out by the researchers, paying attention to aseptic techniques. Based on this value read on the digital screen, the TC levels of individuals were determined. The measurement confidence interval of the device is between 10%-85% (39). It has been reported that the device used is suitable for CVD risk determination (40).

2.3.4. Blood Glucose

According to the American Diabetes Association (ADA), FPG is measured at least eight hour after meals, and postprandial plasma glucose (PPG) levels is measured one to two hours after the start of a meal (41). Individuals' blood glucose value was measured by the researchers, and the ones who had diabetes according to a physician's diagnosis or whose blood glucose was determined to be within the range of diabetic values as a result of the measurements were directly considered to be at high cardiovascular risk (19). Participants were measured using the Accu-Chek Performa Nano measuring device which is calibrated. Later, these parameters were classified based on the recommendations of the ADA (41). This device gives the averages of daily measurements and is an easy and confidential professional system (42). After the coding process of the device was carried out, the fingers of the individuals were cleaned with alcohol cotton. Then, the finger of the procedure was pierced with a lancet, the first blood sample was cleaned with cotton, and the second blood sample was taken into the scope of measurement. After the measurement, the measurements taken two hours after the main meal were recorded as satiety, and the measurements taken before the main meal were recorded as fasting blood sugar.

2.3.5. Blood Pressure

The blood pressures of the individuals were measured by the researchers. Prior to the measurement, individuals were allowed to rest for five minutes. During the measurement, the individuals were not allowed to talk and they were exhorted not to cross their legs. The blood pressures of the individuals were measured twice during a five-minute interval, and a blood pressure value was formed by taking the arithmetic average of these values. In case there was a difference of more than 5 mmHg between the blood pressure values measured twice, the measurements were repeated (33,43). A calibrated aneroid sphygmomanometer (Erka Perfect, Germany) was used to measure participants' SBP and diastolic blood pressure (DBP), and these measurements were classified according to the guidelines of the American Society of Hypertension and the International Society of Hypertension (20,44). Accordingly, those with SBP <120 mm/hg were classified as normotensive, those between 120-139 mm/hg as prehypertensive, and with ≥140 mm/hg as hypertensive. In DBP, <80 mm/hg was classified as normotensive, between 80-89 mm/hg as prehypertensive, and ≥90 mm/hg as hypertensive (44).

2.3.6. Smoking History

The smoking history of the participants was rated on a three point scale from (1) current smoker (≥100 cigarettes in his lifetime and currently smokes), (2) former smoker (≥100 cigarettes in his lifetime but quit smoking), and (3) never smoker (≤100 cigarettes in his lifetime or never smoked) as recommended by the Centers for Disease Control and Prevention (45).

2.4. Data Collection Process

Data of this study was collected between April-October 2018. Researcher applied the Demographic and Medical Information Data Collection Form to the participants in the sample followed by a face-to-face interview using the HeartScore Database (actual) and the Perceived CVD Risk Defining Form. Data collected from participants was entered on the HeartScore website to determine the actual CVD risks of participants for the next ten years (17,18,31). Men in the high-risk group were sent to family physicians while men in very high-risk group were sent to cardiologists. Moreover,

all participants were given written texts with the HeartScore system's suggestions regarding the personal risk level, regardless of the risk level.

2.5. Ethical Considerations

Ethical approval was obtained from the ethics board for clinical research of Akdeniz university, (2018-02/70904504). Institutional permission was also obtained from a local governor (2018-03/522.03-E.89). All individuals provided written informed consent. This study was conducted in accordance with the principles of the Declaration of Helsinki.

2.6. Statistical Analysis

Data was analyzed using SPSS version 23.0 software. First, compliance of variables with normal distribution was evaluated using the Shapiro Wilks test. Second, the Kruskal Wallis test was applied to evaluate more than two quantitative data groups along with descriptive statistical methods (mean, standard deviation, frequency), and the FPG and PPG of participants that influenced their CVD risk perceptions was evaluated. Later, the perceived CVD risk was compared to the actual CVD risk using cross-tabulations (McNemar-Bowker test). Third, the Chi-square test was applied to evaluate the qualitative data in which descriptive characteristics were influenced by participants actual and perceived CVD risk level (except for FPG and PPG). Finally, in the assessment of perceived CVD risk factors, multivariate logistic regression analysis was applied. Coding for the logistic regression analysis was as follows. For the dependent variable. the participant's correct determination of CVD risk was coded as "1," and lower or higher determination of CVD risk than actual (wrong determination) was coded as "0." Independent variables included the following.

Age

Two dummy variables were added since age was considered in three categories. For age dummy variable 1, the 51-60 age range was coded as "1," and the 40-50 and 61-65 age ranges were coded as "0." For age dummy variable 2, the 61-65 age range was coded as "1", and the 40-50 and the 51-60 age range were coded as "0."

CVD History in the Family–Hypertension (HT) and Diabetes mellitus (DM)

They were coded as "1" for Yes and "0" for No.

BMI

Two dummy variables were added since BMI was categorized in three categories. For BMI dummy variable, overweight was coded as "1", and normal weight and obese were coded as "0". For BMI dummy variable 2, obese was coded as "1," and normal weight and overweight were coded as "0."

Physical Activity

Two dummy variables were added since physical activity was considered in three categories. For physical activity dummy variable 1, a low level of activity was coded as "1," and an inactive and sufficient level of activity was coded as "0." For physical activity dummy variable 2, a sufficient level of activity was coded as "1," and an inactive and low level of activity was coded as "0".

3. RESULTS

Actual CVD risks and these affecting variables of participants are shown in Table 1. Accordingly, 60.8% of men were at a high/very high level of actual CVD risk. Very high risk of actual CVD in participants with hypertensive DBP (60.7%) was higher than normotensive (47.3%) and prehypertensive (52.3%). This difference is significant ($p<0.01$).

The risk for obese participants (60.5%) was higher than for normal weight (40.7%) and overweight individuals (52.8%) ($p<0.01$) while a very high actual CVD risk rate in participants with a sufficient level of physical activity (38.9%) was significantly lower than with an inactive level (61%) or a low level (52.4%) of physical activity ($p<0.05$).

The comparison between actual and perceived CVD risk is presented in Table 2. Accordingly, 8.3% were at high and 52.5% of men very high risk for actual CVD risk, but only 13.3% of men perceived their CVD risks as high and only 8% as very high level. Additionally, 60.8% of men were at high or very high risk for CVD over the next ten years. However, 78.8% perceived their CVD risk as low-moderate. Therefore, there were substantial gaps between the perceived and actual risk levels. Moreover, among those at very high risk, 78% perceived themselves at low or moderate CVD risk whereas 20.3% of subjects with low risk for CVD overestimated their risk (wrong pessimism). Another important point is that only 20.3% perceived their CVD risk as a high/very level while 60.8% were at high or very high level of actual CVD risk (Table 2).

Table 1. *Influence of descriptive characteristics of participants at actual CVD risk level*

Descriptive Characteristics		Actual CVD Risk				χ^2	p
		Low Risk (n=64)	Moderate Risk (n=93)	High Risk (n=33)	Very High Risk (n=210)		
		n (%)	n (%)	n (%)	n (%)		
Age range[a]	40-50 years	58 (37.7)	36 (23.4)	0 (0.0)	60 (39.0)		
	51-60 years	6 (3.7)	51 (31.1)	17 (10.4)	90 (54.9)		
	61-65 years	0 (0.0)	6 (7.3)	16 (19.5)	60 (73.2)		
CVD history in family	Yes	14 (20.3)	14 (20.3)	2 (2.9)	39 (56.5)	4.425	0.219
	No	50 (15.1)	79 (23.9)	31 (9.4)	171 (51.7)		
DM[a]	Yes	0 (0.0)	0 (0.0)	0 (0.0)	61 (100)		
	No	64 (18.9)	93 (27.4)	33 (9.7)	149 (44.0)		
HT[a]	Yes	0 (0.0)	0 (0.0)	0 (%0.0)	82 (100)		
	No	64 (20.1)	93 (29.2)	33 (10.4)	128 (40.3)		
SBP[a]	Normotensive	37 (27.0)	34 (24.8)	9 (6.6)	57 (41.6)		
	Prehypertensive	25 (13.4)	51 (27.4)	13 (7.0)	97 (52.2)		
	Hypertensive	2 (2.6)	8 (10.4)	11 (14.3)	56 (72.7)		
DBP	Normotensive	39 (23.6)	41 (24.8)	7 (4.2)	78 (47.3)		
	Prehypertensive	18 (14.1)	32 (25.0)	11 (8.6)	67 (52.3)	23.395	**0.001****
	Hypertensive	7 (6.5)	20 (18.7)	15 (14.0)	65 (60.7)		
BMI	Normal weight	22 (24.2)	20 (22.0)	12 (13.2)	37 (40.7)		
	Overweight	32 (17.8)	47 (26.1)	6 (3.3)	95 (52.8)	24.609	**0.001****
	Obese	10 (7.8)	26 (20.2)	15 (11.6)	78 (60.5)		
Physical activity level	Inactive	20 (13.7)	28 (19.2)	9 (6.2)	89 (61.0)		
	Low level active	23 (14.0)	39 (23.8)	16 (9.8)	86 (52.4)	12.631	**0.048***
	Sufficient level active	21 (23.3)	26 (28.9)	8 (8.9)	35 (38.9)		
Total cholesterol[b]	Normal	63 (16.9)	87 (23.3)	30 (8.0)	193 (51.7)		
	Little high /High	1 (3.7)	6 (22.2)	3 (11.1)	17 (63.0)		
Smoking[a]	Yes	34 (14.9)	58 (25.4)	24 (10.5)	112 (49.1)		
	No	30 (17.4)	35 (20.3)	9 (5.2)	98 (57.0)		
Smoking classification	Current smoker	34 (14.9)	58 (25.4)	24 (10.5)	112 (49.1)		
	Former smoker	2 (14.3)	4 (28.6)	0 (0.0)	8 (57.1)	7.011	0.320
	Never smoker	28 (17.7)	31 (19.6)	9 (5.7)	90 (57.0)		

*χ^2: Chi-square test *p<0.05 **p<0.01 [a]Given that this variable is a risk factor in HeartScore, no analysis was done. [b]Combined with a small high category since there was only one participant with high total cholesterol level. CVD: cardiovascular disease, DM: diabetes mellitus, HT: hypertension, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index*

Table 2. *Comparison of actual and perceived CVD risk*

Perceived CVD Risk	Actual CVD Risk					P*
	Low	Moderate	High	Very high	Total	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Low	32 (8.0)	51 (12.8)	16 (4.0)	115 (28.8)	214 (53.5)	0.001**
Moderate	19 (4.8)	24 (6.0)	9 (2.3)	49 (12.3)	101 (25.3)	
High	7 (1.8)	11 (2.8)	8 (2.0)	27 (6.8)	53 (13.3)	
Very high	6 (1.5)	7 (1.8)	0 (0.0)	19 (4.8)	32 (8.0)	
Total	64 (16.0)	93 (23.3)	33 (8.3)	210 (52.5)	400 (100)	

**The McNemar-Bowker test was used for statistical analysis. **p<0.01*
Actual CVD risk was determined using HeartScore: low risk <1%; moderate risk ≤1-<5%; high risk ≤5-<10%, and very high risk ≥10% of CVD.
CVD: cardiovascular disease

Forty-eight percent of the participants perceive their CVD risks to be lower than they are and 23.8% to perceive it higher than they are. Correct estimation rate of CVD risk was 28.2% (Table 3).

Table 3. *Estimate condition of participants' real and perceived CVD risks*

Estimate condition of participants' real and perceived CVD risks	N	%
Underestimate the risk (↓) (Wrong optimist)	192	48.0
Identifying the risk of correctly (√) (Realistic)	113	28.2
Overestimate the risk (↑) (Wrong pessimistic)	95	23.8

*aChi-Square Test bKruskal Wallis Test *p<0.05 **p<0.01 ***Combined with little high category since there was only one participant with high total cholesterol level. CVD: cardiovascular disease, HT: hypertension, FPG: fasting plasma glucose, PPG: postprandial plasma glucose, BMI: body mass index, DM: diabetes mellitus*

Prevalence of CVD risk factors by perceived CVD risk of men may be seen in Table 4. 71.8% of participants were wrong in their perception in terms of their actual CVD risks (Table 4). Univariate analyses are shown in Table 4; accordingly, age, HT, DM, BMI, family history and physical activity variables have influence on the correct determination of CVD risk. At this point, the rate of individuals in the 40-50 age range have the same approximate determination perception and actual risk of CVD (37%) higher than individuals in the 51-60 age range (27.4%) and the 61-65 age range (13.4%).

It was an a priori expectation that the healthy men in the study would have a more accurate perceived CVD risk compared with the unhealthy men. Accordingly, the results showed this to be the case only in the BMI categories (overweight) (Table 4). The details are as follows. Accurate detection of CVD risk (Table 4) in individuals without HT (30.8%) was higher than for those with HT (18.3%); accurate detection in individuals without DM (30.7%) was higher than for those with DM (14.8%); accurate detection for overweight individuals (35%) was higher than for those of normal weight (29.7%) and those who were obese (17.9%); accurate detection for those without a family history of CVD (37.7%) was higher than for those with a family history of CVD (26.3%); and, finally, accurate detection for individuals with a sufficient activity level (34.4%) was higher than for those who were inactive (28.8%) and those with a low level of activity (24.4%).

It was determined after putting the significant factors found in univariate analyses (shown in Table 4) through logistic regression analysis (Table 5) that participants in the 61-65 age range and with DM failed to determine correct CVD risk. According to this, the 61-65 age range had 0.344 times more failure to determine the actual CVD risk compared to the 40-50 age range and the 51-60 age [odds ratio (OR)=0.34, 95% CI: 0.16-0.73, (p<0.01)]. In addition, individuals with DM had 0.45 times higher rate of failure to determine actual CVD risk compared to those without DM [OR=0.45, 95% CI: 0.20-0.99, (p<0.05)].

Table 4. *Influence of individuals' descriptive characteristics regarding their perception of CVD risk*

Descriptive Characteristics		Perceived CVD Risk			χ^2	p
		Determined below actual risk (Wrong optimists) [n=192]	Determined the same level as actual (Realist) [n=113]	Determined over actual risk (Wrong pessimists) [n=95]		
		n (%)	n (%)	n (%)		
Age range	40-50 years	24 (15.6)	57 (37.0)	73 (47.4)	128.644[a]	**0.001****
	51-60 years	102 (62.2)	45 (27.4)	17 (10.4)		
	61-65 years	66 (80.5)	11 (13.4)	5 (6.1)		
HT	Yes	48 (58.5)	15 (18.3)	19 (23.2)	6.020[a]	**0.048***
	No	144 (45.3)	98 (30.8)	76 (23.9)		
Antihypertensive intake status	Yes	37 (55.2)	13 (19.4)	17 (25.4)	3.194[a]	0.202
	No	155 (46.5)	100 (30.0)	78 (23.4)		
Total cholesterol***	Normal	173 (46.4)	108 (29.0)	92 (24.7)	5.937[a]	0.051
	Little high /High	19 (70.4)	5 (18.5)	3 (11.1)		
Antilipidemic medicine intake	Yes	4 (57.1)	1 (14.3)	2 (28.6)		
	No	188 (47.8)	112 (28.5)	93 (23.7)		
DM	Yes	39 (60.7)	9 (14.8)	15 (24.6)	7.064[a]	**0.029***
	No	155 (45.7)	104 (30.7)	80 (23.6)		
FBG	Ort±SS	132.36±74.29	110.70±25.51	114.38±38.41	1.106[a]	0.575
	Median (min-max)	106.5 (83-508)	104 (71-187)	108 (2-280)		
FBG without DM (n=142)	Normal	26 (44.8)	18 (31.0)	14 (24.1)	2.221[b]	0.695
	Risky	26 (39.4)	17 (25.8)	23 (34.8)		
	Diabetes	8 (44.4)	6 (33.3)	4 (22.2)		
PPG	Ort±SS	151.16±63.84	137.20±36.19	143.48±58.76	1.132[a]	0.568
	Median (min-max)	134.5 (82-453)	130 (94-265)	128 (87-389)		
PPG without DM (n=197)	Normal	59 (46.1)	43 (33.6)	26 (20.3)	1.897[b]	0.755
	Risky	31 (50.0)	19 (30.6)	12 (19.4)		
	Diabetes	5 (71.4)	1 (%14.3)	1 (14.3)		
BMI	Normal	36 (39.6)	27 (29.7)	28 (30.8)	20.896[a]	**0.001****
	Overweight	74 (41.1)	63 (35.0)	43 (23.9)		
	Obese	82 (63.6)	23 (17.8)	24 (18.6)		
Self-CVD history	Yes	35 (48.6)	22 (30.6)	15 (20.8)	0.487[a]	0.784
	No	157 (47.9)	91 (27.7)	80 (24.4)		
Family CVD history	Yes	23 (33.3)	26 (37.7)	20 (29.0)	7.325[a]	**0.026***
	No	169 (51.1)	87 (26.3)	75 (22.7)		
Smoking	Yes	108 (47.4)	59 (25.9)	61 (26.8)	3.116[a]	0.211
	No	84 (48.8)	54 (31.4)	34 (19.8)		
Physical activity level	Inactive level	69 (47.3)	41 (28.8)	35 (24.0)	13.087[a]	**0.011***
	Low-level active	93 (56.7)	40 (24.4)	31 (18.9)		
	Sufficient level active	30 (33.3)	31 (34.4)	29 (32.2)		

Table 5. *Analysis of the factors influencing perceived CVD risk in terms of actual CVD risk*

Variables	B	SE	OR	95% CI	P
Age (51-60 years)	-0.19	0.25	0.82	0.49-1.36	0.444
Age (61-65 years)	-1.06	0.38	0.34	0.16-0.73	**0.006**
Family CVD history	0.45	0.29	1.57	0.89-2.79	0.117
HT	-0.26	0.33	0.77	0.39-1.48	0.435
DM	-0.79	0.40	0.45	0.20-0.99	**0.048**
Overweight	0.31	0.29	1.37	0.77-2.42	0.280
Obese	-0.51	0.34	0.59	0.30-1.16	0.132
Physical activity (Low level active)	-0.32	0.27	0.72	0.42-1.22	0.232
Physical activity (Sufficient level active)	-0.06	0.30	0.94	0.51-1.71	0.845
Constant	-0.483	0.322	0.617		0.133

Multivariate logistic regression model
aActual CVD risk determination situations were included in the logistic regression model in two groups of right and wrong determinations. Moreover, only significant and close-significants were included in the analysis since not all the variables were included.
B: regression coefficient, SE: standard error, OR: odds ratio, CI: confidence interval, CVD: cardiovascular disease, HT: hypertension, DM: diabetes mellitus, BMI: body mass index

4. DISCUSSION

The most important finding of this study was that 71.8% of men were wrong in their perception of their CVD risks. Subsequently, 60.8% of men had a high/very high risk for actual CVD risk but only 20.3% of men perceived their CVD risks as high/very high. Individuals who do not think that they have a CVD risk are those who cannot adapt to risk reduction behaviors such as smoking cessation, etc. From this point forth, this finding is very important. Moreover, the study showed that in terms of substantially influencing the correct perception of CVD risk were factors that included being of a younger age; having a family history of CVD, HT, DM, or BMI; and having a physical activity level while at an older age and being DM. These findings highlights the predictors of risk perception for men for CVD.

In this study, it was observed that the participants had a skewed risk perception regarding CVD risks. About half are optimistic about CVD risks. This distorted perception may prevent them from taking action for risk management. Ideally, an individual's perceptions of their risks should be compatible with their actual risks. Only 28.2% of the participants correctly defined their risks. In previous studies, it has been determined that adults have skewed perceptions of CVD risks (23,25-28, 46-51). It is important to evaluate the 10-year CVD risk of adults and to give recommendations regarding the lifestyle appropriate to their risks in maintaining their health (15,16,18,21).

62.2% of older men perceived their CVD risks as at a low level (Table 4) although age is a CVD risk-increasing factor. In other words, they may be defined as "wrong optimists." Several other studies revealed that older men perceive their CVD risks as lower than their actual risk (23,27,46) while other studies stated that older men perceived a higher CVD risk than the actual risk (25,34,47).

The HeartScore program does not include family history in its risk calculation. A total of 33.3% of men who had a family history of CVD perceived a CVD risks lower than the actual CVD risk (Table 4). Available findings indicated that family history increased the sensitivity of family members. This study revealed that one out of three men with a family history of CVD had an incorrect perception of their CVD risk (Table 4). Contrary to this, conducted studies revealed that men with a family history of CVD tended to perceive their CVD risks at a high level (33,34,47).

Participants with HT were included in the direct high-risk group in the HeartScore calculation (18). However, in this study, more than half of men with HT (58.5%) perceived their risk as lower than the actual risk (Table 4). This finding indicates that a majority of men with HT are not aware of CVD risks. In similar studies, it was stated that men with HT perceive their CVD risks at a very low or zero level (33,46,47). This situation could be related to disease management and lack of CVD risk knowledge. Participants with DM are included in the direct very high-risk group in the HeartScore calculation (18). In this study, more than half of the men with DM (60.7%) perceived themselves to be in the low-risk group (Table 4). Similar studies revealed that men with DM perceive themselves to be in a lower risk group than their actual CVD risk level (27,33,48). It is possible to assert, upon evaluating this and other similar studies as a whole, that men with DM have a distorted CVD risk perceptions.

The correct CVD risk perception of the overweight group (35%) was higher than that of the group with normal weight (29.7%) and those who were obese (17.9%) (Table 4). Another study revealed that obese men have an accurate of risk perception of CVD (49). However, there are also studies that revealed that men with normal weight have a more accurate perception of their CVD risks (25).

This study determined that physically active men have a more correct CVD risk perception (Table 4). It is known that increased physical activity reduces CVD risks. Two other similar studies stated that physically active men perceive their CVD risks to be at a very low risk level (25,26). Hence, it can be said that physically active men have a correct perception of CVD risk.

Logistic regression analysis was performed here, as it is thought that there may be masking or confounding variables in determining the factors affecting the perceived risk of cardiovascular disease. Accordingly, it was determined that elderly (61-65) and individuals with DM misunderstood the cardiovascular disease risks ($p<0.05$). According to HeartScore, age is an important variable and advancing age increases the risk level (49). Contrary to the findings of this study, according to the relevant studies, it was determined that older men consider themselves to be at high risk for CVD (25,34,47,50). The lower educational level of older men

in this study may be a reason for not perceiving the risks correctly.

In various studies, it has been determined that the perceived CVD risks of men diagnosed with DM are at a lower level compared to the actual CVD risks (27,33,47,51). Once the studies in the literature evaluating the perceived CVD risks of men diagnosed with DM are evaluated as a whole, it is evident that the perceived CVD risks of men with diabetes are at a lower level compared to their actual CVD risks. However, it is known and acknowledged that men with DM are at a very high risk level in terms of CVD risk. Therefore, it can be said that men diagnosed with DM have a false perception of risk for CVDs, and it is necessary to provide informative trainings regarding CVD risks to men with DM and to inform them with respect to behaviors that help to reduce CVD risks in this manner.

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5. CONCLUSION

These results showed that more than half of the participants were at a very high level of CVD risk, and approximately one out of two men perceived their CVD risk to be lower than their actual risk. We suggest that the CVD risks and risk perceptions of men in 40-65 age range should be determined periodically. Distorted CVD risk perception may hinder the search for good health. Therefore, more frequent training and consultation should be offered, especially to individuals with diseases that increase CVD risk.

Conflicts of interest: The author declare no conflict of interest

REFERENCES

[1] World Health Organization (WHO). Cardiovascular diseases [Internet]; https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(CVDs) Accessed July 13, 2021.
[2] Balbay Y, Gagnon-Arpin I, Malhan S, Öksüz ME, Sutherland G, Dobrescu A, Villa G, Ertuğrul G, Habib M. Modeling the burden of cardiovascular disease in Turkey. Anatol J Cardiol. 2018;20(4):235-240.
[3] Yin J, Jin X, Shan Z, Li S, Huang H, Li P, Peng X, Peng Z, Yu K, Bao W, Yang W, Chen X, Liu L. Relationship of sleep duration with all-cause mortality and cardiovascular events: a systematic review and dose-response meta-analysis of prospective cohort studies. J Am Heart Assoc. 2017;6(9):e005947.
[4] Onat A, Can G. Prevalence of heart disease in our adults, new coronary events and death from heart, TEKHARF 2017 Leading the medical world in its approach to chronic diseases. Istanbul: Logos; 2017 (Turkish).
[5] Turkish Statistical Institute. Turkey Health Survey [Internet], 2016-2018; http://www.turkstat.gov.tr/PreTabloArama.do?metod=search. Accessed March 5, 2019 (Turkish).
[6] Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, Townsend N. The epidemiology of cardiovascular disease in the UK 2014. Heart 2015;101(15):1182-1189.
[7] Kotseva K, De Bacquer D, De Backer G, Rydén L, Jennings C, Gyberg V, Abreu A, Aguiar C, Conde AC, Davletov K, Dilic M, Dolzhenko M, Gaita D, Georgiev B, Gotcheva N, Lalic N, Laucevicius A, Lovic D, Mancas S, Miličić D, Oganov R, Pajak A, Pogosova N, Reiner Ž, Vulic D, Wood D, On Behalf of The Euroaspire Investigators. Lifestyle and risk factor management in people at high risk of cardiovascular disease. A report from the European Society of Cardiology European Action on Secondary and Primary Prevention by Intervention to Reduce Events (EUROASPIRE) IV cross-sectional survey in 14 European regions. Eur J Prev Cardiol. 2016;23(18):2007-2018.
[8] Kjøllesdal M, Degerud E, Næss Ø. The association between obesity and cardiovascular disease mortality in different strata of socioeconomic position: evidence from pooled Norwegian health surveys. Eur J Public Health. 2019;29(6):1160-1166.
[9] Borodulin K, Vartiainen E, Peltonen M, Jousilahti P, Juolevi A, Laatikainen T, Männistö S, Salomaa V, Sundvall J, Puska P. Forty-year trends in cardiovascular risk factors in Finland. Eur J Public Health. 2014;25(3):539-546.
[10] Li T, Hashimoto H. Household dietary salt consumption and mortality from cardiovascular diseases: an ecological panel analysis in Japan. Eur J Public Health. 2019;29(5):826-832.
[11] Ünal B, Sözmen K, Uçku R, Ergör G, Soysal A, Baydur H, Meseri R, Şimşek H, Gerçeklioğlu G, Doğanay S, Budak R, Kiliç B, Günay T, Ergör A, Demiral Y, Aslan Ö, Çimrin D, Akvardar Y, Tuncel P. High prevalence of cardiovascular risk factors in a Western urban Turkish population: a community-based study. Anatol J Cardiol. 2013;13(1):9-17.
[12] Ministry of Health, Turkey. Health Statistics Yearbook [Internet], 2017; https://dosyasb.saglik.gov.tr/Eklenti/30148,ingilizcesiydijiv1pdf.pdf?0. Accessed February 4, 2019 (Turkish).
[13] Ritchie H, Roser M. "Smoking" [Internet], 2019; https://ourworldindata.org/smoking. Accessed June 10, 2022.
[14] The Substance Abuse and Mental Health Services Administration (SAMHSA), Center for Behavioral Health Statistics and Quality. National Survey on Drug Use and Health. [Internet], 2019; https://www.samhsa.gov/data/sites/default/files/reports/rpt29394/NSDUHDetailedTabs2019/

NSDUHDetTabsSect2pe2019.htm#tab2-19b. Accessed July 21, 2021.
[15] Eray A, Ateş E, Set T. Assessment of cardiovascular disease risk in adults. Turk J Fam Pract. 2018;22(1):12-9 (Turkish).
[16] Ministry of Health, Turkey. Periodic Health Examinations and Screening Tests Proposed in Family Medicine Practice [Internet], 2015; https://dosyaism.saglik.gov.tr/Eklenti/43917,5—aile-hekimligi-uygulamasinda-onerilen-periyodik-saglik-muayeneleri-ve-tarama-testleri-rehberipdf.pdf?0. Accessed August 15, 2019 (in Turkish).
[17] Mossakowska TJ, Saunders CL, Corbett J, MacLure C, Winpenny EM, Dujso E, Payne RA. Current and future cardiovascular disease risk assessment in the European Union: an international comparative study. Eur J Public Health. 2018;28(4):748-754.
[18] Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs FDR, Løchen ML, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren WMM, Binno S; ESC Scientific Document Group. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the sixth joint task force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention and Rehabilitation (EACPR). Eur Heart J. 2016;37(29):2315–2381.
[19] Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT; ESC Scientific Document Group. 2016 ESC/EAS guidelines for the management of dyslipidemias. Eur Heart J. 2016;37(39):2999-3058.
[20] Akgöz A, Gözüm S. Cardiovascular disease risk in Turkish family health centers. J Vasc Nurs. 2019;37(2):117-124.
[21] Ministry of Health, Turkey. Cardiovascular diseases prevention and control program 2015-2020. Ankara: Report no, 988 (Turkish).
[22] Leite L, Baptista R, Leitão J, Cochicho J, Breda F, Elvas L, Fonseca I, Carvalho A, Costa JN. Chest pain in the emergency department: risk stratification with Manchester triage system and HEART score. BMC Cardiovasc Disord. 2015;15(1):48-55.
[23] Ko Y, Boo S. Self-perceived health versus actual cardiovascular disease risks. Jpn J Nurs Sci. 2016;13(1):65-74.
[24] de Lima MM Jr, da Silva GR, Jensem Filho SS, Granja F. Association between perceived lifetime risk of cardiovascular disease and calculated risk in a male population in Brazil. Vasc Health Risk Manag. 2016;12:279-286.
[25] Alwan H, William J, Viswanathan B, Paccaud F, Bovet P. Perception of cardiovascular risk and comparison with actual cardiovascular risk. Eur J Cardiovasc Prev Rehab. 2009;16(5):556-561.
[26] Barnhart JM, Wright ND, Freeman K, Silagy F, Correa N, Walker EA. Risk perception and its association with cardiac risk and health behaviors among urban minority adults: the Bronx Coronary Risk Perception study. Am J Health Promot. 2009;23(5):339-342.
[27] Desgraz B, Collet TH, Rodondi N, Cornuz J, Clair C. Comparison of self-perceived cardiovascular disease risk among smokers with Framingham and PROCAM scores: a cross-sectional analysis of baseline data from a randomized controlled trial. BMJ Open. 2017;7(1):e012063.
[28] Katz M, Laurinavicius AG, Franco FG, Conceicao RD, Carvalho JA, Pesaro AE, Wajngarten M, Santos RD. Calculated and perceived cardiovascular risk in asymptomatic subjects submitted to a routine medical evaluation: the perception gap. Eur J Prev Cardiol. 2015;22(8):1076-1082.
[29] Temu TM, Kirui N, Wanjalla C, Ndungu AM, Kamano JH, Inui TS, Bloomfield GS. Cardiovascular health knowledge and preventive practices in people living with HIV in Kenya. BMC Infect Dis. 2015;15(1):421-429.
[30] Cleland C, Ferguson S, Ellis G, Hunter RF. Validity of the International Physical Activity Questionnaire (IPAQ) for assessing moderate-to-vigorous physical activity and sedentary behavior of older adults in the United Kingdom. BMC Med Res. Methodol 2018;18(1):176.
[31] European Society of Preventive Cardiology. HeartScore Turkey website [Internet]. https://heartscore.escardio.org/2016/Login.aspx?ReturnUrl=%2f2016%2fdefault.aspx%3fmodel%3dturkey&model=turkey. Accessed May 2, 2019.
[32] Kleindorfer DO, Towfighi A, Chaturvedi S, Cockroft KM, Gutierrez J, Lombardi-Hill D, Kamel H, Kernan WN, Kittner SJ, Leira EC, Lennon O, Meschia JF, Nguyen TN, Pollak PM, Santangeli P, Sharrief AZ, Smith SC Jr, Turan TN, Williams LS. 2021 Guideline for the prevention of stroke in patients with stroke and transient ischemic attack. Stroke. 2021;52: e364–e467.
[33] Boo S, Froelicher ES, Yun JH, Kim YW, Jung JY, Suh CH. Perceived and actual risk of cardiovascular disease in patients with rheumatoid arthritis in Korea: a cross-sectional study. Medicine (Baltimore). 2016;95(40): e5117.
[34] Petr EJ, Ayers CR, Pandey A, de Lemos JA, Powell-Wiley TM, Khera A, Lloyd-Jones DM, Berry JD. Perceived lifetime risk for cardiovascular disease (from the Dallas Heart Study). Am J Cardiol. 2014;114(1):53-58.
[35] Saglam M, Arikan H, Savci S, Inal-Ince D, Bosnak-Guclu M, Karabulut E, Tokgozoglu L. International physical activity questionnaire: reliability and validity of the Turkish version. Percept Mot Skills. 2010;111(1):278-284.
[36] World Health Organization (WHO). Guide to Physical Measurements [Internet]. https://www.who.int/ncds/surveillance/steps/Section%204%20Step%202%20Physical%20Measurements.pdf. Accessed May 3, 2019.
[37] Centers for Disease Control and Prevention (CDC). About Adult BMI [Internet]; https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html. Accessed August 1, 2019.
[38] Martinez TLR, Santos RD, Armaganijan D, Torres KP, Loures-Vale A, Magalhães ME, Lima JC, Moriguchi E, Amodeo C, Ortiz J. National alert campaign about increased cholesterol: determination of cholesterol levels in 81, 262 Brazilians. Arq Bras Cardiol. 2003;80(6):635-638.
[39] Roche Diagnostic Turkey. Products [Internet], 2022; https://diagnostics.roche.com/gb/en/home.html Accessed June 6, 2022.
[40] Coqueiro Rda S, Santos MC, Neto Jde S, Queiroz BM, Brügger NA, Barbosa AR. Validity of a portable glucose, total cholesterol, and triglycerides multi-analyzer in adults. Biol Res Nurs. 2014;16(3):288-294.
[41] American Diabetes Association (ADA). Standards of medical care in diabetes. Diabetes Care. 2020;43(suppl 1):S16-S71.
[42] Freckmann G, Schmid C, Baumstark A, Pleus S, Link M, Haug C. System accuracy evaluation of 43 blood glucose monitoring systems for self-monitoring of blood glucose according to DIN EN ISO 15197. J Diabetes Sci Technol. 2012;6(5):1060-1075.

[43] Arıcı M, Birdane A, Güler K, Yıldız BO, Altun B, Ertürk Ş, Aydoğdu M, Özbakkaloğlu M, Ersöz HÖ, Süleymanlar G, Tükek T, Tokgözoğlu L, Erdem Y. Turkish hypertension consensus report. Turk Kardiyol Dern Ars. 2015;43(4):402-409. (Turkish)

[44] Weber MA, Schiffrin EL, White WB, Mann S, Lindholm LH, Kenerson JG, Flack JM, Carter BL, Materson BJ, Ram CV, Cohen DL, Cadet JC, Jean-Charles RR, Taler S, Kountz D, Townsend RR, Chalmers J, Ramirez AJ, Bakris GL, Wang J, Schutte AE, Bisognano JD, Touyz RM, Sica D, Harrap SB. Clinical practice guidelines for the management of hypertension in the community: a statement by the American Society of Hypertension and the International Society of Hypertension. J Clin Hypertens (Greenwich). 2014;16(1):14-26.

[45] Centers for Disease Control and Prevention (CDC). National Health Interview Survey, Adult Tobacco Use Information [Internet]; https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm. Accessed August 16, 2019.

[46] Mohd Azahar NMZ, Krishnapillai ADS, Zaini NH, Yusoff K. Risk perception of cardiovascular diseases among individuals with hypertension in rural Malaysia. Heart Asia. 2017;9(2):1-4.

[47] Frijling BD, Lobo CM, Keus IM, Jenks KM, Akkermans RP, Hulscher ME, Prins A, van der Wouden JC, Grol RP. Perceptions of cardiovascular risk among patients with hypertension or diabetes. Patient Educ Couns. 2004;52(1):47-53.

[48] van der Weijden T, van Steenkiste B, Stoffers HE, Timmermans DR, Grol R. Primary prevention of cardiovascular diseases in general practice: mismatch between cardiovascular risk and patients' risk perceptions. Med Decis Making 2007;27(6):754-61.

[49] Masic I, Dilic M, Raljevic E, Vulic D, Mott D. Trends in cardiovascular diseases in Bosnia and Herzegovina and perspectives with HeartScore Programme. Med Arh 2010;64(5):260-263.

[50] Meischke H, Sellers DE, Sellers DE, Goff DC, Daya MR, Meshack A, Taylor J, Zapka J, Hand MM. Factors that influence personal perceptions of the risk of an acute myocardial infarction. Behav Med 2000;26(1):4-13.

[51] Choi S, Rankin S, Stewart A, Oka R. Perceptions of coronary heart disease risk in Korean immigrants with type 2 diabetes. Diabetes Educ 2008;34(3):484-492.

How to cite this article: Topuz I, Gozum S. A Comparison of Actual Cardiovascular Disease Risks to the Perceptions of Middle-aged Men: A Cross-Sectional Study. Clin Exp Health Sci 2022; 12: 607-617. DOI: 10.33808/clinexphealthsci.984039

Clinical and Experimental Health Sciences

Comparison of Clinical-related Characteristics of Endodontic Patients Before and During the COVID-19 Pandemic

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Received: 27.09.2021 **Accepted:** 22.06.2022

ABSTRACT

Objective: To compare patient-related and clinical-related characteristics of endodontic patients, before and during the COVID-19 pandemic.

Methods: The study population comprised 506 patients (teeth: *n*=674) aged 13–81 years who attended undergraduate dental clinics in the Endodontics Department of the Faculty of Dentistry at Biruni University for endodontic treatment. Patient-related and clinical-related data were compared at two time intervals: before the first COVID-19 case was reported in Turkey and after the first COVID-19 case was reported in Turkey.

Results: There were no significant differences in terms of age and sex of the patients attending the clinics before versus during the pandemic. The incidence of reported pain before the pandemic was significantly lower than that reported during the pandemic *(p*=0.041). Periapical health before the pandemic was significantly better than that during the pandemic (*p*<0.001). The frequency of a diagnosis of asymptomatic irreversible pulpitis before the pandemic was significantly higher than that during the pandemic (*p*<0.001). The frequency of a diagnosis of asymptomatic apical periodontitis was higher during the pandemic than before the pandemic (*p*<0.001).

Conclusion: These results may be explained by patients, other than those with severe symptoms, not wishing to attend endodontics clinics during the pandemic due to concerns about contracting the virus and passing the infection to family members.

Keywords: COVID-19, endodontic diagnosis, PAI score, pandemic, systemic disorders

1. INTRODUCTION

An outbreak of coronavirus disease 2019 (COVID-19) caused by a new coronavirus named severe acute respiratory syndrome coronavirus 2 was reported in Wuhan, Hubei, China in late December 2019 (1). The virus spread throughout China before then spreading to other countries, leading to a pandemic and international health crisis (2). Inhalation and direct contact were suggested as the most likely transmission routes (1). The World Health Organization (WHO) announced COVID-19 a pandemic on March 11, 2020, after which most countries instigated various virus transmission prevention measures, including lockdowns, travel bans and closing services not considered essential (3). By the end of December 2020, vaccination had commenced in a number of countries with the aim of decreasing the spread of COVID-19. Globally, as of March 11, 2021, the WHO had reported over 118.3 million COVID-19 cases and over 2.6 million COVID-19-related deaths (4).

Although vaccination started gradually, people's lives did not return to normal. Many patients did not return to endodontics clinics for treatment, most likely due to concerns about contracting the virus. Clinical education in dentistry faculties resumed in Turkey in October 2020. Treatment in undergraduate dental clinics also resumed at this time. Endodontics clinics are unique among dental clinics in that the patient population tends to comprise individuals with severe odontogenic pain. Pulpal and periapical diseases account for a significant number of all dental emergencies (5,6). Symptomatic irreversible pulpitis, symptomatic apical periodontitis and acute apical abscesses are the most common endodontic emergencies (5-7). Although patients with these disorders may have no clinical symptoms for some time, radiological signs of an endodontic infection may be detected during routine checkups.

The COVID-19 pandemic poses a risk to many individuals, particularly those with systemic disorders. Dental patients with systemic disorders may feel particularly vulnerable to contracting the virus. The dental treatment requirements

DOI: 10.33808/clinexphealthsci.1001197

of individuals with systemic disorders during the pandemic need to be addressed (8).

There are no retrospective studies on clinical-related characteristics of endodontic patients before and during the COVID-19 pandemic. The purpose of the present study was to compare patient-related (age, sex and systemic disorders) and clinical-related (tooth type, symptoms, periapical index (PAI) scores and endodontic diagnoses) characteristics of endodontic patients, before versus during the COVID-19 pandemic.

2. METHODS

The present study was approved by Biruni University Institutional Review Board, Turkey (19.03.2021 – 2021/49-15).

2.1. Patient Selection

The study population comprised 506 patients (teeth: *n*=674) aged 13–81 years who visited student clinics in the Endodontics Department of the Dentistry Faculty at Biruni University for endodontic treatment at two time intervals: between October 7, 2019 and January 10, 2020 (Fall semester of the 2019–2020 academic year and before March 11, 2020 when the first COVID-19 case in Turkey was recorded) and between October 5, 2020 and January 08, 2021 (Fall semester of the 2020–2021 academic year and after March 11, 2020 when the first COVID-19 case in Turkey was recorded). Data on the patient's age, sex and medical and dental histories were obtained from the patient database system. In total, the records of 315 patients (teeth: *n*=408) before the first reported COVID-19 case in Turkey and 191 patients (teeth: *n*=266) after the first reported case were evaluated.

Patients older than 13 years whose endodontic treatment had been performed by fourth – or fifth-year dental undergraduate students and for whom periapical radiographs of the area of interest were available for diagnosis were included in the present study. Pregnant patients, patients with cellulitis, patients with diffuse soft tissue bacterial infections or intra – or extra-oral swellings and patients whose radiographies were difficult to examine were excluded, as well as teeth with periapical cysts, trauma, apical surgery, endo-perio lesions, third molar teeth and retreated teeth.

2.2. Patient Eecords' Assessment

The patients were divided into the following age groups: 13 – to 19-year, 20 – to 29-year, 30 – to 39-year, 40 – to 49-year, 50 – to 59-year and 60+ year. For all patients, the following parameters were recorded: age, sex and systemic disorder history; tooth type; presence of pain and swelling; PAI score; and endodontic diagnosis. Data on medical treatment histories, current meical treatments and current drug use were recorded. Cardiovascular diseases, hypertension, diabetes, chronic kidney disease, respiratory disease, chronic liver disease gastrointestinal disease, obesity, cancer and blood disorders were regarded as systemic disorders.

2.3. Clinical Assessment

The clinical assessment was based on subjective and objective findings. In terms of subjective findings, chief complaints, such as symptoms, duration, location, onset and pain stimuli, were recorded. In relation to objective findings, facial symmetry, sinus tract (if any), soft tissue, caries and restorations were recorded. Pulp tests were performed if necessary, as well as percussion and palpation tests (9). No evoked or spontaneous pain was classified as 'no pain'. Pain that was not spontaneous but provoked by hot, chewing, percussion or palpation was considered 'evoked' pain. Severe, sharp and continuous pain, which kept the patient awake at night and persisted after removal of the stimulus, or deep, continuous, dull and throbbing pain, which increased on biting, was considered 'spontaneous' pain'. Swelling was classified as 'no swelling' or 'swelling', with swelling referring to localized swelling of associated soft tissue.

2.4. Radiographic Assessment

The periapical status was determined using the PAI suggested by Orstavik et al. (10), in which the periapical section on a radiograph is scored as below:

1 = Normal periapical structures

2 = Small changes in bone structure

3 = Changes in bone structure with some mineral loss

4 = Periodontitis with well-defined radiolucent area

5 = Severe periodontitis with exacerbating features

Teeth with more than one root were categorized according to the root with the highest score. The periapical status of all 674 teeth involved in this study were analysed radiographically. The PAI scores were determined based on periapical radiographs taken using a phosphor plate system (Dürr Dental, Bietigheim-Bissingen, Germany), using the parallel technique for standardization. The radiographs were analysed by two experienced endodontists using Picture Archiving and Communication Systems software version 1.1.1.6 for Windows 10 (Microsoft Corporation, Redmont, WA, USA) displayed on a 28-inch Samsung LU28H750UQMXUF monitor (Samsung Electronics, Seoul, South Korea) with 3,840 x 2160 pixel resolution. Before examining the radiographs, for calibration training, each examiner assessed a series 20 radiographs not related to this study. Cohen's kappa was used to assess inter-examiner consensus. A value of 0.89 denoted excellent agreement. Disagreement between the two examiners in the radiographic examinations was resolved through discussion until consensus was reached.

2.5. Diagnostic Assessment

The endodontic diagnoses were based on the criteria of the American Association of Endodontics and American Board of Endodontics (11). According to these criteria, asymptomatic irreversible pulpitis was considered vital inflamed pulp that

failed to resolve in patients without symptoms. Symptomatic irreversible pulpitis was considered vital inflamed pulp that failed to resolve in patients with mild symptoms, which that did not impact on routine activities, or discomfort, which had a moderate or severe impact on routine activities. Asymptomatic apical periodontitis was considered inflammation of pulpal origin or destruction of the apical periodontium in patients with no clinical symptoms. Symptomatic apical periodontitis was classified as inflammation of pulpal origin or destruction of the apical periodontium in patients with discomfort that had a moderate or severe impact on routine activities and responded painfully to chewing, percussion or palpation. An acute apical abscess was classified as an inflammatory reaction to pulpal infection and necrosis, with rapid onset of spontaneous pain and swelling. A chronic apical abscess was considered an inflammatory reaction to pulpal infection and necrosis, with gradual onset and no or mild discomfort, discharge of pus through a sinus tract.

2.6. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY). Descriptive statistical methods (mean, standard deviation and frequency) were used for evaluation of the study data. A chi-square test and Fisher's exact chi-square test were used for comparison of qualitative data. A value of $p<0.05$ was considered significant.

3. RESULTS

Table 1 provides information on the distribution of the patients according to age, sex and presence and type of systemic disorders. There were no significant differences in terms of age, sex or presence and type of systemic disorders before versus during the pandemic ($p=0.619$, $p=0.533$, $p=0.994$ and $p>0.05$, respectively; Table 1).

Table 2 shows the distribution of tooth type, the presence and type of pain, the presence of swelling and periapical health and endodontic diagnoses before and during the pandemic. There was no significant difference between tooth types before versus during the pandemic ($p=0.111$). The incidence of reported pain before the pandemic was significantly lower than that reported during the pandemic ($p=0.041$) (Fig.1). In terms of pain types and swelling, there was no significant difference before versus during the pandemic ($p=0.855$ and $p=1.000$, respectively). Periapical health before the pandemic was significantly better than that than during the pandemic ($p<0.001$) (Fig. 2). The prevalence of a diagnosis of asymptomatic irreversible pulpitis was significantly higher before the pandemic as compared with that during the pandemic ($p<0.001$). The prevalence of a diagnosis of asymptomatic apical periodontitis diagnosis before the pandemic was lower than that during the pandemic ($p<0.001$). There were no statistical differences in terms of the frequencies of symptomatic irreversible pulpitis, symptomatic apical periodontitis, acute apical abscess and chronic apical abscess diagnoses before versus during the pandemic ($p>0.05$) (Fig. 3).

Table 1. *The distribution of the patients according to age, sex, presence and type of systemic disorders in terms of pandemic (n=506)*

		Before Pandemic	During Pandemic	
		n (%)	n (%)	*p*
Age	13-19	59 (18.7)	45 (23.6)	[1]0.619
	20-29	62 (19.7)	44 (23)	
	30-39	73 (23.2)	38 (19.9)	
	40-49	64 (20.3)	32 (16.8)	
	50-59	29 (9.2)	17 (8.9)	
	60+	28 (8.9)	15 (7.9)	
Sex	Male	128 (40.6)	83 (43.5)	[1]0.533
	Female	187 (59.4)	108 (56.5)	
Systemic Disorders	Present	71 (22.5)	43 (22.5)	[1]0.994
	Absent	244 (77.5)	148 (77.5)	
Systemic Disorders	Cardiovascular diseases	9 (12.7)	10 (23.3)	[2]0.226
	Hypertension	36 (50.7)	18 (41.9)	[2]0.470
	Diabetes	9 (12.7)	8 (18.6)	[2]0.555
	Respiratory disease	10 (14.1)	7 (16.3)	[2]0.962
	Chronic kidney disease	4 (5.6)	0 (0%)	[3]0.295
	Chronic liver disease	7 (9.9)	1 (2.3)	[3]0.255
	Gastrointestinal disease	4 (5.6)	0 (0%)	[3]0.295
	Cancer	1 (1.4)	2 (4.7)	[3]0.556
	Obesity	3 (4.2)	3 (7)	[3]0.671
	Blood disorders	4 (5.6)	6 (14)	[3]0.174

[1]*Chi-square test* [2]*Continuity (yates) correction* [3]*Fisher's Exact test*

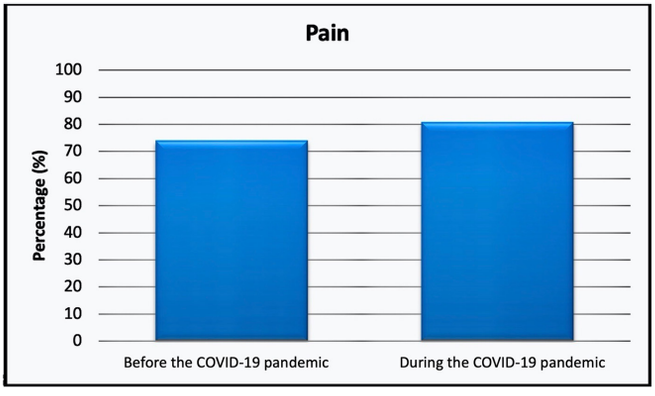

Figure 1. *The frequency of pain in terms of before and during pandemic*

Table 2. *The distribution of tooth type, presence and type of pain, presence of swelling, periapical health and endodontic diagnosis in terms of pandemic (n=674)*

		Before Pandemic	During Pandemic	
		n (%)	n (%)	*p*
Tooth Type	Maxillary anterior	44 (10.8)	36 (13.5)	0.111
	Maxillary premolar	101 (24.8)	55 (20.7)	
	Maxillary molar	102 (25)	51 (19.2)	
	Mandibular anterior	21 (5.1)	10 (3.8)	
	Mandibular premolar	53 (13)	38 (14.3)	
	Mandibular molar	87 (21.3)	76 (28.6)	
Pain	Absent	106 (26)	51 (19.2)	0.041*
	Present	302 (74)	215 (80.8)	
Type of Pain	Evoked	131 (43.4)	95 (44.2)	0.855
	Spontaneous	171 (56.6)	120 (55.8)	
Swelling	Absent	394 (96.6)	257 (96.6)	+1.000
	Present	14 (3.4)	9 (3.4)	
Periapex	Healthy	368 (90.2)	202 (75.9)	<0.001*
	Not healthy	40 (9.8)	64 (24.1)	
Diagnosis	Asymptomatic irreversible pulpitis	124 (30.4)	33 (12.4)	<0.001*
	Symptomatic irreversible pulpitis	85 (20.8)	72 (27.1)	
	Asymptomatic apical periodontitis	108 (26.5)	111 (41.7)	
	Symptomatic apical periodontitis	76 (18.6)	36 (13.5)	
	Acute apical abscess	14 (3.4)	9 (3.4)	
	Chronic apical abscess	1 (0.2)	5 (1.9)	

Chi-square test *+Continuity (yates) correction* **p<0.05*

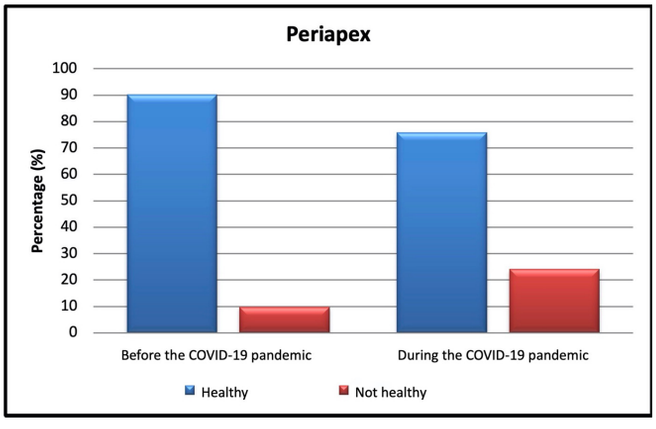

Figure 2. *The status of periapical health in terms of before and during pandemic*

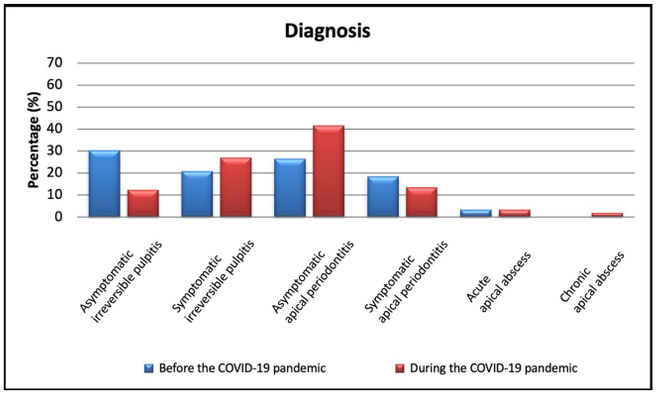

Figure 3. *The distribution of endodontic diagnosis in terms of before and during pandemic*

4. DISCUSSION

Undergraduate clinics in the endodontics department of our dentistry faculty resumed practice in the fall term of 2020–2021 academic year. As shown in this study, a smaller number of patients (n=191) visited these clinics during this period as compared with the same period the previous year (n=315). This finding was in accordance with that found in a research across the United States, which reported a decrease in the number of individuals attending endodontics clinics after the end of lockdowns (12). The decrease in numbers may be due to patients' fears of contracting the virus in the clinics and transmitting it to their families.

In our study, the results revealed no difference in the ages of the patients attending clinics before versus during the pandemic. In terms of sex, more females than males attended the clinics during both time intervals. The latter may be explained by the relatively low percentage of working women in Turkey. In contrast, Yu et al. reported little difference in the percentage of male and female patients attending endodontics clinics in Wuhan during the COVID-19 pandemic (13).

In present study, there was no significant difference in frequency of patients in the same age groups before versus during the pandemic. However, patients in the 30–39 year age group (23.2%) accounted for the majority of endodontic patients attending the clinics before the pandemic, whereas patients in the 13–19 year age group (23.6%) accounted for the majority of patients attending the clinics during the pandemic. This finding may be explained by schools being closed during the pandemic and children having a reduced risk of severe illness if infected with the virus. In contrast to our study, Yu et al. reported that those aged 45–64 years accounted for the majorioty of endodontic patients in their study on visits to endodontics clinics in Wuhan (13).

The presence of a systemic disorder is a major risk factor for severe illness if contaminated with SARS-CoV-2 (14). In the present study, number of patients with systemic disorders attending our endodontics clinics during the pandemic did not decrease significantly as compared with the number before the pandemic. Hypertension was the most frequent systemic disorder both before and during the pandemic. This result was similar to that of Bogari et al., who reported that hypertension was the most common systemic disorder (63.7%) among their endodontic patients (15). As one in every three people worldwide is expected to develop hypertension in their lifetime (15), the high frequency of this disorder in our study is not unexpected. Clinicians have a duty to evaluate risk factors for this systemic disease as part of health care, especially during the pandemic.

In terms of tooth type and endodontic treatment, in our study, maxillary molars most frequently required treatment before the pandemic, whereas mandibular molars most frequently required treatment during the pandemic. Our results also showed that mandibular anteriors were the least likely teeth to require endodontic treatment both before and during the pandemic. Our study results were comparable to those of other studies in terms of tooth types most commonly requiring endodontic treatment. Abuzenada et al. noted that mandibular molars were the most commonly involved tooth considering the endodontic treatment (16). In contrast, Demirci et al. reported that maxillary molars were more vulnerable to caries than mandibular molars (17). Differences in patient profiles with respect to education, income or social class might explain the inconsistency between the results in our study versus those reported in the literature.

Johnson et al. reported that a higher number of patients visited emergency endodontics clinics before the COVID-19 pandemic than during the pandemic (18). In our study, although there was a decrease in the number of individuals visiting undergraduate clinics of our endodontics department during the pandemic, the prevalence of individuals with the complaint of pain increased during the pandemic in comparison with the frequency before the pandemic (80.8% vs. 74%). In terms of evoked and spontaneous pain, the frequency was almost the same before versus during the pandemic in the present study. Both evoked and spontaneous pain are subjective findings. In the absence of confirmation by a clinical examination, subjective findings may not be a reliable indidcator of pain that occurred before and during the pandemic. In this study, the frequency of swelling was the same before the pandemic as during the pandemic. This may be due to swelling being a clinical emergency that caused patients to visit the clinic immediately, irrespective of the presence or absence of the pandemic.

A healthy periapical structure is vital for dental health. During the lockdown, all undergraduate clinics in dentistry faculties in Turkey were closed for at least 6 months. Therefore, treatment plans, both for patients midway through endodontic treatment and those scheduled to commence treatment could not proceed. As can be seen from the results of our study, the periapical health of the patients attending our clinics was significantly worse during the pandemic than before the pandemic. An increase in periapical lesions is to be expected, as no treatment was available while the clinics were closed. Rechenberg et al. pointed to a correlation between PAI scores and pain (19). In this study, PAI scores of the patients increased during the pandemic in comparison with those before the pandemic, indicating an unhealthy periapical apex and pain.

Previous studies reported that symptomatic irreversible pulpitis and symptomatic apical periodontitis were the most common endodontic diseases at the beginning of the pandemic (13,20). In our study, asymptomatic irreversible pulpitis and asymptomatic apical periodontitis were more common than other diseases before and during the pandemic, respectively. The inconsistency in the results might be explained by the earlier studies focusing on events during the first months of the pandemic (13,20). In the present study, patient records pertaining to the fall term of 2019–2020 academic year were taken as the first time interval, and the records of the fall term of 2020–2021 academic year were taken as the second time interval. In addition, in our study, the high frequency of asymptomatic apical periodontitis was clearly associated with the increase in PAI scores during the pandemic.

5. CONCLUSION

In the present study , there was an increased frequency of pain and higher PAI scores detected during the pandemic versus before the pandemic. Furthermore, the frequency of asymptomatic apical periodontitis after the COVID-19 pandemic was higher than normal. These results may be explained by patients, other than those with severe symptoms, not wishing to attend endodontics clinics during the pandemic due to concerns about contracting the virus and passing the infection to family members.

REFERENCES

[1] Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet. 2020;395(10223):470-473. DOI: 10.1016/S0140-6736(20)30185-9

[2] Zhang J, Lin G, Zeng J, Lin J, Tian J, Li G. Challenges of SARS-CoV-2 and lessons learnt from SARS in Guangdong Province, China. J Clin Virol. 2020;126(5):104341. DOI: 10.1016/j.jcv.2020.104341

[3] World Health Organization. Coronavirus disease (COVID-19) outbreak situation. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed March 21, 2020.

[4] World Health Organization. Coronavirus disease (COVID-19) weekly epidemiological update and weekly operational update. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed March 21, 2021.

[5] Yu J, Zhang T, Zhao D, Haapasalo M, Shen Y. Characteristics of Endodontic Emergencies during Coronavirus Disease 2019 Outbreak in Wuhan. J Endod. 2020;46(6):730-735.

[6] Rechenberg DK, Held U, Burgstaller JM, Bosch G, Attin T. Pain levels and typical symptoms of acute endodontic infections: a prospective, observational study. BMC Oral Health. 2016;16(5):61.

[7] Dou L, Vanschaayk MM, Zhang Y, Fu X, Ji P, Yang D. The prevalence of dental anxiety and its association with pain and other variables among adult patients with irreversible pulpitis. BMC Oral Health. 2018;18(6):101.

[8] Dimitroulas T, Bertsias G. Practical issues in managing systemic inflammatory disorders during the COVID-19 pandemic. Mediterr J Rheumatol. 2020;31(2):253-256.

[9] Tan B, Sun Q, Xiao J, Zhang L, Yan F. Pulp status of teeth in patients with chronic advanced periodontitis. Int J Clin Exp Pathol. 2020;13(4):635-641.

[10] Zanini M, Decerle N, Hennequin M, Cousson PY. Revisiting Orstavik's PAI score to produce a reliable and reproducible assessment of the outcomes of endodontic treatments in routine practice. Eur J Dent Educ. 2021;25(2):291-298.

[11] Shinde O, Pawar AM, Banga KS, Atram J, Wahjuningrun DA. Endodontic Emergencies in Mumbai City during COVID-19 Lockdown and Different Phases of Unlock. Int J Environ Res Public Health. 2021;18(14):7314.

[12] Martinho FC, Griffin IL. A cross-sectional survey on the impact oc coronavirus disease 2019 on the clinical practice of endodontists across the United States. J Endod. 2021;47(1):28 – 38.

[13] Yu J, Zhang T, Zhao D, Haapasalo M, Shen Y. Characteristics of endodontic emergencies during coronavirus disease 2019 outbreak in Wuhan. J Endod. 2020;46(6):730-735.

[14] Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. Int J Infect Dis. 2020;94(5):91-95.

[15] Bogari DF, Bakalka GT, Hazzazi LW, Jan AM, Elias WY, McDonald NJ, Alhazzazi TY. The prevalence of hypertension in endodontic clinics: A pilot study. Dentistry 2016;6(4):1000370.

[16] Abuzenada B, Syed GA, Pullishery F, Ahmed T, Sindi A, Khan A, Alogaly J, Alshareef M. Frequency and Distribution of Teeth Requiring Endodontic Treatment in Jeddah Subpopulation: A Retrospective Cross-sectional Study. Ann Med Health Sci Res. 2021;11(5):1428-1430. https://www.amhsr.org/articles/frequency-and-distribution-of-teeth-requiringendodontic-treatment-in-jeddah-subpopulation-aretrospective-crosssectional-study-8102.html

[17] Demirci M, Tuncer S, Yuceokur AA. Prevalence of caries on individual tooth surfaces and its distribution by age and gender in university clinic patients. Eur J Dent. 2010;4(3):270-279.

[18] Johnson RE, Foy TE, Ellingsen TA, Nelson JL, Dillon JK. Odontogenic infections : Disease burden during COVID-19 at a single institution. J Oral Maxillofac Surg. 2021;79(4):830-835.

[19] Rechenberg DK, Munir A, Zehnder M. Correlation between the clinically diagnosed inflammatory process and periapical index scores in severely painful endodontically involved teeth. Int Endod .2021;54(2):172-180.

[20] Patel B, Eskander MA, Ruparel NB. To drill or not to drill: Management of endodontic emergencies and in-process patients during the COVID-19 pandemic. J Endod. 2020;46(11):1559-1569.

How to cite this article: Kaplan T, Sonmez Kaplan S. Comparison of Clinical-related Characteristics of Endodontic Patients Before and During the COVID-19 Pandemic. Clin Exp Health Sci 2022; 12: 618-623. DOI: 10.33808/clinexphealthsci.1001197

Clinical and Experimental Health Sciences

Which Phenotypic Method Is the Most Accurate for Detection of Extended – Spectrum β-Lactamases (ESBLs) in *Escherichia coli* ?

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Received: 27.08.2021 **Accepted:** 12.07.2022

ABSTRACT

Objective: The aim of the study is to determine the Extended-Spectrum β-Lactamases (ESBLs) by three different phenotypic methods of the *Escherichia coli (E. coli)* strains that isolated from various clinical samples.

Methods: A total of 93 *E. coli* samples were isolated from hospitalized patients. Antibiotic susceptibility tests were done by automated system Phoenix 100 (Becton Dickinson, Sparks, MD, USA). ESBL production was tested by double disc synergy test (DDST), combined disc test (CDT) and three-dimensional test (TDT). All statistical analyses were done using statistical packages SPSS Demo Ver 22 (SPSS Inc. Chicago, IL, USA).

Results: In the investigation of ESBL production among *E. coli* species, 87 (93.5%) strains were ESBL positive by DDST, 73 (78.5%) strains were ESBL positive by CDT, 71 (76.3%) strains were ESBL positive by TDT. According to statistical analysis: There were statistical differences between DDST-CDT (p=<0.001) and DDST-TDT (p=<0.001). However, there was no statistical difference between CDT-TDT (p=0.207)

Conclusion: According to our study results, DDST test was more advantageous than CDT and TDT such as was not require additional financial expenditure and time, and can be easily used in routine laboratories. Therefore, routine monitoring of ESBL with DDST should be determined because of the conspicuous prevalence of ESBL forming and multidrug-resistant of *E. coli.*

Keywords: *Escherichia coli*, ESBL, Disc Diffusion Test, *Enterobacteriaceae*

1. INTRODUCTION

Escherichia coli (*E. coli*) is a member of the normal flora which find in the gastrointestinal tract of humans. Since *E. coli* normally does not cause diseases, it is cause diseases when pass to a different tissue and organ. Although gastroenteritis is the main infection, they cause meningitis, peritonitis, septicemia and pneumonia (1). Extended-Spectrum β-Lactamases (ESBLs), a penicillin degrading penicillinase, was first discovered by Abraham and Chain in the late 1930s in a *E. coli* strain. ESBLs are enzymes that hydrolyze oxyimino cephalosporins, which can be inhibited by clavulonic acid (2). Until today, approximately 400 beta lactamase enzymes has been demonstrated, and nearly 150 of them ESBLs. ESBLs are responsible for resistance of broad spectrum cephalosporins and monobactams. ESBLs production and its rapid spread among bacteria has been causing serious problems in recent years (3)

Beta-lactam antibiotics inhibits bacterial cell wall formation by interfering with the protein necessary for cell wall formation where later bacteria are either killed or inhibited from growth. Penicillin-binding protein (PBP) considered as a specific role in the synthesis of peptidoglycan, and beta-lactam antibiotics bind to these PBP which later leads to lysis and death of cells. Beta-lactam antibiotics are primarily divided into four groups: penicillins, cephalosporins, monobactams, carbapenems (4).

Nosocomial infections caused by multidrug resistance of *E. coli* are associated with the highest mortality and the huge treatment costs in to the world (5). According to the WHO, at least 700,000 people were died each year, and it is estimated that drug-resistance could cause 10 million deaths each year by 2050, with a significant socioeconomic impact (6).

Although the antibiotic resistance detection is important, ESBLs producing bacteria are not detected by routinely antibiotic sensitivity tests (7,8). In ESBLs detection and verification tests are based on the demonstration of synergy between a third-generation cephalosporin and a beta-lactamase inhibitor (usually clavulanıc acid) (9).The following phenotypic methods can be used to determine the ESBLs such as double disc synergy test (DDST), combined disc

DOI: 10.33808/clinexphealthsci.987433

test (CDT), three-dimensional test (TDT), E-test, automatize systems and molecular techniques (10).

The aim of the study was determined the ESBLs using three different phenotypic methods (DDST, CDT, and TDT) in *E.co*li strains.

2. METHODS

2.1. Design of Study

The study was conducted in the Microbiology Laboratory at the Department of Medical and Clinical Microbiology, in the Faculty of Medicine, Near East University. Total of 93 *E. coli* strains were included in the study. This study was approved by Near East University Ethics committee of 25.02.2021 / 2021/88.

2.2. Identification and Antibiotic Susceptibility Test (AST)

Bacterial identification and ASTs were performed by full automated system Phoenix 100 (Becton Dickinson, Sparks, MD, USA) in line with the manufacturer's recommendations. AST results were evaluated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria. *E. coli* ATCC 25922 used as negative control.

2.3. Double-Disc Synergy Test (DDST)

The bacterial suspension was prepared in accordance to the manufacturer's standard density (0.45-0.55 McFarland) then spread on Mueller-Hinton Agar (MHA) (Merck, KgaA, Germany) plate. Ceftazidime (CAZ; 30 µg) , ceftriaxone (CRO; 30 µg) (Cat. NO: ASD02300), cefotaxime (CTX; 30 µg), and aztreonam (ATM; 30 µg) (Cat. NO: ASD00700) were placed to plates to a disk contain amoxicillin-clavulanic acid (AMC; 20/10 µg) in the center, positioned at a distance of 20 mm (center to center). After incubation at 35°C overnight, the expansion of the inhibition zone around the cephalosporins or ATM towards the AMC disc, or the presence of a synergy area in which bacteria grows indicate the presence of ESBLs (Fig. 1). DDST has been done according to the EUCAST guidelines (11).

2.4. Combined Disc Test (CDT)

The bacterial suspension was prepared in accordance to the manufacturer's standard density (0.45-0.55 McFarland) then spread on MHA (Merck, KgaA, Germany) plate. CTX (30 µg) discs with cefotaxime/clavulanic acid (CTC; 40 µg) were placed on MHA plates. Plates were incubated at 35°C overnight. Inhibition zones around the discs with and without CTX/ clavulanic acid were measured. If the difference between the discs containing and without CTX/clavulanic acid was greater than or equal to 5 mm, ESBLs was considered positive. CDT has been done according to the EUCAST guidelines (Fig. 1).

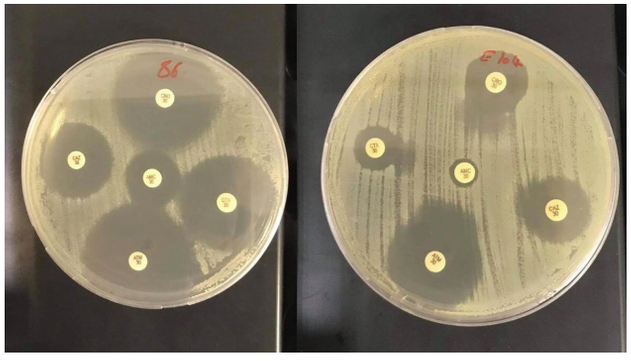

Figure 1. *ESBLs positive by combined disc test*

2.5. Three-Dimensional Test (TDT)

The prepared bacteria suspension with a density of 0.5 McFarland and spread on the agar plate. The medium was cut in a circle, close to the center of the petri dish, and 3 mm away from the antibiotic discs used. The formed medium line was filled with a liquid medium in which the microorganism to be tested was growing. After the inoculations were performed, CAZ (30 µg), CTX (30 µg), CRO (30 µg), and ATM (30 µg) discs were placed. The plate was then incubated at 35°C for 16-18 hours. Heart-shaped distortion of the zone of inhibition around the antibiotic disc was indicated ESBLs production (Fig. 2).

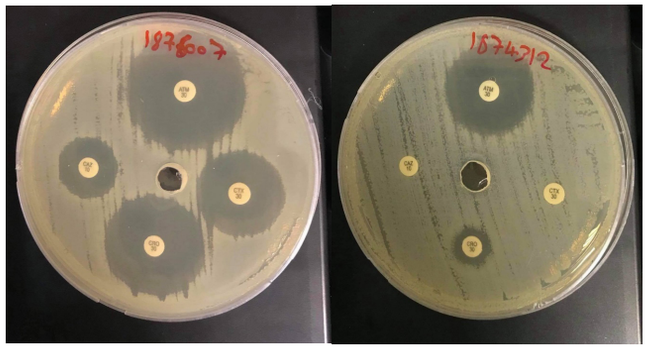

Figure 2. *: ESBLs positive by three dimensional test*

2.6. Statistical Analysis

SPSS (Statistical Package of the Social Sciences) Demo Ver 22 (SPSS Inc., Chicago, IL, USA) program was used for all statistical analysis of the data. Additionally, binary logistic regression was performed for comparison between methods. Relative risks was calculated as odds ratios (ORs) and 95% confidence intervals (CIs) by the use of binary logistic regression.

3. RESULTS

Antibiotic susceptibility test results of 93 *E. coli* strains were the resistance of amikacin were 0%, the resistant of amoxicillin/clavulanic acid were 63.4%, the resistant of ampicillin were 91.4%, the resistant of cefixime were 77.4%, the resistant of ceftazidime were 82.8%, the resistance of ceftriaxone were 81.7%, the resistance of cefuroxime were

63.4% , resistance of ciprofloxacin were 65.6%, the resistance of ertapenem were 0%, the resistance of fosfomycin were 4.3%, the resistance of gentamicin were 23.7%, the resistance of imipenem were 0%, the resistance of meropenem were 0%, the resistance of nitrofurantoin were 2.2%, the resistance of piperacillin/tazobactam were 30.1%, and the resistance of trimethoprim/sulfamethoxazole were 71.0% (Table 1).

Table 1. *Antimicrobial susceptibility results of E. coli strains.*

Antibiotic Name	Susceptible /Resistance	n (%)
Amikacin	Susceptible	93 (100%)
	Resistant	0 (0%)
Amoxicillin Clavulanic Acid	Susceptible	34 (36.6%)
	Resistant	59 (63.4%)
Ampicillin	Susceptible	8 (8.6%)
	Resistant	85 (91.4%)
Cefixime	Susceptible	21 (22.6%)
	Resistant	72 (77.4%)
Ceftazidime	Susceptible	16 (17.2%)
	Resistant	77 (82.8%)
Ceftriaxone	Susceptible	17 (18.3%)
	Resistant	76 (81.7%)
Cefuroxime	Susceptible	34 (36.6%)
	Resistant	59 (63.4%)
Ciprofloxacin	Susceptible	32 (34.4%)
	Resistant	61 (65.6%)
Ertapenem	Susceptible	93 (100%)
	Resistant	0 (0%)
Fosfomycin	Susceptible	89 (95.7%)
	Resistant	4 (4.3%)
Gentamicin	Susceptible	71 (76.3%)
	Resistant	22 (23.7%)
Imipenem	Susceptible	93 (100%)
	Resistant	0 (0%)
Meropenem	Susceptible	93 (100%)
	Resistant	0 (0%)
Nitrofurantoin	Susceptible	91 (97.8%)
	Resistant	2 (2.2%)
Piperacillin/ Tazobactam	Susceptible	65 (69.9%)
	Resistant	28 (30.1%)
Trimethoprim/ Sulfamethoxazole	Susceptible	27 (29.0%)
	Resistant	66 (71.0%)

According to the three different phenotypic test; 87 (93.5%) *E. coli* strains were ESBLs positive by DDST, 73 (78.5%) *E. coli* strains were ESBLs positive by CDT and 71 (76.3%) *E. coli* strains were positive by TDT. The comparative information of all three tests was shown in Table 2.

According to statistical analysis; comparing the DDST with other methods for highest accuracy rate, it was found that there was a statistically significant difference in the results. Accordingly, the difference between DDST-CDR and DDST-TDT was statistically significant (p=<0.001; p=<0.001, respectively), but the difference between CDT-TDT was not significant (p=0.207).

Table 2. *The results of double disc synergy test ,combined disc test, and three dimensional test.*

Method to Detect ESBLs			ESBLs in *E. coli*
DDTS	**Positive**	Number	87
		Percent (%)	93.5%
	Negative	Number	6
		Percent (%)	6.5%
	Total		93 (100%)
CDT	**Positive**	Number	73
		Percent (%)	78.5%
	Negative	Number	20
		Percent (%)	21.5%
	Total		93 (100%)
TDT	**Positive**	Number	71
		Percent (%)	76.3%
	Negative	Number	22
		Percent (%)	23.7%
	Total		93 (100%)

DDT: Double Disc Synergy Test (DDT); CDT: Combined Disc Test (CDT); TDT: Three-dimentional Test; ESBLs: Extended-Spectrum Beta Lactamases

4. DISCUSSION

Beta-lactamase activity is the most important for resistance to beta-lactam antibiotics. The effectiveness of broad-spectrum cephalosporins against to the *Enterobacteriaceae* family is challenged by newly emerging enzymes especially in the enzyme group called ESBLs. ESBLs is responsible for the development of resistance to beta-lactams such as aztreonam, ceftazidime, ceftriaxone, and cefotaxime. In Europe, these enzymes were first identified, then it is appeared in different countries, such as the United States of America and Japan. These spread of resistance were based on the using overuse and unnecessary of the third-generation cephalosporins (12,13). Epidemiological studies of the ESBLs in *E. coli* strains are important informations for public and hospital infection (14) .

Laboratory methods for screening and confirmation of ESBLs should be accurate, simple and rapid. There are several phenotype and molecular tests have been used for determine the ESBL (8). However, there are some limitations for the molecular methods such as experienced staff, costs and the complexity (15). This study aimed to investigate the most accurate phenotype methods for the detection of ESBL positive *E.coli.*

Shaikh et al. indicated that 77.78% of *E. coli* isolates were ESBLs positive by DDST method (16); Mehrgan H. et al. (17) reported that 212 isolates of *E. coli* (49%) were ESBLs positive by DDST. In the study performed by Al-Muhtaseb et. al (18) ESBLs was studied with 59 *E. coli* isolates and ESBLs was positive in 20 samples (34%). Güzel M et al. (19) were found 81 ESBLs *E. coli* strains by CDT methods. Öztürk et. al (20) were reported that DDST and E-test (p=0.187) were found similar but, screening test was significantly effective than DDST (p<0.05) for *E. coli* strains. According to our results; DDST (93.5%) was more accurate than other test for determeing ESBLs.

All phenotyping methods have advantages and disadvantages. The advantage of the TDT is simultaneous determination of antibiotic susceptibility and β-lactamase detection but not specific for determination of the ESBLs (21). For the CDT, the sensitivity is 79-97% and the specificity is 94-100%. Its advantages are easy, routine and cheap. The disadvantage of the CDT is that there is no standardization of the distance between the discs (22).

In our study, there were statistical differences between DDST-CDT (p=<0.001) and DDST-TDT (p=<0.001). However, there was no statistical difference between CDT-TDT (p=0.207). Although, the EUCAST recommends the CDT test for detection of ESBLs by phenotyping methods, our results indicated that DDST had the highest percentage (93.5%) to determine the ESBLs. Limitation of the study were the moleculer techniques might be used for the determination of the ESBLs and then determine the source of the ESBLs either nosocomial nor community.

Infections caused by ESBL positive bacteria often constitute a problem for the therapeutic options and cause treatment failures. Therefore, detection of ESBL positive bacteria should be performed routinely in microbiology laboratories for the appropriate antimicrobial therapy can be instituted and the dissemination of ESBL positive bacteria may be prevented by employing appropriate infection control measures. Although, the molecular methods may provide accurate results in the identification of ESBL genes, their accessibility is often limited, and they are expensive. However, phenotypic methods are easy to perform and interpret. In our study, DDST was found to be a superior method than CDT and TDT for detection of ESBL positive bacteria.

According to studies resullts, we said that there is a missing laboratory knowledge and testing have generated several unresolved issues. The first step is a screening for reduced susceptibility to any of the screening agents such as cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, or aztreonam. Therefore, confirmatory testing which is doing after a positive screening result, is based on tests with combinations of screening agents and the beta-lactamase inhibitor clavulanate. Confirmatory testing may need to one more day to deternine the ESBLs. If the laboratory reports a positive ESBL screening result and the isolate subsequently proves to be ESBL negative, the report could lead to unnecessary use of a carbapenem. On the other hand, if the laboratory withholds the positive screening result and the isolate is subsequently confirmed as ESBL positive, appropriate therapy may have been delayed for a day (22).

Unfortunately, a reporting rule cannot cover all situations. Rather, the need to report a positive screening result should be determined on a case-by-case basis using common sense and experience as guides, taking into account the patient's status, infection control considerations, and the likelihood of a positive confirmatory test (based on prior experience with isolates from the same patient population). Using a reliable, rapid confirmatory test could minimize the time required for the second-step test and lessen this reporting dilemma. Another solution would be including ESBL confirmation testing in the routine susceptibility test.

REFERENCES

[1] Vila J., Sâez-Lôpez, E., Johnson Jr., Römling U., Dobrint U., Cantôn R., Giske Cg., Naas T., Carattoli A., Martinez-Medina M., Bosch J., Retamar P., Rodrîguez-Bano J., Baquero, F., Soto Sm. *Escherichia coli:* an old friend with new tidings. FEMS Microbiology Reviews 2016; 40(4):437.

[2] Abraham E. P., Chain E. An enzyme from bacteria able to destroy penicillin. Nature 1940; 146(3713):837–837.

[3] Paterson D. L., & Bonomo R. A. Extended-spectrum β-lactamases: A clinical update. Clinical Microbiology Reviews 2005; 18(4): 657–686.

[4] Etebu E., Arikekpar I. Antibiotics: Classification and mechanisms of action with emphasis on moleculer perspectives. Int. J. Appl. Microbiol. Biotechnol. Res. 2016; 90-101.

[5] Founou R.C., Founou L.L., Essack S.Y. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS ONE 2017, 12, e0189621.

[6] New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis. Available online: https://www.who.int/news-room/detail/29-04-2019-new-report-calls-for-urgent-action-to-avertantimicrobial

[7] Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS. Prevalence of newer beta-lactamases in Gram-negative clinical isolates collected in the United States from 2001 to 2002. J Clin Microbiol 2006; 44: 3318–3324.

[8] Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 2008; 14 (suppl 1): 90– 103

[9] Livermore DM. Of Pseudomonas, porins, pumps and carbapenems. J Antimicrob Chemother 2001; 47 (3):247-250

[10] Özsoy MF, Öncül O, Yıldırım A, Pahsa A. Genişlemiş spektrumlu beta-laktamazlar: klinik önemi ve getirdiği sorunlar. Flora 2001; 6(Ek1): 3-23.

[11] http://eucast.org. Accession date: 14.03.2022

[12] Livermore, DM. Beta-Lactamases in laboratory and clinical resistance. Clinical Microbiology Reviews 1995; 8(4):557–584.

[13] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005; 18:657-86.

[14] Henshke-Bar-Meir R., Yinnon A. M., Rudensky B., Attias D., Schlesinger Y., & Raveh, D. Assessment of the clinical significance of production of extended-spectrum βlactamases (ESBL) by Enterobacteriaceae. Infection 2006; 34(2): 66–74

[15] Polsfuss S, Bloembeerg GV, Giger J, Meyer V, Böttger EC, Hombach M. Evaluation of a diagnostic flow chart for detection and confirmation of extended spectrum β-lactamasess (ESBL) in Enterobacteriaceae. Clin Microbiol Infect 2012; 18:1194-1204

[16] Shaikh NK, Mundhada SG, Lalngaihzuali R, Ingole KV. Comparison of different phenotypic methods for the detection of extended spectrum β-lactamase (ESBL) in bacterial isolates from tertiary care centre. Int J Cur Res Rev 2016; 8 (11):1*14.

[17] Mehrgan H., & Rahbar M. Prevalence of extended-spectrum β-lactamase-producing Escherichia coli in a tertiary care

hospital in Tehran, Iran. International Journal of Antimicrobial Agents 2008; 31(2): 147–151

[18] Al-Muhtaseb, M. Kaygusuz, A. Kan kültürlerinden izole edilen *Escherichia coli* ve *Klebsiella pneumoniae* suşlarında genişlemiş spektrumlu beta-laktamaz (GSBL) sıklığı. Ankem Derg 2008; 22(4): 175–82.

[19] Güzel M, Gena Y, Aksoy A, Moncheva P, Hristova P. Investigation of three different methods for detection of ESBL production and antibiotic resistance percentage of ESBL producinvg Gram negatrive bacteria. Turk Hij Den Biyol Derg 2015; 72 (2):131-8.

[20] Öztürk C.E., Kaya D., Yücel M., Çalışkan E., Behçet M., Ankaralı H. Comparison of Various Phenotypic Methods in Detection of Extended-spectrum Beta-lactamases. ANKEM Derg 2010; 24 (3):111-116.

[21] Singh RK, Manoj Kumar. A Simplified method of Three Dimensional Technique for the Detection of AmpC Beta-Lactamases. Archives of clinical microbiology 2013; 4(3.1).

[22] Thomson KS. Controversies about extended spectrum and Amp-C beta lactamases. Emerg Infect Dis. 2001; 7 (2): 333-336

How to cite this article: Amro MMY, Guler E, Suer K, Guvenir M. Which Phenotypic Method Is the Most Accurate for Detection of Extended–Spectrum β-Lactamases (ESBLs) in *Escherichia coli*? Clin Exp Health Sci 2022; 12: 624-628. DOI: 10.33808/clinexphealthsci.987433

Clinical and Experimental Health Sciences

The Relationship between Work Environments and Intention to Leave in Nursing: A Cross-sectional and Correlational Study

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Received: 07.09.2021 **Accepted:** 08.06.2022

ABSTRACT

Objective: Unfavorable work environments are among the factors that affect nurses' intention to leave. This study was explored to examine the relationship between nurses' work environments and their intention to leave.

Methods: This cross-sectional and correlational design study was carried out with 547 nurses working in a university, a private hospital, and a teaching hospital between November 2016 and February 2017. The Practice Environment Scale of the Nursing Work Index and a question about intention to leave was used. Descriptive statistics, Pearson correlation and multiple linear regression analysis were used in the analysis of the data.

Results: The mean total score of scale was found to be M= 2.30±0.56 and nurses' perceptions of work environments to be unfavorable. It was found that; 13.9% of the nurses did not intend to leave, 35.5% had a low, and 50.8% had a high intention to leave. It was determined that unfavorable work environments increases intention to leave (R= .370, R^2= .137, p<0.001).

Conclusion: This study found that it was nurses' intention to leave was negatively affected by their work environments. Hospital and nurse managers should be aware of the need to create positive work environments in order to prevent nurses from leaving the profession.

Keywords: Favorable, intention to leave, nursing, shortage, work environment.

1. INTRODUCTION

Considering the fundamental role nurses play in health services, it is clear that creating favorable work environments is important for nurses (1). A healthy and positive work environment has an important place in maintaining patient safety and achieving the desired results in patient care (2). There are many results showing that favorable work environments have beneficial effects in many areas, ranging from job satisfaction to patient outcomes (3,4). On the other hand, it is known that the unfavorable working environment is one of the important factors affecting the intention to leave of the nurses (5,6).

The quality of care, patient safety, and patient/employee satisfaction will increase with the improvement of work environments. In addition, as a result of unfavorable work environments, a global nursing shortage is currently being faced (7). Among the factors that affect nurses leaving the profession, which is the most important cause of loss of workforce in nursing, are the insufficient number of nurses, job dissatisfaction, low wages, work stress, friction with colleagues, heavy workloads, and unfavorable work conditions (8,9,10). In addition to the negative impacts of unwanted departures on patients and nurses, it is also commonly agreed that nurses leaving increases costs (11). One of the strategies to prevent nurses leaving the profession is to improve their work environments. It is necessary to create healthy work environments and develop strategies to persuade nurses to stay in the profession and to develop the leadership skills of all the nurses employed in each institution.

Initial studies on creating healthy work environments for nurses were conducted in Canada and the United States and were opened to discussion in 2007 as the theme of that year's the ICN. In the ICN's report, it was stated that the most important problem triggering the crisis of a shortage of nursing staff was an unhealthy work environment and that this negatively affected both patient and nurse outcomes (12). Prior to this, a project launched by the Registered Nurses Association of Ontario (2003) in 2003 aimed to create,

DOI: 10.33808/clinexphealthsci.991808

"Healthy Work Environments Best Practice Guidelines" (13). The American Organization of Nurse Executives (2003) also examined changes and innovations in nursing work environments to solve the problem of nurse shortages in their study in 2003 (14). In another US study, the American Association of Critical-Care Nurses (2005) in 2005 defined standards for healthy work environments for nurses under six main headings (15).

In research conducted in Turkey, nurses evaluated the different dimensions of unfavorable/moderate work environments (1,4,16). In 2008, the Turkish Nurses Association published a report entitled "Working Conditions of Nurses in Turkey" (17). At the national level, the issue was examined in the "Quality Standards in Health – Hospital Version 5", published in March 2016 (18). However, the 12 standards covered include all health professionals and do not provide a framework for improving nurses' work environments.

One of the aims of creating a healthy work environment for nurses in developed and developing countries is to retain nurses in institutions and in the profession (1). Although there are several reasons for the shortage of nurses, the key factor that alienates nurses from their work environment or the profession is the unfavorable work environment (11). Most studies have concluded that nurses intention to leave nursing due to unfavorable work environments (10,19,20).

The worldwide shortage of nurses has been exacerbated by the impact of the COVID-19 pandemic. According to the report published by ICN in 2022, there is a global need for 5.9 million nurses, and this need is predicted to increase to 13 million in the future (5). In the international literature, there are studies reporting that the rate of thinking about intention to leave of nurses during the pandemic varies between 3.1% and 45.2% (21,22,23). Unfavorable working environments such as exposure to coronavirus infection and illness of nurses working on the front lines due to the pandemic, lack of personal protective equipment, and burnout have shown an increase in the intention to leave (24,25). As stated in the ICN report, unfavorable working environments affect the intention to leave nurses (5). It is important to prevent nurses from leaving the organization where they work, as it affects the continuity and quality of patient care. Therefore, managers and policymakers have a great responsibility in improving the unfavorable working environments, which is one of the factors that cause nurses to intention to leave. Although there are studies in the literature that an unfavorable working environment affects leaving the job, this study aims to contribute to the literature by examining the work environment variables that affect the intention to leave, with a new sample, for this ongoing problem. Additionally, considering that favorable working environments are one of the factors that reduce the intention to leave that this study was carried out to determine the relationship between the working environment of nurses and their intention to leave.

2. METHODS

2.1. Study Design, Setting and Samples

This study was a cross-sectional and correlational design. This study was carried out in a university hospital, a private hospital, and a training hospital, which have the highest number of nurses in the city center where the research was conducted. The university hospital had a total of 450 nurses with a capacity of 1174 beds, the private hospital had a total of 178 nurses with a capacity of 201 beds and the teaching hospital had a total of 380 nurses and a capacity of 598 beds. The universe of the study consisted of all nurses who had worked in these three hospitals for at least six months (n: 823). As a criterion for the samples, it was determined that the nurses had to have worked in the institution for at least six months in order to have valid concerns about the institution and to be able to evaluate the work environments. This study, it was aimed to reach the whole population without using any sampling method, and data were collected from 603 nurses. However, 547 nurses filled in the data collection tools fully.

2.2. Measurements

2.2.1. The Practice Environment Scale of the Nursing Work Index (PES-NWI)

Nurses' work environments were measured with PES-NWI (26). It was translated and adapted to Turkish by Türkmen et al. (2011) (27). The scale consists of 31 items and five sub-dimensions. This instrument uses a 4-point Likert-type scale (*strongly agree* = 1 to *strongly disagree* = 4). All items in the scale are reversed. Scale evaluation is made over the average scores of the total and sub-dimension items and a scale score between 1-4 is obtained. As the scores of individuals from the scale increase, their attitudes towards the work environment also increase in a positive way (26). In the Turkish version, the Cronbach's alpha coefficient was found to be .94, while the Cronbach's alpha coefficient was .95 in this study.

2.2.2. The Intention to Leave

The intention to leave the profession of the participants was measured using a question (28). The scale has five responses. Thinking about leaving ''several times a month'', ''several days a week'' or ''every day'' indicate high levels of intention to leave the profession; ''several times a year'' indicates a low level of intention to leave, and ''never'' signifies no intention to leave.

2.3. Data Collection

Research data were collected between November 2016 and February 2017. All clinics were visited and the participants were informed about the study. The questionnaires were distributed to each nurse by the researcher and it was agreed that they would be completed. The average time to fill out the survey questions is 7-10 minutes.

2.4. Data Analysis

Data analysis was performed using the IBM SPSS version 28.0 software (IBM Corp., Armonk, NY, USA). The results of the research were evaluated at the 95% confidence interval and p<0.05 significance level. Data on the nurses' characteristics and distribution of intention to leave were presented with frequency and percentage. The evaluation of the total and sub-dimensions of the scale was based on mean, standard deviation, minimum, and maximum values. The relationship between intention to leave and the total and sub-dimensions of the work environment scale was evaluated with Pearson Correlation analysis, and work environment scale sub-dimensions predicting intention to leave were presented with multiple linear regression analysis.

2.5. Ethical Consideration

Authorities who adapted the scale used in the study to Turkish were notified by e-mail. The study was approved by the Ethics Committee (IRB No: 2016/702). Written permissions were also obtained from the management of the hospitals where the research was conducted. Informed consent of the nurses is taken orally and the consent is indicated in the prepared questionnaire.

3. RESULTS

The mean age of the nurses was 31.03±7.98, 59.8% were married, and 41.9% were employed in a university hospital. It was determined that the average number of years of nursing experience in hospital was 7.53±7.05, 31.4% worked in surgical units, 29.4% worked in internal medicine units and 87.9% worked as bedside nurses (Table 1).

The distributions of the intention to leave of the nurses are shown in Table 2. It was determined that 13.9% of the nurses did not intention to leave, 35.5% of them had a low intention to leave and 50.6% of them had a strong intention to leave (Table 2).

The mean total PES-NWI score was found to be 2.30 ± 0.56. When the mean scores obtained from the sub-dimensions were examined, the highest score of 2.47±0.64 was for the sub-dimension of "Nursing Foundations for Quality Care", and the lowest score of 1.88±0.62 was for the sub-dimension of "Staffing and Resource Adequacy". A significant and negative way correlation was found between the intention to leave and the total and sub-dimensions of the PES-NWI (Table 3).

According to the results of the regression analysis, a highly significant relationship at a medium level was identified between "Nurse Participation in Hospital Affairs", "Nurse Manager Ability and Leadership", "Nurse Foundations for Quality of Care", "Collegial Nurse–Physician" and "Staffing and Resource Adequacy" and intention to leave (R= .370, R2= .137, p<0.001). These sub-dimensions were determined to explain 13.7% of the total variance in intention to leave. According to standardized regression coefficient (β)" Staffing and Resource Adequacy" and "Collegial Nurse-Physician Relationships" were found to have an effect on intention to leave (p <0.05) (Table 4).

Table 1. *Nurses' characteristics (n: 547)*

Characteristics	n (%)
Age ($\bar{x}$ ±*Sd* = 31.03±7.98)	
19-28	251 (45.9)
29-39	202 (36.9)
40 or more	94 (17.2)
Marital status	
Married	327 (59.8)
Single	220 (40.2)
Working Hospital	
University Hospital	229 (41.9)
Teaching Hospital	186 (34.0)
Private Hospital	132 (24.1)
Years of nursing experience in hospital (*x*±*Sd* = 7.53±7.05)	
5 years or less	279 (51.0)
6-10 years	140 (25.6)
11 years or more	128 (23.4)
Working unit	
Surgical units	172 (31.4)
Internal medicine units	161 (29.4)
Intensive Care Unit	70 (12.8)
Emergency	44 (8.0)
Operating Room	34 (6.2)
Management	34 (6.2)
Outpatient units	32 (5.9)
Position	
Bedside Nurse	481 (87.9)
Senior / Intermediate Manager	34 (6.2)
Other*	32 (5.9)

** Outpatient units and polyclinics*

Table 2. *Distribution of intention to leave in nursing*

Intention to Leave	n (%)
No intention to leave	76 (13.9)
Low intention to leave	194 (35.5)
High intention to leave	277 (50.6)

Table 3. *Correlation between Intention to Leave and the PES-NWI*

	Mean	SD	Min-Max	1	2	3	4	5	6
Intention to Leave (1)	2.76	1.22	1-5						
PES-NWI									
Nurse Participation in Hospital Affairs (2)	2.24	0.63	1-4	-.291*					
Nursing Foundations for Quality of Care (3)	2.47	0.64	1-4	-.216*	.801*				
Nurse Managers Ability and Leadership (4)	2.20	0.68	1-4	-.283*	.830*	.721*			
Staffing and Resource Adequacy (5)	1.88	0.62	1-4	-.344*	.583*	.438*	.608*		
Collegial Nurse-Physician Relationships (6)	2.37	0.74	1-4	-.268*	.641*	.565*	.575*	.461*	
PES-NWI Total score	2.30	0.56	1-3.94	-312*	.946	.902	890	670	722

PES-NWI, Practice Environment Scale of the Nursing Work Index; SD, standard deviation
*Min, Minumum; Max, Maximum. *p>0.01*

Table 4. *Regression analysis for determining the effect of the PES-NWI sub-dimensions of the Intention to Leave*

Variables	*B*	*Standart Error*	*β*	*t*	*p*
Intention to Leave					
Constant	4.412	0.217		20.289	0.000*
Nurse Participation in Hospital Affairs	-0.179	0.170	-0.093	-1.052	0.293
Nurse Foundations for Quality of Care	0.086	0.131	0.045	0.660	0.509
Nurse Managers Ability and Leadership	-0.054	0.135	-0.031	-0.403	0.687
Staffing and Resource Adequacy	-0.477	0.102	-0.243	-4.674	0.000*
Collegial Nurse-Physician Relationships	-0.170	0.086	-0.104	-1.972	0.049*
R= 0.370 R^2= 0.137	F=17.216		p<0.001		

**p <0.05*

4. DISCUSSION

A lack of nurses in health institutions and a high number of nursing staff leaving the profession cause disruption to health care services. In this regard, it is important to determine the relationship between nurses' work environments and their intention to leave, to improve their work environments and, to prevent them from resigning.

As a result of the findings of the study, it was determined that nurses' perceptions of their work environments were negative way. The results of studies in the literature show that nurses perceive their work environments to be unfavorable at a moderate level (20,26,27,29). Unfavorable work environments can lead to a loss of staff, a decrease in the quality of care, and most importantly, an increase in medical errors. Therefore, managers have a major responsibility to improve work environments.

In the study, it was determined that the sub-dimension with the highest score was "Nursing Foundations for Quality of Care". This result is in parallel to other studies which found that this sub-dimension had the highest score (20,26,27). Accordance to the Turkish Health Ministry's service quality standards, using nursing care plans, and offering patient safety training and regular in-service training are thought to lead to high scores in this sub-dimension.

The lowest score in the study was found to be in the sub-dimension of "Staffing and Resource Adequacy". This sub-dimension, which influences work environments, has often been found to have the lowest score in other studies too (26,27,30,31). As the shortage of nurses is both a global and national problem, this may have led to the low scores for the "Staffing and Resource Adequacy" sub-dimension. This is likely to be a result of increasing workload experienced worldwide due to the insufficient number of nurses. In the "Organization for Economic Co-operation and Development (OECD) Health Statistics for the European Union and Turkey" published by the Turkey Ministry of Health the number of nurses per 1000 people between 2000 and 2013 in OECD countries was examined. The average rate in OECD countries was nine per 1000, but in Turkey, unfortunately, it was found to be fewer than two nurses per 1000 (32). These data explain why the nurses in this study found that staffing and resources were insufficient. In addition, the contractual employment of nurses and the fact that they are seen as easily expendable resources may lead to them leaving institutions. In terms of the institutions themselves, the lack of clear definitions of nurses' duties, being obliged to do other professionals' works, and the lack of support staff can lead to them developing a dislike for the profession and may cause them to leave an institution. Moreover, factors such as the inability of the managers to represent their employees, how specific problems are approached, not taking the opinions of the nurses into account when decisions are made and other related policies may affect whether nurses leave the

profession, and consequently lead to there being insufficient numbers of staff.

This study found that 13.9% of the nurses did not intend to leave the profession, 35.5% had a low and 50.8% had a strong intention to leave. In the study conducted by Çaylak and Altuntaş (2017), this number was 35.6% (19). Greinacher et al. (2021) reported that 30% (33), and Ying et al. (2021) reported that 20% of nurses intention to leave (34). According to the results of the study conducted by Lee et al. (2015) in Taiwan, it was determined that 59% of nurses intention to leave the profession (35). These results show that the intention to leave nursing is a problem in both developed and developing countries. The high likelihood of nurses leaving the profession in different countries suggests that the reasons affecting their decision to leave or stay are similar. A strong intention to leave is important in terms of demonstrating nurses' job dissatisfaction. Inadequate employment conditions, negative perceptions of the roles and responsibilities of the profession, inadequate developmental and career opportunities, inequality in wage distribution and managerial problems can all lead to job dissatisfaction which affects nurses' intention to leave.

According to the results of the regression analysis in this study, it was observed that the sub-dimensions of "Staffing and Resource Adequacy and Collegial Nurse-Physician Relationships", which are two variables in the work environment scale, have an effect on intention to leave and that these variables predict intention to leave by 13.7%. In a study, it was determined that the satisfaction rate of the patients who received service in the unit with a sufficient number of nurses and where the collegial nurse-physician relations were good was higher. In stressful environments, conflicts between team members are inevitable and negative situations such as leaving the job are reflected in the organization. As a result of this study, the lack of a sufficient number of employees in the organizations seen and communication problems among colleagues affect the intention to leave. There are studies in the literature reporting that there are different variables that affect the intention to leave. In the regression analysis conducted by Çaylak and Altuntaş (2017), it was found that seven variables affected intention to leave by 16% (19). Kloutsiniotis and Mikhail (2017) study showed that job satisfaction had a 44% negative impact on intention to leave (36), while, in a study by Lavoie-Tremblay et al. (2016), it had an 11% effect on intention to leave and was determined to be affected by weekly working hours, education, experience and the unit worked in (37). In Arslan and Kocaman (2016), study on Turkish nurses' dissatisfaction, the number of years worked in the institution, emotional exhaustion, depersonalization, relations between nurses and physicians and lack of personnel and resources were determined to affect the nurses' intention to leave the profession by 44% (38). In Leineweber et al. (2016) study conducted in 10 European countries, it was concluded that dissatisfaction and unfavorable work environments affected employment and the intention to leave nursing (10).

These studies investigate the effects of a different number of variables on the intention to leave nursing. The results show that there is no single variable which has an effect on the intention to leave and the effect of the variables on intention to leave changes at different rates. For this reason, it is thought that managers should analyze the variables that affect intention to leave well and take precautions accordingly.

5. CONCLUSION

This study was carried out to explore the relationship between nurses' intention to leave the profession and their work environments. As a result of the study:

- Nurses' perceptions of their work environments were unfavorable.
- The most positive perceptions of nurses for work environments were in the sub-dimension for "Nursing Foundations for Quality of Care".
- Nurses' most unfavorable perceptions of nurses were about the adequacy of staffing and resources in their work environments.
- 50.6% of nurses had a high level of intention to leave.
- Nurses' intention to leave is affected by their work environments.

One of the most important reasons for the decrease in the number of nurses in the world and in Turkey is nurses leaving the profession. A heavy workload, insufficient wages, job dissatisfaction and, in particular, an unfavorable work environment are among the factors that affect whether nurses will leave. We are confronted with the problem of a shortage of nurses due to the fact that although existing nurses continue to work, their commitment to the institution is not strong, and that nurses who are new to the profession often leave the profession after a short period of time. Nurse managers should determine nurse and patient ratios at regular intervals, plan nursing according to the patient profile, as well as ensuring that an appropriate number of staff are employed and that appropriate resources are available, as these are the factors most contributing to an unfavorable work environment. When under taking workforce planning, they should also take into account the results of studies about nurses' intention to leave. In addition, nurses' intention to leave should be measured at regular intervals and necessary measures should be taken to identify those who have a strong intention to leave.

Funding

The authors declared that this study has received no financial support

Conflict of Interest

The authors have no conflicts of interest to declare.

REFERENCES

[1] Kocaman G, Yurumezoglu HA, Uncu S, Turkmen E, Goktepe N, Intepeler SS. The development of healthy work environment standards for nurses in Turkey. Journal of Education and Research in Nursing. 2018; 15(1):30-39. http://dx.doi.org/10.5222/HEAD.2018.030

[2] Copanitsanou P, Fotos, N, Brokalaki, H. Effects of work environment on patient and nurse outcomes. Br J Nurs. 2017; 26(3):172-176. https://doi.org/10.12968/bjon.2017.26.3.172

[3] Albashayreh A, Al Sabei SD, Al-Rawajfah OM, Al-Awaisi H. Healthy work environments are critical for nurse job satisfaction: Implications for Oman. Int Nurs Rev. 2019; 66(3):389-395. https://doi.org/10.1111/inr.12529

[4] Samur M, Intepeler SS. Factors influencing nurses' perceptions of occupational safety. Arch Environ Occup Health. 2017; 72(1):45-52. https://doi.org/10.1080/19338.244.2016.1156045

[5] International Council of Nurses (ICN). Sustain and Retain in 2022 and Beyond, 2022. Available from https://www.icn.ch/publications

[6] Dans, M., Lundmark, V. The effects of positive practice environments: Leadership must-knows. J Nurs Manag. 2019; 50(10):7-10. https://doi.org/10.1097/01.NUMA.000.058.0624.53251.29

[7] Dilig-Ruiz A, MacDonald I, Varin MD, Vandyk A, Graham ID, Squires JE. Job satisfaction among critical care nurses: A systematic review. Int J Nurs Stud. 2018; 88:123-134. https://doi.org/10.1016/j.ijnurstu.2018.08.014

[8] Özlük B, Baykal Ü. Organizational citizenship behavior among nurses: The influence of organizational trust and job satisfaction. Florence Nightingale J Nurs. 2020; 28(3):333-340. https://doi.org/10.5152/FNJN.2020.19108

[9] Al-Hamdan Z, Manojlovich M, Tanima B. Jordanian nursing work environments, intent to stay, and job satisfaction. J Nurs Scholarsh. 2017; 49(1):103-110. https://doi.org/10.1111/jnu.12265

[10] Leineweber C, Chungkham HS, Lindqvist R, Westerlund H, Runesdotter S, Alenius L. S, Tishelman C. Nurses' practice environment and satisfaction with schedule flexibility is related to intention to leave due to dissatisfaction: A multi-country, multilevel study. Int J Nurs Stud. 2016; 58: 47-58. https://doi.org/10.1016/j.ijnurstu.2016.02.003

[11] Hayes LJ, O'Brien-Pallas L, Duffield C, Shamian J, Buchan J, Hughes F, Laschinger H. K, North N. Nurse turnover: A literature review-an update. Int J Nurs Stud. 2012; 49(7):887-905. https://doi.org/10.1016/j.ijnurstu.2011.10.001

[12] International Council of Nurses (ICN). Positive practice environments: Quality workplaces = Quality patient care: information and action tool kit, 2007. Available from https://www.twna.org.tw/frontend/un16_commission/webPages_4/IND/1.pdf

[13] Registered Nurses' Association of Ontaria. Pilot evaluation of implementation and update of healthy work environment best practice guidelines: Final report, 2003. Available from https://rnao.ca/sites/rnao-ca/files/HWE_Evaluation_Final_Report.pdf

[14] American Organization of Nurse Executives. Policy statement on mandated staffing ratios, 2003. Available from http://www.aone.org/resources/mandated-staffing-ratios.pdf

[15] American Association of Critical-Care Nurses. AACN standards for establishing and sustaining healthy work environments: A journey to excellence, 2005. Available from http://ajcc.aacnjournals.org/content/14/3/187.full.pdf+html

[16] Tambağ H, Can R, Kahraman Y, Şahpolat M. The effect of the work environment on job satisfaction among nurses. Medical Journal of Bakırköy. 2015; 11(4):143-149. https://doi.org/10.5350/BTDMJB201511402

[17] Bilazer FN, Konca GE, Uğur S, Uçak H, Erdemir F, Çıtak E. 7 gün-24 saat/ hasta başında: Türkiye'de hemşirelerin çalışma koşulları. Ankara: Türk Hemşireler Derneği, 2008. Available from: http://www. turkhemsirelerdernegi.org.tr/menu/hemsirelik-yayinlari/yayinlar/ turkiyede-hemsirelerin-calisma-kosullari.aspx.

[18] Sağlık Bakanlığı. Sağlıkta Kalite standartları – Hastane (Versiyon-5; Revizyon-01), 2016. Available from https://www.saglikaktuel.com/d/file/sks_hastane_versiyon_5_1.pdf

[19] Çaylak E, Altuntas S. Organizational silence among Nurses: The Impact on organizational cynicism and intention to leave work. J Nurs Res. 2017; 25(2):90-98. https://doi.org/10.1097/jnr.000.000.0000000139

[20] Nantsupawat A, Kunaviktikul W, Nantsupawat R, Wichaikhum OA, Thienthong H, Poghosyan L. Effects of nurse work environment on job dissatisfaction, burnout, intention to leave. Int Nurs Rev. 2017; 64(1):91-98. https://doi.org/10.1111/inr.12342

[21] Jo S, Kurt S, Bennett JA, Mayer K, Pituch KA, Simpson V, Skibiski J, Tagagi E, Karaaslan MM, Özlük B, Reifsnider, E. Nurses' resilience in the face of coronavirus (COVID-19): An international view. Nurs Health Sci. 2021; 23(3):646-657. https://doi.org/10.1111/nhs.12863

[22] Said RM, El-Shafei DA. Occupational stress, job satisfaction, and intent to leave: Nurses working on front lines during COVID-19 pandemic in Zagazig City, Egypt. Environ Sci Pollut Res Int. 2021; 28(7):8791-8801. https://doi.org/10.1007/s11356.020.11235-8

[23] Raso R, Fitzpatrick JJ, Masick K. Nurses' intent to leave their position and the profession during the COVID-19 pandemic. J Nurs Adm. 2021; 51(10):488-494. https://doi.org/10.1097/NNA.000.000.0000001052

[24] Varasteh S, Esmaeili M, Mazaheri M. Factors affecting Iranian nurses' intention to leave or stay in the profession during the COVID-19 pandemic. Int Nurs Rev. 2021; 69(2):1-11. https://doi.org/10.1111/inr.12718

[25] Özlük B, Bıkmaz Z. The experiences of nurses diagnosed with COVID-19 in Turkey: A qualitative study. Nurs Health Sci. 2021; 23(4):916-924. https://doi.org/10.1111/nhs.12881

[26] Lake ET. Development of the practice environment scale of the Nursing Work Index. Res Nurs Health. 2002; 25(3):176-188. https://doi.org/10.1002/nur.10032

[27] Türkmen E, Badır A, Balcı S, Topçu SA. The adaptation of the practice environment scale of the nursing work index into Turkish: Reliability and validity study. J Res Dev Nurs. 2011; 13(2):5-20.

[28] HM, Tackenberg P, Müller B, NEXT-Study Group. Working conditions and intent to leave the profession among nursing staff in Europe. HM. Hasselhorn (Ed.). Stockholm: National Institute for Working Life, 2003. Available from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.622.3322&rep=rep1&type=pdf

[29] Lee HF, Chiang HY, Kuo HT. Relationship between authentic leadership and nurse intent to leave: The mediating role of work environment and burnout. J Nurs Manag. 2019; 27(1):52-65. https://doi.org/10.1111/jonm.12648

[30] Lacher S, Geest S, Denhaerynck K, Trede I, Ausserhofer D. The quality of nurses' work environment and workforce outcomes from the perspective of swiss allied healthcare assistants and registered nurses: A cross-sectional survey. J Nurs Scholarsh. 2015; 47(5):458-467. https://doi.org/10.1111/jnu.12151

[31] Leone C, Bruyneel L, Anderson JE, Murrells T, Dussault G, de Jesus ÉH., Sermeus W, Aiken L, Rafferty AM. Work environment issues and intention-to-leave in Portuguese nurses: A cross-sectional study. Health Policy. 2015; 119(12):1584-1592. https://doi.org/10.1016/j.healthpol.2015.09.006

[32] Sağlık Bakanlığı. OECD, Avrupa Birliği Sağlık İstatistikleri ve Türkiye, Hastanelerde Beşeri ve Fiziki Kaynakların İncelenmesi, 2015. Available from https://www.saglikaktuel.com/d/file/35c966a9f1d343909d4d0858bec69333.pdf

[33] Greinacher A, Helaß M, Nikendei C, Müller A, Mulfinger N, Gündel H, Maatouk, I. The impact of personality on intention to leave the nursing profession: A structural equation model. J Clin Nurs. 2021; 31(11-12);1570-1579. https://doi.org/10.1111/jocn.16010

[34] Ying LY, Ramoo V, Ling LW, Nahasaram ST, Lei CP, Leong LK, Danaee, M. Nursing practice environment, resilience, and intention to leave among critical care nurses. Nurs Crit Care. 2021; 26(6):432-440. https://doi.org/10.1111/nicc.12551

[35] Lee YW, Dai YT, McCreary LL. Quality of work life as a predictor of nurses' intention to leave units, organisations and the profession. J Nurs Manag. 2015; 23(4):521-531. https://doi.org/10.1111/jonm.12166

[36] Kloutsiniotis PV, Mihail DM. Linking innovative human resource practices, employee attitudes and intention to leave in healthcare services. Employee Relations. 2017; 39(1):34-53. http://dx.doi.org/10.1108/ER-11-2015-0205

[37] Lavoie-Tremblay M, Fernet C, Lavigne GL, Austin S. Transformational and abusive leadership practices: impacts on novice nurses, quality of care and intention to leave. J Adv Nurs. 2016; 72(3):582-592. https://doi.org/10.1111/jan.12860

[38] Arslan YH, Kocaman G. Predictors of nurses' intentions to leave the organisation and the profession in Turkey. J Nurs Manag. 2016; 24(2):235-43. https://doi.org/10.1111/jonm.12305

How to cite this article: Aydoğmuş S, Özlük B. The Relationship between Work Environments and Intention to Leave in Nursing: A Cross-sectional and Correlational Study. Clin Exp Health Sci 2022; 12: 629-635. DOI: 10.33808/clinexphealthsci.991808

Clinical and Experimental Health Sciences

The Prevalence and Related Factors of Eating Disorders and Eating Attitudes Among Balikesir University Students

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Received: 30.09.2021 **Accepted:** 04.04.2022

ABSTRACT

Objective: Eating disorders are classified as psychiatric diseases that include deterioration in eating behaviors and attitudes and has negative effects on the physical and mental health of the individual. The main objective of the study is to elaborate the psychosocial and psychopathological factors related to eating disorders among university students.

Methods: We have enrolled 199 female and 201 male volunteer students at Balıkesir University Faculty of Medicine. Participants were evaluated with a semi-structured questionnaire prepared by our institution regarding clinical experience and available information sources and according to DSM-V diagnostic criteria. Eating Attitude Scale, Ortho-15 Scale, Maudsley Obsessive Compulsive Question List, Rosenberg Self-Esteem Scale and Body Perception Scale were administered to all participants. Individuals with previously known or concomitant dementia, delirium, mental retardation, psychotic disorder diagnosis, depression with psychotic symptoms, and bipolar depression were not included in the study.

Results: The OCD sub-dimension and cleaning sub-dimension differed significantly according to the gender variable (p<0.05). The body image mean scores were significantly different in terms of the psychiatric diagnosis variable (p<0.05). Participants with psychiatric diagnosis had significantly higher obsessive compulsive disorder suspicion and rumination dimensions and body dissatisfaction scores. When the chronic disease variable was analyzed with the variables of eating attitudes, self-esteem, OCD symptoms and body image, it was found that the eating attitude differed significantly compared to the chronic disease variable (p<0.05). In addition, ortho-cognitive (p<0.05) and ortho-clinical (p<0.05) mean scores were found to differ significantly. The eating attitude scores were higher in people with chronic diseases.

Conclusion: This research showed that eating attitudes changed with sociodemographic characteristics and was correlated with obsessive compulsive disorder symptoms, body image and self-esteem. In this study we found that eating attitudes of university students had a significant relationship with psychopathological and psychosocial factors; such as obsessive compulsive disorder symptoms, body image and self-esteem.

Keywords: Body Image, Self-Esteem, Obcessive Compulsive Disorder, Sociodemographics, Eating Disorders

1. INTRODUCTION

Eating disorders are classified as psychiatric diseases that include deterioration in eating behaviors and attitudes and has negative effects on the physical and mental health of the individual *(1, 2)*. The psychopathology that lies beneath eating disorders are, especially bulimia nervosa *(3)* and anorexia nervosa *(4)* and the main problem is not over or under eating *(5)*; excessive and unrealistic perception and exaggerated preoccupation with weight and appearance *(6)*. The prevalence of eating disorders are increasing in young people due to the fact that eating attitudes are affected by many factors, especially in youth period *(7)*. Gender and age take the first place amongst the factors affecting eating attitudes in the literature. The American Psychiatric Association reports that the incidence of Anorexia Neurosa *(AN)* increases between the ages of 15-19 and 40% of AN cases are in this age group *(7,8)*. The majority of bulimia neurosa *(BN)* cases occur in university youth, which includes late adolescence, and before the age of 25 *(8)*. When the effect of gender is evaluated, it is seen that especially young girls attach more importance to body image and aesthetics than boys and are more prone to eating disorders *(9)*. Other factors affecting the eating attitude are body mass index *(BMI)*, body dissatisfaction and practicing continuous diet *(10, 11)*.

Apart from the factors that negatively affect the eating attitude, the combination of biological *(genetic or neurochemical)*, developmental, cultural, individual, psychological, familial and environmental factors paves the way for the formation of eating disorders *(12, 13)*. Recent studies in the USA indicate

DOI: 10.33808/clinexphealthsci.1002101

that 3% of adolescent individuals have eating disorders *(8)* and quite similarly 2.3% in Turkey *(13)*. In recent studies it has been suggested that eating disorders show their first symptoms especially in childhood and adolescence and later developmental periods may increase the risk of eating psychopathology *(13)*. In previous studies stating *(14,15)* eating disorders were defined as a psychopathology that is handled with more than one theoretical framework and has different risk factors *(16-20)*.

Considering the approaches emphasizing eating behavior and eating psychopathology, it is thought that cognitive behavioral approaches provide important information both in the explanation and effective treatment of eating disorders *(9)*. Cognitive-behavioral approaches have stated that people's attribute on excessive meaning to variables such as body image and weight and determine their own value through these two individual variables may trigger maladaptive eating behaviors *(17-19)*.

Up to date extensive research have been conducted supporting the association of obsessive-compulsive symptoms in eating disorders and eating behavior disorders in obsessive-compulsive disorders *(18,19)*. It is stated that the phenomenology of both disorders is similar, in both disorders there is a rigid, perfectionist structure, it is noteworthy that cognitive distortions and mental overwork are similar, and that once started, the problematic behavior cannot be stopped *(20)*.

Previously it was published that the body image perception of people with eating disorders were similar to the symptoms of Body Dysmorphic Disorder *(BDD)* and those people have an over-evaluative attitude towards their body image. It is thought that OCD and eating disorders, which may have similar symptoms, may be a subject that experts have difficulty in diagnosing in clinical practice and there may be uncertainty about which condition is the trigger or whether it is seen simultaneously *(21-23).*

1.2. Study Hypothesis

Recent studies support that the rate of eating disorders in developing societies are as high as in western societies *(24)*. It is noteworthy that studies on eating disorders are still scarce in Turkey.

Due to the fact that eating disorders are associated with more than one factor and the diversity of risk factors, this study was structured on the examination of eating behavior and related factors in young adult individuals and aimed to investigate the prevalence of eating attitudes and eating disorders in Balıkesir University students.

2. METHODS

This research has been conducted at Balıkesir University Faculty of Medicine between 27/09/2017 – 27/03/2018. The study has been approved by ethics committee *(Date: 04/10/2017 and protocole number 2017/86)*. Informed consent has been obtained from all the participants prior to questionairres.

We have enrolled 199 female and 201 male volunteer students at Balıkesir University Faculty of Medicine. Participants were evaluated with a semi-structured questionnaire prepared by our institution regarding clinical experience and available information sources and according to DSM-V diagnostic criteria. Eating Attitude Scale, Ortho-15 Scale, Maudsley Obsessive Compulsive Question List, Rosenberg Self-Esteem Scale and Body Perception Scale were administered to all participants.

The sociodemographic data form and all other scales were filled by the participant. Individuals with previously known or concomitant dementia, delirium, mental retardation, psychotic disorder diagnosis, depression with psychotic symptoms, and bipolar depression were not included in the study.

2.1. Statistical Analysis

SPSS 20 program was used in the statistical analysis of our study. The distribution of the data was determined by using appropriate statistical methods *(Descriptive Statistics and Normality Analysis)* as well as visual graphics. Independent Sample t-Test, One-Way Analysis of Variance *(ANOVA)*, Tukey test or Mann Whitney U test were used for pairwise comparisons of groups. In the evaluation of categorical data, cross tables were made and chi-square test was used for comparisons. In addition, intergroup linkage analysis and comparison tests were performed. $P<0.05$ was accepted as statistical significance level, and it was studied at 95% confidence level.

The sample size has been calculated via Cochran (1977) sampling calculation method within 95% CI and 5% SE on minimum number of participants generating statistical significance *(Cochran, W.G. (1977) Sampling Techniques. 3rd Edition, John Wiley & Sons, New York)*.

3. RESULTS

In order to examine the demographic variables, we have conducted a frequency analysis on their percentage distributions. The findings are given in *Table 1*. According to the initial analysis 67.8% of the participants were between the ages of 18-21 *(n=271)*, 30% *(n=120)* were between the ages of 22-25 and 2.3% *(n=9)* were between the ages of 25-30. A minority of the subjects *(3.3%, n=13)* reported that they had previous psychiatric diagnosis and 96.8% *(n=387)* declared none. The rate of chronic disease was 5.3% *(n=21).* When the Body Mass Index ranges of the participants were examined, 10% *(n=40)* were underweight, 69.8% *(n=279)* were normal, 16.5% *(n=66)* were overweight, and 3.5% were obose *(n=15)*.

Table 1. *Frequency Distribution of Participants' Demographic Variables*

Demographic Variables (N=400)		N	%
Age	18-21 age	271	67,8
	22-25 age	120	30,0
	25-30 age	9	2,3
Gender	Female	199	49,8
	Male	201	50,3
Class	1	119	29,8
	2	80	20,0
	3	59	14,8
	4	79	19,8
	5	45	11,3
	6	18	4,5
Marrital Status	Single	397	99,3
	Married	3	,8
Living with	Alone	43	10,8
	With family	69	17,3
	Dormitory	209	52,3
	House Friend	79	19,8
Where she/he spent most of her life	Village/Town	22	5,5
	District/Small town	145	36,3
	City	233	58,3
Income Level	<1000 TRY	269	67,3
	1000-2000 TRY	118	29,5
	2000-3000 TRY	10	2,5
	>3000 TRY	3	,8
Psychiatric Disease	Yes	13	3,3
	No	387	96,8
Chronic Disease	Yes	21	5,3
	No	379	94,8
Alcohol	Yes	88	22,0
	No	312	78,0
Smoking	Yes	73	18,3
	No	327	81,8
BMI	Thin	40	10,0
	Normal	279	69,8
	Over weight	66	16,5
	Obese	15	3,5
	Total	400	99,8
Total		**400**	**100,0**

BMI values were calculated by the researcher in line with the participants' height and weight information.

When the gender variable was examined with the variables of eating attitudes, self-esteem, OCD symptoms and body image, it was found that the OCD sub-dimension and cleaning sub-dimension differed significantly according to the gender variable *(p<0.05)*. The cleanliness sub-dimension was found to be higher in women than in men. In addition, cognitive dimension *(p<0.05),* clinical dimension *(p<0.05)* and emotional dimension *(p<0.05)*, which were the sub-dimensions of ORTO-15 applied to the participants in order to measure their eating attitudes *(p<0.05)* differed significantly. It was found that the mean scores of orthorexia in terms of sub-dimensions were significantly higher than the mean scores of women *(Table 2)*.

During the analysis of the psychiatric diagnosis variable with eating attitudes, self-esteem, OCD symptoms and body image variables, the OCD sub-dimensions of doubt *(p<0.05)* and rumination *(p<0.05)* were used for psychiatric diagnosis. The body image mean scores were significantly different in terms of the psychiatric diagnosis variable *(p<0.05)*. Participants with psychiatric diagnosis had significantly higher OCD suspicion and rumination dimensions and body dissatisfaction scores.

When the chronic disease variable was analyzed with the variables of eating attitudes, self-esteem, OCD symptoms and body image, it was found that the eating attitude differed significantly compared to the chronic disease variable *(p<0.05).* In addition, ortho-cognitive *(p<0.05)* and ortho-clinical *(p<0.05)* mean scores were found to differ significantly. The eating attitude scores were higher in people with chronic diseases.

In Table 3, the first model, mean scores of EAT-40 *(β= .257)* and ORTO-15 *(β= – .224)* were found to significantly predict Maudsley total scores. It was observed that the participants' EAT-40 scores *(t=5.198; p<0.05)* were significant at the rate of 15% and the ORTO-15 score explained 15% of the Maudsley total scores. In the second model, Self-Esteem and Body Perception mean scores were included in addition to the YTT-40 and ORTO-15 score averages, and the significance level of the explanatory relationship of eating attitudes in the model was examined. In line with the results, it was determined that 19% of the participants explained the Maudsley total score when the self-esteem and body image variables of the YTT-40 and ORTO-15 mean scores were included.

Table 2. *Eating Attitudes and Correlation Analysis of Related Factors*

1	2	3	4	5	6	7	8	9	10	11			
Maudsley Control Sub-dimension			1										
Maudsley Cleaning			0.400**	1									
Maudsley slowness			0.652**	0.369**	1								
Maudsley doubt			0.555**	0.334**	0.500**	1							
Maudsley rumination			0.644**	0.325**	0.662**	0.512**	1						
Eating Habit			0.250**	0.257**	0.272**	0.197**	0.274**	1					
Orto-15 Cognitive			0.100*	0.204**	-088	0.123*	0.135**	0.251**	1				
Orto-15 Clinic			0.249**	0.140**	0.241**	0.193**	0.360**	0.413**	0.408**	1			
Orto-15 Emotional			0.173**	0.302**	0.156**	-0.212**	0.232**	0.268**	0.621**	0.414**	1		
Self Respect			0.174**	0.153**	0.189**	0.112*	0.249**	0.221**	0.049	0.159**	0.012	1	
Body Perception			0.090	0.141**	0.089	0.133**	0.261**	0.096	-0.072	0.187**	0.084	0.151**	1

*** p<0,01*

** p<0,05*

The numbers given in the column define the scale in the row.

Table 3. *Linear Regression Analysis of Eating Attitude, Self-Esteem, Body Image and OCD Symptoms*

Predictors		*Predicted*	*B*	*β*	*R*	R^2	*Adjusted* R^2	*t*	*p*
1	(Constant)		19.75					8.465	0
	Eating Behaviour	Maudsley Total	0.178	0.257	0.398	0.159	0.154	5.198	0*
	Orto-15		-0.231	-0.224				-4.519	0*
2	(Constant)		11.890					4.361	0
	Eating Behaviour	Maudsley Total	0.147	0.213	0.453	0.206	0.198	4.293	0*
	Orto-15		0.224	-0.217				-4.455	0*
	Rosenberg		0.350	0.155				3.322	0.001*
	Body Perception		0.037	0.137				3.002	0.003*

P<0,05 associated variable: OCD

4. DISCUSSION

In this study, we have aimed to analyze the eating attitudes and related factors in university students. The findings obtained in the study could be elaborated as the distribution of sociodemographic variables, and secondly, examining the clinical variables such as eating attitude, obsessive-compulsive symptoms, self-esteem and body image. The age distribution was found to be in the range of 18-25. It has been reported that the participants mostly stayed in dormitories because they were university students. The participants stated that they mostly live in districts and metropolitan cities. The rate of participants with psychiatric and chronic diseases was lower than those without. When the distribution of the Body Mass Index *(BMI)* variable, which is important in terms of the research subject, is examined in line with the classification, it was seen that the participants have the highest rate of being normal weight and second group was overweight.

The Maudsley sub-dimensions were were found to be positively associated with eating attitude. This shows that OCD symptoms were high and eating psychopathology increaseed in the same direction. Another study conducted by Herrera Giménez *(14)* suggested that bulimia nervosa cases may be at risk of emotional eating attacks due to excessive emphasis on body image and weight. In a study conducted by Hovardaoğlu *(15)*, body image was examined in schizophrenic and depressive patients. Accordingly, it was thought that body image could be an important factor in the clinical sample.

On the other hand, in another study conducted by Brechan and Kvalem *(16)*, it was concluded that depressive symptoms and body dissatisfaction were associated with disordered eating behavior. In another study conducted by Shafran, Fairburn, Robinson, and Lask *(17)*, it was stated that the need for self-control or avoidance of people regarding body image is associated with health eating behavior. It was suggested by Reas and colleagues *(18)* that avoidance behavior is frequently observed in overweight individuals as a result of the importance they attach to body image, and emotional eating behavior can be observed as a result of the emotional load caused by this situation. In a national study conducted by Hudson et al. *(20)*, it was concluded that the prevalence of eating disorders was significantly associated with body image dissatisfaction.

It was observed that Maudsley OCD sub-dimensions were negatively related to orthorexia sub-dimensions. This showed that there was an inverse linear relationship between the psychopathological course of healthy living and nutritional behavior and obsessive-compulsive disorder. In addition, it was thought that healthy living and nutritional behaviors did not have high scores on the basis of averages and this was due to the fact that this situation cannot be evaluated from a psychopathological point of view. It was found that the Maudsley slowness and rumination sub-dimensions were positively related to body image, and the participants who reported obsessive-compulsive behavior in these sub-dimensions had high body image scores. As stated by Shafran et al. *(17)* and Reas et al. *(18)*, it can be thought that body-checking behavior may progress at a psychopathological level, and because this condition progresses in an obsessive-compulsive state, a situation that can be associated with OCD may occur for individuals *(25-30)*. In this direction, it was seen that the findings obtained in the study were consistent with other studies in the literature. In addition, the high mean scores of the Rosenberg Self-Esteem Scale, which states that high scores lead to low self-esteem, and Maudsley OCD sub-dimensions were positively related, and low self-esteem and OCD symptoms were high. Low self-esteem was also found to be associated with eating attitude, and high scores indicating inconsistency of eating attitudes and high self-esteem scores were linear.

In line with the results obtained, it was determined that the maladaptive and psychopathological course of the eating attitude explained the OCD level. In the other step of the analysis, the level of explanation of the OCD total score by the research variables in the explanatory relationship of body image and self-esteem was examined, and it was found that the explanatory power of eating attitudes increased significantly with the inclusion of body image and self-esteem. In this direction, it has been determined that body dissatisfaction and low self-esteem of individuals have a mediating function in the relationship between eating attitude and OCD symptoms. It was seen that the findings are similar to the research results in the literature in the field of eating psychopathology. As stated by Castellini et al. *(31)*, body image and low self-esteem are common among the consequences of eating disorders. Adams et al. *(32)*, published that discomfort due to body image was associated with individuals' self-esteem. Low self-esteem

was observed in individuals who were dissatisfied with their appearance or who are worried about it. Wichstrøm and von Soest *(33)*, on the other hand, argued that this situation has a two-way relationship and each of them has factors that can affect each other. In their prospective study, it was concluded that the relationship between adolescents' self-esteem and body dissatisfaction was mutual. On the other hand, Ward and Hay *(34)* suggested that body dissatisfaction may accompany depressive symptoms and dysfunctional coping methods in depression, coping and body dissatisfaction, and this may be related to low self-esteem. In our country, Öyekçin *(35)* stated that in the etiological evaluation of eating disorders, self-esteem and, accordingly, body image, which is shaped by the way a person evaluates himself or herself, may be an important factor in the emergence of eating disorders *(35, 36)*.

Limitations of the Study

One of the limitations of the research is that it was carried out only on students studying at a university in Balıkesir. Therefore, the generalizability level of these results to the whole of Turkey or to a wider region is low. The fact that the questionnaire forms distributed in this study were not in digital media and were applied in print both increased the financial burden and limited the number of people reached. The questionnaire to be prepared in the digital environment will make it easier to reach a large number of university students and reduce the financial burden.

5. CONCLUSION

This research showed that eating attitudes changed with sociodemographic characteristics and was correlated with obsessive compulsive disorder symptoms, body image and self-esteem.In this study we found that eating attitudes of university students had a significant relationship with psychopathological and psychosocial factors; such as obsessive compulsive disorder symptoms, body image and self-esteem. The increase in scores of eating attitude and OCD symptoms were associated with image dissatisfaction and low self-esteem. Regarding the demographics of study population, one can say that Balıkesir University Faculty of Medicine students' outcomes were consistent with other studies.

REFERENCES

[1] Stefano Erzegovesi, Laura Bellodi. Eating Disorders, CNS Spectr. 2016;21(4):304-309.

[2] Treasure J, Duarte TA, SchmidManer U. Eating Disorders, Lancet. 2020;395(10227):899-911.

[3] Hoek HW, van Hoeken D. Review of the prevalence and incidence of eating disorders, Int J Eat Disord. 2003;34(4):383-396.

[4] Toker DE, Hocaoğlu Ç. Yeme bozuklukları ve aile yapısı: Bir gözden geçirme. Düşünen Adam; 2009, 22(1-4):36-42. (Turkish).

[5] Tasca GA, Balfour L. Attachment and eating disorder: A review of current research. Int J Eat Disord. 2014;47(7):710-717

[6] Chow CM, Ruhl H, Tan CC, Ellis L. Fear of fat and restrained eating: negative body talk between female friends as a moderator, Eat Weight Disord. 2019;24(6):1181-1188.

[7] Tam CK, Ng CF, Yu CM, Young BW. Disordered eating attitudes and behaviours among adolescents in Hong Kong: prevalence and correlates, J Paediatr Child Health. 2007;43(12):811-817.

[8] Farahani SJ, Chin YS, Nasir MTM, Amiri P. Disordered Eating and its Association with Overweight and Health-Related Quality of Life Among Adolescents in Selected High Schools of Tehran. Child Psychiatry Hum Dev. 2015;46(3):485-492.

[9] Murphy R, Straebler S, Cooper Z, Fairburn CG. Cognitive behavioral therapy for eating disorders, Psychiatr Clin North Am. 2010;33(3):611-627

[10] Hilbert A, Pike KM, Goldschmidt AB, Wilfley DE, Fairburn CG, Dohm FA, Walsh BT, Weissman RS. Risk factors across the eating disorders, Psychiatry Res. 2014;220(1-2):500-506.

[11] Polivy J, Herman C. Sociocultural idealization of thin female body shapes: An introduction to the special issue on body image and eating disorders. Journal of Social and Clinical Psychology, 2004;23(1): 1-6

[12] Castillo M, Weiselberg E. Bulimia Nervosa/Purging Disorder, Curr Probl Pediatr Adolesc Health Care, Curr Probl Pediatr Adolesc Health Care. 2017;47(4):85-94.

[13] Kartal FT, Ayhan NY. Relationship between eating disorders and internet and smartphone addiction in college students, Eat Weight Disord. 2021;26(6):1853-1862.

[14] Giménez MH. Bulimia nervosa: emotions and making decisions. Rev Psiquiatr Salud Ment.2011;4(2):88-95

[15] Batista M, Antić LZ, Žaja O, Jakovina T, Begovac I. Predictors of eating disorder risk in anorexia nervosa adolescents. Acta Clin Croat. 2018;57(3):399-410

[16] Brechan I, Kvalem IL. Relationship between body dissatisfaction and disordered eating: Mediating role of self-esteem and depression. Eat Behav. 2015;17:49-58

[17] Shafran R, Fairburn CG, Robinson P, Lask B. Body checking and its avoidance in eating disorders. Int J Eat Disord. 2004;35(1):93-101.

[18] Reas DL, Grilo CM, Masheb RM, Wilson GT. Body checking and avoidance in overweight patients with binge eating disorder. Int J Eat Disord. 2005;37(4):342-346.

[19] Casale AD, Sorice S, Padovano A, Simmaco M, Ferracuti S, Lamis DA. Rapinesi C, Sani G, Girardi P, Kotzalidis GD, Pompili M, Psychopharmacological Treatment of Obsessive-Compulsive Disorder (OCD), Curr Neuropharmacol. 2019;17(8):710-736.

[20] Hudson JI, Hiripi E, Pope HG, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. Biol Psychiatry. 2007;61(3):348-358.

[21] Bartz JA, Hollander E. Is obsessive–compulsive disorder an anxiety disorder? Prog Neuropsychopharmacol Biol Psychiatry. 2006;30(3):338-352.

[22] Barbarich N. Is there a common mechanism of serotonin dysregulation in anorexia nervosa and obsessive compulsive disorder? Eat Weight Disord. 2002;7(3):221-231.

[23] Wu KD. Eating disorders and obsessive–compulsive disorder: A dimensional approach to purported relations. J Anxiety Disord. 2008;22(8):1412-1420.

[24] Guerdjikova AI, Mori N, Casuto LS, McElroy SL. Binge Eating Disorder, Psychiatr Clin North Am. 2017;40(2):255-266.

[25] Alpaslan AH, Soylu N, Avcı K, Coşkun KS, Kocak U, Taş HU. Disordered eating attitudes, alexithymia and suicide probability among Turkish high school girls. Psychiatry Res. 2015;226(1):224-229.

[26] Westmoreland P, Krantz MJ, Mehler PS, Medical Complications of Anorexia Nervosa and Bulimia, Am J Med. 2016;129(1):30-37.

[27] Peterson K, Bratman RF. Anorexia nervosa in adolescents: An overview. Nursing. 2019;49(10):24-30.

[28] Donini LM, Marsili D, Graziani MP, Imbriale M, Cannella C. Orthorexia Nervosa: Validation of a diagnosis questionnaire. Eat Weight Disord. 2005;10(2):e28-32.

[29] Smith KE, Mason TB, Lavender JM. Rumination and eating disorder psychopathology: A meta-analysis, Clin Psychol Rev. 2018;61:9-23.

[30] Brochu JP, Meilleur D, DiMeglio G, Taddeo D, Lavoie E, Erdstein J, Pauzé R, Pesant C, Thibault I, Frappier JV. Adolescents' perceptions of the quality of interpersonal relationships and eating disorder symptom severity: The mediating role of low self-esteem and negative mood, Eat Disord. 2018;26(4):388-406.

[31] Castellini G, Sauro CL, Mannucci E, Ravaldi E, Rotella CM, Faravelli C, Ricca V. Diagnostic crossover and outcome predictors in eating disorders according to DSM-IV and DSM – V proposed criteria: A 6-year follow-up study. Psychosom Med. 2011;73(3):270-279.

[32] Adams KE, Tyler JM, Calogero R, Lee J. Exploring the relationship between appearance-contingent self-worth and self-esteem: The roles of self-objectification and appearance anxiety. Body Image. 2017;23:176-182.

[33] Wichstrøm L, von Soest T. Reciprocal relations between body satisfaction and self-esteem: A large 13-year prospective study of adolescents. Adolesc. 2016;47:16-27.

[34] Ward RM, Hay MC. Depression, coping, hassles, and body dissatisfaction: Factors associated with disordered eating. Eat Behav. 2015;17:14-18.

[35] Oyekcin DG, Deveci A. Yeme bozukluklarının etiyolojisi. Current Approaches in Psychiatry, 2012, 4(2):138-153. (Turkish).

[36] Ozdin S, Karabekiroolu A, Özbalcı GS, Aker AA. The effect of cognitive symptoms in binge eating disorder on depression and self-esteem: a cross-sectional study, t Weight Disord. 2021;26(5):1483-1489

How to cite this article: Oguz EG, Karlidere T. The Prevalence and Related Factors of Eating Disorders and Eating Attitudes Among Balikesir University Students. Clin Exp Health Sci 2022; 12: 636-641. DOI: 10.33808/clinexphealthsci.1002101

Clinical and Experimental Health Sciences

Assesment of Physicians' Attitudes Towards COVID-19 Vaccine

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Received: 06.10.2021 **Accepted:** 21.01.2022

ABSTRACT

Objective: The aim of this study is to physicians attitudes towards the COVID-19 vaccine.

Methods: Our research is a cross-sectional study, which was conducted between January 4th and February 26th, 2021. A Google Forms questionnaire was prepared according to the literature. The first part of the two-part questionnaire included the sociodemographic characteristics of the physicians and some variables thought to be related to COVID-19. In the second part, questions from the "Attitudes Towards COVID-19 Vaccine Scale (ATCVS)" were included. Multiple linear regression analysis with variables that were significant in univariate analysis was used for further analysis.

Results: Three hundred fifteen (71.9%) of a total of 438 participants stated that they were assigned to units related to COVID-19 during the pandemic. With more than 10 years of work in the profession, physicians had a more positive attitude towards vaccination than those with 1 – 5 years' experience. Most of the physicians reported that there were insufficient studies on these newly developed vaccines. Possible adverse effects, uncertainty about the efficacy and safety of the vaccine, and beliefs that people are not at risk for severe disease were prominent.

Conclusions: Despite all the uncertainties about the efficacy, safety, and long-term adverse effects of newly developed COVID vaccines, it was determined that 79% of the physicians approached the vaccine positively. The most important reason for vaccine hesitation was the insufficient studies about COVID-19 vaccines.

Keywords: COVID-19 vaccines, pandemic, vaccine hesitancy

1. INTRODUCTION

In addition to the mask, social distance and hygiene, we now have a new weapon against to COVID-19 with the production of the vaccine. Vaccination is one of the most successful public health practices in human history (1). The main aims of the vaccination are to stop the transmission of the virus inter individuals and to control the disease by minimizing deaths (2). Thanks to the developing technology, research and development studies have been integrated into immunization and new-generation vaccination (3). While vaccine studies about COVID-19, which is one of the newest agendas of the medical world, continue in many laboratories, studies have evolved to a different stage with new mutations. The new vaccines developed against COVID-19 are expected to be a global weapon that will both reduce virus spread and limit the effect of the virus (4).

Despite great progress in vaccination technologies in the last century, many vaccine-preventable diseases are re-spreading again, which is why the World Health Organization (WHO) defines vaccine hesitancy as a major threat to global health (5). Vaccination hesitation is an important public health problem, and resistance in the control of vaccine-preventable diseases also undermines the power of healthcare professionals. Concerns about the COVID-19 vaccine are mostly due to insufficient information about new vaccines and possible adverse effects, especially in the long term (6). Given the fact that COVID-19 vaccines are new, concerns about the vaccine's efficacy and adverse effects have caused public health professionals to worry about whether enough people will get vaccinated (7).

Several surveys of intention to receive a COVID-19 vaccine, when one becomes available, have been administered throughout the pandemic by academics, politics and researchers. In a meta-analysis study of global acceptance of the Covid-19 vaccine, the estimated acceptance rate was 68.4% (8). In a study on the public's view of the vaccine and the factors affecting this situation from Turkey, it was

DOI: 10.33808/clinexphealthsci.1005495

reported that only 41.2% of the participants approached it positively (9).

People obtaining information from reliable sources about the risks and benefits of the vaccine and how it works and protects them could help solve the problem of infodemia. Therefore, it is expected that both physicians and public health professionals will be ready to anticipate, understand, and respond to patients' questions and concerns to combat common misinformation and increase confidence in the vaccine.

Discussions on the efficacy and safety of the COVID-19 vaccines have yet to put an end to doubts in society as a whole, including healthcare professionals (7). In this study, it is aimed to evaluate physicians' attitudes towards the COVID-19 vaccine.

2. METHODS

2.1. Study Design

Our research is a cross-sectional study, which was conducted between January 4th and February 26th, 2021. The questionnaire used in our research was prepared using Google Forms. It was sent across Turkey using social media in various support groups and physician practices using Facebook and WhatsApp. All physicians aged 23 years and over were included in the study.

Assuming a positive attitude rate of 50% towards the COVID-19 vaccine, 95% confidence interval and 5% margin of error, a simple random sampling method was used and the required sample size was estimated as 384 participants. A total of 438 physicians agreed to participate in the study and constituted the study group.

2.2. Data Collection

In our study, a Google Forms questionnaire was prepared according to the literature and was used as the data collection tool (10-12). The first part of the two-part questionnaire included the sociodemographic characteristics of the physicians and some variables thought to be related to COVID-19. In the second part, questions from the "Attitudes Towards COVID-19 Vaccine Scale (ATCVS)" were included.

In the study, the ATCVS was used to evaluate the attitudes of physicians towards the COVID-19 vaccine. The scale, developed by Geniş et al. in 2020, consists of nine questions in 5-point Likert form. The scale has two sub-dimensions as positive and negative attitude. Positive attitude has four questions and negative attitude has five questions. Items in the negative attitude sub-dimensions are scored inversely. A value between 1-5 is obtained by adding the item scores in the scale sub-dimension then dividing the total score by the number of items in that sub-dimension. High scores from the positive attitude sub-dimension indicate that the attitude towards vaccination is positive. The items in the negative attitude sub-dimension are calculated after reversing, and the higher scores in this sub-dimension indicate that the negative attitude towards vaccination is less (10).

2.3. Statistical Analysis

The data were evaluated using the SPSS version 15.0 statistical package program. Descriptive statistical analysis was performed for all variables examined in the study. Normality was tested using the Kolmogorov-Smirnov test. The Mann-Whitney U test and the Kruskal-Wallis test were used for statistical analysis. Multiple linear regression analysis with variables that were significant in univariate analysis was used for further analysis. Statistical significance was considered for p-values of ≤0.05.

2.4. Ethical Considerations

After obtaining permission for scientific research studies on COVID-19 from the Ministry of Health Scientific Research Platform (F.number: 2020-12-24T16_50_38) for conducting the study, ethical permission was sought and granted by Eskisehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (Date: 12.01.2021, E-25403353-050.99-146299)

3. RESULTS

Of the total 438 participants, 310 (70.8%) were women and 128 (29.2%) were men. The mean age was 35.4 ± 8.5 (range, 24 – 71) years. One hundred ninety-eight (45.2%) of the physicians were specialists, and 37.7% (n = 165) were physicians who had practiced medicine for 10 years or more. Three hundred fifteen (71.9%) of the physicians stated that they were assigned to COVID-19–related units during the pandemic, 31.7% (n = 100) of whom were assigned to two or more units during this period. These units were outpatient clinics (29.5%, n = 130), inpatient wards (25.1%, n = 111), fillation (16.1%, n = 71) which is the name given to the process of determining what causes any infectious disease, emergency wards (13.4%, n = 59), intensive care (10.2%, n = 45), and others such as laboratory service, and home care service (5.7%, n = 25).

In the study, positive attitudes towards the COVID-19 vaccine were higher in the 24 – 30 years' age group and the specialist physicians compared with other physician groups. It was found that with more than 10 years of work in the profession, physicians had a more positive attitude towards vaccination than those with 1 – 5 years' experience. The distribution of physicians' ATCVS scores according to their sociodemographic characteristics is given in (Table 1).

The physicians in the study who had an influenza vaccine during the pandemic period had a higher positive attitude than those who did not. Physicians who recommended the vaccine to their patients had a more positive attitude towards the vaccine and less negative attitude than indecisive physician. In addition, it was determined that physicians who

were considering getting the COVID-19 vaccine had less negative attitudes towards the vaccine (Table 2).

Ninety-two (21.0%) of the physicians answered 'No' or 'Not decided yet' to the question, 'Do you consider getting a COVID-19 vaccine?' The common reason for hesitating about the vaccine was 'Not enough information studies about the vaccines' (Figure 1).

Table 1. *Distribution of physicians' ATCVS scores according to their sociodemographic characteristics*

Sociodemographic characteristics		n (%)	ATCVS Positive Attitude Median (min-max)	ATCVS Negative Attitude Median (min-max)
Age Group**	24-30	156 (35.6)	4.0 (1.3-5.0)[a]	3.6 (1.0-5.0)[a]
	31-40	186 (42.5)	4.3 (1.0-5.0)[b]	4.0 (1.0-5.0)[b]
	41 and more	96 (21.9)	4.5 (1.0-5.0)[b]	4.0 (1.8-5.0)[b]
	z/Kw; p		**15.415; <0.001**	**33.498; <0.001**
Sex*	Female	310 (70.8)	4.1 (1.0-5.0)	3.8 (1.0-5.0)
	Male	128 (29.2)	4.0 (1.0-5.0)	4.0 (1.0-5.0)
	z/Kw; p		-0.880; 0.379	1.356; 0.175
Title**	General practitioner	91 (20.8)	4.0 (1.0-5.0)[a]	3.8 (2.0-5.0)[a]
	Assistant physician	149 (34.0)	4.0 (1.0-5.0)[a]	3.6 (1.0-5.0)[a]
	Specialist physician	198 (45.2)	4.5 (1.0-5.0)[b]	4.0 (1.0-5.0)[b]
	z/Kw; p		**16.949; <0.001**	**27.223; <0.001**
Professional experience (year)**	1-5	162 (37.0)	4.0 (1.0-5.0)[a]	3.6 (1.0-5.0)[a]
	6-10	111 (25.3)	4.0 (1.0-5.0)[a,c]	4.0 (2.0-5.0)[b]
	More than 10	165 (37.7)	4.5 (1.0-5.0)[c]	4.0 (1.0-5.0)[b]
	z/Kw; p		**9.484; 0.009**	**31.284; <0.001**
History of chronic illness *	Yes	90 (20.5)	4.0 (1.0-5.0)	4.0 (1.0-5.0)
	No	348 (79.5)	4.0 (1.0-5.0)	3.8 (1.0-5.0)
	z/Kw; p		0.298; 0.766	-1.283; 0.199

** Mann-Whitney U, ** Kruskal-Wallis, a, b, c; The difference between groups that do not have the same letter in each column is significant (p <0.05)*

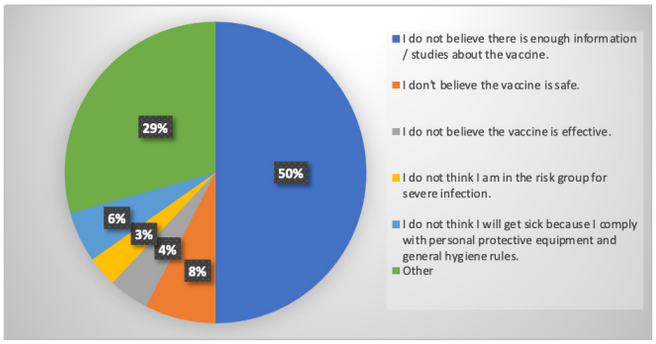

Figure 1. *Reasons for Physicians to Hesitate About Getting the COVID-19 Vaccine*

The sources of information about the COVID-19 vaccine are shown in Figure 2; the most common source was 'articles/ publications/literature information (32.4%), followed by the internet/social media (24.5%).

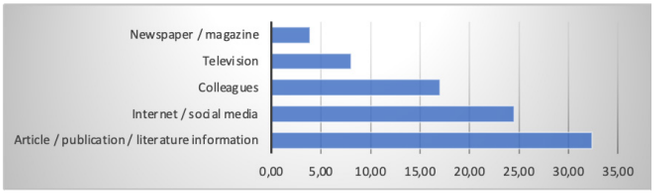

Figure 2. *The sources of information about the COVID-19 vaccine*
** Numbers were evaluated based on the answers given, not individuals.*

According to the results of the multiple linear regression analysis created with variables that were significant in the univariate analyses, it was found that the status of recommending the COVID-19 vaccine to patients and considering having the COVID-19 vaccine were variables that affected the positive and negative attitude towards the vaccine. The results of the multiple linear regression models for the subdimension scores of ATCVS are given in Table 3.

Table 2. *Distribution of physicians' ATCVS scores according to selected variables related to COVID19*

Selected variables related to COVID-19		n (%)	ATCVS Positive Attitude Median (min-max)	ATCVS Negative Attitude Median (min-max)
Influenza vaccination at pre-pandemic period*	Yes	179 (40.9)	4.0 (1.0-5.0)	3.8 (1.0-5.0)
	No	259 (59.1)	4.0 (1.0-5.0)	3.8 (1.0-5.0)
	z/Kw; p		-0.282; 0.778	-0.045; 0.964
Influenza vaccination at the pandemic period **	Yes	171 (39.0)	4.3 (1.0-5.0)[a]	4.0 (1.0-5.0)
	No	244 (55.7)	4.0 (1.0-5.0)[b]	3.8 (1.0-5.0)
	Indecisive	23 (5.3)	4.0 (1.3-5.0)[a,b]	3.8 (2.8-4.6)
	z/Kw; p		**11.609; 0.003**	4.213; 0.122
COVID-19 infection nearby *	Yes	412 (94.1)	4.0 (1.0-5.0)	3.8 (1.0-5.0)
	No	26 (5.9)	4.0 (1.0-5.0)	4.0 (2.8-5.0)
	z/Kw; p		-1.370; 0.171	1.222; 0.222
Have COVID-19 infection *	Yes	72 (16.4)	4.0 (1.0-5.0)	4.0 (2.0-5.0)
	No	366 (83.6)	4.0 (1.0-5.0)	3.8 (1.0-5.0)
	z/Kw; p		0.994; 0.320	-0.388; 0.698
Recommending COVID-19 vaccine to patients **	Yes	362 (82.6)	4.3 (1.0-5.0)[a]	4.0 (1.0-5.0)[a]
	No	17 (3.9)	2.0 (1.0-5.0)[b]	3.2 (1.8-5.0)[a,b]
	Indecisive	59 (13.5)	3.0 (1.0-5.0)[b]	3.4 (1.6-5.0)[b]
	z/Kw; p		**-9.622; <0.001**	**34.399; <0.001**
Willingness for COVID-19 vaccine **	Yes	346 (79.0)	4.5 (1.0-5.0)[a]	4.0 (1.0-5.0)[a]
	No	49 (11.2)	2.5 (1.0-5.0)[b]	3.4 (1.8-5.0)[b]
	Indecisive	43 (9.8)	3.0 (1.8-5.0)[b]	3.4 (1.6-4.8)[b]
	z/Kw; p		**91.827; <0.001**	**28.971; <0.001**

** Mann-Whitney U, ** Kruskal-Wallis, a, b, c; The difference between groups that do not have the same letter in each column is significant (p<0.05)*

Table 3. *Multiple linear regression models for the subdimension scores of ATCVS*

Sociodemographics and variables related to COVID-19	Positive Attitude Sub-dimension	Negative Attitude Sub-dimension
	β (95% CI)	β (95% CI)
Age	0.012 (-0.020-0.044)	0.009 (-0.014-0.031)
Title	0.003 (-0.015-0.021)	0.009 (-0.003-0.022)
Professional experience (years)	-0.008 (-0.035-0.019)	0.016 -0.003-0.035)
Influenza vaccination in the pandemic period	-0.013 (-0.036-0.010)	-
Recommending COVID-19 vaccine to patients	0.060*** (0.038-0.082)	0.022** (0.006-0.038)
Willingness for COVID-19 vaccine	0.048*** (0.023-0.072)	0.019* (0.001-0.036)
R^2 F	0.18 17.319***	0.10 11.258***

*p: *≤ 0.05; **≤ 0.01; ***≤ 0.001; CI: Confidence interval, ß: Unstandardize beta, R2: Adjusted R2, F: Test value*

4. DISCUSSION

Despite all the uncertainties about the efficacy, safety, and long-term adverse effects of newly developed COVID vaccines, it was determined that 79% of the physicians approached the vaccine positively. In addition, the physicians' willingness to be vaccinated and recommending vaccines to their patients were found to be effective variables in positive and negative attitudes towards vaccination. It was stated that the most important reason for vaccine hesitation was the insufficient studies about COVID-19 vaccines.

All activities related to vaccination and vaccine reliability studies that will prevent vaccine hesitation are the primary duties of all healthcare professionals, especially public health experts. Vaccination is our strongest weapon against COVID-19 infection, after hygiene and distancing; and the first vaccinations have been initiated in healthcare workers in many countries (13). During the pandemic, health workers have taken on the treatment and follow-up of infected patients, but also took an active part in the fillation and public education studies. Healthcare workers in Turkey volunteered for the Phase III CoronaVac trial, one of the newly developed vaccines, and vaccination studies were initiated in September 2020 (14).

In the study, 79.0% of the 438 physicians stated that they were considering being vaccinated, 11.2% stated that they did not want to be vaccinated, and 9.8% stated that they were undecided about the vaccine. Phase III of the CoronaVac study was still ongoing at the time the survey was collected; the high numbers of negative and hesitant answers about vaccination could be attributed to the lack of clarity on the data and vaccination studies have not yet accelerated. However, our study was in line with the results of COVID-19 vaccine studies among healthcare workers and the literature (15, 16). The common hesitation (50.0%) about vaccination was related to 'the lack of sufficient information and studies' about the vaccine'. The reasons for those with vaccine hesitation without any explanation (29.0%) could be different financial or political reasons or just a fear of unknown adverse effects of the vaccine. In addition, the government was sharing only the daily number of patients with the public, without any knowledge about the number of infected people until November 25th, 2020, which may have caused different perceptions of the severity of the epidemic among both society and physicians (17).

Positive attitude towards the vaccine increasing with age among the physicians was found in our study, like in many studies in the literature (18-20). Although female physicians were found to have a more positive attitude towards vaccination, the difference was not statistically significant.

Although most of the physicians (71.9%) worked in units related to COVID-19, it was observed that the attitudes of physicians working in non-COVID units were more positive, but there was no statistical difference between the physicians' desire for vaccination in our study. The negative attitudes of the physicians who had recently entered the profession towards the vaccine were less, the positive attitudes of the physicians who had more experience and working more than 10 years was higher towards the vaccine. Based on a COVID-19 study by Dror et al, it was reported that internal medicine branches looked more positively upon vaccines than surgical branches and healthcare workers who did not work in COVID units refused to get the vaccine more (15). In another study about COVID-19 by Shaw et al, it was reported that the desire for vaccination was lower in healthcare workers who were charged with primary patient care (20). Kose et al. reported that a group of people, mostly comprising university students, who had tetanus, pneumococcal, and influenza vaccines, also had a higher willingness for the COVID-19 vaccine (6). Among the participating physicians in our study, those who had influenza vaccines, especially during the pandemic period, had higher positive attitudes towards the COVID-19 vaccine. It is thought that the previous vaccination experiences of healthcare professionals may also influence their behaviors about newly developed vaccines (6, 20,21). It may be due to the fear of being confused about the effects and tracking or discerning the symptoms of COVID and influenza or having the disease more severely, if they have both infections simultaneously.

In our study, 82.6% of the physicians answered 'Yes' to the question, 'Would you recommend the COVID-19 vaccine to your own patients?'. The physicians who responded positively to this question also had more positive attitudes towards the COVID-19 vaccine. On the other hand, it is an important step for physicians to share their own vaccination experiences, which will encourage their patients to get vaccinated (18). The recommendation of a physician is considered the only force in vaccination acceptance (22-24).

In the study, 79.0% of the physicians who stated that they were considering being vaccinated had more positive attitudes towards vaccination. It is thought that physicians who are hesitant about vaccination will clarify their positions in line with the results of scientific research. It may be possible that physicians will have significant influence in the vaccination of the public if they have reliable evidence-based medicine. For example, studies have shown that the willingness to get vaccinated with the influenza vaccine and the trust towards the influenza vaccine were higher (13, 25, 26). The most important issue that physicians have to overcome is to increase vaccination rates and to prevent vaccination hesitation with education on vaccination and different vaccination campaigns.

Age, title, professional experience, and influenza vaccination rates during the pandemic period had a significant impact on ATCVS scores in the multiple linear regression model, only willingness to be vaccinated and recommending the vaccine to patients were still significant after adjusting for confounding factors. The WHO defines vaccine hesitation as a global health problem (24), and many studies have shown that there is a serious distrust among the public against these newly developed vaccines. Lack of information transparency regarding new vaccines, different conspiracy theory beliefs, and distrust towards drug campaigns cause a suspicious approach to vaccination among the public (27-29).

Vaccine hesitation has been identified as one of the greatest challenges in the fight against COVID-19 (15). Previous studies have established that the most common reasons for hesitation about COVID-19 vaccines among healthcare professionals are insufficient information about vaccines, unknown efficacy, and unknown adverse effects (1, 7, 19, 20). The results of our study are similar; physicians reported that there were insufficient studies on these newly developed vaccines. Our study adds that possible adverse effects, uncertainty about the efficacy and safety of the vaccine, and beliefs that people are not at risk for severe disease were prominent.

The present study has some limitations including the small sample size; the participants' were all physicians, most of them were women, a homogeneous group with similar characteristics; and the study was a descriptive-cross-sectional study. The collection of data via social media and in a very short period prevented reaching many physicians. At the time when the survey forms were collected, many vaccination studies had not yet been completed. Nevertheless, the study also has strengths. Conducting the study with a group with a high level of education and knowledge about the disease made the results more reliable. This study is one of the pioneering studies in which the views of physicians who can lead society in the acceptance of vaccines against COVID-19 vaccines are evaluated.

5. CONCLUSION

With time, more vaccine studies will have a positive effect on the vaccine hesitation and awareness of physicians. It is a priority issue for the authorities, physicians, and healthcare professionals to quickly conduct training to increase vaccine acceptance among the public, as well as COVID-19 vaccination studies. Thanks to vaccination programs and education, the desired community immunity will be gained by vaccination of a sufficient part of the population and infodemia with vaccine hesitation will be decreased. It is thought that this study on physicians will be a guide for both other healthcare professionals and community-based studies, and will play an important role in increasing confidence in COVID vaccines with an evidence-based medicine perspective.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Conflicts of interest

All the authors have no financial disclosures.

REFERENCES

[1] Neumann-Böhme S, Varghese NE, Sabat I, Barros PP, Brouwer W, van Exel J, Stargardt T. Once we have it, will we use it? A European survey on willingness to be vaccinated against COVID-19. Eur J Health Econ 2020;21(7):977–982.

[2] Wang W, Wu Q, Yang J, Dong K, Chen Xinghui, Bai X, Yu H. Global, regional, and national estimates of target population sizes for covid-19 vaccination: descriptive study. BMJ 2020;371.

[3] Koff WC, Schenkelberg T. The future of vaccine development. Vaccine 2020;38(28):4485–4486.

[4] Kennedy J, Leask J. Social media platforms have a moral duty to ban misinformation about vaccines: Two leading thinkers on vaccine hesitancy and misinformation debate this crucial question. Index Censorsh 2020;49(4):76–79.

[5] Puri N, Coomes EA, Haghbayan H, Gunaratne K. Social media and vaccine hesitancy: new updates for the era of COVID-19 and globalized infectious diseases. Hum Vaccin Immunother 2020; 16(11): 2586–2593.

[6] Kose S, Mandiracioglu A, Sahin S, Kaynar T, Karbus O, Ozbel Y. Vaccine hesitancy of the COVID-19 by health care personnel. Int J Clin Pract 2021;75(5).

[7] Meyer MN, Gjorgjieva T, Rosica D. Healthcare worker intentions to receive a COVID-19 vaccine and reasons for hesitancy: A survey of 16,158 health system employees on the eve of vaccine distribution. PsyArXiv.2020. DOI: 10.31234/osf.io/ge6uh.

[8] Wang W, Wu Q, Yang J, Dong K, Chen X, Bai X, Yu H. Global, regional, and national estimates of target population sizes for covid-19 vaccination: descriptive study. BMJ 2020; 371: m4704.

[9] Mert O. Türkiye'de insanların COVID-19 aşısına bakışı. Dicle Tıp Dergisi 2021; 48: 583–594.(Turkish)

[10] Geniş B, Gürhan N, Koç M, Geniş Ç, Şirin B, Çirakoğlu OC, Coşar B. Development of perception and attitude scales related

with COVID-19 pandemia. Pearson Journal of Social Sciences-Humanities 2020; 5: 306–328.

[11] Çiftci F, Şen E, Demir N, Çiftci O, Erol S, Kayacan O. Beliefs, attitudes, and activities of healthcare personnel about influenza and pneumococcal vaccines. Hum Vaccin Immunother 2018; 14(1): 111–117.

[12] Hofmann F, Ferracin C, Marsh G, Dumas R. Influenza vaccination of healthcare workers: a literature review of attitudes and beliefs. Infection 2006; 34(3), 142-147.

[13] Huang P. First COVID-19 Vaccine Doses To Go To Health Workers, Say CDC Advisers. Shots-Health News from NPR 2020. Published [05 November 2020]. Accessed [06 April 2021]. https://www.npr.org/sections/health-shots/2020/11/05/931844298/first-covid-19-vaccine-doses-to-go-to-health-workers-say-cdc-advisers

[14] Tirumalaraju D. Sinovac begins Phase III trial of Covid-19 vaccine in Turkey [Internet]. Clinical Trials Arena 2020. Published [23 September 2020]. Accessed [01 April 2021]. https://www.clinicaltrialsarena.com/news/sinovac-covid-vaccine-turkey/.

[15] Dror AA, Eisenbach N, Taiber S, Morozov NG, Mizrachi M, Zigron A, Sela E. Vaccine hesitancy: the next challenge in the fight against COVID-19. Eur J Epidemiol 2020;35(8):775–9.

[16] Sallam M. COVID-19 vaccine hesitancy worldwide: a concise systematic review of vaccine acceptance rates. Vaccines 2021; 9(2):160.

[17] British Broadcasting Corporation. Covid-19: Türkiye'de Temmuz ayından beri ilk kez günlük koronavirüs vaka sayısı açıklandı. Published [26 November 2020]. Accessed [20 April 2021]. https://www.bbc.com/turkce/haberler-turkiye-55082062

[18] Kuter BJ, Browne S, Momplaisir FM, Feemster KA, Shen AK, Green-McKenzie J, Offit PA. Perspectives on the receipt of a COVID-19 vaccine: A survey of employees in two large hospitals in Philadelphia. Vaccine 2021; 39(12), 1693-1700.

[19] Freeman D, Loe BS, Chadwick A, Vaccari C, Waite F, Rosebrock L, Lambe S. COVID-19 vaccine hesitancy in the UK: the Oxford coronavirus explanations, attitudes, and narratives survey (Oceans) II. Psychol Med 2020; 1–15.

[20] Shaw J, Stewart T, Anderson KB, Hanley S, Thomas SJ, Salmon DA, Morley C. Assessment of US health care personnel (HCP) attitudes towards COVID-19 vaccination in a large university health care system. Clin Infect Dis. 2021; 73(10):1776-1783. doi: 10.1093/cid/ciab054.

[21] Kwok KO, Li KK, Wei WI, Tang A, Wong SYS, Lee SS. Influenza vaccine uptake, COVID-19 vaccination intention and vaccine hesitancy among nurses: A survey. nt J Nurs Stud 2021;114:103854.

[22] Gargano LM, Herbert NL, Painter JE, Sales JM, Morfaw C, Rask K, Hughes JM. Impact of a physician recommendation and parental immunization attitudes on receipt or intention to receive adolescent vaccines. Hum Vaccin Immunother 2013; 9(12), 2627-2633.

[23] Hofstetter AM, Robinson JD, Lepere K, Cunningham M, Etsekson N, Opel DJ. Clinician-parent discussions about influenza vaccination of children and their association with vaccine acceptance. Vaccine 2017; 35(20), 2709-2715.

[24] World Health Organization. Ten threats to global health in 2019. Accessed [22 April 2021]. https://www.who.int/news-room/spotlight/ten-threats-to-globalhealth-in-2019

[25] Hunt C, Arthur A. Student nurses' reasons behind the decision to receive or decline influenza vaccine: a cross-sectional survey. Vaccine 2012; 30(40), 5824-5829.

[26] Abu-Gharbieh E, Fahmy S, Rasool BA, Khan S. Influenza vaccination: healthcare workers attitude in three Middle East countries. Int J Med Sci 2010; 7(5), 319.

[27] Vergara RJD, Sarmiento PJD, Lagman JDN. Building public trust: a response to COVID-19 vaccine hesitancy predicament. J Public Health (Oxf) 2021;43(2):e291–2. DOI: 10.1093/pubmed/fdaa282.

[28] Mallapaty S, Ledford H. COVID-vaccine results are on the way-and scientists' concerns are growing. Nature 2020; 16-17.

[29] Montagni I, Ouazzani-Touhami K, Mebarki A, Texier N, Schück S, Tzourio C. Acceptance of a Covid-19 vaccine is associated with ability to detect fake news and health literacy. J Public Health (Oxf) 2021;43:695–702.

How to cite this article: Oznur Muz FN, Onsuz MF, Metintas S, Arslantas D, Unsal A, Oktar D, Yucel E, Tepetas M. Assesment Of Physicians' Attitudes Towards COVID-19 Vaccine. Clin Exp Health Sci 2022; 12: 642-647. DOI: 10.33808/clinexphealthsci.1005495

Clinical and Experimental Health Sciences

Development and Validation of a Rapid HPLC-DAD Method for Determination of Favipiravir in Pharmaceutical Formulation

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Received: 08.09.2021 **Accepted:** 29.09.2021

ABSTRACT

Objective: The aim of this work was to develop and validate a rapid and simple high-performance liquid chromatography method with a diode-array detector (HPLC-DAD) for determination of favipiravir in bulk and tablet formulations.

Methods: The chromatographic analysis was performed at 30 °C with a Poroshell 120EC-C18 column (4.6 x 50 mm, 2.7 µm). The mobile phase was a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (90:10, v/v). The run time was 5 min at a flow rate of 0.5 mL/min.

Results: The proposed method was successfully validated in terms of precision, accuracy, linearity, robustness, limits of detection (LOD) and quantification (LOQ) parameters. The calibration plot was linear over a concentration range of 10-100 µg/mL. The LOD and LOQ values were found to be 0.58 µg/mL and 2.03 µg/mL, respectively. The average recovery values were found to vary from 99.45 percent to 104.29 percent.

Conclusion: As a result, it was concluded that the developed method can be used successfully in the determination of favipiravir in pharmaceutical preparations.

Keywords: COVID-19 treatment, HPLC, validation, favipiravir, SARS-CoV-2

1. INTRODUCTION

The Chinese government notified the WHO in December 2019 of pneumonia hospitalized patients with an unknown etiology. These patients were eventually identified as COVID-19, with SARS-Cov-2 as the causal virus. WHO declared a pandemic of Coronavirus illness in March 2020 (1-3). By the end of July 2021, the total number of cases recorded globally has surpassed 200 million, with 4 million fatalities. There is currently no COVID-19 therapy that has been scientifically proved to be effective and safe. Off-label treatments have already been approved for the treatment of various illnesses in Europe, the United States (USA), and our nation and have been demonstrated to be efficacious in vitro in SARS-CoV. One of these drugs used in this treatment is favipiravir (FAV) (4-10). When the literature studies on the analysis of favipiravir by HPLC are examined, it is seen that there are two studies based on gradient elution, and the analysis time of favipiravir in these studies was 21 and 60 minutes, respectively (11,12). Another HPLC techniques for determining FAV in pharmaceutical dosage forms was recently published (1,13). The proposed study is more sensitive than the two previously reported methods (1.20/3.60 and 0.985/2.986 µg/mL), with lower LOD and LOQ (0.58/2.03 µg/mL). So, it is predicted that this validate, and rapid method because it is inexpensive and allows rapid and sensitive analysis. The proposed method is rapid, accurate, very simple, and sensitive for quantification of favipiravir in pharmaceutical formulations. This method employs a simple mobile phase without the need of a buffer, requires no complicated sample preparation procedures, and has higher sensitivity than some of the previously described methods.

2. METHODS

2.1. Chemicals and Reagents

Bulk favipiravir and its pharmaceutical formulation (Favimol labelled content of FAV 200 mg) were kindly supplied by Dr. Şükran Özdatlı. HPLC-grade acetonitrile and formic acid were purchased from Merck. Millipore's Milli-Q water purification

DOI: 10.33808/clinexphealthsci.992869

technology was used to create HPLC-grade water. All the other reagents were of analytical grade.

2.2. Instruments

The Agilent 1260 Infinity HPLC system (Agilent Technologies, USA) was utilized for the chromatographic operations, which included a solvent pump, auto-sampler, column compartment, and DAD detector. The wavelength of detection was 323.0 nm. The chromatographic analysis was performed at 30 °C with a Poroshell 120EC-C18 column (4.6 x 50 mm, 2.7 µm) and a mobile phase A and B composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively. The flow rate was maintained at 0.5 mL/min.

2.3.Preparation of Stock and Working Solutions

Stock solutions of 1.0 mg/mL of FAV was prepared using an ultra-pure water in volumetric flask. The solution was warmed at 37 ℃ and shake it in the ultrasonic bath for a while for obtaining a higher solubility. Working standard solutions containing 10–100.0 µg/mL of FAV were generated by diluting this solution with ultra-pure water.

2.4. Method Validation

The ICH guidelines were followed for developing and validating the analytical method. System appropriateness, linearity, specificity, precision, accuracy, robustness, the limit of detection (LOD) and quantification (LOQ) were all addressed as validation parameters.

2.5. Specificity

In order to determine the specificity of the method, the presence of interference from the mobile phase was investigated with the help of a diode array detector. For this purpose, besides the chromatogram of the mobile phase, the UV spectrum and peak purity index values obtained from the FAV peak were examined.

2.6. Linearity

A six-point calibration plot for FAV was used to assess linearity. The standard solutions, ranging in concentration from 10 to 100 µg/mL, were used to prepare standard calibration. Under optimum chromatographic conditions, the standard solutions were injected three times into the HPLC system. The regression line's slope and Y-intercept values were calculated.

2.7. Limits of Detection and Quantitation (LOD and LOQ)

The LOD and LOQ values were calculated as 3.3 and 10 times the ratio of the calibration plot standard deviation to its slope, respectively.

2.8. Precision

Precision was determined at three distinct concentrations (n=5) by calculating intra-day and interday (repeatability was determined by examining the standard solution on five separate days) variations of the method. In precision research, on the same day and for five days, five repetitions of standard solution at three concentrations (30, 50, 70 µg/mL) were injected into the system. The relative standard deviation was used to get the accuracy value (RSD).

2.9. Accuracy

To test the accuracy of the suggested method, recovery studies were carried out using the spiking method. The sample solutions of known quantity (20 µg/mL) were spiked with 80, 100, and 120 percent of three different amounts of bulk and calculating favipiravir recovery for each concentration from equation of the calibration plot.

2.10. Robustness

A robustness study was conducted to assess the impact of certain modifications in chromatographic conditions. Column temperature, mobile phase flow rate and ratio are all variables. The system appropriateness parameters were verified after each change by injecting the sample solution into HPLC system and comparing the results to those obtained with the initial chromatographic settings.

3. RESULTS

3.1. Chromatographic Conditions

The analysis was performed in a C18 column (4.6 x 50 mm, 2.7 µm) using a solvent system of 0.1 % formic acid in water: 0.1 % formic acid in acetonitrile (90:10, v/v) by isocratic elution. The flow rates of the mobile phase were maintained at 0.5 mL/min. From the UV spectra of standard solution, the wavelength corresponding to maximal absorbance (max) was determined to be 323 nm (Figure 1). The column temperature was kept at 30 °C during the procedure. Table 1 presents the conditions of HPLC system.

Table 1. *Optimized chromatographic conditions*

Parameter	Chromatographic conditions
Instrument	Agilent 1260 Infinity HPLC system
Column	Poroshell 120EC-C18 4.6 x 50 mm, 2.7 µm
Mobile phase [A:B (90:10, v/v)]	Mobil-phase A: 0.1% formic acid in water Mobil-phase B: 0.1% formic acid in acetonitrile
Flow rate	0.5 mL/min
Detection wavelength (DAD)	323 nm
Runtime	5 min
Column temperature	30 °C
Volume of injection	10 µL
Retention time	2.40 min

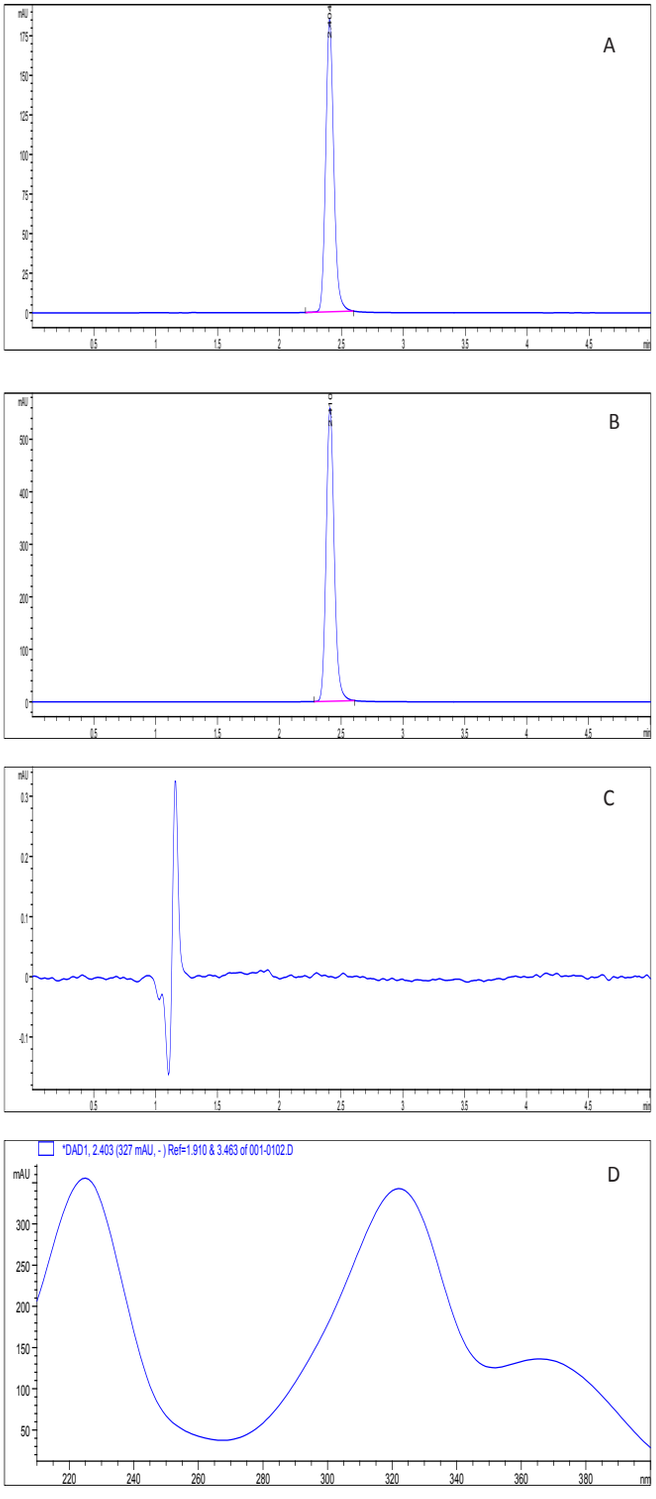

Figure 1. *(A) Chromatogram (standard solution, 40 µg/mL, λ:323 nm). (B) Chromatogram (sample solution, 100 µg/mL, λ: 323 nm). (C) Chromatogram (Blank solution, λ: 323 nm). (D) Absorbance spectra of FAV.*

3.2. Method Validation

Specificity

In the analysis, only the mobile phase injection was performed, and it was determined that there was no peak in the minutes when favipiravir was seen. Whether there was any interference with the favipiravir peak obtained when working with the developed method was determined with the help of a diode sequential detector. The spectra and peak purity index values show that there is no interference, and the peaks are pure (Figure 2).

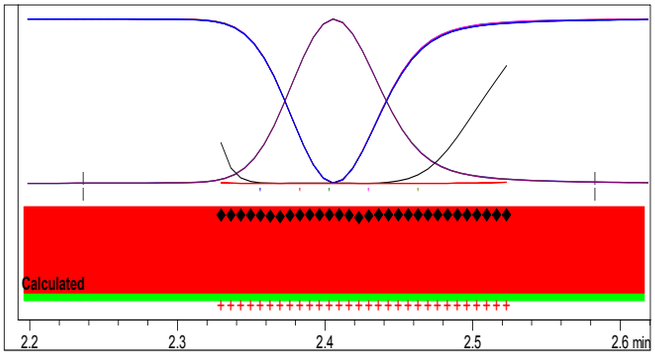

Figure 2. *Peak purity of FAV. (Purity factor: 999.963 threshold: 999.991 noise threshold: 0.016)*

3.3. Linearity

To prepare standard solutions, the stock standard solution of FAV was diluted appropriately with ultra-pure water. Under chromatographic working conditions, the standard solutions were injected three times into the chromatographic system. Regression analysis was used to assess the suggested method's linearity at six concentration levels ranging from 10 to 100 µg/mL. The parameters of the regression analyze of the calibration plot are given in the Table 2, and the calibration plot drawn between the indicated concentration values and the average peak areas is given in the Figure 3. The LOQ and LOD were calculated using a recommended formula (ICH Q2 (R1) as follows (14):

LOD = 3.3 SD / slope ; LOQ = 10 SD/slope

Table 2. *Regression analysis and LOD/LOQ values of the proposed method*

Parameter	Value
Linearity range (µg/mL)	10-100
Slope	23.375
Intercept	25.924
Correlation coefficient	0.9998
SE of intercept	10.0322
SD of intercept	4.0948
LOD/LOQ (µg/mL)	0.58/2.03

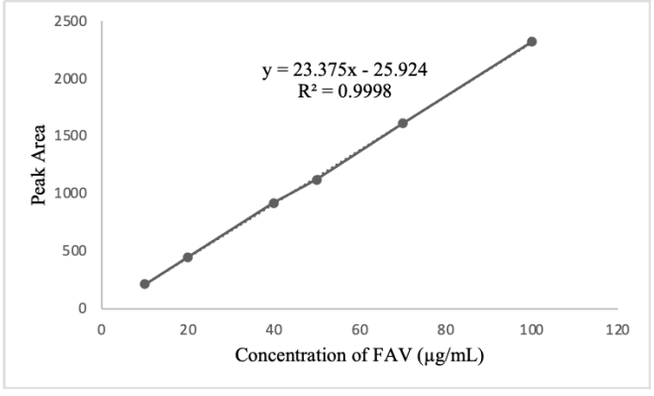

Figure 3. *Calibration curve of standard working solutions of favipiravir*

3.4. Precision

A sequence of FAV analyses was done for five consecutive days (n=5) in the precision trials. The intra-day and inter-day precision RSD were found to be <1%, indicating that the method was accurate enough. In the analyzes performed on the same day, the relative standard deviation was calculated between 0.06-0.14%. The relative standard deviation obtained in the analyzes performed on different days was found to be between 0.14-0.58%. Table 3 summarizes the findings.

Table 3. *Intra-day and Inter-day precision data of FAV*

Std. Conc.µg/ml	Taken conc. (µg/mL)	Found conc. (µg/mL) ± SD	Peak area RSD (%)
	30	30.0325±0.0371	0.1371
Intra-day	50	49.9796±0.0445	0.0909
	70	70.5806±0.0394	0.0574
	30	29.9302±0.0417	0.1394
Inter-day	50	49.4170±0.0627	0.1269
	70	69.0084±0.3973	0.5757

3.5. Accuracy

The method's accuracy was demonstrated using the standard addition technique. In this approach, a certain amount (20 µg/mL) of solution was mixed with 80, 100, and 120 percent of three distinct levels of bulk. Percentage recoveries for the FAV was computed. As a result of the trials, the recovery values were found in the range of 99.45-104.29%. Table 4 shows the findings of the recovery results.

Table 4. *Recovery data*

Spiked level (%)	Added conc. (µg/mL)	Found conc. (µg/mL)	Recovery%	Average Recovery %± SD	RSD%
	36	37.6877	104.6882		
80	36	37.5136	104.2045	104.2976	0.2766
	36	37.4400	104.0001		
	40	40.5147	101.2869		
100	40	40.4229	101.0572	101.0416	0.2048
	40	40.3123	100.7808		
	44	43.7792	99.4983		
120	44	43.7623	99.4599	99.4457	0.0499
	44	43.7561	99.3791		

3.6. Robustness

According to the data, flow rate and mobile phase concentration have little influence on FAV chromatographic separation behavior. FAV retention period is unaffected by changes in the flow rate or mobile phase ratio. The technique did not alter much when the column temperature was changed. Table 5 shows the findings of this investigation, given as a percent RSD.

Table 5. *Robustness of the proposed HPLC method for favipiravir**

Flow rate		Composition of mobile phase		Column temperature	
0.4 mL/min	0.6 mL/min	A:B (95:5)	A:B (85:15)	28 °C	32 °C
108.60±0.43	99.98±0.87	98.28±0.17	102.81±0.46	100.03±0.20	100.47±1.82

**The studies were performed with the 50 µg/mL standard solution and the results were provided in mean recovery%±RSD%*

3.7. Application of the Method to the Tablet Formulation

The developed method has been successfully used for the determination of favipiravir in pharmaceutical formulations. Table 6 shows the results of the favipiravir test on the commercially available tablet. The amounts mentioned on the pill labels are closely connected to the findings achieved. This demonstrates the utility of the content assessment approach.

Table 6. *Results of analysis of FAV*

Formulation	Original tablet solution (µg/mL)	Amount found±SD (µg/mL)	% RSD
FAV tablets	100	102.9175±0.1170	0.1137

Average of 6 determinations; SD: standard deviation; RSD: relative standard deviation

4. DISCUSSION

When the analyzes of favipiravir in pharmaceutical preparations were examined, it was seen that there were very few chromatographic analysis methods. The UV spectrum obtained from the FAV peak with a diode array detector was examined and it was decided that the wavelength was 323 nm. When operating under these conditions, the retention time for FAV was found to be 2.4 minutes. After the appropriate chromatographic conditions were determined, the method validation study was carried out. For this purpose, studies were carried out to determine the Selectivity, Linearity, Accuracy, Precision, LOD, LOQ and robustness parameters. The method was found to be linear in the concentration range of 10-100 µg/mL. In order to determine the reproducibility of the method, three different concentrations of samples were studied on the same day and on different days. As a result, the RSD values of the analyzes performed on the same day were determined as 0.06-0.14%, and the RSD values of the analyzes performed on different days were determined as 0.14-0.58%. To determine the accuracy of the developed method, the standard addition method was applied. In the study, increasing concentrations of standard FAV solutions were added to the capsule solution containing FAV. As a result of the trials, the recovery values were found in the range of 99.45-104.29%. LOD and LOQ values for the developed method were determined as 0.58 µg/mL and 2.03 µg/mL, respectively. After the validation process was completed, the developed method was used for FAV quantification in FAV capsules and as a result, the average FAV amount was found to be 102.92%.

When the validation findings were compared to the ICH limits, the suggested technique yielded good system appropriateness values. The precision values in terms of RSD percent were lower than 1% with high recoveries in the tablet analysis. The suggested approach was compared to selected previous research on the determination of FAV in tablet formulations by HPLC-UV method in the literature. The r^2 values for all the compared techniques were more than 0.999. The proposed technique has the least duration

of retention. The proposed study is more sensitive than the two previously reported methods [1.20/3.60 µg/mL (1) and 0.985/2.986 µg/mL (13)], with lower LOD and LOQ (0.58/2.03 µg/mL). So, this method is inexpensive and allows more rapid and sensitive analysis than two related methods in literature.

5. CONCLUSION

The proposed method is simple, rapid, accurate, and sensitive for quantification of favipiravir in pharmaceutical formulations. This method employs a simple mobile phase without the need of a buffer, requires no complicated sample preparation procedures, and has higher sensitivity than some of the previously described methods. Hence this proposed method can be widely used in quality control laboratories for determination of favipiravir.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: D.T, Design: D.T., Analysis or Interpretation: D.T., Literature Search: D.T., Writing: D.T.

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

Financial Disclosure: The author declared that this study received no financial support.

REFERENCES

[1] Ibraam EM, Heba E, Fathalla B, Adel EI. Green micellar solvent-free HPLC and spectrofluorimetric determination of favipiravir as one of COVID-19 antiviral regimens Microchemical Journal 2021;165 (2021): 106189.

[2] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease, (COVID-19): A review. JAMA 2020; 324 (2019): 782–793.

[3] Acquavia A, Foti L, Pascale R, Nicolo A, Brancaleone V, Cataldi TR. Detection and quantification of Covid-19 antiviral drugs in biological fluids and tissues. Talanta. 2021; 224 (2021): 121862.

[4] Delang L; Abdelnabi R, Neyts J. Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res. 2018; 153: 85–94.

[5] China patent (CN104914185A). HPLC method for measuring related substances in Favipiravir. 16.09.2015.

[6] Madelain JG, Nguyen THT, Jacquot F, Oestereich L, Kadota T. Favipiravir pharmacokinetics in nonhuman primates and insights for future efficacy studies of hemorrhagic fever viruses, Antimicrob. Agents Chemother.2017; 61 (1): (2017), https://doi.org/10.1128/AAC.01305-16.

[7] Smee DF, Hurst BL, Egawa H, Takahashi, K, Kadota T, Furuta Y. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virusinfected cells, J. Antimicrob. Chemother. 2009;64 (2009): 741–746.

[8] Nguyen THT, Guedj J, Anglaret X, Laou´enan C, Madelain V, Taburet AM. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted, PLoS Negl. Trop. Dis. 2017;11 (2): e0005389.

[9] Gowen BB, Sefing EJ, Westover JB, Smee, DF, Hagloch J, Furuta Y, et al., Alterations in favipiravir (T-705) pharmacokinetics and biodistribution in a hamster model of viral hemorrhagic fever. Antiviral Res. 2015;121 (2015): 132–137.

[10] Safa MM, Ahmed A H, Sherin F H, Amira H. Kamal Experimental design approach for development of spectrofluorimetric method for determination of favipiravir; a potential therapeutic agent against COVID-19 virus: Application to spiked human plasma Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.2021; 249 (2021): 119241

[11] Nguyen THT, Guedj J, Anglaret X, Laou´enan C, Madelain V, Taburet AM. Favipiravir pharmacokinetics in Ebola-Infected patients of the JIKI trial reveals concentrations lower than targeted, PLoS Negl. Trop. Dis. 2017;11 (2): e0005389.

[12] A kind of Favipiravir has the HPLC assay method of related substance, Google Patents, CN104914185B (2015) Chinese article.

[13] Bulduk I. HPLC-UV method for quantification of favipiravir in pharmaceutical formulations, Acta Chromatographica. 2020; 1-7, DOI: 10.1556/1326.2020.00828.

[14] ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.; 2018 July 7. Available from: http://www.ema.europa.eu/docs/en_GB/document_library /Scientific_guideline/2009/09/WC500002662.pdf [Website].

How to cite this article: Taskin D. Development and Validation of a Rapid HPLC-DAD Method for Determination of Favipiravir in Pharmaceutical Formulation. Clin Exp Health Sci 2022; 12: 648-652. DOI: 10.33808/clinexphealthsci.992869

Clinical and Experimental Health Sciences

Cynara Scolymus (Artichoke) Improves Liver Regeneration after Partial Liver Resection in Ratsß

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Received: 21.12.2021 **Accepted:** 28.02.2022

ABSTRACT

Objective: Liver regeneration is necessary to restore hepatic mass and functional capacity after partial hepatectomy (PH). Cynara scolymus (CS) is a pharmacologically important plant that contains phenolic acids and flavonoids, and experimental studies have indicated that it has antioxidant and hepatoprotective effects. The aim of this study was to investigate the role of CS in liver regeneration after PH in rats.

Methods: A total of 36 Wistar albino rats weighing 280.5 ± 18.6 g were used. CS leaf extract was administered after partial hepatectomy. The rats were sacrificed at postoperative day 14, and the histological changes were assessed. The mitotic index (MI), nucleus size, hepatocyte size, and binucleation rate (BR) of hepatocytes were assessed using hematoxylin-eosin (H&E) staining.

Results: The rats that received CS extract had significant differences in liver regeneration markers, including the hepatocyte size, mitotic index, and Ki-67 proliferation index ($p<0.05$). The average increase in liver mass in 14 days was higher in the CS group, but the differences between the groups were not significant (1.70±0.2 g in sham group versus 2±0.8 g in CS group, $p=0.75$).

Conclusions: The results indicate that CS leaf extract promoted hepatocellular proliferation and hypertrophy, which resulted in accelerated liver regeneration.

Keywords: Cynara Scolymus, Artichoke, liver regeneration, liver resection, liver histology

1. INTRODUCTION

The liver can regenerate, which allows it to withstand extended hepatectomy. Nevertheless, insufficient remnant liver is one of the most common causes of death after hepatectomy (1). It is well known that insufficient remnant liver size after liver resection or a living donor liver transplant (LDLT) may result in an inability to meet the metabolic demand of the body and can cause liver failure (2).

Liver regeneration is necessary to restore hepatic mass and functional capacity after partial hepatectomy (3). To increase liver regeneration, it is crucial to obtain enough liver mass in order to prevent complications related to liver failure. It has been shown that hepatocyte proliferation is the main factor in liver regeneration (4). It has been reported that intracellular reactions which play role in kinetics of cell proliferation have influences in the liver regeneration (5). Moreover, studies showed that liver regeneration after hepatectomy is regulated by the immune system (6), and the inhibition of lipid peroxidation and increased glutathione peroxidase activation are reported to increase during liver regeneration.

Commonly known as artichoke, *Cynara scolymus* (CS) is a pharmacologically important plant that contains phenolic acids and flavonoids, and experimental studies have indicated that it has antioxidant activity (7). Many studies have shown that CS has liver-protective effects by inhibiting lipid peroxidation and increasing glutathione peroxidase activity (8, 9). Nevertheless, no study has explained the effects of CS on liver regeneration after hepatectomy. The goal of this study was to investigate the effect of CS in liver regeneration after hepatectomy.

2. METHODS

The study was performed using 6-month-old Wistar albino female rats weighing 280.5 ± 18.6 g, which were obtained from Zonguldak Bulent Ecevit University Animal Laboratory. All experimental work related to the study was

DOI: 10.33808/clinexphealthsci.1008534

carried out according to the standards of national guidelines (28914/February 15, 2014). The study was approved by the Ethical Review Board at Zonguldak Bulent Ecevit University, Zonguldak, Turkey (protocol number 2018-12-07/06). The animals were kept in a cage and had free access to food and water. The animals were kept in a day/night cycle at room temperature.

The study was designed using three groups of n=8 rats each, but 36 rats were obtained in case any rat died during hepatectomy or right after. In total, 12 rats died during or right after the procedure. Thus, the study was started with three groups of 8 rats each.

In the control group, rats did not have surgery and received regular standard food and water. In the sham group, rats underwent hepatectomy but received standard regular food and water. In the CS Group, rats underwent hepatectomy and received CS extracts (by oral gavage) in addition to standard food and water.

Partial hepatectomy was performed by the removal of a part of the middle and left lateral lobes of the liver. CS leaf extract was administered at a dose of 0.16 mg/kg/day two times **orally** to rats in the CS group. At postoperative day 14, the rats were sacrificed. By the end of the study, one rat in the control group and two rats in both the sham and CS groups died.

The average liver weight was calculated for each group. The mitotic index (MI), nucleus size, hepatocyte size, and binucleation rate (BR) of hepatocytes were assessed using hematoxylin-eosin (H&E) staining. For morphological evaluation Olympus CX43 model microscope was used. The mitotic activity was determined using a microscope at 10 high-power fields (HPFs) (X40). The sizes of the nuclei and hepatocytes were determined using 3 HPFs. In this study a photograph of each area belonging to a HPF was taken by the camera attachment of confocal microscope. As we know in every HPF nearly 500 cell can be obtained. The microscopic image of each HPF was transferred to the image J analysis program. In this program, the nucleus and cell size were measured individually for each hepatocyte. All values were summed numerically and divided by the total number of cells within the area to calculate the mean value. This calculation was done separately for both nucleus and cell size. Proliferation index for hepatocytes were evaluated by Ki67 immunohistochemistry. Immunohistochemistry was performed with monoclonal ready-to-use antibody against human Ki-67 antigen (Clone 30-9; Ventana, Tucson, AZ, USA). Rabbit Monoclonal Primary antibody. Staining was performed using a Roche Ventana Automatic Device. The proliferation index was calculated based on the evaluation of 100 hepatocytes with positive stained nuclei**.** We accepted brown staining of nucleus of tumor cells as positive for Ki67. The intensity was varying cell to cell. We considered any degree of intensity as positive staining. The negative tumor cells were blue. In this evaluation detecting tumor cells are important. Because tumor cells are mixed with stromal and lymphoid cells. We evaluated the areas with the most intense staining by scanning the areas. These areas were assessed and minimum 500 cells were counted. In these areas positive staining cells were deterimined. Finally Ki67 proliferation indeks was calculated like this; number of positive staining cells/ number of tumor cells x100 (10)

2.1. Statistical Analyses

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) was used for the statistical analyses. Data are expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) was applied to test the differences between groups. Significant differences between two groups were determined using a post-hoc Tukey test, and $p < 0.05$ was considered significant.

3. RESULTS

The average size of livers in the control group was 9.92 ±1.3 g, which is considered as an average weight in all groups. The liver index was calculated as a percentage (%) of the rats' liver weight (g) with respect to the body weight (g), which was 3.54%. The average hepatectomy liver weight in the sham and CS groups was 2.2 ±0.7 g, which is 22.2% of the total liver weight. The average size of livers at the end of the study in sham group was 9.41±0.8g (94.9% of the original liver), while that of the CS group was 9.71± g (97.8%). The average increase of liver mass in 14 days was higher in the CS group than the sham group, but the difference was not significant (1.70±0.2 g in sham group versus 2±0.8 g in CS group, p=0.75). No significant difference was found in the liver index in the sham group versus the CS group (3.36% versus 3.46%, p=0.67).

The effect of CS on the histological morphology after hepatectomy was analyzed. The rates of steatosis, inflammation, sinusoidal dilatation, congestion, and hydropic degeneration in liver cells between three groups were compared (Table 1). These findings were assigned grades of 1 of 4 based on the literature (11). H&E staining indicated that steatosis did not occur in any group, but inflammation was slightly higher in both the sham and CS groups. Although there was no differences in sinusoidal dilatation between groups, congestion and the degree of hydropic degeneration were higher in both the sham and CS groups (Table 1).

Table 1. *The histological liver changes in groups*

Groups	Steatosis	Inflammation	Sinusoidal dilatation	Congestion	Hydropic dejeneration
C	0	0	+	+	0
S	0	+	+	++	+
CS	0	+	+	++	+

None: 0 Minimal: + Mild: ++ Moderate: +++ High: ++++

In further comparisons between experimental groups, the changes were significantly different between all three of

them (Table 2). Histological alterations are shown in Figure 1. H&E staining revealed that the mean size of nuclei was significantly higher in the CS group than both the control group (10.4±0.6 vs. 7.75 ±0.2 µm, p<0.0001) and the sham group (10.4±0.6 vs. 8.33±0.75 µm, p=0.0068). The mean size of nuclei was significantly higher in the sham group than the control group (8.33±0.75 vs. 7.75 ±0.2 µm, p=0.18). The mean size of hepatocytes was significantly higher in the CS group than in both the control group (480.6 ±38 µm vs. 315.9±80, p=0.0021) and sham group (480.6 ±38 µm vs. 265.8± 18.9 µm, p=0.0003). However, no significant difference was found between the sham group and control group (315.87±80.4 vs. 265.8± 19 µm, p=0.456).

The mean MI was significantly higher in the CS group than both the control group (4.7±1.2 vs. 2.25±1.3, p=0.015) and sham group (4.7±1.2 vs. 1.5±0.7, p= 0.348)). However, no statistically difference was found between the sham group and the control group (2.25±1.3 vs. 1.5±0.7, p= 0.348). Statistically significant differences were found in the BR in the CS group versus the sham group (29.2±6 vs. 12±5%, p= 0.003) and versus the control group (29.2±6 vs. 6.5 ±2, p=0.0001), as well as between the control group and sham group (12±5% vs. 6.5 ±2.1, p= 0.042)

The Ki-67 proliferation index rate was compared between groups, and the differences were significant (Table 2, Figure 2). The mean rate of the Ki-67 proliferation index was 2% in the control group, while it was 3% in the sham group, but the differences were not significant (2% and 3%, p=0.114). The mean rate of the Ki-67 proliferation index was 8% in the CS group, and there was a significant difference between the CS group and sham group (8 % vs. 3% p=0.043).

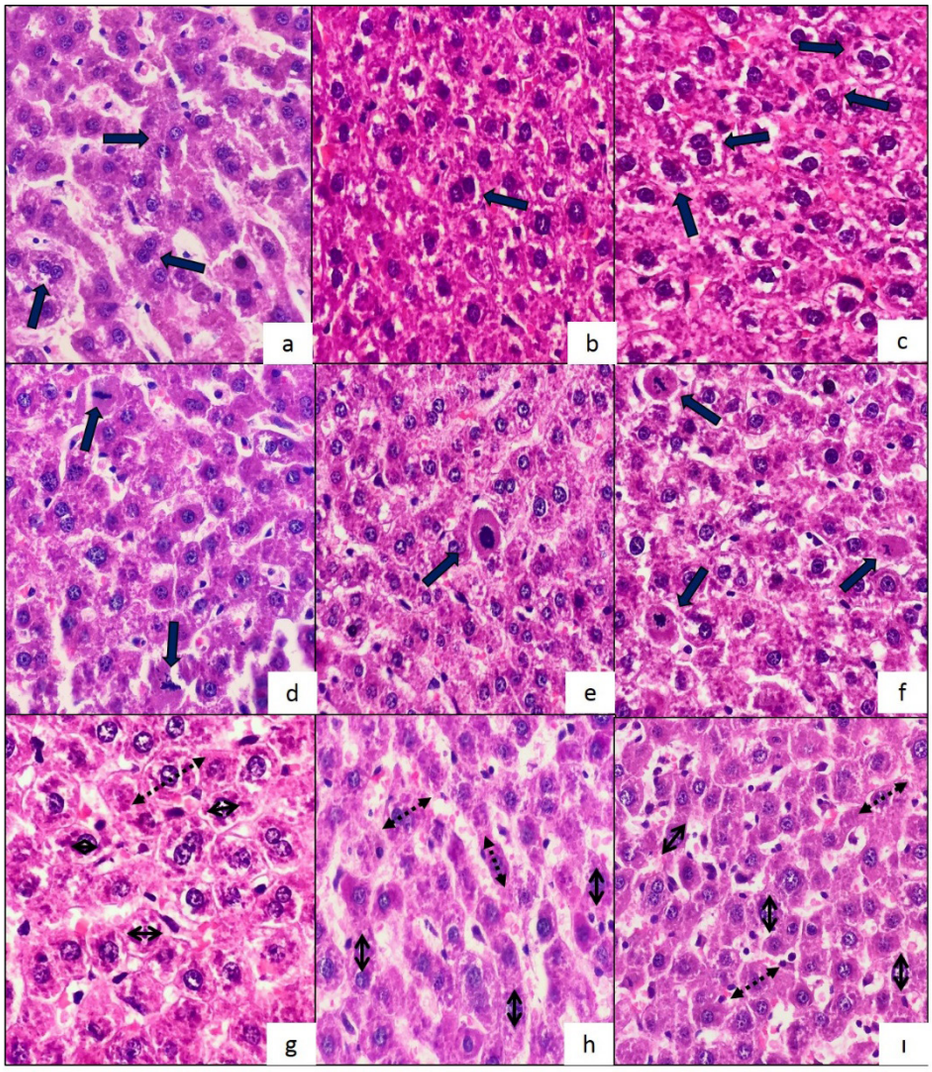

Figure 1. *Histomorphological findings of each group and also their comparison with each other (HEx400). Figures 1a-c show binucleation rates for group C, S and CS, respectively. Figures d-f show the mitotic figure for each groups in the same order. Group CS have the most mitotic figure among the groups as we see in figure f. Figures g-ı demonstrate the nuclear and hepatocyte size.*

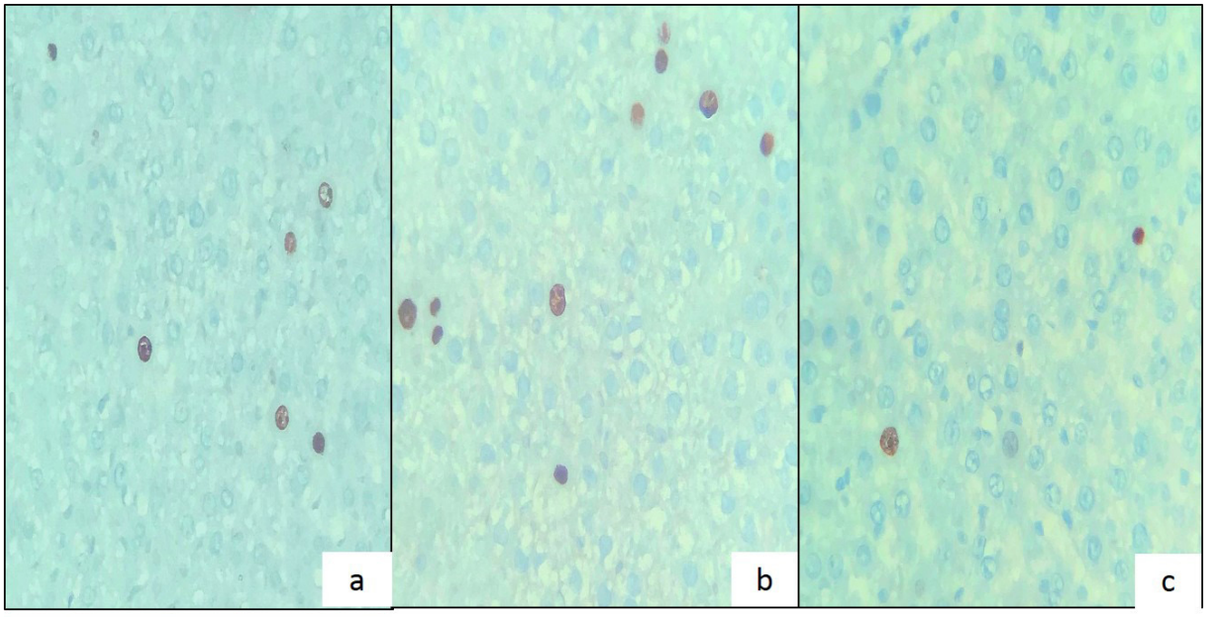

Figure 2. *a-c Ki67 proliferation index in groups C, S and CS, respectively (Ki67 immunohistochemistry x400).*

Table 2. *The comparison of histological changes in liver regeneration between groups (stained with H&E).*

Histological parameters (mean±SD)	C Group	S Group	CS Group	P
Size of nucleus (µm)	7.75 ±0.2	8.33±0.75	**10.4±0.6**	**<0.001**
Size of hepatocyte (µm)	315.9±80	265.8± 19	**480.6 ±38**	**<0.001**
Mitotic index (MI)%	2.25±1.3	1.5±0.7	**4.7±1.2**	**<0.001**
Binucleation rate (BR)%	6.5 ±2.1	12±5	**29.2±6**	**<0.001**
Ki 67 proliferation index (%)	2%	3 (%	**8 %**	**<0.001**

4. DISCUSSION

Currently, the main obstacle in liver transplantation is organ shortage. In order to overcome this obstacle, LDLT has been accepted as an option, particularly in Eastern countries, but the rate has slightly increased in Western countries as well. The main concern in LDLT is providing enough functional liver mass to maintain liver function in both the donors and recipients. The main success behind LDLT is liver regeneration. Liver regeneration not only makes LDLT available to those who need it, it also provides an option to surgeons to perform liver resection for advanced liver cancers that require extensive liver regeneration. However, it carries the risk of the remnant liver mass not being enough to maintain liver function. Both LDLT and liver resection for liver cancer rely upon liver regeneration to get enough liver mass (12).

Various strategies have been developed in models of hepatectomy to increase liver regeneration after hepatectomy in both humans and rats (13, 14). This study was performed to evaluate the role of CS in liver regeneration. It has been reported that CS has many health benefits (15), such as antioxidant activity. For example, one study reported that CS has liver-protective effects and downregulates oxidative stress in acute DZN-induced liver injury in rats (16). Another study showed liver-protective effects in liver damage induced by paracetamol (17). Nevertheless, research has still been needed to fully understand the effects of CS on liver regeneration after partial hepatectomy.

Although the normal liver is dormant with only a little hepatocyte proliferation, after partial hepatectomy, it was shown that the remaining liver undergoes a series of rapid endothelial, inflammatory, and epithelial changes (18). Multiple important mechanisms and factors control normal liver regeneration, such as IL-6, TNF, hepatocyte growth factor (HGF), epidermal growth factor (EGF), and thyroid hormone, which have been previously reported in detail and are not discussed here in depth (18, 19). The novel findings in this study are the CS-related histological changes of the liver after hepatectomy in rats. The changes were found to involve significant increases in the mean nucleus size, hepatocyte size, MI, BR, and Ki 67 proliferation index. Although changes were noted in all rats after partial hepatectomy compared to the control group, they were significantly higher in the CS group.

A previous study reported that by 7–10 days after hepatectomy, a rat's liver largely regrows to about normal size (93%) through hyperplasia of the remnant lobes (18). In this study, by 14 days, the average size of the liver at the end of the study in the sham group was 94.9% of the native liver size, but the recovery was higher (97.8%) in the CS group. The reason why there was not a significant difference between groups could be that the time needed to complete all regeneration is reported to be about 20 days (18). A second reason could be that we did not do 70% liver resection, as was done in a previous study; instead, we performed 22.2% resection (18).

It has been reported that the proliferation of hepatocytes induces regeneration of the liver (20). Proliferation and hypertrophy almost equally contribute to regeneration (21). The sizes of hepatocytes and nuclei are reported to increase significantly in regeneration (21, 22). This is consistent with our study, which showed increased hepatocyte size and nuclear size, and the changes were significantly higher in rats receiving CS. The number of binuclear hepatocytes and binuclear-to-total hepatocyte ratio are considered as signs of liver regeneration in other studies (22). However, the present study was not consistent with earlier studies, and we found that even the BR was higher in both the sham group and CS groups, but it was significantly higher in rats receiving CS.

A wide range of markers are used to describe hepatic regeneration criteria (18, 23). MI, which is used in the present study, is the ratio between the number of cells in mitosis and the total number of cells. Our study showed that MI was significantly higher in rats receiving CS. Previous studies reported using immunofluorescence staining of Ki-67 as a proliferation marker (21, 24). Ki-67 is expressed from the G1 to the M phase. Its expression was found to be higher in both the sham and CS groups compared to the control group. However, the expression of Ki-67 was significantly increased in rats that received CS compared with rats that did not.

CS is cultivated in many parts of the world because of its nutritional benefits and medicinal properties (25). It has shown beneficial effects in diseases of the biliary tract, digestive action, scurvy, anemia, and atherosclerosis (26, 27). However, this study is the first to our knowledge to demonstrate experimentally that CS increases liver regeneration after liver resection in rats.

There are some limitations in this study. We found out that CS increases liver regeneration by changing hepatocyte histology, but we did not investigate the exact mechanism of CS that creates the histological changes. Thus, studies are needed for further investigation. In conclusion, the present study showed that CS increases liver regeneration after partial liver resection with increases in both proliferation and hypertrophy in the remnant liver.

Acknowledgment

We would like to thank Veterinary Surgeon Osman Cengil for his great assistance in laboratory. This study was not financially supported by any institution or company

All authors declared no conflict of interest and funding.

REFERENCES

[1] Chapelle T, Op de Beeck B, Driessen A, Roeyen G, Bracke B, Hartman V, Huyghe I, Morrison S, Ysebaert D, Francque S. Estimation of the future remnant liver function is a better tool to predict post-hepatectomy liver failure than platelet-based liver scores. Eur J Surg Oncol. 2017;43(12):2277-2284.

[2] Asencio J.M, Vaquero J, Olmedilla L, Garcia Sabrido JL: "Small-for-flow" syndrome: shifting the "size" paradigm. Med Hypotheses 2013;80(5):573-537.

[3] Clavien PA. Liver regeneration: A spotlight on the novel role of platelets and serotonin. Swiss Med Wkly. 2008; 138(25-26): 361-370.

[4] Thorgeirsson SS: Hepatic stem cells in liver regeneration. FASEB J. 1996; September 10(11):1249-56.

[5] Michalopoulos GK. Liver regeneration after partial hepatectomy: Critical analysis of mechanistic dilemmas. The American Journal of Pathology 2010; 176 (1): 2-13

[6] Naseem S, Hussain T, Manzoor S. Interleukin-6: A promising cytokine to support liver regeneration and adaptive immunity in liverpathologies. Cytokine Growth Factor Rev. 2018;39:36-45.

[7] Salekzamani S, Mameghani ME, Rezazadeh K. The antioxidant activity of artichoke (Cynara scolymus): A systematic review and meta-analysis of animal studies. Phytother Res. 2019;33(1):55-71.

[8] Colak E, Ustuner MC, Tekin N, Colak E, Burukoglu D, Degirmenci I, Guney HV. The hepatocurative effects of Cynara scolymus. Leaf extract on carbon tetrachloride-inducedoxidative stress and hepatic injury in rats. Springerplus. 2016;5:216.

[9] Pérez-García F, Adzet T, Cañigueral S. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Radic Res. 2000;33(5):661–665.

[10] Kinra P, Malik A. Ki 67: Are we counting it right?. Indian J Pathol Microbiol 2020;63:98-99

[11] Matsuo K, Murakami T, Kawaguchi D, Hiroshima Y, Koda K, Yamazaki K, Ishida Y, Tanaka K. Histologic features after surgery associating liver partition and portal vein ligation for staged hepatectomy versus those after hepatectomy with portal vein embolization. Surgery. 2016;159(5):1289-1298.

[12] Lauterio, A. , Di Sandro S, Concone G, De Carlis R, Giacomoni A, De Carlis L. Current status and perspectives in split liver transplantation. World J. Gastroenterol. 2015; 21: 11003–11015.

[13] Ren, W. Wang X, Zhang A, Li C, Chen G, Ge X, Pan K, Dong JH. Selective bowel decontamination improves the survival of 90% hepatectomy in rats. J. Surg. Res. 2015,195: 454–464.

[14] M Ninomiya, K Shirabe, T Terashi, H Ijichi, Y Yonemura, N Harada, Soejima Y, Taketomi A, Shimada M, Maehara Y. Deceleration of regenerative response improves the outcome of rat with massive hepatectomy. Am. J. Transplant.2010; 10:1580–1587.

[15] Ben Salem M, Affes H, Ksouda K, Dhouibi R, Sahnoun Z, Hammami S, Zeghal KM. Pharmacological Studies of artichoke leaf extract and their health benefits. Plant Foods Hum Nutr. 2015;70(4):441-453.

[16] Ahmadi A, Heidarian E, Ghatreh-Samani K. Modulatory effects of artichoke (Cynara scolymus L.) leaf extract against oxidative stress and hepatic TNF-α gene expression in acute diazinon-induced liver injury in rats. J Basic Clin Physiol Pharmacol. 2019;30(5):/j/jbcpp.2019.30.

[17] Sümer E, Senturk GE, Demirel ÖU, Yesilada E. Comparative biochemical and histopathological evaluations proved that receptacle is the most effective part of Cynara scolymus against liver and kidney damages. J Ethnopharmacol. 2020;249:112458.

[18] Michalopoulos, G. K. & DeFrances, M. C. Liver regeneration. Science 1997; 276: 60–66.

[19] Michalopoulos, G. K. Liver regeneration. J. Cell. Physiol. 2007; 213: 286–300.

[20] Dusabineza AC, Van Hul NK, Quinones JA, Starkel P, Najimi M, Leclercq IA. Participation of liver progenitor cells in liver regeneration: lack of evidence in the AAF/PH rat model. Lab. Invest. 2012; 92: 72–81.

[21] Miyaoka Y, Ebato K, Kato H, Arakawa S, Shimizu S, Miyajima A . Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. Curr Biol. 2012;22(13):1166-1175.

[22] Miyaoka Y, Miyajima A. To divide or not to divide: revisiting liver regeneration. Cell Div. 2013;8(1):8.

[23] Akçan A, Kucuk C, Ok E, Canoz O, Muhtaroglu S, Yilmaz N, Yilmaz Z. The effect of amrinone on liver regeneration in experimental hepatic resection model. J Surg Res. 2006;130:66–72.

[24] Wan HF, Li JX, Liao HT, Liao MH, Luo L, Xu L, Yuan KF, Zeng Y. Nicotinamide induces liver regeneration and improves liver function by activating SIRT1. Mol Med Rep. 2019;19(1):555-562.

[25] Lattanzio V, Paul AK, Lansalta V, Cardinali A. Globe artichoke: a functional food and source of nutraceutical ingredients. J Funct Foods. 2009; 1:131–144.

[26] Gebhardt, Fausel M. Antioxydant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. Toxicol In Vitro. 1997; 144:279–286.

[27] Kraft K. Artichoke leaf extract–recent findings reflecting effects on lipid metabolism, liver, and gastrointestinal tracts. Phytomedicine. 1997; 43:69–78.

How to cite this article: Gunay Y, Kaymaz E. Cynara Scolymus (Artichoke) Improves Liver Regeneration after Partial Liver Resection in Rats. Clin Exp Health Sci 2022; 12: 653-658. DOI: 10.33808/clinexphealthsci.1008534

Clinical and Experimental Health Sciences

Investigation of Changes in Liver Microanatomy in the Steatosis Model Created by Permanent Canula in Rats

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Received: 13.10.2021 **Accepted:** 17.12.2021

ABSTRACT

Objective: The knowledge of nonalcoholic fatty liver disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH) is limited to the findings from available suitable models for this disease. A number of rodent models have been described in which relevant liver pathology develops in an appropriate metabolic context. In this experimental study, it was aimed to create a new liver fat model by giving fat from the portal vein of rats and to visualize the changes in the liver with advanced microscopic techniques.

Methods: 28 female rats were used in the study. Permanent intraabdominal cannulas were inserted into the portal vein of the rats. Rats were randomly divided four group. Intralipid 20% substance was injected through cannula to the experimental groups during the test period. Control group received saline at the same rate. At the end of the experiment, the animals were visualized with a laser speckle microscope and livers were divided into sections according to the stereological method. The sections were painted with Hematoxylin-Eosin, Oil red o, Masson trichoma, Bodipy, Nile red. Sections were evaluated under a microscope.

Results: Ballooning, inflammation and fibrosis were observed in the 2 week intralipid group. In the 1 week intralipid group, the rate of parenchyma decreased while the sinusoid rate increased, and sinusoid rate increased significantly in the 2 week intralipid ($p<0.05$).

Conclusion: According to the findings, steatohepatitis was detected in the 2 week intralipid, whereas only steatosis was observed in the 1 week intralipid. Thus, it was concluded that the newly formed rat model causes steatosis.

Keywords: NAFLD, Steatosis, Portal vein, Confocal microscope, Laser speckle.

1. INTRODUCTION

The prevalence of nonalcoholic fatty liver disease (NAFLD) is between 4% and 47% in different populations (1,2).

Alcoholic and nonalcoholic fatty liver is a disease characterized by the accumulation of triglycerides in hepatocytes. In nonalcoholic steatosis, not only fat, but also intralobular inflammation, hepatocellular ballooning, and advanced fibrosis are seen (3). Nonalcoholic steatosis is the most common chronic liver disease in the world (4), a metabolic disease that occurs in 10-35% of the world population and more than 50% of obese people (5).

Animal studies for the nonalcoholic steatosis model not only explain the pathogenesis of steatosis, but also provide important information for testing the therapeutic effects of various agents. However, these animal models need to coincide with both the pathophysiology and histopathology of human nonalcoholic steatosis (6,7). Currently, there are many animal models for nonalcoholic steatosis. None of these models demonstrates the entire process of human steatosis. Therefore, new models are needed to provide information about the pathogenesis and treatment of nonalcoholic steatosis (4).

The aim of this study was to create a non-alcoholic steatosis test model in rat liver by injecting fat from the portal vein using a permanent cannula to create a similar model of human fatty liver. In addition, it was aimed to detect the changes in liver tissue in more detail by making necessary stains after steatosis. At the same time, it is aimed to show uptake of fatty acid and possible accumulation of fat in intracellular fluid with fluorescent dyes to be applied and to detect all structural changes in liver tissue quantitatively by the latest stereological methods.

2. METHODS

The ethical approval of the study was obtained from Istanbul Medipol University Animal Experiments Local Ethics

DOI: 10.33808/clinexphealthsci.948391

Committee (date: 14.04.2014 and decision number: 17). A total of 28 female Sprague-Dawley rats weighing 250-300 g were used in the study. The animals were obtained from Istanbul Medipol University Medical Research Center (MEDITAM). All experiments were done in Regenerative and Restorative Medicine Research Center (REMER). The animals were maintained at the controlled temperature of 23 ± 1°C, humidity of 55 ± 5%, in a 14-hour light/10-hour dark cycle. Throughout the study, the animals were provided with soy-free, in-house-prepared pelleted feed and filtered drinking water, ad libitum. Experimental groups (n=14) and control groups (n=14) were divided into 1-week intralipid (n=7), 1-week saline (S) (n=7) and 2-week intralipid (n=7), 2-week saline (n=7).

One-week experiment group was injected intralipid 20% substance + saline through the portal cannula for one week (1w/intralipid). One-week control group received saline in the portal cannula (1w/saline). Two-weeks experiment group was injected intralipid 20% + saline from the portal cannula for two weeks (2w/intralipid), while the control group was injected with saline for two weeks (2w/saline).

2.1. Surgical Procedures

Before surgery, animals were anesthetized. For surgical operations, the Ritsma protocol was used (8) (Fig. 1 A, B, C.). The edges of the cannula were adhered to the vessel with tissue adhesive (9). The reservoir with the cannula was placed in the pocket we created (Fig. 1 D, E).

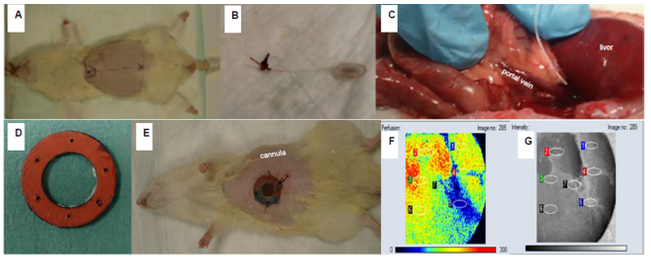

Figure 1. *Preparation of the rats for surgical operation and insert the cannula into the portal vein and blood flow image.*

A. Shaving and incision. **B.** Catheter preparation. **C.** Inserting the cannula into the portal vein. Abdominal imaging window developed by the materials in our laboratory and **D.** placement of the rat in the portal vein with cannula. **E.** Laser speckle microscopy shows the imaging process of the liver. **F.** amount of perfusion (blue: low flux, green: medium flux, red: high flux). **G.** measured areas in liver.

2.1.2. Intralipid injection:

After surgical procedures, intralipid ® 20% substance from portal vein was given to the experimental groups in the form of 2.4 g fat / kg + saline every day during the experiment period, while steatosis was aimed to occur in the control groups 0.9% saline administered daily during the same period.

2.1.3.Live microscopic examination of steatosis model developed rats:

Blood flow in the liver was evaluated with laser speckle microscopy while the rats were under anesthesia. The distance between the rat and the microscope objective was adjusted and the measurement started (Fig. 1 G, H). For this purpose, the same areas were used to measure on the same lobe of each rat. All rats were imaged between the 5th and 10th minutes and the values were recorded.

2.1.4.Examination of steatosis model rats by biochemical, histological and stereological methods:

At the end of the experiment, all rats were anesthetized, blood was taken from the heart and euthanized than liver tissue was collected (10,11).

2.1.5. Biochemical Analyses:

Aspartate aminotransaminase (AST), Alanine aminotransferase (ALT) serum levels were determined. Rat Eliza kits (Sigma) were used for the samples. The solutions were prepared following the protocols in the assay kits.

2.1.6. Fluorescent Staining:

Nile red staining and Bodipy staining; The stock solution was prepared before staining and then imaged on a confocal microscope (Zeiss LSM 780 NLO Multi Photon and Confocal Microscope; Zeiss Axio Observer Z1, fully motorized inverted microscope).

2.1.7. Histological investigation

For histological investigation, 8 µm sections were taken from frozen liver tissues in cryostat. Images were taken with axio zoom microscope.

2.1.8. Oil Red O staining (ORO)

With this dye, the presence of lubrication under fluorescence microscopy was evaluated.

2.1.9. Hemotoxylin-Eosin staining:

Hemotoxylin and eosin staining were performed on sections taken at 5 µm size. It was evaluated whether there was ballooning and whether the lubrication was macrovesicular or microvesicular.

2.1.10. Masson trichoma staining:

For staining, 8 µm sections were taken from the tissues with cryostat. Fibrosis in liver tissue was evaluated with stained sections.

2.2. Morphometric Analyses

In our study, morphometric evaluations were performed to obtain quantitative results. For this purpose, after the blood collection from rats, removed liver lobes were divided into 6-8 sections and tissue fragments were taken according to systematic random sampling method (12). A piece of tissue of 1 cm^3 was taken for each animal. Subsequently the fractions were frozen at – 80 ° C. Sections of frozen tissue were then taken in 5 µm sections on the cryostat. Sections were selected by systematic random sampling method

(one out of every 16 sections). All sections were stained with Hematoxylin-Eosin. Mean diameter of central veins in liver and liver sinusoids to liver parenchyma ratio (Vv) were calculated on these sections. To estimate the mean diameter of central veins, major (a) and minor (b) diameters of central veins were measured and the formula $(D = \sqrt{a.b})$ was used However, sinusoids to parenchyma ratio was determined by point counting method (13).

2.3. Statistical Analyses

Mean, standard deviation, median lowest, highest, frequency and ratio values were used in descriptive statistics of the data. Distribution of variables was measured by Kolmogorov Simirnov Test. Mann-Whitney u test was used for the analysis of quantitative independent data. The chi-square test was used for the analysis of qualitative independent data, and the Fischer test was used when the chi-square test conditions were not met. SPSS 22.0 program in analysis.

3. RESULTS

In the 1w/intralipid group, steatosis, including mostly microvesicular-weighted macrovesicles, was observed. Hepatocellular ballooning was not observed. There were lobular inflammations. In the 2w/intralipid group; steatosis was predominantly macrovesicular. There was lobular inflammation. Hepatocellular ballooning was observed. Perisinusoidal fibrosis and portal fibrosis were also present. Lipogranulomas were large in size. Steatosis ballooning and lobular inflammation were not seen in the control group (Fig.3) (Table 1).

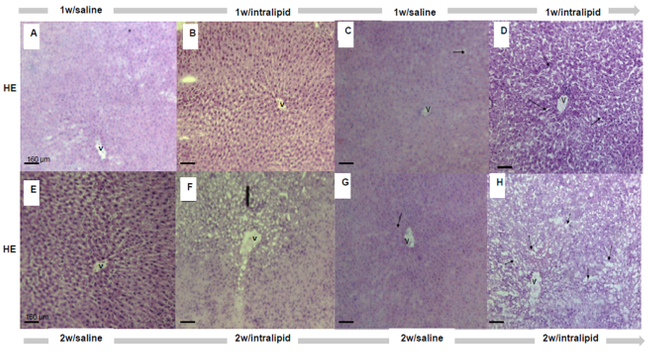

Figure 2. *Histological investigation of central vein and sinusoid/ parencyma.*

A. 1w/saline central vein. **B.** 1w/intralipid central vein **C.** 1w/saline sinosoid/ parencyma. **D.** 1w/intralipid sinusoid/parencyma. **E.** 2w/saline central vein. F. 2w/intralipid central vein. **G.** 2w/saline sinosoid/parencyma. **H.** 2w/intralipid sinusoid/parencyma. V: central vein, narrow: sinusoid (HE staining).

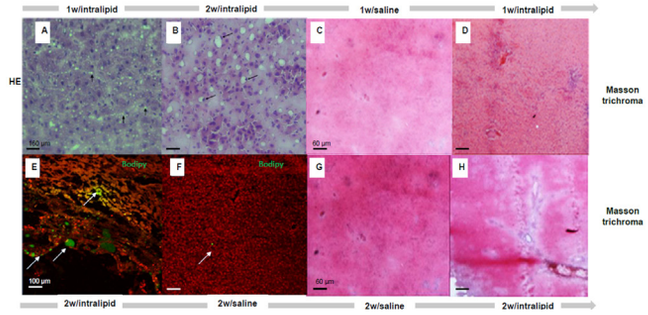

Figure 3. *Ballooning, fibrosis and fat accumulation images.*

A. 1w/intralipid. **B.** 2w/intralipid (short black arrows show microvesicular ballooning, long black arrows show macrovesicular ballooning HE). **C.** 1w/ saline. **D.** 1w/intralipid (less periportal fibrosis). **G.** 2w/saline. **H.** 2w/intralipid (pericinusoidal and periportal fibrosis) Masson trichroma. **E.** 2w/intralipid. **F.** 1w/intralipid; the green spots are the foci where the bodipy fluorescent dye radiates. Shows intracellular fat accumulation (Bodipy staining).

Table 1. *Summary of Results.*

	1w/saline	1w/intralipid	2w/saline	2w/intralipid	p
Mean central vein diameters (μ) Med±s.d.	5.8 ± 0.7	8.1 ± 2.6	5.8 ± 0.7	10.7 ± 2.8	**0.00**[m]
Sinusoids / parenchyma (Vv) Med±s.d	4.5 ± 2.8	14.4 ± 5.5	4.4 ± 1.3	22.4 ± 3.9	**0.00**[m]
Ballonning (n=42 sample each group)	(+) n-(%) 0; 0.0%	(+) n-(%) 0; 0.0%	(+) n-(%) 0; 0.0%	(+) n-(%) 8; 19.0%	**0.000**[X²]
Fibrosis (n=42 sample each group)	Perisinusoidal; 0.0%	Perisinusoidal; 0.0%	Perisinusoidal; 0.0%	Perisinusoidal; 11.9%	**0.000**[X²]
	Periportal; 0.0%	Periportal; 14.3%	Periportal; 0.0%	Periportal; 88.1%	
Fat granule rate (n=42 sample each group)	%5; 0.0%	%5; 33.3%	%5; 0.0%	%5; 28.6%	**0.000**[X²]
	%15; 0.0%	%15; 0.0%	%15; 0.0%	%15; 14.3%	
	> %50; 0.0%	> %50; 0.0%	> %50; 0.0%	> %50; 57.1%	
Fatty acid release (n=42 sample each group)	(+) n-(%) 0; 0.0%	(+) n-(%) 32; 76.2%	(+) n-(%) 0; 0.0%	(+) n-(%) 37; 88.1%	**0.000**[X²]
Intracellular fat accumulation	(+) n-(%) 0; 0.0%	(+) n-(%) 0; 0.0%	(+) n-(%) 0; 0.0%	(+) n-(%) 42; 100%	**0.000**[X²]
ALT(U/L) Med±s.d.	96.9 ± 42.4	81.6 ± 32.9	83.6 ± 34.7	117.5 ± 58.8	0.25[m]
AST(U/L) Med±s.d.	188.3 ± 95.4	217.0 ± 97.5	162.6 ± 38.5	343.4 ± 194.3	**0.05**[m]
Blood flow (mm/min) Med±s.d.	162.1±22.4	109.6 ± 48.3	160.5 ± 44.1	120.1 ± 10.5	0.089[k]

Liver histopathological and biochemical parameters in control and experimental groups. [m] Mann-Whitney U test. [X²] Chi-square test. [K] Kruskal-Wallis (Mann-Whitney U test). (p <0.05)

3.1. Histological and Fluorescent Staining Findings

Ballooning / fibrosis results (Fig. 1, 3) and Oil red o, Nile red and Bodipy staining results are shown in Table 1 (Fig. 3, 4).

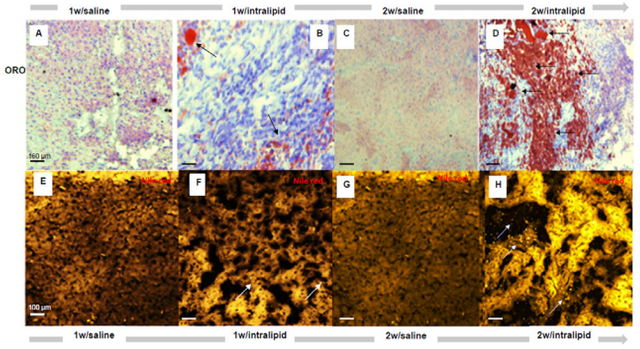

Figure 4. *Lipid droplet and fatty acids images.*

A. 1w/saline. **B.** 1w/intralipid. **C.** 2w/saline. **D.** 2w/intralipid. **G.** 2w/saline (short black arrows shows oil granules, ORO). **E.** 1w/saline. **F.** 2w/intralipid. **G.** 2w/saline.**H.** 2w/intralipid, white arrows show fatty acids, the golden yellow spots are the foci where the nile red dye radiates. (Nile red staining).

3.2. Morphometric, Biochemical and Liver Perfusuion (Blod flow) Findings

Results are shown in the Table 1 (Fig. 2, 3).

4. DISCUSSION

The knowledge of NAFLD and NASH is limited by the suitable models for this disease at the end of previous studies. A number of rodent models have been described in which relevant liver pathology develops in an appropriate metabolic context. These models play a key role in research on NASH. To date, not a single rodent model has been able to explain the progression of this disease in humans, but these models can mimic the characteristics of the disease in humans. Therefore, it is important that researchers select appropriate rodent models (14).

When rats were evaluated macroscopically, large or small lipogranuloma was observed in all of the 2w/intralipid (Fig. 4 A-D). Lipogranulomas are not necessary for the diagnosis of NAFLD, but since it is a frequently observed finding in NASH (15), it has been valuable data indicating that our model may have positive results macroscopically.

Morphometric examinations in our study: mean central vein diameter was found to be larger in both 1w/intralipid and 2w/intralipid than control group rats. When the 2w/intralipid and 1w/intralipid were compared (Fig. 2 A-D), the mean central vein diameter was larger in the 2w/intralipid compared to the other group, indicating that the mean central vein diameter increased as fat was fed from the portal vein. However, it was observed that the vessel diameters in the portal area increased more in the 2w/intralipid compared to the 1w/intralipid. There was no such study on the diameter of the central vein in parallel with our study. Shih et al. 2016 (16), stated that the change in central vein diameter did not correlate with blood flow in their transplantation study.

When the sinusoid / parenchyma ratio was calculated morphologically; The rats in the 1w/intralipid group were found to have increased sinusoidal space compared to the control group, but decreased liver parenchyma. In the 2w/intralipid group, it was observed that the sinusoidal space increased and liver parenchyma decreased more than the 1w/intralipid group. It is noteworthy that the parenchyma decreases and the sinusoidal space increases as the duration of the experiment increases. Noorafhsan et al. 2005 (17), in their study of diabetic rats reported that changes in the volume of sinusoid volume to changes in hepatocyte volume. Altunkaynak and Ozbek 2009 (12), female rats in their study of high fat content in the increase in sinusoidal volume due to necrosis parenchyma loss and decreased the density of hepatocytes were connected. Again, Yahyazadeh et al. 2017 (18), in their high-fat diet study, stated that feeding with fatty diet causes vascular dilatation in the liver and this may be due to inflammatory changes. Brancatelli et al. (19) attributed the cause of sinusoidal dilatation to increased venous pressure.

Ballooning and Mallory-Equivalent bodies represent liver cell damage and are critical in making the diagnosis of NASH (20). In our study, ballooning was observed only in the 2w/intralipid group (Fig. 3), whereas no ballooning was observed in the one-week experimental group. This indicates the presence of steatohepatitis in the 2w/intralipid group.

NASH is part of the NAFLD spectrum, characterized by lobular inflammation and progressive perisinusoidal fibrosis (21). Liver fibrosis is a liver disease that can result in the accumulation of extracellular matrix proteins, mainly collagen, and subsequent cirrhosis, portal hypertension, hepatic failure and HCC due to chronic liver damage (22).

In our study, inflammation was detected in 2w/intralipid groups, but not in the majority of the 1w/intralipid groups. This inflammation in the 2w/intralipid groups is mainly caused by lobular infiltration. This is an important symptom of steatohepatitis (23), indicating that steatohepatitis occurs in the 2w/intralipid group. When sections were stained with Masson trichoma stain used to show fibrosis, fibrosis was observed in some of the 1w/intralipid groups. Both periportal and perisinusoidal fibrosis were present in the two-week experimental group. In the 1w/intralipid group, periportal fibrosis was seen in some regions alone. However, the presence of both periportal and perisinusoidal fibrosis in the 2w/intralipid group is an indication that the model we developed was going to steatohepatitis.

When oil red o stained sections were evaluated, oil droplets were observed in experimental groups compared to control groups in our study. When the 1w/intralipid group and the 2w/intralipid group were compared, the percentage of fat droplets on the cross-section in the 2w/intralipid group was higher than the 1w/intralipid group. In addition, when comparing the size of the oil droplets in the hematoxylin-eosin stained sections, an image was obtained with microvesicular oil drops, mostly on the basis of macrovesicular weight in the 2w/intralipid group. In the 1w/intralipid group, a few rats in the group had less macrovesicular weighted fat droplets than the 2w/intralipid group, while the other

rats had microvesicular weighted fat droplets, including macrovesicles. Although microvesicular fat droplets have a poor prognosis in the long term, they are known to carry a great risk for the development of steatohepatitis and liver damage (24). In our study, we can say that simple steatosis occurs in the animals in the 1w/intralipid group and the risk of steatohepatitis is high. The presence of severe steatosis in rats in the 2w/intralipid group was determined by staining.

When all these histological findings were evaluated together; In the 1w/intralipid group, non-alcoholic fatty liver was evaluated; mild steatosis (> 5%) as well as periportal inflammation and no ballooning. In 2w/intralipid group, severe steatosis (33% – 66%), lobular inflammation was observed as 2-4 foci per foci, with several ballooned hepatocytes in sections. Given these data, it is possible to mention the presence of NAFLD in both experimental groups. When non-alcoholic steatohepatitis is graded, it is possible to mention only mild steatosis in the 1w/intralipid group and panacinar steatosis in the 2w/intralipid group. Since fibrosis was not observed in the 1w/intralipid group except portal fibrosis, staging could not be performed, whereas the 2w/intralipid group showed stage 2 characteristics. In the light of these data, steatosis has occurred in our 1w/intralipid group, but it can be said that the 2w/intralipid group has gone to steatohepatitis.

Abnormal intrahepatic fat accumulation (steatosis) in the form of cytoplasmic lipid droplets is an early pathophysiological feature of alteration of liver metabolism. For this reason, when we evaluated the fatty acid intake by Bodipy staining to detect the fat droplets, the foci of radiation were determined in certain areas in 2w/intralipid groups. This radiation showed us that fatty acid intake and accumulation were present in the 2w/intralipid group.

In order to detect intracellular fat accumulation by Nile red staining, fluorescence radiation from the stained sections was evaluated under a microscope. The abundance and scarcity of the focal points were examined from the sections where there was radiation. According to the results, more radiant focal points were detected in some rats in the 1w/intralipid group, and less in some rats in the 2w/intralipid group. Thus, more intracellular fat accumulation was observed in the 2w/intralipid group than in the 1w/intralipid group. Foci with radiation are often more frequent in or near the portal area, especially where cell infiltration occurs.

The accumulation of end products of enzymatic reactions in the liver may contribute to liver microvascular damage and NAFLD progression by further enhancing liver dysfunction. Since the end products of the enzymatic reaction are essentially advanced glycation end products, it has been disclosed that by stimulating the receptor, it exacerbates liver damage, fibrosis and inflammation, which in turn activates oxidative and inflammatory pathways (24).

In the studies to date, microcirculation change of NAFLD has been mentioned (25, 26). For this purpose, in our study, blood flow was evaluated by laser speckle for microcirculation of liver blood flow. Although no significant difference was found between the one week groups, it was observed that blood flow slowed down in some parts of the liver lobe compared to other groups in the 1w/intralipid group. Seifalian et al. 1999 (26) , in their study found that decreased microcirculation in the steatosis group and as a result of hepatic fat infiltration of the liver blood flow and parenchymal microcirculation were interpreted as slowed. McCuskey et al. 2004 (27), in their study of steatohepatitis with dietary fat in the flow of fat to go down to the direction of fibrosis interpreted their studies. In a NAFLD study performed by Pereira et al. 2017 (28), with a high-fat diet, it was found that liver blood flow decreased by up to 47%. This situation is similar to our study. When the 2w/intralipid groups were compared with the saline groups, it was observed that blood flow decreased in the portal vein fat treated groups compared to the saline groups and a significant difference was observed. Our results coincide with other studies (26,28).

As a result of the ALT evaluation of the blood samples of the experimental groups, no statistically significant difference was observed between the groups. However, according to the AST results, there was no difference between the 1w/intralipid group and the control group, whereas the AST values of the 2w/intralipid group were higher than the other groups. When the results were examined, the AST value of the 2w/intralipid group was found to be significant compared to the saline group. AST / ALT ratio of 1.33 and above is indicative of steatosis (29). In fact, the increase in AST is directly proportional to fibrosis (30). When we look at all of our groups, the ratio is over 1.33. Surprisingly, this rate was high in our control groups. This model, which we developed because we observed fibrosis formation due to the fact that AST was higher in the 2w/intralipid group compared to other groups, shows that this model is similar to other steatohepatite models (16). At the same time, as the increase in AST is a determinant factor in the blood after liver tissue damage, this result confirms the histological data of our study.

When we compare the data obtained in our study with other models, we can say that we created a new model for NAFLD studies by showing the presence of fatty acids in hepatocytes in addition to ballooning, steatohepatitis and fibrosis in our model.

The formation mechanism of NAFLD has been discussed in all studies until today and it has been stated that not all models made reflect the same spectrum in humans. Therefore, we set out to create a new model closest to the human NAFLD. As a matter of fact, in our study, when we look at the general parameters in this model, we observed that NAFLD was formed by administering oil directly from the portal vein as well as parenteral models. With this new model, we think that may be opened a new door in NAFLD formation mechanism studies.

We recommend that researchers focus on the development of liver carcinoma, the last step of NAFLD. Since cardiovascular diseases are seen in people with NAFLD, it is necessary to determine the presence of cardiovascular diseases in the lubrication performed with this model. At the same time, other studies in which our model will be used can be obtained from different parameters and molecular evaluation of NAFLD to provide new information

about the mechanism of this disease. With this information can help in the development of drug therapy.

Conflicts of interest: None

REFERENCES

[1] Michael C. Kew. Obesity as a cause of hepatocellular carcinoma. Ann Hepatol. 2015; 14 (3):299-303.

[2] Ooi GJ, PaBurton PR, Doyle L, Wentworth JM, Bhathal PS, Sikaris K, Cowley MA, Roberts SK, Kemp W, O'Brien PE, Brown WA. Modified thresholds for fibrosis risk scores in nonalcoholic fatty liver disease are necessary in the obese. Obes Surg. 2017; 27: 115-125.

[3] Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014; 20(42): 15539–15548.

[4] Takahashi Y, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2012; 18(19): 2300–2308.

[5] Botham KM, Napolitano M. Bravo E. The emerging role of disturbed CoQ metabolism in nonalcoholic fatty liver disease development and progression. Nutrients 2015;7(12):9834-9846.

[6] Schattenberg JM, Galle PR. Animal models of non-alcoholic steatohepatitis of mice and man. Dig Dis. 2010; 28 (1): 247-254.

[7] Hebbard L, George J. Animal models of nonalcoholic fatty liver disease Nat. Rev. Gastroenterol. Hepatol. 2011; 8:35.

[8] Ritsma L, Steller EJA, Ellenbroek, SIJ, Kranenburg O, Borel Rinkes IHNM, Rheenen JV. Surgical implantation of an abdominal imaging window for intravital microscopy. Nat Protoc. 2013; 8 (3): 583-594.

[9] Gervaz P, Scholl B, Gillet M. Permanent access tothe portal vein in rats: experimental model. European Surgical Research 2000; 32: 203-206.

[10] Meisel JA, Le HD, Meijer VE, Nose V, Gura KM, Mulkern RV, Sharif MRA, Puder M. Comparison of 5 intravenous lipid emulsions and their effects on hepatic steatosis in a murine model. Journal of Pediatric Surgery 2011; 46: 666-673.

[11] Kalish BT, Le HD, Gura KM, Bistrian BR, Puder M. A metabolic analysis of two intravenous lipid emulsions in an murine model. Plos one 2013; 8(4): e59653.

[12] Altunkaynak BZ, Ozbek E. Overweight and structural alterations of the liver in female rats fed a high-fat diet: A stereological and histological study. Turk J Gastroenterol. 2009; 20(2): 93-103.

[13] Bedi KS, Thomas YM, Davies CA, Dobbing J. Synapse-to-neuron ratios of the frontal and cerebellar cortex of 30-day-old and adult rats undernourished during early postnatal life. J Comp Neurol. 1980; 193:49–56.

[14] Imajo K, Yoneda M, Kessoku T, Ogawa Y, Maeda S, Sumida Y, Hyogo H, Eguchi Y, Wada K, Nakajima A. Rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Int J Mol Sci. 2013; 14 (11); 21833.

[15] Brunt EM. Nonalcoholic fatty liver disease: what the pathologist can tell the clinician. Dig Dis. 2012; 1: 61-68.

[16] Shih TH, Huang CE, Lee YE, Chen CL, Wang CH, Huang CJ, Cheng KW, Wu SC, Juang SE, Jawan B, Yang SC. Preoperative portal vein velocity/size and effect on living donor liver transplantation recipient hemodynamics during inferior vena cava clamping. Transplant Proc. 2016; 48(4): 1049-1051.

[17] Noorafshan A, Esmail-Zadeh B, Bahmanpour S, Poost-Pasand A. Early stereological changes in liver of Sprague-Dawley rats after streptozotocin injection. Indian J Gastroenterol. 2005; 24: 104-107.

[18] Yahyazedeh A, Altunkaynak BZ, Akgül N, Akgül HM. A histopathological and stereological study of liver damage in female rats caused by mercury vapor. Biotech Histochem. 2017; 92(5): 338-346.

[19] Brancatelli G, Furlan A, Calandra A, Dioguardi Burgio M. Hepatic sinusoidal dilatation. Abdom Radiol (NY). 2018 doi: 10.1007/s00261.018.1465-1468.

[20] Sakamoto M, Tsujikawa H, Effendi K, Ojima H, Harada K, Zen Y, Kondo F, Nakano M, Kage M, Sumida Y, Hashimoto E, Yamada G, Okanoue T, Koike K. Pathological findings of nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. Pathol Int. 2017; 67 (1): 1-7.

[21] Fan JG, Qiao L. Commonly used animal models of non-alcoholic steatohepatitis. Hepatobiliary Pancreat Dis Int. 2009; 8 (3): 233-240.

[22] Lu XY, Xi T, Lau WY, Dong H, Xian ZH, Hua Yu, Zhu Z, Shen F, Wu MC, Cong WM. Pathobiological features of small hepatocellular carcinoma: Correlation between tumor size and biological behavior. J Cancer Res Clin Oncol. 2011;137 (4): 567-575.

[23] Leung C, Herath CB, Jia Z, Goodwin M, Mak KY, Watt MJ. Dietary glycotoxins exacerbate progression of experimental fatty liver disease. J Hepatol. 2014; 60: 832-838

[24] Choi YJ, Lee CH, Lee KY, Jung SH, Lee BH. Increased hepatic fatty acid uptake and esterification contribute to tetracycline-induced steatosis in mice. Toxicol Sci.2015; 145(2): 273-282.

[25] Hirooka M, Koizumi Y, Miyake T, Ochi H, Tokumoto Y, Tada F, Matsuura B, Abe M, Hiasa Y. Nonalcoholic fatty liver disease: portal hypertension due to outflow block in patients without cirrhosis. Radiology 2015; 274(2): 597-604.

[26] Seifalian AM, Piasecki C, Agarwal A, Davidson BR. The effect of graded steatosis on flow in the hepatic parenchymal microcirculation. Transplantation 1999; 68(6): 780-784.

[27] Pereira ENGDS, Silvares RR, Flores EEI, Rodrigues KL, Ramos IP, Silva IJ, Machado MP, Miranda RA, Pazos-Moura CC, Gonçalves-de-Albuquerque CF, Faria-Neto HCC, Tibiriça E, Daliry A. Hepatic microvascular dysfunction and increased advanced glycation end products are components of non-alcoholic fatty liver disease. PLoS One 2017;12(6): e0179654.

[28] McCuskey RS, Ito Y, Robertson GR, McCuskey MK, Perry M, Farrell GC. Hepatic microvascular dysfunction during evolution of dietary steatohepatitis in mice. Hepatology 2004;40(2):386-393.

[29] Oh MK, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2008; 28: 503–522.

[30] Çolak Y, Tuncer I. Nonalcoholic liver diseases and steatohepatitis. J Ist Faculty Med 2010; 73:3.

How to cite this article: Nalbant A, Sakul BU, Yucel F. Investigation of Changes in Liver Microanatomy in the Steatosis Model Created by Permanent Canula in Rats. Clin Exp Health Sci 2022; 12: 659-664. DOI: 10.33808/clinexphealthsci.948391

Clinical and Experimental Health Sciences

Evaluation of the Relationship of Fatigue, Anxiety and Depression Levels in Individuals with the Precautions Taken in the COVID-19 Pandemic Process

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Received: 18.10.2021 **Accepted:** 04.04.2022

ABSTRACT

Objective: This study aims to evaluate the effects of the COVID-19 process and the measures taken on fatigue, anxiety and depression levels in individuals, and the factors that may cause this effect.

Methods: The study is of cross-sectional type. A total of 281 participants who applied to the pandemic outpatient clinic were included in the study. Data collection form with 27 questions and Hospital Anxiety and Depression Scale (HADS) with 14 questions were used in the study. Relationships between data were evaluated with t-test and chi-square test in independent groups. The importance levels of the factors affecting the anxiety and depression scores were determined by the Chaid Analysis. Statistically, cases with p<.05 were considered significant.

Results: A total of 281 participants, with a mean age of 40.09±12.35 years and 56.2% women, were included in the study. The mean depression score of the participants in HADS was 7.43±3.85. The mean anxiety score was 8.11±4.04. 61.6% of the participants had depression and 31.0% had anxiety. According to Chaid's analysis, the most effective factor on anxiety and depression scores was that the fear of contracting the COVID-19 infection exhausted the individual.

Conclusion: As a result of the research, it was found that the frequency of depression and anxiety was high in patients who applied to the pandemic outpatient clinic. It was determined that more than half of the participants were afraid of catching an infection and this fear caused fatigue in them. Mental health should not be ignored during the pandemic period and both physical and mental health of people should be protected with holistic approaches.

Keywords: Anxiety, depression, fatigue, Hospital Anxiety and Depression Scale, prevention.

1. INTRODUCTION

On 31 December 2019, the World Health Organization (WHO) China Country Office reported cases of pneumonia of unknown etiology in Wuhan, China (1,2). On January 7, 2020, the causative agent of these pneumonia cases was identified as a new coronavirus (2019-nCoV) that has not been detected in humans before. Later, the name of the 2019-nCoV disease was accepted as Coronavirus disease-19 (COVID-19) (3). COVID-19 infection, whose main clinical symptoms include fever, weakness, myalgia, dry cough and shortness of breath, was defined as a global epidemic (pandemic) by the World Health Organization on March 11, since it was seen in 113 countries outside of China, where the epidemic started (3). The first COVID-19 case in Turkey was seen on 11 March 2020 (4). In the process since the first case was detected, the main country strategy regarding the epidemic has been to reduce the incidence of cases and slow the rise in the epidemic curve with public health measures (4).

The COVID-19 pandemic affects societies not only in the field of health, but also in the economic and psychosocial field. In the face of such a major global epidemic, many people worry about the health of themselves and their families, and may face difficulties related to work or financial issues (5). Not knowing when the epidemic will end, fear of catching infection, lack of a definitive treatment for the infection, exposure to the ever-changing flow of information about the pandemic and COVID-19 infection, weakening of interpersonal relations due to social isolation adversely affect the mental health of the society. These effects may worsen the diagnosed psychiatric disorder or pre-existing psychiatric disorders (6). Studies show that the frequency of disorders such as anxiety, depression, fear, stress and sleep problems increases during the COVID-19 epidemic (6-9).

Psychosocial factors play an important role during epidemics. For example, societies' attitudes towards masks and social distance, their perspective on vaccination have important effects on the spread of infection or its rapid control (10). At the beginning of the pandemic, the diagnosis, treatment and prevention of COVID-19 infection were focused on and intensive studies were carried out in these areas. With the prolongation of the pandemic period, attention was drawn

DOI: 10.33808/clinexphealthsci.1011262

to mental health and studies on the effects of the pandemic on human psychology increased (11).

Fatigue, one of the known symptoms of COVID-19, is a common complaint in the general population, as well as a common symptom of various physical and psychiatric disorders. Studies have shown that fatigue is affected by many factors, including age, gender, physical condition, psychological conditions, and personality traits (12). Rules such as physical and social isolation, constant use of masks, and paying more attention to hygiene rules than usual, which entered our lives with the COVID-19 pandemic, can cause fatigue (13,14).

This study aims to determine the effects of knowledge, attitudes, behaviors and precautions regarding COVID-19 on the fatigue, anxiety and depression levels felt in individuals who applied to the pandemic outpatient clinic. It also aims to determine the risks and protective factors related to felt fatigue, anxiety and depression levels.

2. METHODS

2.1. Study Type

The study is a cross-sectional designed epidemiological study.

2.2. Ethical Approval

The Turkish Ministry of Health, General Directorate of Health Services approved the study protocol (Approval Date/Number: 22.01.2021-01-22-T18_28_33). In addition, permission was obtained from the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine (Date: 5 February 2021, No: 2021/3068), and the hospital's chief physician. Before starting the study, all participants were informed about the details of the study and their verbal consent was obtained.

2.3. Sample Size

The population of the study consisted of patients who applied to the pandemic outpatient clinic of Necmettin Erbakan University Meram Medical Faculty Hospital. Based on the-chi-square test in the G Power program, the sample size was calculated to be at least 207 with 0.05 type 1 error, 95% power, medium effect size (0.3), and maximum degree of freedom of 4. Data from the study sample were obtained by simple random sampling method.

2.3. Data Collection Tools

After the literature review, a data collection form was prepared for the research. The data collection form consists of 41 questions and 3 parts. The first part of the form, consisting of 9 questions, is the personal information form created to obtain the demographic information of the participants. The form includes questions about age, gender, educational status, marital status, occupation, income status and presence of chronic disease.

The second part consists of 18 questions. In this section, sources of information about the pandemic, feeling tired against the measures taken during the pandemic process, and being afraid of contracting COVID-19 were questioned. In this section, information questions about the pandemic were also given and the participants were asked to answer these questions.

The second part, consisting of 14 questions, questions the measures taken during the COVID-19 pandemic and the effects of these measures on individuals. The third part with 14 questions includes the Hospital Anxiety and Depression Scale (HADS). HADS is a self-administered scale. It was first developed by Zigmond and Snalth (15), and its Turkish validity and reliability study was performed by Aydemir et al. (16). The scale consists of 14 questions, 7 of which measure anxiety and 7 of which measure depression. For Turkish people, the anxiety cut-off score was 10 and the depression cut-off score was 7. The minimum score that can be obtained from both the anxiety and depression subscales of the scale is 0, and the maximum score is 21 (16).

2.4. Procedure

Study data were collected after ethical approval was obtained. The study was conducted on male and female patients who applied to the pandemic outpatient clinic between 01.03.2021 and 15.04.2021 and gave verbal consent to participate in the study, and 281 people were included. Participants who did not give verbal consent to participate, had mental disorders that would prevent communication, and had deficiencies in the data collection form were not included in the study. Data collection forms were filled by the participants in accordance with the pandemic rules. Each form was completed in an average of 15 minutes.

2.5. Statistical Analysis

Data entry and statistical analysis were performed using the SPSS for Windows version 18.0 (SPSS Inc. Chicago, IL, USA) package program. In summarizing numerical data; arithmetic mean±standard deviation and median (1st quarter-3rd quarter) values were used. The numbers and percentages were used to summarize categorical data. In the evaluation of numerical data conforming to the normal distribution, independent-samples t-test was used. The relationship between the anxiety and depression scores of the participants was evaluated with Pearson Correlation analysis. One-way Anova test was used to determine whether the anxiety and depression levels of the participants differed according to the information sources. Post hoc LSD test was performed to determine from which group the significant difference originated. The importance levels of the factors affecting the anxiety and depression scores of the participants were determined by the Chaid Analysis. The distribution

of categorical data was evaluated with the chi-square test. Statistically, cases with p<.05 were considered significant.

3. RESULTS

3.1. Sociodemographic Characteristics of the Participants

A total of 281 people, with a mean age of 40.09±12.35 years and 56.2% women were included in the study. Sociodemographic characteristics of the patients are presented in Table 1.

Table 1: *Sociodemographic characteristics of the participants.*

Demographic Variables		n	%
Gender	Female	158	56.2
	Male	123	43.8
Marital Status	Married	213	75.8
	Single	68	24.2
Educational Status	Middle School and Below	83	29.5
	High School and Above	198	70.5
Job	Officer	64	22.8
	Employee/Small Business	69	24.6
	Not Working	87	31.0
	Other	61	21.7
Family Structure	Nuclear Family	225	80.1
	Extended Family	34	12.1
	Alone	22	7.8
Living Place	Provincial Center	211	75.1
	Town/Village	70	24.9
Chronic Disease	No	188	66.9
	Yes	93	33.1
Change In Income During The Pandemic Period	Decreased	119	42.3
	Unchanged/Increased	162	57.7

The patients included in the study received the most information about the precautions to be taken against COVID-19 infection from television/newspaper (n=124; 44.1%), social media/internet (n=109; 38.8%) and health personnel (n=37; 13.2%) and other people (n=11; 3.9%). A total 205 (73.0%) participants stated that the measures taken against transmission routes (such as mask, distance, disinfection) during the COVID-19 pandemic caused physical, social or psychological fatigue. Of the patients who applied to the pandemic outpatient clinic, the rate of those who stated that they were afraid of contracting COVID-19 infection was 82.6% (n=232). Of these 232 patients who stated that they were afraid, 61.2% (n=172) stated that this fear tired out them, and 48.0% (n=135) believed that this fear and fatigue would be overcome by vaccination.

3.2. Prevalence of Depression and Anxiety of Participants

According to the HAD scale, 61.6% of the participants had depression and 31.0% had anxiety. The mean depression score of 281 participants from HADS was 7.43±3.85; the mean anxiety score was 8.11±4.04. There was a statistically significant positive and high correlation between the anxiety and depression scores of the participants (r=0.611, p<.001).

3.3. Investigation of Depression-Related Characteristics of Participants

When the characteristics of the patients that may be related to depression were investigated, we determined that the depression scores of those living in rural areas were higher than those living in the urban (Table 2).

Table 2: *Factors that may affect participants' tendency to depression.*

Features		Depression score					
		<7 points		≥7 points			
		n	%	n	%	$\chi 2$	p
Living place	Provincial Center	91	43.1	120	56.9	**7.886**	**.005**
	Town/Village	17	24.3	53	75.7*		
Change In Income During The Pandemic Period	Decreased	39	32.8	80	67.2	2.796	.095
	Unchanged/ Increased	69	42.6	93	57.4		
Did the measures taken during the pandemic period cause physical or psychological fatigue in you?	Yes	64	31.2	141	68.8*	**16.673**	**<.001**
	No	44	57.9	32	42.1		
Are you afraid of contracting COVID-19 infection?	Yes	77	33.2	155	66.8*	**15.465**	**<.001**
	No	31	63.3	18	36.7		
Does the fear of getting sick tire you? (n=232)	Yes	46	26.7	126	73.3*	**12.460**	**<.001**
	No	31	51.7	29	48.3		
Can the fear of getting sick go away with the vaccine? (n=232)	Yes	53	39.3	82	60.7	**5.364**	**.021**
	No	24	24.7	73	75.3*		
I support the curfew restrictions.	Yes	86	38.2	139	61.8	0.021	.884
	No	22	39.3	34	60.7		
When I come into contact with someone outside, I remember the COVID-19 disease.	Yes	93	36.6	161	63.4	3.700	.054
	No	15	55.6	12	44.4		
I apply personal hygiene measures after contact.	Yes	103	38.3	166	61.7	0.055	.518
	No	5	41.7	7	58.3		
I clean the products bought after shopping with soap or disinfectant.	Yes	40	28.0	103	72.0*	**13.469**	**<.001**
	No	68	49.3	70	50.7		
If I go to the markets, I pay attention to the time I spend there.	Yes	88	36.1	156	63.9*	**4.394**	**.036**
	No	20	54.1	17	45.9		
If I catch COVID-19, I think I will recover quickly with effective treatment.	Yes	84	42.4	114	57.6	**4.510**	**.034**
	No	24	28.9	59	71.1*		
I think the mask rule is exaggerated.	Yes	16	40.0	24	60.0	0.048	.826
	No	92	38.2	149	61.8		
I am tired of washing my hands frequently due to the pandemic.	Yes	23	25.3	68	74.7*	**9.849**	**.002**
	No	85	44.7	105	55.3		
I am tired of using masks due to the pandemic.	Yes	29	28.2	74	71.8*	**7.260**	**.007**
	No	79	44.4	99	55.6		
Do you see physical distancing as isolation?	Yes	89	37.7	147	62.3	0.325	.569
	No	19	42.2	26	57.8		

* indicates the group from which the statistical difference originates.

There was no statistically significant difference between depression and other variables such as gender, age, marital status, educational status, occupation, family structure, presence of chronic disease, and sources of information about the disease.

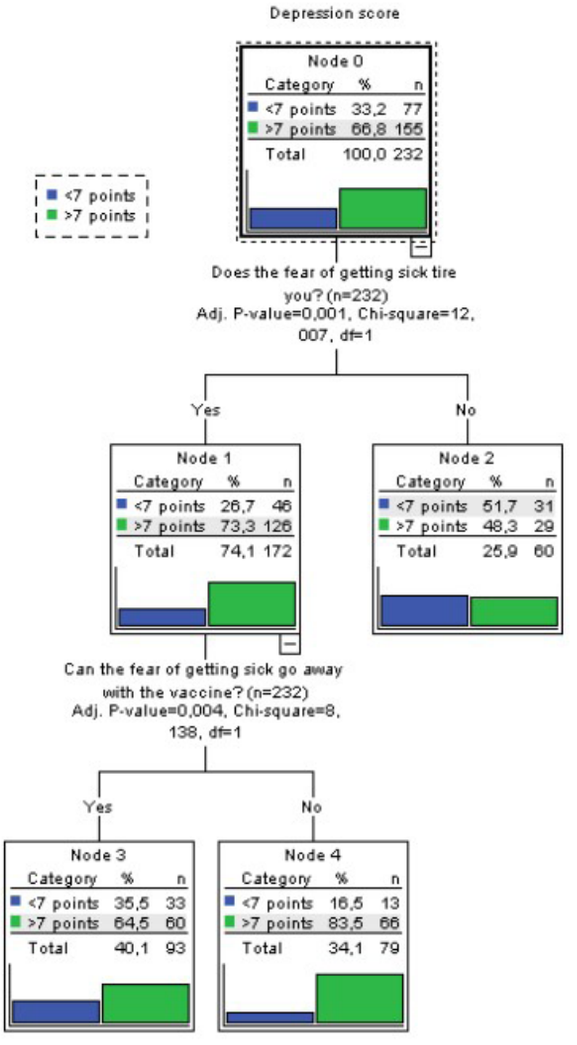

Figure 1. *The results of the Chaid analysis of the factors affecting the depression score.*

The independent variables affecting the depression score as a result of the Chaid analysis are given in Figure 1. According to the Chaid analysis, 61.6% of the participants had a depression score of 7 and above. We found that the most important factor on the depression score of the participants included in the study was that the fear of catching the COVID-19 infection exhausted the participants. While the depression score of 73.3% of those who thought that the fear of COVID-19 infection made them tired was 7 and above, the depression score of 56.9% of those who thought that the infection did not tire them was below 7. Among the participants who stated that the fear of catching the COVID-19 infection exhausted them, 83.5% of those thought that this fear would not be cured by vaccination had a depression score of 7 and above, while this rate was 64.5% of those who thought that they would be cured by vaccination.

3.4. Investigation of Anxiety-Related Characteristics of Participants

When the anxiety-related characteristics of the participants were investigated, it was observed that the mean age of those with high anxiety scores (36.68±11.59) was statistically significantly lower than the mean age of those with low anxiety scores (41.61±12.40) (t=3.141, p=.002). In addition, it was determined that the anxiety scores of the participants whose education level was secondary school or below were significantly lower than those whose education level was high school and above (Table 3).

A statistically significant difference was found between anxiety scores according to information sources about COVID-19 infection (F=7.195, p<.001). The difference is that the anxiety score of those who get information from television or newspaper ($\bar{x}$=7.22) is lower than those who get information from social media ($\bar{x}$=9.18) and other people ($\bar{x}$=10.72), and those who get information from social media ($\bar{x}$=9.18) have a lower anxiety score than those who get information from social media ($\bar{x}$=9.18). It was due to the fact that the employees ($\bar{x}$=7.16) were higher than those who had information. There was no statistically significant difference between anxiety and other variables such as gender, marital status, place of residence, education level, occupation, family structure and presence of chronic disease (p>.05).

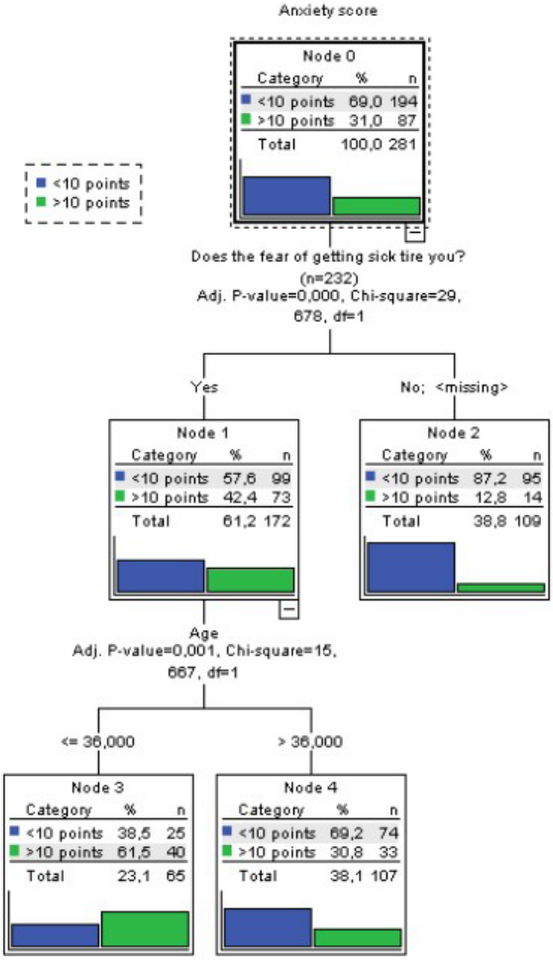

Figure 2. *The result of the Chaid analysis of the factors affecting the anxiety score.*

The result of the Chaid analysis of the independent variables affecting the anxiety score is given in Figure 2. According to the Chaid analysis, 69.0% of the participants had an anxiety score below 10, and 31.0% had an anxiety score of 10 and above. In the study, it is observed that the most important factor on the anxiety score is the fear of contracting the COVID-19 infection. While 42.4% of those who thought they had a fear of contracting COVID-19 infection had an anxiety score of 10 and above, 12.8% of those who thought they were not tired had an anxiety score of 10 and above. Of the participants who stated that the fear of contracting the COVID-19 infection exhausted them, 61.5% of those aged 36 and under had an anxiety score of 10 and above, while only 30.8% of those younger than 36 had an anxiety score of 10 and above.

4. DISCUSSION

It is inevitable that the COVID-19 pandemic, which affects large masses and causes more than 5 million deaths, will have psychological effects on almost all individuals. In this study, we aimed to evaluate the effects of the COVID-19 process and the measures taken on the fatigue, anxiety and depression levels in individuals, and the factors that may cause this effect. Approximately three-quarters of the 281 people who applied to the pandemic outpatient clinic and were included in the study stated that they felt physically, socially or psychologically tired against the measures taken due to the COVID-19 pandemic. In addition, 82.6% of the participants were afraid of getting an infection. In the study conducted by Morgül et al. in Istanbul with 3672 participants, it was found that 64.1% of the participants had psychological fatigue (5). In this study, it is thought that the reason for the high level of fatigue is that the study was conducted only on patients who applied to the pandemic outpatient clinic, fatigue was a subjective perception and patients were left to their own statements.

At the end of the study, the prevalence of depression was found to be 61.6% and the prevalence of anxiety was 31.0% in patients who applied to the pandemic outpatient clinic. In various studies conducted during the pandemic period, the lowest prevalence of depression was found to be between 19.0% and 57.4% (17-21). During the pandemic period worldwide, the prevalence of anxiety was found to be the lowest 14.0% and the highest 45.1% (17-21). According to the global health estimates published by WHO in 2017, it was estimated that the global population diagnosed with depression and anxiety levels were 4.4% and 3.6% in 2015, respectively (22). These results indicated that the rates are higher than the general population (22) both in this study and in other studies conducted during the pandemic period. This shows that the pandemic period has significant effects on people's mental health. Depression and anxiety occur with a complex interaction of social, psychological and biological factors. People with adverse living conditions (unemployment, age, psychological trauma) are more likely to suffer from psychiatric disorders (23). Increasing unemployment during the pandemic period, losing relatives due to infection, social isolation, fear of death, etc. The increase in conditions that predispose to mental diseases can be associated with the increase in the prevalence of these diseases.

In a study conducted in China, the prevalence of depression in urban residents was found to be lower than in rural areas (24). Similar results were obtained in our study. It was thought that the reason for this was that people experienced and accepted the COVID-19 pandemic faster due to the high risk of disease and the number of cases in the city center.

According to research by Naragon and Wu, a moderate level of anxiety, which is a response to acute stress, is seen as beneficial and cause more attention to COVID-19 precautions (25,26). Similarly, in this study, participants unafraid of contracting COVID-19 infection have a statistically significant and lower anxiety score compared to those who are afraid of getting caught. A similar significant and lower anxiety score was observed when those who do not pay attention to the time they spend in the markets, participant think that the mask rule is exaggerated compared to those who do not think and those who are not tired of washing their hands frequently.

Similar to the literature, among the patients included in the study; the anxiety scores of those with secondary education and below were statistically significant and lower than those with high school or higher education (27). It was thought that this was due to the fact that individuals with a high level of education paid more attention to the scientific and academic warnings published about the COVID-19 pandemic and saw the COVID-19 infection as a more serious problem.

In the study, the most important independent variables on depression were the fear of contracting COVID-19 infection, tiring individuals, and the thought of whether this fear could be cured by vaccination. On anxiety, these variables were the fatigue of individuals by fear of contracting COVID-19 infection and age. In a study investigating the prevalence of anxiety and depression during the COVID-19 pandemic in Pakistan, it was reported that the prevalence of anxiety is high under the age of 35 (19). Similarly, studies have found that fear of being infected is associated with anxiety and depression (17,18). The first time individuals encounter a situation that threatens public health, such as a pandemic, increases the fear of contracting the disease. The inability to find a definitive treatment for COVID-19 infection, its different course in each individual, and the inability to predict the future make this fear permanent and have an impact on mental health.

Our study had some limitations. First, due to the cross-sectional design of the study, the long-term causal relationships between various factors associated with anxiety and depression could not be evaluated. Second, our study findings may not be generalizable to the entire urban population of the country, as this study was conducted in a specific hospital and a specific outpatient clinic. Third,

HADS, which is used to evaluate the prevalence of anxiety and depression, is a self-administered screening tool and is not used for diagnostic purposes. Therefore, studies using diagnostic tools such as the Structured and Clinical Interview for DSM or the Mini International Neuropsychiatric Interview are recommended to confirm our findings. Despite the above limitations, this study provides important data to contribute to the literature on the prevalence of anxiety and depression during the COVID-19 pandemic and the factors that may be associated with it.

5. CONCLUSION

As a result of the research, it was found that the frequency of depression and anxiety was high in patients who applied to the pandemic outpatient clinic. The prevalence of depression was 61.6% and the prevalence of anxiety was 31.0% in 281 people included in the study. The most important variable that affected the anxiety and depression of the participants was that the fear of contracting COVID-19 exhausted them. Giving detailed, up-to-date and accurate health information to individuals with fear and the feeling of fatigue due to it, anxiety and depression prevalence, which is higher than the literature, and explaining the scope and limitations of the measures taken for protection (hand washing, mask, distance) can reduce the depression and anxiety levels of the pandemic on people. Controlling these levels is important in terms of taking individual precautions and awareness of the control measures implemented throughout the country. Vaccination, which is the most important method in ending the pandemic process, may be one of the most important factors in reducing the level of depression in humans. As a result, a holistic approach to the pandemic should be provided, and both the mental and physical health of the people should be taken into account.

Funding: The author(s) received no financial support for the research, authorship and/or publication of this article.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] World Health Organization. (2020 b, March 11). WHO Director General's opening remarks at the media briefing on COVID-19. Accessed [10 August 2021]. https https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—-11-march-2020

[2] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet 2020;395(10223):497-506.

[3] World Health Organization. (2020 a). Corona virus disease (COVID-2019): Situation report-138. Accessed [10 August 2021]. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200606-covid-19-sitrep-138.pdf?sfvrsn=c8abfb17_4

[4] T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. COVID-19 genel bilgiler, epidemiyoloji ve tanı rehberi. 2020. (Turkish)

[5] Morgul E, Bener A, Atak M, Akyel S, Aktaş S, Bhugra D, Ventriglio A, Jordan R. COVID-19 pandemic and psychological fatigue in Turkey. The International Journal of Social psychiatry. 2020;67(2):128-135.

[6] Pierce M, Hope H, Ford T, Hatch S, Hotopf M, John A, Kontopantelis E, Wessely S, Manus S, Mabel K. Mental health before and during the COVID-19 pandemic: A longitudinal probability sample survey of the UK population. The Lancet Psychiatry 2020;7(10):883-892.

[7] Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. The outbreak of COVID-19 coronavirus and its impact on global mental health. International Journal of Social Psychiatry 2020;66(4):317-320.

[8] Chen S, Bonanno GA. Psychological adjustment during the global outbreak of COVID-19: A resilience perspective. Psychological Trauma: Theory, Research, Practice, and Policy. 2020;12(1):51-54.

[9] Gıca Ş, Akkubak Y, Aksoy ZK, Küçük A, Cüre E. Effects of the COVID-19 pandemic on psychology and disease activity in patients with ankylosing spondylitis and rheumatoid arthritis. Turkish Journal of Medical Sciences 2021;51(4):1631-1639.

[10] Choi J. The psychology of pandemics: Preparing for the next global outbreak of infectious disease. Newcastle upon Tyne, UK: Cambridge Scholars Publishing. Asian Communication Research 2020;17(2):98-103.

[11] Mamun MA, Griffiths MD. First COVID-19 suicide case in Bangladesh due to fear of COVID-19 and xenophobia: Possible suicide prevention strategies. Asian Journal of Psychiatry 2020;51:102073.

[12] Finsterer J, Mahjoub SZ. Fatigue in healthy and diseased individuals. American Journal of Hospice and Palliative Medicine 2014;31(5):562-575.

[13] Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, Wu j, Du H, Chen T, Li R, Tan H, Kang L, Yao L, Huang M, Wang H, Wang G, Liu Z, Hu S. Factors associated with mental health outcomes among health care workers exposed to coronavirus disease 2019. JAMA 2020;3(3):1-12.

[14] Taylor S. The psychology of pandemics: Preparing for the next global outbreak of infectious disease. Cambridge Scholars Publishing.2019.

[15] Zigmond AS, Sanlth RP. The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica 1983;67:361-370.

[16] Aydemir Ö, Güvenir T, Küey L, Kültür S. Hastane Anksiyete ve Depresyon Ölçeği Türkçe formunun geçerlilik ve güvenilirlik çalışması. Türk Psikiyatri Dergisi 1997;8:280-287. (Turkish)

[17] Wang C, Pan R, Wan X, Tan Y, Xu L, Ho CR. Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. International Journal of Environmental Research and Public Health 2020;17(5):1729.

[18] Choi EPH, Hui BPH, Wan EYF. Depression and anxiety in Hong Kong during COVID-19. International Journal of Environmental Research and Public Health 2020;17(10):3740.

[19] Amin F, Sharif S, Saeed R, Durrani N, Jilani D. COVID-19 pandemic-knowledge, perception, anxiety and depression among frontline doctors of Pakistan. BMC Psychiatry 2020;20(1):1-9.

[20] Solomou I, Constantinidou F. Prevalence and predictors of anxiety and depression symptoms during the COVID-19 pandemic and compliance with precautionary measures: Age and sex matter. International Journal of Environmental Research and Public Health 2020;17(14):4924.

[21] Özdin S, Bayrak Özdin Ş. Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: The importance of gender. International Journal of Social Psychiatry 2020;66(5):504-511.

[22] World Health Organization. Global Health Estimates. Depression and Other Common Mental Disorders. (2017). Accessed [10 August 2021]. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf

[23] World Health Organization. Depression. (2020). Accessed [10 August 2021]. https://www.who.int/news-room/fact-sheets/detail/depression

[24] Zhang J, Zhu L, Li S, Huang J, Ye Z, Wei Q, Du C. Rural–urban disparities in knowledge, behaviors, and mental health during COVID-19 pandemic: A community-based cross-sectional survey. Medicine 2021;100(13).

[25] Naragon-Gainey K, McMahon TP, Chacko TP. The structure of common emotion regulation strategies: A meta-analytic examination. Psychological Bulletin 2017;143(4):384.

[26] Wu DY, Guo M, Gao YS, Kang YH, Guo JC, Jiang XL, Chen F, Liu T. Clinical effects of comprehensive therapy of early psychological intervention and rehabilitation training on neurological rehabilitation of patients with acute stroke. Asian Pacific Journal of Tropical Medicine 2012;5(11):914-916.

[27] Moghanibashi-Mansourieh A. Assessing the anxiety level of Iranian general population during COVID-19 outbreak. Asian Journal of Psychiatry 2020;51:102076.

How to cite this article: Kucukkendirci H, Yucel M, Durduran Y. Evaluation of the Relationship of Fatigue, Anxiety and Depression Levels in Individuals with the Precautions Taken in the COVID-19 Pandemic Process. Clin Exp Health Sci 2022; 12: 665-671. DOI: 10.33808/clinexphealthsci.1011262

Clinical and Experimental Health Sciences

Comparison of the Effect of Denture Cleansers on Long-Term Water Sorption and Solubility of Polyetheretherketone with other Denture Base Materials

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Received: 19.10.2021 **Accepted:** 05.04.2022

ABSTRACT

Objective: In this in vitro study, the effect of three denture cleansers (DCs) after immersion in a chemical solution applied to polyetheretherketone (PEEK) and other denture base materials (DBMs) on long-term water sorption and solubility was compared.

Methods: Disk-shaped specimens (50±1.0-mm diameter and 0.5±0.1-mm thickness) were prepared from four DBMs (n=48). All specimens were randomly subdivided into four storage media groups (n=12): Corega tablet (CT), Protefix tablet (PT), 1% sodium hypochlorite (NaOCl) solution (SH), and control (distilled water, DW). Storage media were renewed thrice a day for 120 days and simulated for 1-year use of overnight immersion. Water sorption and solubility (μg/mm^3) of DBMs before and after immersion in storage media were examined and obtained data were statistically analyzed using a multivariate analysis of variance, followed by multiple comparisons by a posthoc Tukey's test ($p < 0.05$).

Results: From statistical analysis, the effect of different DCs on the water sorption and solubility of DBMs revealed a statistical difference ($p<0.05$). The PEEK group exhibited a statistical difference in mean water sorption values among all cleanser groups ($p<0.05$). For the PEEK group, a statistical difference was observed in the DW group among SH and CT groups in terms of the mean solubility values ($p<0.05$), while a statistically significant difference was not observed in the PT group among SH and CT groups ($p> 0.05$).

Conclusion: DCs affect PEEK and other DBMs in terms of water sorption and solubility in the long-term follow-up.

Keywords: Denture base material, denture cleanser, PEEK, solubility, water sorption

1. INTRODUCTION

Owing to the increase in the life expectancy and number of elderly individuals, implant or tooth-supported removable prostheses are used in dentistry as an alternative to fixed prosthetic restorations (1,2). There is a 13–29% incidence in partial or complete removable prostheses among adults (2). Polymers such as polymethyl methacrylate (PMMA), polyamide, and polyetheretherketone (PEEK) can be used in removable prostheses (3,4). Owing to its low density, aesthetics, cost-effectiveness, and facile manipulation, PMMA has been used in removable prostheses for a long time. However, its water sorption, solubility, impact and bending strength, residual monomer, and polymerization shrinkage still need to be improved (4). On account of these disadvantages, high elasticity polyamides have been widely used due to their high impact strength, reduction of polymerization shrinkage and associated deformation, and absence of residual monomers. However, this material has been reported to exhibit various issues such as water sorption, surface roughness, bacterial contamination, discoloration, and difficulty in polishing (5,6).

Biocompatible metals such as cobalt-chromium or titanium are preferred framework materials in removable prostheses (1). However, even titanium, which is known to be corrosion resistant, can compromise their biocompatibility as it causes galvanic corrosion with the combination of different metals in the oral environment in the case of polymetallism (7-9). In addition to the risks of hypersensitivity and corrosion, other disadvantages of metal-framework removable prostheses include aesthetic problems with a metal appearance, adverse tissue reactions, loss of abutment teeth, and biofilm production. In addition, it is difficult and expensive to produce metal frameworks in removable prostheses by using computer-aided design/computer-aided manufacturing (CAD-CAM) systems (1,9-11).

DOI: 10.33808/clinexphealthsci.1011897

Owing to the disadvantages of metal frameworks in removable prostheses, the development of a high-performance polymer was investigated using metal-free materials, such as PEEK, for application in removable prostheses (1,10). Compared to metal frameworks, polymer-based frameworks exhibit advantages such as better aesthetics (translucency and color), lower cost, higher elasticity, light-weight nature, lower water sorption and solubility, and ease of manufacturing and repair (1,6,9-12). Particularly, polymers exhibit significant advantages in removable prosthetic treatment due to their design control, manufacturing flexibility, and reproducible precision potential with CAD-CAM systems. In addition, mechanical and physical properties of PEEK are similar to those of bone and tooth hard tissues, thereby permitting the more stable and atraumatic production of removable prostheses. In addition, PEEK polymer is resistant to heat and can offer denture disinfection with autoclave (1,3,10). However, additional studies are needed for the application of PEEK in removable prostheses.

It is key to ensure prosthetic hygiene and protect the health of the oral mucosa of implant or tooth-supported removable denture users. Otherwise, oral and systemic infections may develop in these individuals (3,13). Hence, complete or partial removable dentures should be cleaned regularly and efficiently to prevent oral and systemic diseases. Denture cleaning with chemical solutions is an important factor that contributes to the oral health, prosthesis lifespan, and overall quality of patients. Chemical denture cleansers are categorized into different groups according to their chemical compounds such as alkali peroxides, acids, enzymes, and alkali hypochlorites (3,14). Ideally, chemical DCs with different contents in the market should be effective for discoloration and biofilm production without causing the physical and mechanical damage of denture base materials (DBMs) (15). In an ideal case, DBMs should contain insoluble components and exhibit low water sorption. However, these materials may be exposed to saliva, food, water, and cleansers throughout their lifetime, leading to water sorption and loss of other soluble components. In addition, these factors may affect the color stability of DBMs and may not satisfy the aesthetic expectation of patients (16).

DBMs have been evaluated in terms of color stability, surface roughness, hardness, water sorption, and solubility (3,5,12-17). However, these studies that compare the effect of DCs, PEEK, and other DBMs in terms of water sorption and solubility are not available. In only one study, Liebermann et al. (17) have evaluated the effect of different aging regimes/times of sodium chloride, artificial saliva, physiological saliva, and distilled water in the range of 1–180 days on different CAD/CAM polymers based on PEEK, hybrid and nanohybrid composite resins, and PMMA in terms of surface roughness, water sorption, solubility, Martens hardness, and indentation modulus. In this study, storage media did not exhibit any effect on the surface roughness and water sorption, and water sorption significantly increased with the storage period. In addition, PEEK exhibited the lowest water sorption and solubility values.

The aim of this in vitro study was to compare the effect of PEEK and other DBMs on water sorption and solubility after immersion three cleansers storage media. The null hypothesis was that different cleansers will not affect the water sorption and solubility of the DBMs.

2. METHODS

In this study, the effect of three DCs on the water sorption and solubility of PEEK (PK group), injection-molded polyamide (PA group), auto-polymerized (AP group), and heat-polymerized resin PMMA (HP group) was examined (Figure 1). Forty-eight disc-shaped specimens (50±1.0-mm diameter and 0.5±0.1-mm thickness) were prepared from each DBM group(n=48) according to EN ISO 20795 and randomly subdivided into four storage media groups (n=12): Corega tablet (CT), Protefix tablet (PT), 1% sodium hypochlorite (NaOCl) solution (SH), and control (distilled water, DW). Table 1 lists the material type, composition, and manufacturer for the materials and solutions used herein.

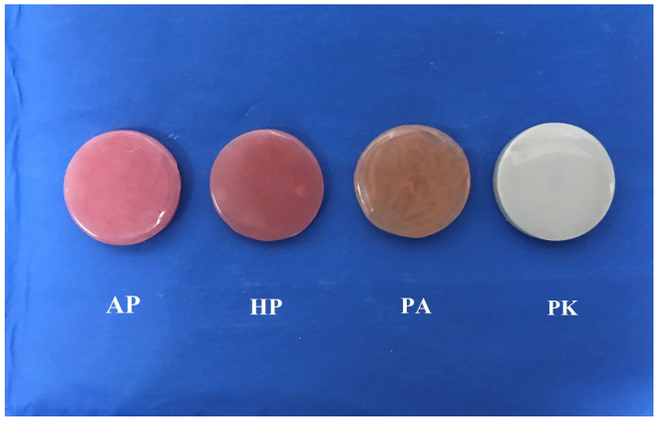

Figure 1. *Denture base material specimens. AP: Auto-polymerized, HP: heat-polymerized resin polymethyl methacrylate (PMMA), PA: Injection-molded polyamide, PK: Polyetheretherketone (PEEK)*

In PA, AP, and HP groups, 144 disc-shaped stainless-steel mold specimens (50±1.0-mm diameter and 0.5±0.1-mm thickness) were prepared by a CNC device (Takisawa Machine Tool Co., Okayama, Japan) for the preparation of DBM specimens. AP and HP group specimens were prepared according to the manufacturer's recommendations. Group HP specimens were polymerized under pressure in a hot water bath at 100°C for 20 min. Group PA specimens were prepared in accordance with the manufacturer's recommendations by using a micro-injection molding system at 280°C for 15 min. After polymerization, the specimens were stored in distilled water at 37°C for 24 h for the elimination of the residual monomer. The excess base resin was trimmed using a tungsten steel bur using a hand piece at a low speed.

PK specimens were designed in the stereolithography (STL) format using AutoCAD software (Autodesk, San Rafael, CA, USA). STL files were transferred to a CAD/CAM milling machine (Ceramill Motion 2, Amann Girrbach AG, Koblach,

Austria), and the specimens were milled from a PEEK dental disk (Juvora Dental Disc; Juvora, London, UK).

All specimens were polished using 600-grit, 800-grit, and 1200-grit waterproof silicon carbide paper by using a polishing device (EcoMet 30; Buehler Ltd., Lake Bluff, IL, USA). They were then polished with a high gloss agent (KMG; Candulor AG, Zurich, Switzerland). A digital caliper (IP54 Digital caliper, SHAN, Columbus, OH, USA) was utilized to ensure a uniform specimen sizes (50-mm diameter and 0.5-mm thickness). The specimens were ultrasonically cleaned for 10 min and dried with a paper towel. All specimens were thermally cycled for 5000 cycles between 5°C and 55°C with a 20-s dwell time and a 10-s transfer time from one bath to another bath (Thermocycler THE 1100; SD Mechatronik Feldkirchen-Westerham, Germany).

After thermal cycling, the same procedure was applied for two effervescent cleanser tablets (Corega and Protefix, respectively) using cleanser solutions prepared according to manufacturer's recommendations by adding one tablet to 200 mL of warm tap water. All specimens were placed in storage environments to simulate 8 h of overnight use. The solutions were renewed thrice a day, and the specimens were washed each time and placed back in the storage media. This procedure was repeated for 120 days to simulate 1 year of use. All experimental processes were performed by the same operator to maintain standardized operations.

Weight measurements were performed using a precision scale (XB 220A; Precisa, Zurich, Switzerland) with an accuracy of 0.1 mg until a constant mass value was obtained on the weight scale screen before immersion and recorded as "m_1" for each specimen. After 120 days of immersion, the specimens were washed with distilled water, gently dried, and the second immersion measurements were performed, and the weight after water sorption were recorded as "m_2." The thickness and diameter of all specimes were measured with a digital caliper with an accuracy of 0.01 mm. All specimens were dried in a desiccator with silica gel at 37°C for 24 h and weighed again (m_3). V is the volume of the specimens in mm^3. The water sorption (W_{sp}) and solubility (W_{sl}) values obtained in $\mu g/mm^3$ were calculated for each specimen by the following formulas:

Water sorption: $(\Delta W_{sp}) = (m_2 - m_1) / V$

Solubility: $(\Delta W_{sl}) = (m_3 - m_1)/V$

2.1. Statistical Analysis

Data were evaluated using a statistical software program (IBM SPSS Statistics, v20.0; IBM Corp). Normality analysis of the data was performed by the Kolmogorov–Smirnov distribution test. Data exhibited a normal distribution. Data for water sorption and solubility values were statistically analyzed using a multivariate analysis of variance, followed by multiple comparisons by a post-hoc Tukey's test. The statistical significance level was set at 0.05.

3. RESULTS

Tables 1 and 3 list the water sorption and solubility values of all DBM specimens in all cleanser baths, respectively (Figure 2). As a result of the two-way analysis of variance performed according to the obtained data, the effect of different DCs on the water sorption and solubility of the DBMs was statistically significant ($p<0.05$). For the AP group, there was a statistically significant difference in water sorption values among DW, CT, and PT groups ($p <0.05$). For the HP group, a statistically significant difference in the mean water sorption values among all cleanser groups was observed ($p<0.05$). For the PA group, a statistically significant difference in the mean water sorption values between all cleanser groups ($p<0.05$) was observed. For the PK group, a statistically significant difference in mean water sorption values between all cleanser groups ($p<0.05$) was observed.

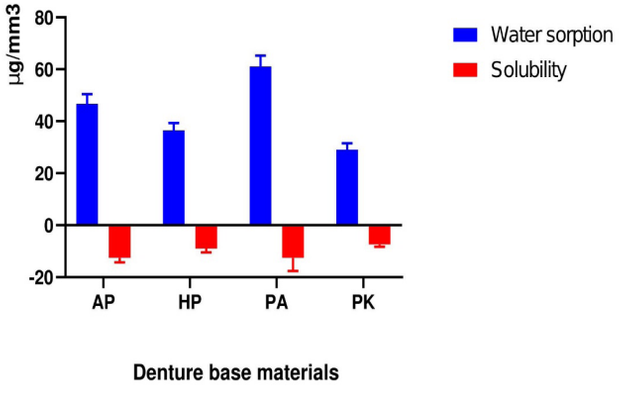

Figure 2. *Mean values of water sorption and solubility of the tested materials in $\mu g/mm^3$.*

AP and PA groups showed higher mean solubility values in cleanser baths. The PK group showed higher mean solubility values than the other DBM groups ($p<0.05$).

Table 1. *Product names, manufacturers, composition properties of test materials, and procedures used in this study.*

Product	Type	Manufacturer
SR Triplex Hot	Heat-polymerized PMMA	Ivoclar Vivadent AG., Schaan, Leichenstein
SR Triplex Cold	Auto-polymerized PMMA	Ivoclar Vivadent AG., Schaan, Leichenstein
Deflex	Injection molded polyamide	Nuxen SRL, Buenos Aires, Argentina
PEEK	Unfilled PEEK CAD/CAM disc	Juvora Dental Disc; Juvora, London, UK
Corega	Potassium Monopersulfate; Sodium Bicarbonate; Sodium Lauryl Sulfoacetate; Sodium Perborate Monohydrate; Sodium Polyphosphate	Stafford-Miller Limited, Waterford, Ireland
Protefix	Sodium bicarbonate, Potassium caroate, Sodium perborate, Citric acid, Sodium laurylsulfate, Aroma	Queisser Pharma, Flensburg, Germany
1% NaOCl	Sodium hypochlorite	Aklar Kimya, Ankara, Turkey

PMMA: Polymethyl methacrylate, PEEK: Polyetheretherketone, CAD/CAM: Computer-aided design/computer-aided manufacturing, NaOCl: Sodium hypochlorite.

Table 2. *Mean water sorption values(μg/mm³) of all specimens in different denture cleansers.*

	Mean ± SD			
Groups	**DW**	**CT**	**PT**	**SH**
AP	26.08±3.12	$57.23±4.72^{A}$	$53.03±4.08^{AB}$	$26.35±3.20^{BC}$
HP	24.74±3.12	$42.58±2.74^{aA}$	$31.70±2.18^{aAB}$	$47.31±3.13^{aABC}$
PA	$63.07±5.19^{ab}$	$85.37±3.93^{abA}$	$43.49±4.51^{abAB}$	$52.62±3.14^{abABC}$
PK	$27.09±1.72^{c}$	$21.60±2.68^{abcA}$	$36.36±2.91^{abcAB}$	$31.23±2.64^{abcABC}$
TOTAL	35.24±16.61	51.70±23.71	41.14±8.82	39.38±11.39

SD: Standart Deviation, AP: Auto-polymerized, HP: heat-polymerized resin polymethyl methacrylate (PMMA), PA: Injection-molded polyamide, PK: Polyetheretherketone (PEEK), CT: Corega tablet, PT: Protefix tablet, SH: Sodium hypochlorite (NaOCl) solution, and DW: Distilled water.

Within the same column or row, the same superscripted letters indicate significant differences (p< 0.05). a:AP, b: HP, and c:PA. Statistically significant differences between denture base material specimens (within the same denture cleanser). A:DW, B: CT, and C:PT. Statistically significant differences between denture cleansers (within the same denture base material specimens).

Table 3. *Mean solubility values (μg/mm³) of all specimens in different denture cleansers.*

	Mean ±SD			
Groups	**DW**	**CT**	**PT**	**SH**
AP	9.40±1.70	$13.15±2.04^{A}$	$16.19±2.25^{AB}$	$9.50±0.97^{BC}$
HP	8.45±1.61	$8.13±1.42^{a}$	$10.73±2.38^{ABa}$	$8.64±0.71^{C}$
PA	$16.59±3.84^{ab}$	$10.99±7.53^{ab}$	$11.64±2.06^{ab}$	$10.91±6.99^{ab}$
PK	$8.41±1.69^{c}$	$6.47±0.83^{Aabc}$	$7.72±1.10^{Babc}$	$6.82±0.66^{Aabc}$

SD: Standart Deviation, AP: Auto-polymerized, HP: heat-polymerized resin polymethyl methacrylate (PMMA), PA: Injection-molded polyamide, PK: Polyetheretherketone (PEEK), CT: Corega tablet, PT: Protefix tablet, SH: Sodium hypochlorite (NaOCl) solution, and DW: Distilled water.

Within the same column or row, the same superscripted letters indicate significant differences (p< 0.05). a:AP, b:HP, and c:PA. Statistically significant differences between denture base material specimens (within the same denture cleanser). A:DW, B:CT, and C:PT. Statistically significant differences between denture cleansers (within the same denture base material specimens).

4. DISCUSSION

In this study, significant differences were observed between DBMs in terms of water sorption and solubility after immersion in three cleanser solutions. The null hypothesis that DCs would have no effect on the water sorption and solubility of DBMs was rejected.

Water sorption and solubility, clinically acceptable values of DBMs are determined by international specifications. The International Organization for Standardization (ISO) specification EN ISO 20795 for DBMs proposes the calculation of water sorption and solubility in units of μg/mm³ according to the volume of the specimens (18). In some studies, water sorption and solubility values were evaluated using the surface area in units of mg/cm² obtained from DBM specimens in specification no.12 in accordance with the American Dental Association (ADA) (19). In another method suggested by Kazanji and Watkinson (20), it is beneficial to determine the long-term water sorption and solubility of DBMs in percentages; however, it does not provide complete standardization. In this study, the effect of DCs on DBMs was evaluated for a long term of 120 days using EN ISO 20795 for specimen preparation.

DBMs should ideally contain insoluble components and exhibit low water sorption. However, their lifetime exposure to factors such as saliva, pH, food, water, and cleanser can lead to the loss of water sorption, plasticizers, and other soluble ingredients (16). The maximum acceptable ISO standard for DBMs is 32 μg/mm³ for water sorption and 1.6 μg/mm³ for solubility (18). According to the results obtained herein, water sorption and solubility values were greater than the ISO values in some groups, possibly because 1 year of use of overnight immersion coincides with a 120-day cleanser bath.

In this study, the same polishing and smoothing process was applied to surfaces of all specimens for standardization before the experiment. Bollen et al. (21) have reported that the surface roughness of PMMA is affected by polishing abrasives used during standardization. As PMMA can be easily polished, its initial roughness is less. However, polyamides exhibit a fibrous, semi-flexible structure as well as low surface hardness (22). Although the Vickers hardness number of PEEK and PMMA materials is similar, PEEK exhibits a surface topography different from that of PMMA (17). Therefore, a different surface polishing procedure may be required. In parallel, Kurahashi et al. (23) have reported that a clinically acceptable surface roughness can be achieved using a soft polishing brush and agent for greater than 3 min for polishing PEEK. Heimer et al. (24) have evaluated the effects of laboratory and chairside polishing methods on the surface roughness and surface free energy of PEEK, auto-polymerized PMMA, and a composite resin and reported that compared to laboratory methods, chairside polishing methods for PEEK render lower surface roughness values. In this study, the same polishing process was applied to all DBMs. Unfortunately, there is no completely acceptable procedure for polishing PEEK compared to other DBMs.

Durkan et al. (25) have evaluated the effect of the 20-day application of DCs on the surface roughness, hardness, and color stability of a butadiene styrene copolymer PMMA, heat-polymerized PMMA, and two polyamides (Deflex and Valplast, respectively) and reported that polyamides significantly affect surface roughness, hardness, and color stability after immersion in a cleanser bath. In addition, in this study, DC-containing sodium perborate (Corega, Protefix) increase the surface roughness of polyamides and PMMA. Aging or wear of DBMs depends on several factors such as discoloration, water sorption, solubility, surface roughness, and hardness. Accordingly, in this study, DCs are thought to cause surface roughness in the DBMs with a polished surface after 120 days of bath, thereby leading to high water sorption and solubility values.

Song et al. (26) have evaluated the physical and mechanical properties of four injection-molded DBMs (i.e., polyamide, polyester, acrylic resin, and polypropylene, respectively), and the water sorption and solubility values of these materials were in the range of 6.17–24.38 μg/mm^3 and 0.76–3.11 μg/mm^3, respectively. The higher water sorption and solubility values in their study compared to this study can be explained by measuring M_2 and M_3 in the long-term. In addition, Nguyen et al. (27) have reported that polyamide does not reach saturation for 8 weeks and continues to absorb water, which is in agreement with result of present study.

Liebermann et al. (17) performed an in vitro study and reported that the solubility of PEEK in physiological saliva and distilled water is less than those of PMMA and composite resin-based materials. In our study, in parallel with the results of this study, the solubility values of the PK group in distilled water were found to be similar to the HP group. In addition, lower solubility values of PK were found in cleanser baths compared to other DBMs. In our study, higher water sorption and solubility values were observed in comparison with those obtained in this study primarily because DCs affect surfaces of PEEK and PMMA. The water sorption and solubility of dental polymers can cause molecular imbalance, which can affect their mechanical properties, dimensional stability, and biocompatibility, thereby resulting in crack formation and subsequent reduction in mechanical properties. water sorption not only affects its physical and mechanical properties but also reduces surface hardness and elastic modulus (17,28). In this study, the water sorption and solubility values of the PEEK group can be attributed to the molecular imbalance occurring on the PEEK surface. Owing to the lack of studies on the effect of DCs on the water sorption and solubility of PEEK, comparison with other DBMs for PEEK is difficult. However, the effect of the PEEK surface topography on water sorption and solubility should be examined in future studies.

Zissis et al. (29) have evaluated the release of residual monomers by the gas-liquid chromatography of four DBMs (i.e., three heat-polymerized PMMA and one auto-polymerized PMMA) and one hard liner over 1 week, 12 months, and 38 months after curing and reported that the release of residual monomers in heat-polymerized PMMA is less than that in auto-polymerized PMMA. In addition, in this study, a statistically significant amount of the residual monomer in auto-polymerized PMMA was reported in the first 12 months. In particular, 1 week after curing, about 2.5% of the residual monomer was observed. Several studies have reported a relationship between residual monomer and water sorption. In the case of a residual monomer, less monomer conversion occurs, possibly leading to increased water sorption and solubility (30,31). In our study, parallel to these studies, a significant difference was observed in the water sorption and solubility in the AP group compared to the HP group depending on the residual monomer amount in chemical cleanser baths.

While DC tablets are a highly recommended hygiene practice by dental health professionals, Axe et al. (32) have reported that only ~24% of removable prosthesis users utilize this approach frequently. In a systematic review, Papadiochou et al. (33) have evaluated hygiene practices in removable prostheses and reported that brushing the prosthesis is the most common cleaning method in removable prostheses and that >50% of removable prosthesis users do not remove their prosthesis at night. In this study, considering the above-mentioned oral hygiene habits, the use of DCs and simulating an 8-h overnight immersion per day is an ideal practice, but it may be partly a limitation of this study in practical terms.

One of the limitations of this study is that the specimens were produced and tested under ideal conditions that may not reflect actual clinical conditions. Other limitations of this study include the inability to completely simulate the oral environment, such as temperature, humidity, pH, bacterial acids, and denture biofilm, possibly affecting water sorption and solubility values. The possible effects of the compounds in DCs on PEEK are not completely known. In addition, with respect to the PEEK polymer, possible effects of different DCs in terms of discoloration, surface roughness, and hardness should be examined in future studies.

5. CONCLUSION

Within the limitations of this in vitro study, the following conclusions were drawn: The water sorption and solubility of DBMs increased due to DCs with different contents during long-term follow-up of 120 days. However, future experimental and clinical studies that investigate the effect of DCs with different contents on the color stability and surface topography of PEEK are required to confirm the results of this study.

Conflicts of interest

None declared.

REFERENCES

[1] Campbell SD, Cooper L, Craddock H, Hyde TP, Nattress B, Pavitt SH, Seymour DW. Removable partial dentures: The clinical need for innovation. J Prosthet Dent 2017;118(3):273-280.
[2] Zitzmann NU, Hagmann E, Weiger R. What is the prevalence of various types of prosthetic dental restorations in Europe? Clin Oral Implants Res 2007;18(3):20-33.
[3] Ozyilmaz OY, Kara O, Akin C. Evaluation of various denture cleansers on color stability and surface topography of polyetherketoneketone, polyamide, and polymethylmethacrylate. Microsc Res Tech 2021;84(1):3–11.
[4] Zafar MS. Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update. Polymers (Basel) 2020;12(10):2299.
[5] Yunus N, Rashid AA, Azmi LL, Abu Hassan MI. Some flexural properties of a nylon denture base polymer. J Oral Rehabil 2005;32(1): 65–71.
[6] Vojdani M, Giti R. Polyamide as a denture base material: A literature review. J Dent Shiraz 2015;16(1):1-9.
[7] Sicilia A, Cuesta S, Coma G, Arregui I, Guisasola C, Ruiz E, Maestro A. Titanium allergy in dental implant patients: a clinical study on 1500 consecutive patients. Clin Oral Implants Res 2008;19(8):823-835.
[8] Foti B, Tavitian P, Tosello A, Bonfil JJ, Franquin JC. Polymetallism and osseointegration in oral implantology: Pilot study on primate. J Oral Rehabil 1999;26(6):495-502.
[9] Schwitalla A, Muller WD. PEEK dental implants: a review of the literature. J Oral Implantol 2013;39(6):743-749.
[10] Wiesli MG, Ozcan M. High-performance polymers and their potential application as medical and oral implant materials: a review. Implant Dent 2015;24(4):448-457.
[11] Schwitalla AD, Spintig T, Kallage I, Muller WD. Flexural behavior of PEEK materials for dental application. Dent Mater 2015;31(11):1377-1384.
[12] Takabayashi Y. Characteristics of denture thermoplastic resins for non-metal clasp dentures. Dent Mater J 2010;29(4):353-361.
[13] Machado AL, Breeding LC, Vergani CE, da Cruz Perez LE. Hardness and surface roughness of reline and denture base acrylic resins after repeated disinfection procedures. J Prosthet Dent 2009;102(2):115-122.
[14] Alam M, Jagger R, Vowles R, Moran J. Comparative stain removal properties of four commercially available denture cleaning products: an in vitro study. Int J Dent Hyg 2011;9(1):37-42.
[15] Felipucci DN, Davi LR, Paranhos HF, Bezzon OL, Silva RF, Pagnano VO. Effect of different cleansers on the surface of removable partial denture. Braz Dent J 2011;22(5):392-397.
[16] Hong G, Murata H, Li Y, Sadamori S, Hamada T. Influence of denture cleansers on the color stability of three types of denture base acrylic resin. J Prosthet Dent 2009;101(3):205-213.
[17] Liebermann A, Wimmer T, Schmidlin PR, Scherer H, Löffler P, Roos M, Stawarczyk B. Physicomechanical characterization of polyetheretherketone and current esthetic dental CAD/CAM polymers after aging in different storage media. J Prosthet Dent 2016;115(3):321-328.
[18] International Organization for Standardization (ISO). Dentistry-Base polymers-Part 1: Denture base polymers. ISO 20795-1:2013. Available from: URL:https://www.iso.org/standard/62277.html. September 12, 2021
[19] Council on dental materials and devices. Revised American dental association specification no. 12 for denture base polymers. J Am Dent Assoc 1975;90(2), 451-458.
[20] Kazanji MN, Watkinson AC. Soft lining materials: their absorption of, and solubility in, artificial saliva. Br Dent J 1988;165(3):91-94.
[21] Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater 1997;13(4):258-269.
[22] Köroğlu A, Şahin O, Dede DÖ, Deniz ŞT, Karacan Sever N, Özkan S. Efficacy of denture cleaners on the surface roughness and Candida albicans adherence of sealant agent coupled denture base materials. Dent Mater J 2016;35(5):810-816.
[23] Kurahashi K, Matsuda T, Ishida Y, Ichikawa T. Effect of polishing protocols on the surface roughness of polyetheretherketone. J Oral Sci 2020;62(1):40-42.
[24] Heimer S, Schmidlin PR, Stawarczyk B. Effect of different cleaning methods of polyetheretherketone on surface roughness and surface free energy properties. J Appl Biomater Funct Mater 2016;14(3):248-255.
[25] Durkan R, Ayaz EA, Bagis B, Gurbuz A, Ozturk N, Korkmaz FM. Comparative effects of denture cleansers on physical properties of polyamide and polymethyl methacrylate base polymers. Dent Mater J 2013;32(3):367-375.
[26] Song SY, Kim KS, Lee JY, Shin SW. Physical properties and color stability of injection-molded thermoplastic denture base resins. J Adv Prosthodont 2019;11(1):32-40.
[27] Nguyen LG, Kopperud HM, Øilo M. Water sorption and solubility of polyamide denture base materials. Acta Biomater Odontol Scand 2017;3(1):47-52.
[28] Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater 2006;22(3):211-222.
[29] Zissis A, Yannikakis S, Polyzois G, Harrison A. A long term study on residual monomer release from denture materials. Eur J Prosthodont Restor Dent 2008;16(2):81-84.
[30] Jagger RG. Effect of the curing cycle on some properties of a polymethylmethacrylate denture base material. J Oral Rehabil 1978;5(2):151-157.
[31] Umemoto K, Kurata S. Basic study of a new denture base resin applying hydrophobic methacrylate monomer. Dent Mater J 1997;16(1):21-30.
[32] Axe AS, Varghese R, Bosma M, Kitson N, Bradshaw DJ. Dental health professional recommendation and consumer habits in denture cleansing. J Prosthet Dent 2016;115(2):183-188.
[33] Papadiochou S, Polyzois G. Hygiene practices in removable prosthodontics: A systematic review. Int J Dent Hyg 2018;16(2):179-201.

How to cite this article: Demirci F, Tanik A. Comparison of the Effect of Denture Cleansers on Long-Term Water Sorption and Solubility of Polyetheretherketone with other Denture Base Materials. Clin Exp Health Sci 2022;12:672-677. DOI:10.33808/clinexphealthsci.1011897

Clinical and Experimental Health Sciences

The Effect of Antimicrobial Policy Implementation on Carbapenem Resistance: A University Hospital Experience

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Received: 19.10.2021 **Accepted:** 21.02.2022

ABSTRACT

Objective: The resistance of Gram-negative bacteria to antibiotics is a global issue that leads to increased mortality and treatment costs. The aim of this study is to see how a newly formed carbapenem control team affected the prevalence of carbapenem-resistant Gram-negative rods and antibiotic consumption expenses in 2017 compared to the year before.

Methods: The rate of carbapenem antibiotic usage in Intensive Care Units and Bone Marrow Transplantation services, as well as the findings of culture materials obtained from various body parts of the same patients, between January 1, 2016, and December 31, 2017 were assessed.

Results: While there was an ordinary restriction on carbapenem consumption in 2016, carbapenem consumption has been more restricted in 2017. The carbapenem-resistant Gram-negative bacteria patterns of culture materials are examined and compared with Defined Daily Dose data of carbapenems. After the restriction, a significant decrease in the consumption of carbapenems was detected. The decline in carbapenem-resistant Gram-negative bacteria and decreasing antibiotic consumption were found to have a moderately positive correlation (r=0.641, p=0.02). A 60.9% decrease was observed in carbapenem costs after carbapenem restriction, on the other hand, an increase in other unrestricted antibiotics was apparent.

Conclusion: Antimicrobial restriction policies can help minimize the rate of carbapenem-resistant Gram-negative rods, which is a serious problem in healthcare. We demonstrated that a decrease in carbapenem-resistant Gram-negative rods isolation rates can lead to a decrease in healthcare-associated infections. Although there is no decrease in the direct antibiotics cost, a drop in carbapenem-resistant may lower the expenses of drastic consequences of infections with carbapenem-resistant and its cost. we can conclude that the Antibiotic Control Policy should be modified based on this new information.

Keywords: *Carbapenem, Resistance, Gram-Negative Bacteria*

1. INTRODUCTION

Antibiotics are among the most important medications used in healthcare today, yet their inappropriate usage has resulted in major problems such as Antimicrobial Resistance. In 2018, Irrational antibiotic use was reported in many hospitals, according to a cross-national study that included Eastern Europe and Central Asia, with Turkey ranking as one of the largest consumers of systemic antimicrobials (1).

The global antibiotic consumption rate is estimated to be 14.3 defined daily doses (DDD) per 1000 population per day in 2018, a 46 percent increase from 2000 (2). In 2011, data on antimicrobial use in 13 non-European Union countries ranged from 15.3 to 42.3 defined daily dose/1000 inhabitants per day (DID), including Turkey (42.3 DID), with data from Turkey limited to outpatient use (3). A recent systematic review that measured the point prevalence and inappropriate antibiotic prescription in Turkey highlighted high consumption rates and inappropriate antibiotic use over the last 15 years, necessitating the implementation of strict and effective stewardship in all Turkish hospitals (4). Antimicrobial restriction policies are intended to reduce morbidity and mortality, as well as the expense of treatment, the length of hospitalization, and the rate of resistance microorganisms, while also improving quality of life (5-6).

Gram-negative rods (GNRs) are among the most frequent causes of community-acquired and healthcare-related infections. The consumption of antibiotics excessively and unnecessarily came along with emerging antimicrobial resistance. Carbapenem group antimicrobials are the last resort to treat multidrug-resistant (MDR) GNRs until recent years(7). Overuse of carbapenems resulted in carbapenem resistance (CR) in *Enterobacterales* especially *Klebsiella* spp (8, 9). In the last ten years, reports of an increase in the incidence of carbapenem-resistant Gram-negative rods (CRGNR) infections such as urinary tract, bloodstream, and ventilator-associated pneumonia, as well as the rate of

DOI: 10.33808/clinexphealthsci.1010571

colonization with these microbes, have been reported in the hospital setting studies (10-12). These infections can be seen as sporadic cases or hospital outbreaks. Unfortunately, in addition to their higher toxicity, such as nephrotoxicity, the therapy choices for infections caused by CRGNRs are severely limited. More effective and less toxic agents have been introduced into clinical practice in recent years, although widespread use is still not available in most countries. Thus controlling the emergence and spread of carbapenem resistance and rational use of antimicrobials is essential for the prevention of such infections and their severe complications(11, 12). In Turkey, the antibiotic restriction policy and Rational Drug Use National Action Plan were implemented in 2003 and 2014, respectively(13-16). These actions resulted in decreasing restricted antibiotic consumption by 26.7% while increasing unrestricted antibiotics and decreasing the prevalence of extended-spectrum beta-lactamase-producing *K. pneumoniae* (17, 18). GNRs are opportunistic pathogenic microorganisms that cause epidemics in hospital settings, particularly in ICUs. Inappropriate antibiotic use in hospitals has led to the development of resistance to many antibiotics used in GNRs. Carbapenem consumption has an important place in various resistance mechanisms. This study aims to see how a newly formed carbapenem control team affected the prevalence of carbapenem-resistant Gram-negative rods and antibiotic consumption expenses in 2017 compared to the year before at Medipol Mega University Hospital in 2017.

2. METHODS

We planned a quasi-experimental study and aimed to evaluate the effect of the newly established carbapenem (imipenem-cilastatin, meropenem, ertapenem) control team (infectious diseases specialist, clinical pharmacist, and infection control nurse) on the antibiotic policy of our hospital on the resistance pattern of GNRs and costs of total antibiotic consumption. Two different annual periods were analyzed. The first one was between Jan-Dec 2016 (Group 1) which there was no restriction or intervention except standard antibiotic policies and the latter was between Jan-Dec 2017 (Group 2) which was intervened by the carbapenem control team after training healthcare staff in the included departments. The carbapenem restriction was made by setting a carbapenem-team approval system in the intervention period. Our center is a third-level university hospital settled in Istanbul and has 515 beds. All ICUs have 104 beds (Adult ICU, Cardiovascular Surgery ICU, and neonatal ICU) and bone marrow transplantation units have 25 beds (adult and pediatric) all of them were included in the study. Coronary ICU was not included because of the short hospitalization period and no carbapenem use. The study included all hospitalized patients in these departments over the specified time periods who were given carbapenems and found CR GNRs in their various samples, regardless of whether they were infected or colonized. In the event of clinical necessity, samples were obtained after consulting with an infectious diseases specialist. Patients who have a history of prior hospitalization and carbapenem use or were found carbapenem-resistant Enterobacteriaceae (CRE) in the screening cultures were excluded.

The ethics committee of Medipol University gave their consent (NO: 328, Date: 15.09.2017), as well as the institution's permission. All data (culture results, hospitalization days, antibiotic consumption amount) was obtained from the hospital information system. The unit price of antibiotics was calculated based on the Turkish Drug Guidelines list. Antibiotic consumption index (ACI) was calculated based on ATC/DDD method suggested by World Health Organization (WHO).

$$ACI = \frac{Total\ amount\ of\ antimicrobial\ used}{DDD * Hospitalized\ days} \chi 1000$$

With this study, we set out to address three main questions: (1) Are the CR GNRs rates going to decrease after intervention? (2) Is the consumption of carbapenems going to decrease after intervention? (3) Is there any effect of the intervention to decrease the consumption of antibiotics other than carbapenems?

Descriptive statistics and the compliance of variables to normal distribution were examined in the study. Shapiro-Wilk test was performed to assess the normality of frequencies. Paired samples t-test was used to compare normally distributed data. Spearman correlation analysis was used to examine the relationship between the resistant number by years and the amount of consumption per patient. Mann-Whitney U test was used to compare inpatients, patient days, urinary catheter days, ventilator days, and central catheter days by years. The chi-square test was used to compare qualitative data. Results of quantitative data are given in mean ± standard deviation/median (min-max) and qualitative data are also presented as frequency and percentage. The significance level was set at $p<0.05$.

3. RESULTS

A total of 1604 samples were taken and cultured within both annual periods. A total of 680 CR GNRs were isolated eventually. Demographic and descriptive characteristics of groups were given in Table 1.

Table 1 Demographic and descriptive characteristics of groups

	Group 1	Group 2	Total	P
Age	47.5 (12-73) [0-98]	53 (23-68) [0-96]	51 (21-70) [0-98]	N/A
Gender (M/F)	171/113 (60/40)	162/99 (62/38)	333/212 (61/39)	N/A
Admitted patients N (%)	47 (23.5 – 69.3)	63.5 (20.5 – 74)		0.6
Hospital-Days	450 (267-540)	452 (251 – 544.5)	N/A	0.6
Urinary catheter days	72 (0-331)	61 (0-341.8)	N/A	0.9
Ventilator Days	194 (0-308)	206 (0-355)	N/A	0.6
Central Catheter Days	221 (152-277)	235 (153-294)	N/A	0.5
ACI – Carbapenem Class per patient	2239.94 ± 104.056	853.64 ± 215.16	N/A	**<0.00**

Data was given in median (IQR) [Min-Max] or N (%) or Mean±SD. Group 1: Jan-Dec 2016 with no intervention; Group 2: Jan-Dec 2017 after intervention; ACI: Antibiotic consumption index

The most common GNRs found in culture materials taken before the intervention was *Acinetobacter* spp. 149 (96%) *Klebsiella* spp. 151 (48%), *Pseudomonas aeruginosa* 88 (47%) while after intervention it was *Acinetobacter* spp. 131 (92%), *Pseudomonas aeruginosa* 72 (40%) *Klebsiella* spp. 56 (25%), in order. It was found that GNRs isolation rates (mean ± SE: 34±3 vs 23±3) after intervention decreased (p= 0.012). The number of isolated Gram-negative bacilli was given in Table2.

Table 2 Number of isolated Gram-negative bacilli.

	Months												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
Group 1	**36**	**34**	**36**	**46**	**37**	**21**	**47**	**44**	**19**	**26**	**32**	**33**	**411**
Acinetobacter spp.	10	15	16	10	15	3	19	22	8	9	11	11	149
Enterobacter spp			1	3	6	3	2	1					16
Escherichia coli		1		1		1						1	4
Klebsiella spp.	14	13	13	23	11	12	17	8	7	7	15	11	151
Morganella spp.												1	1
Proteus mirabillis							1					1	2
Pseudomonas aeruginosa	12	5	6	9	5	2	8	13	4	10	6	8	88
Group 2	**49**	**17**	**10**	**23**	**25**	**15**	**14**	**17**	**19**	**27**	**26**	**27**	**269**
Acinetobacter spp.	21	3	4	15	20	9	6	10	8	11	13	11	131
Enterobacter spp		1					1				2	1	5
Escherichia coli	2	1									1	1	5
Klebsiella spp.	15	5	5	4		1	4	3	2	10	4	3	56
Proteus spp									1		1	1	3
Pseudomonas aeruginosa	11	7	1	4	5	5	3	4	8	6	4	10	72
Serratia marcescens											1		1
Total	**85**	**51**	**46**	**69**	**62**	**36**	**61**	**61**	**38**	**53**	**58**	**60**	**680**

Different types of samples were evaluated, carbapenem-resistant GNRs occurrence in blood cultures and bronchoalveolar lavage samples were reduced (Table 3). Aside from that, there were no significant differences.

While the average amount of expenditure before the intervention was 67.927 Turkish Lira (TL), it was 76.554 TL after the intervention, with no statistically significant difference between the median values (p> 0.05). While the total amount of spending before the restriction was 10.089.963 TL, the total amount of spending after the restriction was 7.376.913 TL. Following carbapenem restriction, costs for carbapenem, lincosamides, and first-generation cephalosporins decreased by 60.9%, 56.3%, and 51.5%, respectively, while costs for second-generation cephalosporins, macrolides, penicillin, and beta-lactamase inhibitors increased by 61.9%, 52.7%, and 48.9%, respectively.

Table 3 Carbapenem resistant Gram negative rods isolated from culture materials.

Sample	Group	Resistant n (%)	Sensitive n (%)	X^2	P
Blood	**G 1**	108 (58)	117 (52)	16.465	**0.001***
	G 2	63 (29.2)	153 (70.8)		
Abscess	**G 1**	2 (66.7)	1 (33.3)	-	-
	G 2	1 (50)	1 (50)		
Bronchoalveolar lavage	**G 1**	28 (63.6)	16 (36.4)	7.582	**0.006***
	G 2	26 (35.6)	47 (64.4)		
Sputum	**G 1**	29 (63)	17 (37)	0.007	0.933
	G 2	8 (57.1)	6 (42.9)		
CSF	**G 1**	4 (100)	0	-	-
	G 2	0	1 (100)		
Tissue Biopsy	**G 1**	4 (44.4)	5 (55.6)	0.525	0.301
	G 2	1 (20)	4 (80)		
Urine	**G 1**	27 (26.5)	75 (73.5)	0.183	0.669
	G 2	14 (22.2)	49 (77.8)		
Catheter tip	**G 1**	30 (57.7)	22 (42.3)	0.776	0.379
	G 2	21 (46.7)	24 (53.3)		
Endotracheal aspirate	**G 1**	147 (49)	153 (51)	2.719	0.12
	G 2	109 (42.4)	148 (57.6)		
Sterile fluid	**G 1**	14 (50)	14 (50)	1.751	0.186
	G 2	11 (30.6)	25 (69.4)		
Wound swab	**G 1**	18 (50)	18 (50)	1.272	0.259
	G 2	15 (34.9)	28 (58.2)		

The difference in carbapenem class antibiotic consumption index (ACI) between the two groups was statistically significant (Table 1), and carbapenem class antibiotic restriction was moderately correlated with a decrease in CR GNR isolation rate (r=0.641, p=0.02).

4. DISCUSSION

Carbapenem class antibiotics are potent options for extended-spectrum beta-lactamases. ESBL producing GNRs yet increasing carbapenem resistance limits its efficacy in daily clinical practice (19). Our findings of frequently isolated GNRs were *Acinetobacter* spp. (96%), *Klebsiella* spp. (48%) and *Pseudomonas aeruginosa* (47%). This finding is similar to what has been found in other ICUs (20-22). The difference of means of annual isolated carbapenem-resistant strains in the non-carbapenem restricted and carbapenem restricted periods was statistically significant in our study. In a 12-year study, Ogutlu et al. concluded that intermittent carbapenem restriction had a significant impact on carbapenem resistance in *Acinetobacter spp* (23).

Our finding of decreased CR of GNRs isolated in blood and bronchoalveolar lavage fluid cultures after restriction of carbapenems, is supported by a multi-center study conducted between 2001-2005, and published in 2011. Altunsoy et al analyzed antimicrobial susceptibility of 14.223 blood culture isolates and antibiotic consumption rate. They found that carbapenem restriction has a considerable role in the reduction of carbapenem-resistant Pseudomonas spp. and *Acinetobacter spp* (24). In our study, carbapenem restriction

via an ID approval system resulted in a significant reduction in ACI. Similarly, Inan et al. discovered that ID consultation improves rational antibacterial selection (25). Another study found that analyzing prospective active surveillance data before and after antimicrobial use reduced antibiotic use and promoted rational antimicrobial use (26). According to a Saudi Arabian study, a three-month carbapenem-free period reduced Pseudomonas aeruginosa meropenem resistance from 74.1 percent to 30 percent and imipenem resistance from 76 percent to 38.5 percent (p=0,01) in comparison to the previous three months. Therefore this research may conclude that, even short-term carbapenem restriction periods may interfere with carbapenem resistance in Pseudomonas aeruginosa, (PSA) (27). Pakyz et al. studied the effects of carbapenem restriction implementation on carbapenem-resistant PSA in 22 university hospitals in the United States of America. In the study, the period from 2002 to 2006 was evaluated retrospectively and it was reported that significantly less carbapenem was consumed in 8 hospitals while carbapenem restriction was implemented (p = 0.04) and significantly lower isolation rate of carbapenem-resistant PSA (p = 0.01) (28). As a result, it has been shown that carbapenem restriction can reduce carbapenem resistance rates in Pseudomonas aeruginosa.

In our study, after carbapenem restriction, 60.9% reduction in the cost of carbapenem use, 56.3% in lincosamides, and 51.5% in 1st generation cephalosporins were observed, yet the cost of second-generation cephalosporins 61.9%, macrolides 52.7%, and Penicillin and beta-lactamase inhibitors increased by 48.9%. According to Arda et al., the adoption of an antimicrobial approval system resulted in a 19.6% reduction in total antibiotic expenditures in the restricted antibiotic groups, but an increase in unrestricted antibiotics (18). A point prevalence survey conducted by Özgenç et al. in 8 universities training and research hospitals in the Aegean region, found that the rate of rational antibiotic use of antibiotics was significantly higher because of the antibiotic restriction practices (29). According to all these studies conducted in our country, antibiotic consumption rates, as well as the expenses of various resistant microbes and antibiotic costs, can be lowered when antibiotic restriction policies are implemented.

5. CONCLUSION

Antimicrobial restriction policies can help minimize the rate of CR GNRs, which is a serious problem in healthcare. We demonstrated that a decrease in CR GNRs isolation rates can lead to a decrease in healthcare-associated infections. Although there is no decrease in the direct antibiotics cost, a drop in CR may lower the expenses of drastic consequences of infections with CR and its cost. Antimicrobial stewardship based on ID consultation, limitation regulations, and local epidemiologic data results in reasonable antibiotic usage and superior clinical practices.

Funding: The authors have no funding sources to declare.

Conflict of interest: The authors do not have any conflict of interest to declare.

REFERENCES

[1] Robertson J, Iwamoto K, Hoxha I, Ghazaryan L, Abilova V, Cvijanovic A, Pyshnik H, Darakhvelidze M, Makalkina L, Jakupi A, Dzhakubekova A, Carp A, Cizmovic L, Rachina S, Radonjic V, Yusufi S, Aksoy M, Ibragimova M, Godman B, Kluge H, Pedersen HB. Antimicrobial Medicines Consumption in Eastern Europe and Central Asia – An Updated Cross-National Study and Assessment of Quantitative Metrics for Policy Action. Front Pharmacol 2019;9:1156.

[2] Browne AJ, Chipeta MG, Haines-Woodhouse G, Kumaran EPA, Hamadani BHK, Zaraa S, Henry NJ, Deshpande A, Reiner RC Jr, Day NPJ, Lopez AD, Dunachie S, Moore CE, Stergachis A, Hay SI, Dolecek C. Global antibiotic consumption and usage in humans, 2000-18: a spatial modelling study. Lancet Planet Health 2021 Dec;5(12):e893-e904.

[3] Versporten A, Bolokhovets G, Ghazaryan L, Abilova V, Pyshnik G, Spasojevic T, Korinteli I, Raka L, Kambaralieva B, Cizmovic L, Carp A, Radonjic V, Maqsudova N, Celik HD, Payerl-Pal M, Pedersen HB, Sautenkova N, Goossens H; WHO/Europe-ESAC Project Group. Antibiotic use in eastern Europe: a cross-national database study in coordination with the WHO Regional Office for Europe. Lancet Infect Dis 2014;14(5):381-387.

[4] Selcuk A. The point prevalence and inappropriateness of antibiotic use at hospitals in Turkey: a systematic review and meta-analysis. J Chemother 2021;33(6):390-399.

[5] Hayashi Y, Paterson DL. Strategies for reduction in duration of antibiotic use in hospitalized patients. Clin Infect Dis 2011;52(10):1232-1240.

[6] Slama TG, Amin A, Brunton SA, File TM Jr, Milkovich G, Rodvold KA, Sahm DF, Varon J, Weiland D Jr. A clinician's guide to the appropriate and accurate use of antibiotics: the Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria. Am J Med 2005;118 Suppl 7A:1S-6S.

[7] Chang HJ, Hsu PC, Yang CC, Kuo AJ, Chia JH, Wu TL, Lee MH. Risk factors and outcomes of carbapenem-nonsusceptible Escherichia coli bacteremia: a matched case–control study. J Microbiol Immunol Infect 2011;44(2):125-130.

[8] Şeker F. *Bir üniversite hastanesinde karbapenem kısıtlamasının karbapenem dirençli gram negatif çomaklar üzerine etkisi* (Master's thesis, İstanbul Medipol Üniversitesi Sağlık Bilimleri Enstitüsü). Avilable from: URL: https://acikerisim.medipol.edu.tr/xmlui/handle/20.500.12511/7182

[9] MacKenzie FM, Forbes KJ, Dorai-John T, Amyes SG, Gould IM. Emergence of a carbapenem-resistant Kiebsiella pneumoniae. Lancet 1997;350(9080):783.

[10] Kwak YG, Choi SH, Choo EJ, Chung JW, Jeong JY, Kim NJ, Woo JH, Ryu J, Kim YS.Risk factors for the acquisition of carbapenem-resistant Klebsiella pneumoniae among hospitalized patients. Microb Drug Resist 2005;11(2):165-169.

[11] Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant Klebsiella pneumoniae strains in an Israeli hospital. Antimicrob Agents Chemother. 2007;51(8):3026-3029.

[12] Koh TH, Wang GC, Sng LH. Clonal spread of IMP-1-producing Pseudomonas aeruginosa in two hospitals in Singapore. J Clin Microbiol 2004;42(11):5378-5380.

[13] TİTCK. Antimikrobiyal Kullanımının Yönetimi: Sorun Nedir? 2016 (Cited 2021 April 02). Available from: URL: http://www.akilciilac.gov.tr/wp-content/uploads/2016/03/aik-bltn-181.pdf. (accessed on 10 oct 2021).

[14] Keçik M, editor Akılcı Antibiyotik Kullanımı, Türkiye Verileri ve Hedefler. ANKEM; 2016. (Turkish)

[15] TİTCK. Akılcı İlaç Kullanımı Ulusal Eylem Planı. In: UAİTSDA, editor. Website: TİTCK; 2014. (Turkish)

[16] TC.Resmi.Gazete. Mali Yılı Bütçe Uygulama Talimatı. Mevzuatı Geliştirme ve Yayın Genel Müdürlüğü; 2003. (Turkish)

[17] Hoşoğlu S, Esen S, Ozturk R, Altindis M, Ertek M, Kaygusuz S, Caylan R, Demirdag K, Sencan I, Ertem GT, Aslan S, Bosnak V, Aygun P, Erol S, Celen MK. The effect of a restriction policy on the antimicrobial consumption in Turkey: a country-wide study. Eur J Clin Pharmacol.2005;61(10):727-731.

[18] Arda B, Sipahi OR, Yamazhan T, Tasbakan M, Pullukcu H, Tunger A, Buke C, Ulusoy S. Short-term effect of antibiotic control policy on the usage patterns and cost of antimicrobials, mortality, nosocomial infection rates and antibacterial resistance. J Infect. 2007;55(1):41-48.

[19] Drew RJ, Turton JF, Hill RL, Livermore DM, Woodford N, Paulus S, Cunliffe NA. Emergence of carbapenem-resistant Enterobacteriaceae in a UK paediatric hospital. J Hosp Infect. 2013;84(4):300-304.

[20] Ergönül Ö, Aydin M, Azap A, Başaran S, Tekin S, Kaya Ş, Gülsün S, Yörük G, Kurşun E, Yeşilkaya A, Şimşek F, Yılmaz E, Bilgin H, Hatipoğlu Ç, Cabadak H, Tezer Y, Togan T, Karaoğlan I, İnan A, Engin A, Alışkan HE, Yavuz SŞ, Erdinç Ş, Mulazimoglu L, Azap Ö, Can F, Akalın H, Timurkaynak F. Healthcare-associated Gram-negative bloodstream infections: antibiotic resistance and predictors of mortality. J Hosp Infect.2016;94(4):381-385.

[21] Mathur P, Varghese P, Tak V, Gunjiyal J, Lalwani S, Kumar S, Misra MC. Epidemiology of blood stream infections at a level-1 trauma care center of India. J lab physicians. 2014;6(1):22-27.

[22] Tabah A, Koulenti D, Laupland K, Misset B, Valles J, Bruzzi de Carvalho F, Paiva JA, Cakar N, Ma X, Eggimann P, Antonelli M, Bonten MJ, Csomos A, Krueger WA, Mikstacki A, Lipman J, Depuydt P, Vesin A, Garrouste-Orgeas M, Zahar JR, Blot S, Carlet J, Brun-Buisson C, Martin C, Rello J, Dimopoulos G, Timsit JF. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study. Intensive Care Med 2012;38(12):1930-1945.

[23] Ogutlu A, Guclu E, Karabay O, Utku AC, Tuna N, Yahyaoglu M. Effects of Carbapenem consumption on the prevalence of Acinetobacter infection in intensive care unit patients. Ann Clin Microbiol Antimicrob. 2014;13(1):1-6.

[24] Altunsoy A, Aypak C, Azap A, Ergönül Ö, Balık I. The impact of a nationwide antibiotic restriction program on antibiotic usage and resistance against nosocomial pathogens in Turkey. Int J Med Sci. 2011;8(4):339.

[25] Asuman İN, DAĞLI Ö, Akçay SŞ, Engin DÖ, Karagül E, Özyürek SÇ. Antibiotic use and cost in a teaching hospital in İstanbul. J Microb Infect Diseases. 2011;1(03):128-33.

[26] Tunger O, Karakaya Y, Cetin CB, Dinc G, Borand H. Rational antibiotic use. J Infect Dev Ctries. 2009;3(2):88-93.

[27] Abdallah M, Badawi M, Amirah MF, Rasheed A, Mady AF, Alodat M, Alharthy A. Impact of carbapenem restriction on the antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolates in the ICU. J Antimicrob Chemother. 2017;72(11):3187-3190.

[28] Pakyz AL, Oinonen M, Polk RE. Relationship of carbapenem restriction in 22 university teaching hospitals to carbapenem use and carbapenem-resistant Pseudomonas aeruginosa. Antimicrob agents chemother. 2009;53(5):1983-1986.

[29] Ozgenç O, Genç VE, Ari AA, El Sibel, Saçar S, Ozunlu H, Akgul A, Demirturk N, Cetin CB, Sungur M, Coşkuner SA, Avci M, Ergonul O. Evaluation of the therapeutic use of antibiotics in Aegean Region hospitals of Turkey: a multicentric study. Indian J Med Microbiol. 2011;29(2):124.

How to cite this article: Derin O, Seker F, Aksoy N, Yiğit P, Yılmaz M, Mert A. The Effect of Antimicrobial Policy Implementation on Carbapenem Resistance: A University Hospital Experience. Clin Exp Health Sci 2022; 12: 678-682. DOI: 10.33808/clinexphealthsci.1010571

Clinical and Experimental Health Sciences

Informed Consent in Dentomaxillofacial Radiology: A Cross-Sectional Study

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Received: 20.10.2021 **Accepted:** 30.03.2022

ABSTRACT

Objective: This study assessed the opinions and attitudes of dentomaxillofacial (DMF) radiology physicians worldwide about informed consent in terms of oral radiology applications.

Methods: DMF radiology physicians in various countries were invited to this study via e-mail. The participants answered their demographic information (gender, age, years of experience, title, institution, and country), and questions about informed consent. The Pearson chi-square and Fisher's exact tests were used for statistical analysis

Results: From 22 countries, 46 male (51.7%) and 43 female (48.3%) DMR radiology physicians completed the questionnaire. More than half of the participants (53.9%) were working in the university hospital, and the highest number of participants (32.6%) was from the European region. Most of the surveyors (70.8%) stated that consent is required in dental radiology. No statistically significant difference was found in the radiographic methods (intraoral, panoramic/extraoral, and cone-beam CT) applied in terms of obtaining consent ($p > 0.05$). While middle-aged physicians (30 –45 age) thought that patients should not be informed about the risk of radiation causing cancer, experienced participants (45 age and above) stated that information should be given about the cancer risk ($p < 0.05$).

Conclusion: The results of this study showed that most of the DMF radiology physicians stated that they have responsibility for getting informed consent and only one-third of the participants inform patients about the risks of radiation.

Keywords: Dental radiology, informed consent, ethics

1. INTRODUCTION

Physicians are responsible for providing all information about the risks, benefits and alternative methods of diagnosis and treatment procedures to be applied to patients (1). Informed consent is the provision of sufficient information to a patient to make consciously a choice, the physician to inform the patient about the risks and alternative treatments, and the patient's approval or rejection of the medical intervention to be applied because of this information (1-3).

Radiographic procedures have an important place in diagnosis and treatment planning in dental practice. The widespread of medical imaging and the trend of overuse with unnecessary reviews that do not provide any health benefits seem to confirm the hypothesis that radiological research is widely trivialized (4). Legal and ethically valid patient consent is required before any patient intervention, including diagnostic radiographic procedures (5). In terms of radiation dose, risks in dentomaxillofacial (DMF) imaging applications are generally lower than in medical applications (6). However, patients should be informed about radiology practices in dentistry. They should be provided with information about why the radiographic examination is required, which techniques can be used, the benefits, risks, duration, and cost of the technique (3,7). In addition to conventional dental imaging such as intraoral and panoramic radiography, the patient's hesitation increases in cone-beam CT applications, which have recently become widespread and whose radiation dose is higher than conventional imaging. The dentists should explain to the patient that there is a procedure that will provide much more benefit than the minimal biological risk associated with the procedure to be performed (8).

To the best of our knowledge, there are published one study evaluating the perceptions and attitudes of DMF radiologists in Turkey about informed consent (7). This study evaluates the opinions and attitudes of DMF radiology physicians worldwide about informed consent in terms of oral radiology applications.

DOI: 10.33808/clinexphealthsci.1012561

2. METHODS

This study protocol was conducted with the principles of the Helsinki Declaration. Before the research, ethical approval was received from Pamukkale University Ethics Committee (No: 60116787-020/37899, Date of approval: 25/06/2020).

2.1. Participants

DMF radiology physicians [research assistants (postgraduate education, Ph.D., or specialty education, etc.), specialists, and lecturers (professor, associate professor, assistant professor)] in various countries were invited to the study via e-mail. A link to the online survey website (Google Forms; Alphabet Co., Mountain View, CA) where the questions were prepared was sent to the surveyors. Participation in the survey was voluntary. The surveyors completed their demographic information (gender, age, years of experience, title, institution, and country), and answered questions about informed consent.

2.2. Survey

The questionnaire in this study consisted of the first part with demographic data and the second part with 10 questions about informed consent. The studies by Kurt et al. (9), Karsli et al. (10), and Akay et al. (7) were used in the preparation of the survey questions. Questions about informed consent and answer options are given in Table 1.

Table 1. *Questions and answers directed to the participants*

Question no	Question	Answer no	Answer
1	How would you evaluate your knowledge on informed consent?	a	Sufficient
		b	Partially sufficient
		c	Insufficient
2	Is it necessary to obtain informed consent from patients at the dental radiology clinic?	a	Yes
		b	No
		c	Not sure/No idea
3	How do you think informed consent should be?	a	Written (signed by patient and physician)
		b	Verbal
		c	Verbal and written
4	Do you get informed consent from your patients before dental radiography at your institution?	a	Yes
		b	No
5	#+ In which radiographic methods do you get patient consent?	a	Intraoral imaging
		b	Panoramic/Extraoral imaging
		c	Cone-beam CT
6	# Whom are these informed consent forms given to or explained the patients?	a	Dentomaxillofacial radiologist
		b	Radiology technician
		c	Secretariat/Physician assistant
7	Do you think that every patient or their relatives should be informed about the risk of cancer caused by radiation?	a	Yes
		b	No
		c	Not sure/No idea
8	Are patients or patients' relatives informed about radiation before the procedure in your radiology department?	a	Yes
		b	No
		c	Not sure
9	Who should provide information about the risk of cancer caused by radiation?	a	Radiology department staff
		b	The referring physician
10	Do you think dentomaxillofacial radiologists have the responsibility for getting informed consent?	a	Yes
		b	No
		c	Not sure/No idea

 # Answered by the participants who stated that informed consent was obtained from the patients before dental radiography.
+ Participants have marked more than one option

2.3. Statistical Analysis

As a result of the power analysis performed by considering the answer categories of the questionnaire questions and the descriptive characteristics of the participants, the sample size was determined as 89 for the medium effect size and the significance level of 0.05, and the statistical power of 90%. The measurement reliability of the questionnaire was evaluated with Cronbach's alpha coefficient and the survey was reported as reliable (Cronbach's alpha = 0.71). The Pearson chi-square and Fisher's exact tests were used for data analysis. For analysis, SPSS (IBM Corp.; New York, NY) was used, and the statistical significance level was determined as $p < 0.05$.

3. RESULTS

From 22 countries, 46 male (51.7%) and 43 female (48.3%) dentists completed the survey. Most of the participants (46.1%) were in the 31-45 age range, while 36.0% of DMF radiology physicians had lower experience (1-8 years). Most of the physicians (70.8%) were DMF radiology specialists. While more than half of the participants (53.9%) were working in the university hospital, the highest number of participants (32.6%) was from the European region. Twenty-seven (30.3%) of DMF radiologists from South America, eleven (12.4%) from the Far East and ten (11.2%) from North America participated in the survey.

Most of the surveyors (70.8%) stated that obtaining informed consent from the patient is necessary at the dental radiology clinic. A significant portion of the participants (41.6%) stated that they did not obtain informed consent before dental radiography procedures in their clinics. The percentage of informed consent for cone-beam CT was higher than for conventional imaging methods. Half of the participants (49.4%) stated that patients should be informed about the cancer risk due to radiation. Details of the answers for the surveyors are shown in Table 2 (Subtitle of 'total participants').

Comparison of views on informed consent by gender, title, and institution are shown in Table 2. Male surveyors thought that the consent should be written while females stated that forms should be both verbal and written ($p > 0.05$). The effect of title on the opinions of radiologists about informed consent was found statistically insignificant ($p > 0.05$). Most of the surveyors (55.5%) from the private institutions stated that informed consent forms were given by secretariat/physician assistants. However, radiologists from the university hospital reported that informed consent forms were given by DMF radiologists (39.3%) and radiology technicians (35.7%) mostly ($p < 0.05$).

Table 3 shows the comparison of views by age, and experience. Most radiologists aged between 30 and 45 (43.9%) felt that patients shouldn't be informed about the risk of cancer. Otherwise, most radiologists aged 45 and above (67.5%) felt that patients should be informed about the risk of cancer ($p < 0.05$). The surveyors who have experienced 1-8 years and 9-20 years had similar opinions about the evaluation of their knowledge, sufficient (approx. 50.0%) and partly sufficient (approx. 50.0%). However, the fact that most of the radiologists with 21 years or more experience (86.2%) thought that their knowledge about informed consent was sufficient gave a statistically significant result ($p < 0.05$). Most of the radiologists who have experienced 1-8 years (72.7%), said that informed consent forms were given by secretariat/physician assistants. However, radiologists who have experienced 21 years and above reported that informed consent forms were given by DMF radiologists (38.9%) and radiology technicians (44.4%) mostly ($p < 0.05$).

While the South Americans radiologists stated that the written consent form would be sufficient, the Europeans and Far Easterners stated that it should be both written and verbal. Details are shown in Table 4.

Table 2. *Comparison of views on informed consent by gender, title, and institution*

		Gender			Title				Institution				
Item	Answer	Female	Male	p-value	Research assistant	Specialist	Lecturer	p-value	Private	State hospital	University hospital	p-value	Total participants
1	a	27 (62.8%)	27 (58.7%)	0.693	5 (50.0%)	39 (61.9%)	10 (62.5%)	0.763	14 (58.3%)	9 (52.9%)	31 (64.6%)	0.674	**54 (60.7%)**
	b	14 (32.6%)	18 (39.1%)		5 (50.0%)	22 (34.9%)	5 (31.3%)		9 (37.5%)	8 (47.1%)	15 (31.3%)		**32 (35.9%)**
	c	2 (4.7%)	1 (2.2%)		0 (0.0%)	2 (3.2%)	1 (6.3%)		1 (4.2%)	0 (0.0%)	2 (4.2%)		**3 (3.4%)**
2	a	32 (74.4%)	31 (67.4%)	0.473	6 (60.0%)	47 (74.6%)	10 (62.5%)	0.464	17 (70.8%)	13 (76.5%)	33 (68.8%)	0.834	**63 (70.8%)**
	b	6 (14.0%)	11 (23.9%)		2 (20.0%)	13 (20.6%)	2 (12.5%)		6 (25.0%)	3 (17.6%)	8 (16.7%)		**17 (19.1%)**
	c	5 (11.6%)	4 (8.7%)		2 (20.0%)	3 (4.8%)	4 (25.0%)		1 (4.2%)	1 (5.9%)	7 (14.6%)		**9 (10.1%)**
3	a	22 (51.2%)	15 (32.6%)	**0.023***	3 (30.0%)	25 (39.7%)	9 (56.3%)	0.356	9 (37.5%)	7 (41.2%)	21 (43.8%)	0.879	**37 (41.6%)**
	b	0 (0.0%)	6 (13.0%)		1 (10.0%)	5 (7.9%)	0 (0.0%)		1 (4.2%)	0 (0.0%)	5 (10.4%)		**6 (6.7%)**
	c	21 (48.8%)	25 (54.3%)		6 (60.0%)	33 (52.4%)	7 (43.8%)		14 (58.3%)	10 (58.8%)	22 (45.8%)		**46 (51.7%)**
4	a	26 (60.5%)	26 (56.5%)	0.830[a]	4 (40.0%)	36 (57.1%)	12 (75.0%)	0.197	13 (54.2%)	9 (52.9%)	30 (62.5%)	0.698	**52 (58.4%)**
	b	17 (39.5%)	20 (43.5%)		6 (60.0%)	27 (42.9%)	4 (25.0%)		11 (45.8%)	8 (47.1%)	18 (37.5%)		**37 (41.6%)**
5	a	17 (65.4%)	21 (80.8%)	0.669	4 (100.0%)	24 (66.7%)	10 (83.3%)	0.280	9 (69.2%)	6 (66.6%)	23 (76.7%)	0.554	**38 (29.2%)**
	b	19 (73.1%)	22 (84.6%)	0.832	4 (100.0%)	27 (75.0%)	10 (83.3%)	0.342	10 (76.9%)	8 (88.9%)	23 (76.7%)	0.878	**41 (31.6%)**
	c	25 (96.2%)	26 (100.0%)	0.999	4 (100.0%)	35 (97.2%)	12 (100.0%)	0.187	13 (100.0%)	8 (88.9%)	30 (100.0%)	0.508	**51 (39.2%)**
6	a	6 (30.0%)	6 (30.0%)	0.838	0 (0.0%)	7 (25.0%)	5 (50.0%)	0.251	1 (11.1%)	0 (0.0%)	11 (39.3%)	**0.007***	**12 (30.0%)**
	b	5 (25.0%)	8 (40.0%)		2 (100.0%)	8 (28.6%)	3 (30.0%)		3 (33.3%)	0 (0.0%)	10 (35.7%)		**13 (32.5%)**
	c	9 (45.0%)	6 (30.0%)		0 (0.0%)	13 (46.4%)	2 (20.0%)		5 (55.5%)	3(100.0%)	7 (25.0%)		**15 (37.5%)**
7	a	25 (58.1%)	19 (41.3%)	0.141	6 (60.0%)	27 (42.9%)	11 (68.8%)	0.141	7 (29.2%)	8 (47.1%)	29 (60.4%)	0.120	**44 (49.4%)**
	b	11 (25.6%)	21 (45.7%)		2 (20.0%)	25 (39.7%)	5 (31.3%)		11 (45.8%)	6 (35.3%)	15 (31.3%)		**32 (36.0%)**
	c	7 (16.3%)	6 (13.0%)		2 (20.0%)	11 (17.5%)	0 (0.0%)		6 (25.0%)	3 (17.6%)	4 (8.3%)		**13 (14.1%)**
8	a	16 (37.2%)	16 (34.8%)	0.194	4 (40.0%)	19 (30.2%)	9 (56.3%)	0.146	6 (25.0%)	4 (23.5%)	22 (45.8%)	0.272	**32 (36.0%)**
	b	18 (41.9%)	26 (56.5%)		4 (40.0%)	33 (52.4%)	7 (43.8%)		14 (58.3%)	9 (52.9%)	21 (43.8%)		**44 (49.4%)**
	c	9 (20.9%)	4 (8.7%)		2 (20.0%)	11 (17.5%)	0 (0.0%)		4 (16.7%)	4 (23.5%)	5 (10.4%)		**13 (14.1%)**
9	a	27 (62.8%)	30 (65.2%)	0.829[a]	6 (60.0%)	41 (65.1%)	10 (62.5%)	0.943	16 (66.7%)	7 (41.2%)	34 (70.8%)	0.087	**57 (64.0%)**
	b	16 (37.2%)	16 (34.8%)		4 (40.0%)	22 (34.9%)	6 (37.5%)		8 (33.3%)	10 (58.8%)	14 (29.2%)		**32 (36.0%)**
10	a	28 (65.1%)	24 (52.2%)	0.454	5 (50.0%)	36 (57.1%)	11 (68.8%)	0.595	12 (50.0%)	13 (76.5%)	27 (56.3%)	0.215	**52 (58.4%)**
	b	9 (20.9%)	14 (30.4%)		3 (30.0%)	17 (27.0%)	3 (18.8%)		7 (29.2%)	2 (11.8%)	14 (29.2%)		**23 (25.9%)**
	c	6 (14.0%)	8 (17.4%)		2 (20.0%)	10 (15.9%)	2 (12.5%)		5 (20.8%)	2 (11.8%)	7 (14.6%)		**14 (15.7%)**

*Pearson's chi-square test, a: Fisher's Exact test, *: significant at the 0.05 level. Details of the questions and answers are given in Table 1.*

Table 3. *Comparison of views on informed consent by age and experience*

		Age				Experience (years)			
Item	**Answer**	**22-30**	**30-45**	**45 and above**	**p-value**	**1-8**	**9-20**	**21 and above**	**p-value**
1	**a**	3 (37.5%)	23 (56.1%)	28 (70.0%)	0.339	16 (50.0%)	13 (46.4%)	25 (86.2%)	**0.014***
	b	5 (62.5%)	16 (39.0%)	11 (27.5%)		15 (46.9%)	14 (50.0%)	3 (10.3%)	
	c	0 (0.0%)	2 (4.9%)	1 (2.5%)		1 (3.1%)	1 (3.6%)	1 (3.4%)	
2	**a**	4 (50.0%)	31 (75.6%)	28 (70.0%)	0.338	24 (75.0%)	19 (67.9%)	20 (69.0%)	0.889
	b	3 (37.5%)	7 (17.1%)	7 (17.5%)		6 (18.8%)	6 (21.4%)	5 (17.2%)	
	c	1 (12.5%)	3 (7.3%)	5 (12.5%)		2 (6.3%)	3 (10.7%)	4 (13.8%)	
3	**a**	3 (37.5%)	18 (43.9%)	16 (40.0%)	0.237	15 (46.9%)	11 (39.3%)	11 (37.9%)	0.081
	b	1 (12.5%)	0 (0.0%)	5 (12.5%)		1 (3.1%)	0 (0.0%)	5 (17.2%)	
	c	4 (50.0%)	23 (56.1%)	19 (47.5%)		16 (50.0%)	17 (60.7%)	13 (44.8%)	
4	**a**	5 (62.5%)	22 (53.7%)	25 (62.5%)	0.701	19 (59.4%)	13 (46.4%)	20 (69.0%)	0.223
	b	3 (37.5%)	19 (46.3%)	15 (37.5%)		13 (40.6%)	15 (53.6%)	9 (31.0%)	
5	**a**	3 (60.0%)	14 (63.6%)	21 (84.0%)	0.236	11 (57.9%)	11 (84.6%)	16 (80.0%)	0.236
	b	3 (60.0%)	17 (77.3%)	21 (84.0%)	0.535	13 (68.4%)	12 (92.3%)	16 (80.0%)	0.481
	c	5 (100.0%)	21 (95.5%)	25 (100.0%)	0.563	18 (94.7%)	13 (100.0%)	20 (100.0%)	0.225
6	**a**	0 (0.0%)	3 (18.8%)	9 (40.9%)	**0.019***	1 (9.1%)	4 (36.4%)	7 (38.9%)	**0.040***
	b	1 (50%)	3 (18.8%)	9 (40.9%)		2 (18.2%)	3 (27.3%)	8 (44.4%)	
	c	1 (50%)	10 (62.5%)	4 (18.2%)		8 (72.7%)	4 (36.4%)	3 (16.7%)	
7	**a**	4 (50.0%)	13 (31.7%)	27 (67.5%)	**0.011***	17 (53.1%)	11 (39.3%)	16 (55.2%)	0.467
	b	4 (50.0%)	18 (43.9%)	10 (25.0%)		9 (28.1%)	12 (42.9%)	11 (37.9%)	
	c	0 (0.0%)	10 (24.4%)	3 (7.5%)		6 (18.8%)	5 (17.9%)	2 (6.9%)	
8	**a**	3 (37.5%)	11 (26.8%)	18 (45.0%)	0.155	13 (40.6%)	5 (17.9%)	14 (48.3%)	0.172
	b	4 (50.0%)	26 (63.4%)	14 (35.0%)		14 (43.8%)	18 (64.3%)	12 (41.4%)	
	c	1 (12.5%)	4 (9.8%)	8 (20.0%)		5 (15.6%)	5 (17.9%)	3 (10.3%)	
9	**a**	3 (37.5%)	28 (68.3%)	26 (65.0%)	0.248	22 (68.8%)	16 (57.1%)	19 (65.5%)	0.633
	b	5 (62.5%)	13 (31.7%)	14 (35.0%)		10 (31.3%)	12 (42.9%)	10 (34.5%)	
10	**a**	4 (50.0%)	25 (61.0%)	23 (57.5%)	0.296	21 (65.6%)	15 (53.6%)	16 (55.2%)	0.262
	b	1 (12.5%)	9 (22.0%)	13 (32.5%)		4 (12.5%)	9 (32.1%)	10 (34.5%)	
	c	3 (37.5%)	7 (17.1%)	4 (10.0%)		7 (21.9%)	4 (14.3%)	3 (10.3%)	

*Pearson's chi-square test, a: Fisher's Exact test, *: significant at the 0.05 level. Details of the questions and answers are given in Table 1.*

Table 4. *Comparison of views on informed consent by region*

		Region						
Item	**Answer**	**Europe**	**Middle East**	**Far East**	**South America**	**North America**	**Others**	**p-value**
1	**a**	12 (41.4%)	6(100.0%)	9 (81.8%)	16 (59.3%)	7 (70.0%)	4 (66.7%)	0.081
	b	17 (58.6%)	0 (0.0%)	2 (18.2%)	9 (33.3%)	2 (20.0%)	2 (33.3%)	
	c	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	1 (10.0%)	0 (0.0%)	
2	**a**	22 (75.9%)	5 (83.3%)	7 (63.6%)	14 (51.9%)	9 (90.0%)	6 (100.0%)	0.326
	b	5 (17.2%)	1 (16.7%)	3 (27.3%)	8 (29.6%)	0 (0.0%)	0 (0.0%)	
	c	2 (6.9%)	0 (0.0%)	1 (9.1%)	5 (18.5%)	1 (10.0%)	0 (0.0%)	
3	**a**	13 (44.8%)	3 (50.0%)	1 (%9.1)	16 (59.3%)	4 (40.0%)	0 (0.0%)	**0.001***
	b	2 (6.9%)	0 (0.0%)	4 (%36.4)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	c	14 (48.3%)	3 (50.0%)	6 (%54.5)	11 (40.7%)	6 (60.0%)	6 (100%)	
4	**a**	16 (55.2%)	4 (66.7%)	5 (45.5%)	15 (55.6%)	7 (70.0%)	5 (83.3%)	0.664
	b	13 (44.8%)	2 (33.3%)	6 (54.5%)	12 (44.4%)	3 (30.0%)	1 (16.7%)	
5	**a**	10 (62.5%)	1 (25.0%)	5 (100.0%)	15 (100.0%)	4 (57.1%)	3 (60.0%)	0.482
	b	12(75.0%)	1 (25.0%)	5 (100.0%)	15 (100.0%)	4 (57.1%)	4 (80.0%)	0.480
	c	15 (93.8%)	4(100.0%)	5 (100.0%)	15 (100.0%)	7 (100.0%)	5 (100.0%)	0.613
6	**a**	2 (50.0%)	2(50.0%)	3 (60.0%)	3 (20.0%)	1 (14.3%)	1 (20.0%)	0.450
	b	1 (25.0%)	2 (50.0%)	2 (40.0%)	4 (26.7%)	2 (28.6%)	2 (40.0%)	
	c	1 (25.0%)	0 (0.0%)	0 (0.0%)	8 (53.3%)	4 (57.1%)	2 (40.0%)	
7	**a**	12 (41.4%)	1 (16.7%)	4 (36.4%)	18 (66.7%)	6 (60.0%)	3 (50.0%)	0.132
	b	10 (34.5%)	4 (66.7%)	7 (63.6%)	7 (25.9%)	3 (30.0%)	1 (16.7%)	
	c	7 (24.1%)	1 (16.7%)	0 (0.0%)	2 (7.4%)	1 (10.0%)	2 (33.3%)	
8	**a**	9 (31.0%)	3 (50.0%)	6 (54.5%)	9 (33.3%)	4 (40.0%)	1 (16.7%)	0.699
	b	16 (55.2%)	2 (33.3%)	5 (45.5%)	14 (51.9%)	3 (30.0%)	4 (66.7%)	
	c	4 (13.8%)	1 (16.7%)	0 (0.0%)	4 (14.8%)	3 (30.0%)	1 (16.7%)	
9	**a**	19 (65.5%)	4 (66.7%)	5 (45.5%)	15 (55.6%)	10 (100.0%)	4 (66.7%)	0.147
	b	10 (34.5%)	2 (33.3%)	6 (54.5%)	12 (44.4%)	0 (0.0%)	2 (33.3%)	
10	**a**	16 (55.2%)	4 (66.7%)	3 (27.3%)	17 (63.0%)	6 (60.0%)	6 (100.0%)	0.122
	b	5 (17.2%)	2 (33.3%)	6 (54.5%)	7 (25.9%)	3 (30.0%)	0 (0.0%)	
	c	8 (27.6%)	0 (0.0%)	2 (18.2%)	3 (11.1%)	1 (10.0%)	0 (0.0%)	

*Pearson's chi-square test, *: significant at the 0.05 level. Details of the questions and answers are given in Table 1.*

4. DISCUSSION

For a patient referred for radiological imaging, obligations such as non-maleficence and the benefits of the procedure occur. While acknowledging that ionizing radiation can cause harm, it should provide diagnostic benefits to the patient (11). The risk of dental imaging is lower than medical imaging (6). However, information should be given to patients about radiological applications. Furthermore, with active information, patients also provide the authority to oversee their health care (12). There is no obvious consensus on whether to obtain informed consent or which consent should be obtained from patients exposed to ionizing radiation during radiological examinations (4). The failure to obtain proper informed consent is medical malpractice. This process should provide enough complete information to allow the patient to understand the consequences of the decision and to allow the patient to make an informed decision (1). In this context, it should be presented to patient information about why the application of imaging is necessary, which techniques can be used, the benefits, risks, cost, and duration of the techniques. Consent is an effective and mutual communication method between the patient and the physicians. Both verbal and written consent accepted express consent forms (13). It is a general legal and ethical principle to obtain valid consent before starting a treatment or physically examining a patient or conducting research involving human participants (2). Lee et al. (14) reported that only 7% of the patients were informed about the risks of computed tomography scans. The main purpose of the current study was to obtain the opinions of DMF radiologists in dental radiology practices regarding informed consent. In this study, 70.8% of the participants were required informed consent in dental radiological procedures, while 19.1% of the participants stated that it was not necessary.

To inform the patient, the dentist must accurately explain to the patient the diagnosis of the problem, the proposed way of treating or managing it with other probable approaches (including no management), and the risks/benefits (8). Radiographic examinations should be performed after reviewing the patients' medical and dental histories and a detailed clinical examination. There is a justification principle in the choice of radiographic method. Radiography is performed when the expected diagnostic benefit outweighs the biological risk of exposure to ionizing radiation (8). In Europe, the Euratom law emphasizes the necessity to justify the need for a radiological examination before a patient is referred to a radiologist (12).

In radiology practice, obtaining mandatory written informed consent from patients in every medical imaging procedure (using ionizing radiation) may cause some problems. These problems are the following; unnecessary anxiety in patients especially after being informed, the refusal of patients to radiological procedures required for individuals (12). There is often a limited patient-physician (radiologist) relationship in radiology clinics (15). Semelka et al. (12) argue that informed consent should be required for procedures with higher radiation exposure. They suggested the necessity of this approval for procedures containing high dose radiation, especially those containing 1 mSv and above, such as CT, positron emission tomography, and fluoroscopy (12). In the literature, the application of "informed consent" in dental radiology procedures can be considered "implied consent" has been reported. It is argued that the patient's obligation to inform is not lifted with implied consent, but in interventions involving low risks, the illumination can be too narrow or even neglected (3). The results of this study showed that a significant portion of the participants (41.6%) did not obtain informed consent before dental radiography procedures in their clinics. Approximately one-quarter of the DMF radiology specialists reported that they obtained consent from patients before intraoral radiography applications, 31.6% before panoramic radiography/extraoral radiography procedures, and 39.2% before cone-beam CT scans.

It is the responsibility of physicians to provide all information about the risks, benefits, and alternatives of applications to be made in the decision-making process of patients (1). Previous studies showed that the awareness of physicians is insufficient for the doses and risks of radiation (16). In a study about informing the patients about the risks and benefits of radiological examinations, the authors reported that physicians requesting radiography did not have sufficient information about the radiation dose (15). Written or verbal information given to the patient may vary depending and the dose and the risk (12). In a previous study, Wright (6) emphasized the requirement for the physician requesting radiography to explain the risks of radiation doses in panoramic radiography and cone-beam CT examinations, as well as the necessity to inform the patient about the radiologic risks for the physicians who keep cone-beam CT or panoramic radiographs in their facilities.

To the best of our knowledge, there is a published study evaluating the knowledge and awareness of DMF radiologists regarding informed consent in dental radiology practices. In the study conducted in Turkey, it was reported that most participants did not inform patients about radiation (7). In radiography implementations, three different professional roles are usually played by the physician who makes the request, the radiologist, and the radiology technician. Considering the involvement of both referring physicians and radiology staff (radiologists and technicians) in the medical imaging procedure, the question arises of who should explain the risk of ionizing radiation (11). However, it has been reported that the informed consent process for medical imaging examinations containing ionizing radiation should start with the referring physician and that explanation support should be provided by the radiologist (17). In our study, while most participants (64.0%) stated that the radiology personnel should provide information to the patient about the radiation risk, 36.0% of the participants reported that referring physicians should make an explanation.

Consent is an effective and mutual communication method between the patient and the physician (13). Three methods are generally used to obtain consent from patients: implied consent, verbal consent, written consent (2,3). Both verbal and written consent is express consent forms (13). Generally, obtaining written informed consent applies to all invasive procedures and all applications that fall within the patient's physical space. The most common applications of obtaining written consent in radiology are mostly before interventional radiology procedures (12). The fact that the informed consent form was signed by both the physician and the patient does not indicate that the physician alone fulfills the ethical/legal responsibilities (9). In our study, approximately half of the participants (51.7%) stated that the informed consent form should be in a written (signed by patient and physician) form from the patients, and 41.6% of them said that it should be both written and verbal.

5. CONCLUSIONS

The results of this study showed that most of the DMF radiology physicians said that they have responsibility for getting informed consent. However, a significant portion of the participants stated that they did not obtain informed consent before dental radiography procedures in their clinics. It was found that only one-third of the participants inform patients about the radiation risks. Further studies can be conducted to compare the views and attitudes of DMF radiologists and medical radiologists by increasing the sample size regarding informed consent in radiology. In addition, the results of this study showed that it is necessary to focus on continuing education on this subject.

Acknowledgement: This study was presented at the 23rd International Congress of DentoMaxilloFacial Radiology, 28 April – 1 May 2021, Gwangju, South Korea.

Conflict of interest: All authors declare that they have no conflict of interest.

Funding: None.

Ethical approval: Ethical approval for the study was received from Pamukkale University Ethics Committee (Number: 60116787-020/37899; Date of approval: 25/06/2020). This study was conducted by following the Declaration of Helsinki.

Informed consent: Informed consent was obtained from the participants for inclusion in the study.

REFERENCES

[1] Carpeggiani C, Picano E. The radiology informed consent form: recommendations from the European Society of Cardiology position paper. J Radiol Prot 2016;36:175–186.

[2] Kakar H, Gambhir RS, Singh S, Kaur A, Nanda T. Informed consent: corner stone in ethical medical and dental practice. J Family Med Prim Care 2014;3:68–71.

[3] Harorlı A, Çaglayan F. Diş hekimliği radyolojisinde aydınlatılmış onam. Turk Klin Oral Maxillofac Rad Special Topics 2019;5:57–68.

[4] Doudenkova V, Pipon JCB. Duty to inform and informed consent in diagnostic radiology: how ethics and law can better guide practice. HEC Forum 2016;28:75–94.

[5] Hadley LC, Watson T. The radiographers' role in information giving prior to consent for computed tomography scans: A cross-sectional survey. Radiography 2016;22:252–257.

[6] Wright B. Contemporary medico-legal dental radiology. Aust Dent J. 2012;57:9–15.

[7] Akay G, Ozdede M, Karadag O. The perceptions and attitudes of Dentomaxillofacial radiologists towards informed consent. Yeditepe J Dent 2021;17:123–127.

[8] Geist JR. Informed refusal in oral and maxillofacial radiology: Does it exist? Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:A8–A10.

[9] Kurt E, Turker T. The evaluation of dentists' views about informed consent who works in university hospital. Cumhuriyet Dent J 2015;18:56–70.

[10] Karsli T, Kalra MK, Self JL, Rosenfeld JA, Butler S, Simoneaux S. What physicians think about the need for informed consent for communicating the risk of cancer from low-dose radiation. Pediatr Radiol 2009;39:917–925.

[11] Younger CWE, Douglas C, Warren-Forward H. Ionizing radiation risk disclosure: when should radiographers assume a duty to inform? Radiography (Lond) 2018;24:146–150.

[12] Semelka RC, Armao DM, Elias J Jr, Picano E. The information imperative: is it time for an informed consent process explaining the risks of medical radiation? Radiology 2012;262:15–18.

[13] Baheti AD, Thakur MH, Jankharia B. Informed consent in diagnostic radiology practice: Where do we stand? Indian J Radiol Imaging 2017;27:517–520.

[14] Lee CI, Haims AH, Monico EP, Brink JA, Forman HP. Diagnostic CT scans: assessment of patient, physician, and radiologist awareness of radiation dose and possible risks. Radiology 2004;231:393–398.

[15] Cardinal JS, Gunderman RB, Tarver RD. Informing patients about risks and benefits of radiology examinations: a review article. J Am Coll Radiol 2011;8:402–408.

[16] Brown N, Jones L. Knowledge of medical imaging radiation dose and risk among doctors. J Med Imaging Radiat Oncol 2013;57:8–14.

[17] Younger CWE, Douglas C, Warren-Forward H. Informed consent guidelines for ionising radiation examinations: A Delphi study. Radiography (Lond) 2020;26:63-70.

How to cite this article: Akay G, Ozdede M, Karadag Atas O. Informed Consent in Dentomaxillofacial Radiology: A Cross-Sectional Study. Clin Exp Health Sci 2022; 12: 683-690. DOI: 10.33808/clinexphealthsci.1012561

Clinical and Experimental Health Sciences

The Effects of Cyanoacrylate on Clinical Healing and Self-Reported Outcomes Following Free Gingival Graft Surgery: A Randomized Clinical Study

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Received: 20.10.2021 **Accepted:** 08.03.2022

ABSTRACT

Objective: This study aims to reveal the effects of cyanoacrylate application at the recipient bed and the donor site in free gingival graft surgery on graft dimensions, clinical healing parameters and patient-based outcomes.

Methods: Free gingival graft operations were conducted on individuals who were randomly assigned to control or test groups. In the test group, the graft was stabilized and the donor site was coated with cyanoacrylate. In the control group, 6/0 polyvinylidene fluoride sutures were used for stabilization, while the donor site was left untreated. Variables including re-epithelization, post-operative complications, pain, bleeding and quality of life at recipient site, and color match, graft dimensions at donor site were assessed for up to 6 months.

Results: Twenty-three individuals completed the study. No differences were observed in any variable between groups except horizontal dimension loss of the graft, which was more abundant in the cyanoacrylate group at six months ($p>0.05$, $p<0.05$; respectively).

Conclusion: According to our results, cyanoacrylate can be used safely for free gingival graft surgery, but does not surpass conventional suturing with polyvinylidene fluoride.

Keywords: Cyanoacrylates, sutures, tissue grafts, patient comfort

1. INTRODUCTION

Although debatable, the presence of an 'adequate' band of keratinized mucosa, is thought to enhance the integrity of the periodontium, and to provide sufficient biofilm control (1). Hence, in cases where a lack of gingiva hinders oral hygiene practice in the region or elicits pain in chewing, soft tissue augmentation can be implemented. Free gingival grafts (FGGs) are widely preferred for augmentation since the procedure ensures predictable results (2). FGG was first described by Björn in 1963 while Sullivan and Atkins (1968) helped the technique to become widespread by stating the details and the major principles of the surgery and the expected wound healing (3). The graft is generally harvested from the palatal mucosa, which provides sufficient tissue and allows easy access for the surgeon. Although it is a relatively safe and secure procedure, secondary healing of the donor site may cause some complications such as bleeding, tissue necrosis, delayed wound healing, post-operative pain and loss of sensation. Hence, researchers focus on minimizing these complications, eventually to increase patient comfort and enhance the success of the surgery (4,5).

Cyanoacrylates (CAs) are widely used in medicine as tissue adhesives. Comprehensive hemostatic, bacteriostatic and bactericidal effects can attach tissues firmly and presumably allow healing with less scar formation (6,7). The monomer form of CA is liquid, but it starts to polymerize the moment it contacts body fluids, gaining imminent adhesive properties which allow easy application of the material (8). By considering these features, surgeons can save time and effort with CA when compared to conventional wound closure. In particular, in situations where classical suturing techniques are insufficient to ensure tissue integrity and haemostasia, the use of CA can be very beneficial. CA can also arguably act as a mechanical barrier and accelerate wound healing and epithelial keratinization (9). This may theoretically enhance healing and eventually help patients to go through a more comfortable post-operative period.

Oral health-related quality of life is a patient-based measure, exemplifying the impact of oral status on an individual's life quality (10). It is an acknowledged indicator of therapeutical need and treatment outcomes (11), whereas patient-based results do not always conform to clinical conclusions. The aim

DOI: 10.33808/clinexphealthsci.1012775

of the study is to compare n-butyl-cyanoacrylate use in free gingival graft to conventional suturing with polyvinylidene fluoride regarding clinical healing parameters and self-reported outcomes following surgery.

2. METHODS

The study's accordance to the ethical guidelines of Helsinki Declaration was approved by the Clinical Research Ethics Committee of Istanbul Biruni University 2015-KAEK-43-18-01. The study was retrospectively registered in ClinicalTrials.gov (NCT04854902).

Sample size was calculated with G*Power 3: two-tail; α=0.05; power (1-β)=0.8. Required size of *n*=11 for each group was determined with the actual power of 0.815. Twenty-three systemically healthy, non-smoking volunteers over 18 years of age, lacking keratinized mucosa in the mandibular anterior region (width ≤ 1mm) with progressive gingival recession and/or discomfort in chewing or with oral hygiene practice, were recruited in Biruni University, Faculty of Dentistry, Turkey between September 2018 and June 2020. Pregnant or lactating patients, and patients on medication were excluded. Prior to enrolment, informed consent was obtained from the participants.

The participants were added to the cyanoacrylate group (CA) or to the suture group (S), based on computer-aided randomization. The surgery and 3rd day evaluation of the donor site were conducted by the same clinician (MY), while the rest of the evaluations were performed by another examiner (BK). Patient follow-up was performed for 6 months after surgery.

2.1. Parameters

Donor site assessment:

Initial mucosal thickness: The mucosal thickness of the donor area was evaluated under local anesthesia at the beginning of surgery. The measurement was conducted with the help of a 15 endodontic reamer which was inserted into the palatal mucosa 5 mm apical of the gingival margin of the second premolar and read with an electronic caliper.

Re-epithelization: The donor site was evaluated at first, second – and third-week intervals following the operation. The completion of epithelization was visually controlled and recorded dichotomously. Hydrogen-peroxide was applied to the donor area with the help of an injector, and it was accepted as complete when no foaming occurred.

Donor site complications: Complications such as pus formation or necrosis in the donor site were recorded on the third day, and at one and two weeks following the operation.

Paresthesia / hyperesthesia in the donor area: At one month control, the donor site was gently rubbed with the help of a periodontal probe, and the patients were asked to point out any difference between the symmetrical reference areas on either side, where the probe was first applied.

Self-reported outcomes:

Pain: The patients were asked to evaluate the level of pain they felt and accordingly mark a visual analogue scale (VAS), which ranged from 0 (no pain) to 10 (unbearable), at weekly intervals within the first four weeks following surgery. The number of painkiller intake was recorded.

Post-operative comfort and quality of life: The patients were asked to fill in Oral Health Impact Profile (OHIP-14) forms at baseline, 3 days, and 1 and 4 weeks (12).

Secondary bleeding: The patients were asked to report any excessive bleeding following surgery.

The evaluation of the graft

Graft dimensions: Mesio-distal (horizontal) and apico-coronal (vertical) width of the graft was measured using a Williams periodontal probe (Hu Friedy), at 1, 3 and 6 months. Measurements were rounded to the closest mm.

Color match: The color harmony of the graft with neighboring keratinized mucosa was evaluated on a scale ranging from 0 (no color match) to 10 (excellent color match).

2.2. Procedure

Dental prophylaxis, oral hygiene training and motivation, and, if necessary, occlusal rehabilitation were performed before the surgical procedure. The surgery was conducted under local anesthesia (Ultracain D-S, Sanofi Aventis) with two identical 15C surgical blades, each used separately for recipient bed preparation and for harvesting.

At the mucogingival junction in the recipient site, an initial horizontal incision was performed. A split-thickness flap was prepared and extended apically until a sufficient area (~7x13 mm) was procured. Immobile connective tissue/periosteum was left on the recipient bed where remaining muscles and loose connective tissue were excised. A 5x10 mm graft was harvested from the hard palate with the guide of a sterile aluminum foil, between the first premolar and the first molars, leaving 2 mm of safety distance with the gingival margins of adjacent teeth. Adipose tissues and irregularities were removed, paying attention to keeping the thickness at ~1.5 mm and the graft size as planned. Immediately after, wet gauze pressure was applied for 5 minutes to control hemorrhage in the donor site.

In the control group, the coronal part of the graft was sutured to the recipient bed with 6/0 polyvinylidene fluoride (PVDF; Trofilen, Dogsan), and a horizontal matrix suture was placed as specified by Holbrook & Ochsenbein (13). In the test group, cyanoacrylate (Indermil, Connexicon Medical) was applied only to the coronal and lateral edges of the graft for stabilization (14). Thereafter, in both groups, 5 minutes of gauze pressure was applied to minimize granulation tissue formation and to increase the surface contact between the graft and the underlying connective tissue. The donor site was left untreated in the control group, while in the test

group a thin layer of cyanoacrylate was applied as a dressing, fully covering the wound.

Chlorhexidine digluconate (0.12%) mouthwash (Klorhex, Drogsan) was prescribed for use two times per day for two weeks. Flurbiprofen tablets (Majezik, Sanovel Pharmaceutical) were prescribed, and the patients were warned to note how many pills they took during the following time period. The participants were asked not to perform oral hygiene practice in the operation area for two weeks, and a soft-diet was recommended. Sutures were removed and the surgical sites were washed with saline at the end of the first post-operative week. The final evaluation of the graft dimensions was conducted at post-operative 6 months.

2.3. Statistical Evaluation

The Statistical Package for the Social Sciences (SPSS 21, Inc., Chicago, IL, USA) was used for statistical analysis. First, descriptive statistics for each variable were calculated. Prior to hypothesis testing, data were examined via the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances according to parametric test assumptions. The Mann-Whitney U-Test was conducted to test the difference between the study groups and data violating the assumptions associated with parametric distribution, while the Student's t-Test for Independent Samples was used for the data meeting the assumptions. The Freidman test was applied to examine gradual changes in the measurements over time. The Dunn multiple comparisons test was used for post-hoc testing procedure. The Spearman correlation coefficient was performed to examine the relationship between variables. $P<0.05$ was considered as statistically significant.

3. RESULTS

28 individuals were recruited, while one patient was excluded during the recall sessions due to SARS-CoV-2 infection. 13 patients in CA (mean age 44 ± 9.31; 13 females) and 14 patients in S (mean age 33.36 ± 7.92; 9 females, 5 males) – a total of 27 patients (mean age 38.48 ± 10.04; 22 females, 5 males) – completed the study.

None of the patients reported any excessive post-operative bleeding. No sign of necrosis or infection was seen in the surgical areas in any timeframe.

Post-operative pain scores (VAS) in the first week were found to be highest, while they decreased gradually thereafter in both groups, with no statistically significant difference at any time point ($p>0.05$) (Table 1). Two patients reported ongoing slight pain at the fourth week. The number of painkillers used in the first post-operative week was also similar in both groups (CA: 3.85 ± 3.58; S: 3.93 ± 3.83; $p>0.05$), while all the patients, without any exceptions, reported that they had stopped taking painkillers following the first week. There was a negative and moderately significant correlation between mucosal thickness and pain scores in the first week ($r= 0.429$, $p<0.05$).

Table 1. *Pain (VAS) and OHIP-14 scores over time*

	VAS *(Mean ± SD)*			**OHIP-14** *(Mean ± SD)*		
	Cyanoacrylate	**Control**	*P-value*	**Cyanoacrylate**	**Control**	*P-value*
Baseline	-	-	-	3.77 ± 4.8 ^{b}	5.79 ± 7.01 ^{ab}	0.28
3rd day	-	-	-	5 ± 4.76 ^{b}	8.5 ± 7.84 ^{ab}	0.18
1st week	2.54 ± 3.45 ^{a}	2.64 ± 2.31^{a}	0.375	4.15 ± 4.51 ^{ab}	8.14 ± 7.03 ^{a}	0.105
2nd week	1.23 ± 2.42 ^{ab}	0.71 ± 0.99^{b}	0.867	-	-	-
3rd week	0 ± 0 ^{b}	0.21 ± 0.43 ^{b}	0.35	-	-	-
4th week	0.15 ± 0.55 ^{ab}	0.14 ± 0.36 ^{b}	0.83	1.08 ± 2.22 ^{a}	4.14 ± 7.95 ^{b}	0.325
*P-value**	0.001	<0.001		0.007	0.016	

VAS: Visual analogue scale scores; OHIP-14: oral health impact profile-14. SD: standard deviation. a, b: Values in the same column with different superscripts represent statistical differences at investigated timeframes in each individual group. P-value*: The significance of the differences between recall sessions in each group. P-value: The significance of the difference between groups

Table 2. *The graft dimensions*

	Graft dimensions *(Mean ± SD; mm)*					
	Horizontal dimension		*P-value*	**Vertical dimension**		*P-value*
	Cyanoacrylate	**Control**		**Cyanoacrylate**	**Control**	
Baseline	10 ± 0 ^{a}	10 ± 0 ^{a}		5 ± 0 ^{a}	5 ± 0 ^{a}	
1st month	9.38 ± 0.87 ^{ab}	9.64 ± 0.93 ^{ab}	0.375	4.69 ± 0.63 ^{ab}	4.57 ± 0.51 ^{ab}	0.488
3rd month	8.85 ± 1.21 ^{bc}	9.36 ± 1.08 ^{bc}	0.239	4.46 ± 0.66 ^{ab}	4.36 ± 0.5 ^{b}	0.583
6th month	8.15 ± 1.34 ^{c}	9.29 ± 1.27 ^{c}	0.017	4.38 ± 0.65 ^{b}	4.36 ± 0.5 ^{b}	0.83
*P-value**	<0.001	0.004		0.002	<0.001	

SD: standard deviation. a, b: Values in the same column with different superscripts represent statistical differences at investigated timeframes in each individual group. P-value*: The significance of the differences between recall sessions in each group; P-value: The significance of the difference between groups

OHIP-14 scores of CA and control also did not show any statistically significant difference with each other at any time point, with a decrease in both groups at the end of the first month relative to pre-operative values (p>0.05) (Table 1). This decrease was statistically significant only in the cyanoacrylate group (p<0.05). There was no loss of sensation in the donor sites of the subjects at the first month recall.

The mean mucosa thickness of the CA group was found to be 3.9 mm ± 0.91 mm, while that of the S was 4.05 mm ± 0.92 mm (p> 0.05). Epithelization of the donor site was not completed in any patient in either group in the first post-operative week. At the second week recall, four patients (30.8%) in the cyanoacrylate group and three patients (21.4%) in the control group showed no more foaming at the site, whereas at the third week the remaining subjects finished re-epithelization. Mean donor site tactile scores evaluating paresthesia at the first month were 9.85 ± 0.55 in cyanoacrylate and 9.21 ± 1.81 in the suture group (p>0.05).

In both study groups, a statistically significant decrease in vertical and horizontal dimensions occurred (CA: 36 ± 8.25 mm^2; S: 40.64 ± 8.09 mm^2) whereas only at the 6th month was the change in horizontal dimensions of the control significantly less than CA (p<0.05) (Table 2). No significant difference was found between the groups in any time period evaluated in terms of color harmony (p>0.05) (Table 3).

Table 3. *Color harmony of the graft with the neighboring keratinized tissue at the recipient site*

	Color harmony *(Mean ± SD)*		
	Cyanoacrylate	**Control**	*P-value*
1st week	4.92 ± 2,18 [b]	5.21 ± 1,48 [b]	0.867
2nd week	6.15 ± 1,14 [ab]	6.21 ± 1,31 [a]	0.905
1st month	6.62 ± 0,77 [a]	6.14 ± 1,03 [a]	0.185
3rd month	6.31 ± 1,03 [ab]	5.86 ± 0,86 [ab]	0.169
6th month	6.15 ± 0,9 [ab]	5.71 ± 0,91 [ab]	0.116
*P-value**	0.015	0.006	

SD: standard deviation. a, b: Values in the same column with different superscripts represent statistical differences at investigated timeframes in each individual group. P-value*: The significance of the difference between recall sessions in each group; P-value: The significance of the difference between study groups

4. DISCUSSION

Suturing is considered to be effective in wound closure, but it can be time consuming. In addition, suture removal can provoke anxiety and pain in many patients. Cyanoacrylates can be used as an alternative with the advantages of fast and easy application, and of their hemostatic, bacteriostatic and bactericidal features (15). There are various forms of CA, based on the length and complexity of the chains (16). N-Butyl-2-Cyanoacrylate has been reported not to cause any immediate or long-term systemic harm, hence is deemed safe and suitable for oral surgery (17).

This study was designed to compare the clinical and patient-based results of cyanoacrylate and conventional closure with PVDF in FGG surgery, evaluating pain, quality of life, graft dimensions, re-epithelization and post-operative complications. These parameters were investigated all together, since they are all interrelated. Psychosocial factors are known to have an impact on wound healing and pain, while concurrent periodontal treatment may affect the quality of life (18). Hence, life quality was the major variable of the planned study, yet relatively low OHIP-14 scores were detected in both groups, at all sessions. This indicates that neither of the techniques drastically affect life quality. Hence, the post-operative period of both can be considered comfortable, while healing parameters come to prominence when considering which technique to apply.

Although limited in number, available studies investigating cyanoacrylate in palatal wound coverage reported less post-operative pain and less painkiller intake as a result (19,20). Tavelli and colleagues reported that only suturing the palatal site caused significantly more pain (19). Accordingly, palatal application of cyanoacrylate appears to result in better outcomes in this regard. They proposed that coating the wound with a gelatin sponge combined with cyanoacrylate constitutes the best option in reducing post-operative pain and discomfort. Stavropoulou *et al.* (5), on the other hand, reported no statistically significant difference in pain when comparing cyanoacrylate with 6-0 polytetrafluoroethylene (PTFE) sutures in the donor site of subepithelial connective tissue grafts. This can be related to the suture material used in their study inducing low inflammatory response. We left the donor site untreated in the control group, in order not to provoke more redundant inflammation, which possibly affected our results. We cannot be precisely sure of this because there are two separate surgical sites with the potential to elicit pain, and this can be considered a limitation of our study. Nevertheless, no difference was noticed between the groups regarding pain perception following surgery. This may also be due to the suture material (PVDF) used in our study, which shows relatively less plaque accumulation and bacterial contamination, procuring minimal inflammatory response (21, 22).

Immobility is particularly important during the healing of free gingival graft, while it ensures the nourishment and survival of the graft without hindrance (3). Assuming that it can be achieved with both sutures and adhesives (15), the severity of inflammatory response can be indicative in recovery performance. Suture materials can cause inflammation and foreign body reaction in the oral mucosa (23). Meanwhile, dental biofilm and debris accumulation in the surgical area and on the suture thread can adversely influence healing (21). As stated above, N-Butyl-2-Cyanoacrylate has been shown to have bacteriostatic and bactericidal effects. However, the data in the available literature regarding the effects of cyanoacrylates in oral surgery are contradictory. Some researchers reported an increase in inflammatory biomarker levels following cyanoacrylate use when compared to various suture materials such as poliglecaprone, silk,

and polyglactin, eventually causing poor recovery (24). On the contrary, according to a study evaluating its use in flap surgery, cyanoacrylate resulted in less inflammatory response and enhanced clinical and histological healing when compared to silk sutures (25). It has also been speculated that cyanoacrylate promotes haemostasia and rapid clinical re-epithelialization and resolution of inflammation (26,27). Yet, according to the results of another study, it does not seem to accelerate epithelization (24). These conflicting results substantiate the need of further research regarding cyanoacrylate use in periodontal surgery.

In this study, no impact of cyanoacrylate on the re-epithelization rate of the donor site was observed. In a study comparing the effects of platelet-rich fibrin and butyl-cyanoacrylate on palatal wound healing, no significant difference between cyanoacrylate (26.1%) and open wound (12.2%) groups was detected in the second week (20). Similar results for complete epithelization were observed in our study (CA: 30.8%; S: 21.4%) in the second week recalls, demonstrating no significant difference.

According to our results, free gingival graft shrinks gradually with time compared to baseline, but this decrease became statistically significant only at 3 months in both study groups. We observed relatively more reduction in the horizontal dimension at 6 months in the cyanoacrylate group. This can be related to the greater horizontal dimension of the graft, expressing the difference between the groups more significantly. Similarly, prior publications disclosed much more abundant dimension loss in width than length, which can be due to recipient bed treat or other yet unknown factors (28, 29). There are two prior publications evaluating the effects of cyanoacrylate use on graft shrinkage, with conflicting results: in one study comparing cyanoacrylate with 7-0 propylene and 5-0 propylene sutures, the researchers observed a decrease in graft size in all groups, while cyanoacrylate showed significantly less shrinkage in all control sessions (14). In addition, they reported less pain with cyanoacrylate. On the other hand, Barbosa *et al*. stated that the use of cyanoacrylate, when compared to conventional suturing, did not differ in regard to graft shrinkage, concluding that it has no impact on healing (28). Our results are not compatible with either study, suggesting a greater decrease of width in the cyanoacrylate group. The horizontal matrix suture used in our control group to stabilize the graft more stringently might have had an impact on this result, but that is yet to be proven. This is more likely due to differences in study design and measurement tools, suggesting that further standardized studies be conducted.

Another finding of our study concerns the proportionally decreasing pain scores with palatal mucosa thickness. Burkhardt *et al.* reported that thicker mucosa reduced pain, which is in accordance with our results (30). In line with this finding, further studies can be conducted in which study groups are formed according to palatal thickness and donor site treatment. Although our data did not indicate any statistically significant effect of gender and age, another limitation of our study is the confined number of subjects, particularly regarding distribution. Another limitation of our study is that the color match and graft size were evaluated conventionally, whereas the digital methods can provide objective and consistent results comparing surgical approaches, regarding particularly graft dimensions (31). However, VAS scoring of color match by blinded examiners and measuring graft dimensions with the help of a periodontal probe, the methods applied in our study are commonly used in similar study designs (32, 33).

5. CONCLUSIONS

Within the limitations of our study, it can be concluded that cyanoacrylate use in free gingival graft does not outperform conventional suturing with PVDF with regard to healing outcomes and post-operative pain or life quality. Thus, both materials can be used in free gingival graft operations without any significant superiority to each other. Consecutive research conducted in larger populations and comparing different cyanoacrylate forms and suture materials could be beneficial.

Acknowledgements

The authors would like to thank to Prof. Sermet Şahin and to Nazli Gül Kınoğlu for their valuable help.

Conflict of interest

The authors have declared no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Zucchelli G, Mounssif I. Periodontal plastic surgery. Periodontol 2015;68(1):333-368.

[2] Miller PD. Root coverage using a free soft tissue autograft following citric acid application. Part 1: Technique. Int J Periodontics Restorative Dent 1982;2(1):65-70.

[3] Sullivan HC, Atkins JH. Free autogenous gingival grafts. I. Principles of successful grafting. Periodontics 1968;6(3):121-129.

[4] Zucchelli G, Mounssif I, Mazzotti C, et al. Does the dimension of the graft influence patient morbidity and root coverage outcomes? A randomized controlled clinical trial. J Clin Periodontol 2014;41(7):708-716.

[5] Stavropoulou C, Atout RN, Brownlee M. A randomized clinical trial of cyanoacrylate tissue adhesives in donor site of connective tissue grafts. J Periodontol 2019;90(6):608-615.

[6] Eaglstein WH, Sullivan T. Cyanoacrylates for skin closure. Dermatol Clin 2005;23(2):193-198.

[7] Montanaro L, Arciola CR, Cenni E. Cytotoxicity, blood compatibility and antimicrobial activity of two cyanoacrylate glues for surgical use. Biomaterials 2000;22(1):59-66.

[8] Miller GM, Dannenbaum R, Cohen DW. A Preliminary Histologic Study of the Wound Healing of Mucogingival Flaps

When Secured With the Cyanoacrylate Tissue Adhesives. J Periodontol 1974;45(8.2):608-618.

[9] Ghoreishian M, Gheisari R, Fayazi M. Tissue adhesive and suturing for closure of the surgical wound after removal of impacted mandibular third molars: A comparative study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108(1):e14-16.

[10] Petersen PE. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century – The approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol 2003;31Suppl 1:3-23.

[11] Ng SKS, Leung WK. A community study on the relationship of dental anxiety with oral health status and oral health-related quality of life. Community Dent Oral Epidemiol 2008;36(4):347-356.

[12] Ozcelik O, Haytac MC, Seydaoglu G. Immediate post-operative effects of different periodontal treatment modalities on oral health-related quality of life: A randomized clinical trial. J Clin Periodontol 2007;34(9):788-796.

[13] Holbrook T, Ochsenbein C. Complete coverage of the denuded root surface with a one-stage gingival graft. Int J Periodontics Restorative Dent 1983;3(3):8-27.

[14] Gümüş P, Buduneli E. Graft stabilization with cyanoacrylate decreases shrinkage of free gingival grafts. Aust Dent J 2014;59(1):57-64.

[15] Borie E, Rosas E, Kuramochi G. Oral Applications of cyanoacrylate adhesives: A literature review. BioMed Res Int 2019; Mar 17:8217602.

[16] Toriumi DM, Raslan WF, Friedman M. Histotoxicity of Cyanoacrylate Tissue Adhesives: A Comparative Study. Arch Otolaryngol Neck Surg 1990;116(5):546-550.

[17] Inal S, Yilmaz N, Nisbet C. Biochemical and histopathological findings of N-Butyl-2-Cyanoacrylate in oral surgery: an experimental study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006 Dec;102(6):e14-17.

[18] Åslund M, Suvan J, Moles DR. Effects of Two different methods of non-surgical periodontal therapy on patient perception of pain and quality of life: A randomized controlled clinical trial. J Periodontol 2008;79(6):1031-1040.

[19] Tavelli L, Asa'ad F, Acunzo R. Minimizing patient morbidity following palatal gingival harvesting: A randomized controlled clinical study. Int J Periodontics Restorative Dent 2018;38(6):e127-134.

[20] Ozcan M, Ucak O, Alkaya B.Effects of platelet-rich fibrin on palatal wound healing after free gingival graft harvesting: A comparative randomized controlled clinical trial. Int J Periodontics Restorative Dent 2017;37(5):e270-278.

[21] Parirokh M, Asgary S, Eghbal MJ.A scanning electron microscope study of plaque accumulation on silk and PVDF suture materials in oral mucosa. Int Endod J 2004;37(11):776-781.

[22] Kakoei S, Baghaei F, Dabiri S.A comparative in vivo study of tissue reactions to four suturing materials. Iran Endod J 2010;5(2):69-73.

[23] Tabanella G. Oral tissue reactions to suture materials: A review. J West Soc Periodontol Periodontal Abstr 2004;52(2):37-44.

[24] Muglali M, Ylmaz N, Inal S. Immunohistochemical comparison of indermil with traditional suture materials in dental surgery. J Craniofac Surg 2011;22(5):1875-1879.

[25] Kumar MS, Natta S, Shankar G.Comparison between silk sutures and cyanoacrylate adhesive in human mucosa:A clinical and histological study. J Int Oral Health 2013 Oct;5(5):95-100.

[26] Camacho-Alonso F, López-Jornet P, Bermejo-Fenoll A. Effects of scalpel (with and without tissue adhesive) and cryosurgery on wound healing in rat tongues. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100(3):e58-63.

[27] Camacho-Alonso F, López-Jornet P. Clinical-pathological study of the healing of wounds provoked on the dorso-lingual mucosa in 186 albino rats. Otolaryngol – Head Neck Surg 2007;136(1):119-124.

[28] Barbosa FI, Corrêa DS, Zenóbio EG.Dimensional changes between free gingival grafts fixed with ethyl cyanoacrylate and silk sutures. J Int Acad Periodontol 2009;11(2):170-176.

[29] Silva CO, Ribeiro ÉDP, Sallum AW.Free gingival grafts: Graft shrinkage and donor-site healing in smokers and non-smokers. J Periodontol 2010;81(5):692-701.

[30] Burkhardt R, Hämmerle CHF, Lang NP. Self-reported pain perception of patients after mucosal graft harvesting in the palatal area. J Clin Periodontol 2015;42(3):281-287.

[31] Lee JS, Jeon YS, Strauss FJ, Jung HI, Gruber R. Digital scanning is more accurate than using a periodontal probe to measure the keratinized tissue width. Sci Rep 2020;Feb 28;10(1):3665.

[32] Aslroosta H, Morshedzadeh G, Moslemi N.Clinical outcomes of free gingival graft following recipient bed preparation with Er,Cr:YSGG laser versus scalpel: A split-mouth randomized clinical trial. Photobiomodul Photomed Laser Surg 2021;39(6):425-433.

[33] Khalil S, Habashneh R, Alomari S. Local application of hyaluronic acid in conjunction with free gingival graft: a randomized clinical trial. Clin Oral Invest 2022;26(2):2165–2174.

How to cite this article: Yilmaz M, Kayaaltı-Yuksek S, Karaduman B. The Effects of Cyanoacrylate on Clinical Healing and Self-Reported Outcomes Following Free Gingival Graft Surgery: A Randomized Clinical Study. Clin Exp Health Sci 2022; 12: 691-696. DOI: 10.33808/clinexphealthsci.1012775

Clinical and Experimental Health Sciences

A Multi-center Retrospective Analysis of Healthcare Workers after COVID-19: Epidemiological and Clinical Features

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Received: 21.10.2021 **Accepted:** 02.02.2022

ABSTRACT

Objective: Concerns regarding the high-level risk of infection among healthcare workers (HCWs) increased after COVID19 was declared as a pandemic in March 2020. Inadequate infection control owing to a shortage of personal protective equipment or an inconvenient usage of infection control measures may play a significant role in transmission to/among healthcare personnel. The study aimed to determine the characteristics and outcomes of COVID-19 patients who are healthcare workers along with possible transmission routes of COVID-19 in four different healthcare facilities in Istanbul.

Methods: All hospital records were reviewed retrospectively. Demographic and clinical characteristics of HCWs were documented, and all infected HCWs were subjected to a phone-based mini-questionnaire and three-dimensional test (TDT). All statistical analyses were done using statistical packages SPSS Demo Ver 22 (SPSS Inc. Chicago, IL, USA).

Results: Clinical features of COVID-19 were similar to the general public's characteristics. The most frequent symptoms were cough, fever, and headache. HCWs with the O blood group tend to have asymptomatic COVID-19 infection. Hospital workers other than medical professionals have a lack of convenience of infection control measures. The median duration of PCR negativity was 9 days. HCWs who had a sore throat at the beginning of COVID-19 have a longer PCR-positive duration.

Conclusion: Understanding the clinical features or characteristics of asymptomatic COVID-19 carriers may aid in the implementation of a feasible screening program for early detection. It is strongly advised that proper infection control precautions, education, and auditing of nonclinical staff be implemented. As a result, transmission among healthcare workers can be avoided.

Keywords: COVID-19, healthcare workers, infection control, asymptomatic, Household contact.

1. INTRODUCTION

After COVID19 was introduced as a pandemic into the World in March 2020, concerns about the high-level risk of infection among healthcare workers increased (1-4). Moreover, personal protective equipment (PPE) was unreachable on some occasions and in some countries. Even while appropriately provided PPE, HCWs might not have enough awareness and proper education to use them. Shortage of both PPE itself and knowledge of using them increases the risk of infection and even death (5-8). According to a Chinese study from the early pandemic period, the majority of virus transmission happened in hospitals (9). Cross-transmission among employees could be a significant route, and asymptomatic carriers, in particular, could play a significant role in this situation (10). As a result, detecting asymptomatic carriers early is critical for infection management in hospital settings. Despite the findings of a Chinese study that concluded that the main infection route for HCWs was the hospital setting (9), Triebel et al. suggested that a screening program of asymptomatic HCWs should be implemented during possible new infection waves, as the HCWs appear to be infected in the general population rather than hospitals (11).

The study aimed to determine the characteristics and outcomes of COVID-19 patients who are healthcare workers along with possible transmission routes of COVID-19 in four different healthcare facilities in Istanbul.

DOI: 10.33808/clinexphealthsci.1012880

2. METHODS

The conducted study was approved by the Istanbul Medipol University Ethics Committee (date 18.03.2021/No 364). It obtained data from four different healthcare centers in Istanbul, Turkey. One of the hospitals is a university hospital, while the others are secondary care facilities. During the initial wave of the pandemic, all these healthcare centers accepted COVID-19 patients and provided COVID-19 testing to their own healthcare workers in accordance with Ministry of Health guidelines (12). Though the COVID-19 Scientific Committee modified the guideline when new scientific knowledge became available, during the study period all patients were given hydroxychloroquine, with favipiravir, tocilizumab, convalescent plasma, and anticoagulant therapy added if needed following hospitalization. Nasopharyngeal swabs for COVID-19 rt-PCR test were taken by a healthcare professional and performed in authorized laboratories with the primers provided by the Ministry of Health. If necessary, thorax computerized tomography was used in the diagnostic process and was reported by the radiologists according to the Ministry of Health guideline (13).

All hospital records provided by occupational health, safety boards, and infection control committees were reviewed retrospectively. Demographic and clinical characteristics of HCWs were documented and all the infected HCWs were subjected to a phone-based mini-questionnaire.

A questionnaire containing 4 questions (Cronbach's alpha= 0.41) was applied to evaluate knowledge and compliance of infection control measures. All data were recorded in the study have been collected by using online Microsoft Forms®. All collected data were analyzed with SPSS 22® (IBM, USA) software for statistical analysis. Chi-square or Fisher's exact tests were used to compare proportions and Student's t-test and Wilcoxon Sum rank test to compare means of parametric data. For variables that were not normally distributed, the Mann-Whitney U test was used. A two-sided α-value of less than 0.05 was considered statistically significant. All data was given in (mean ± SD) if appropriate. Median, interquartile range, min/max, and percentiles were also used.

Definitions

1. Asymptomatic infection: A patient with positive SARS CoV-2 test result and declared no symptoms and/or without any findings in chest imaging.

2. Non-clinical staff: Hospital workers who don't have any degree in medical sciences/professions.

3. RESULTS

The study included a total of 161 participants from different age groups, education levels, comorbidity frequencies, and blood types. Demographic and workplace/duty characteristics are given in Table 1.

Ninety participants (56%) out of 161 HCWs recruited to the study have A Rh-positive blood type while the rest of participants have blood groups of 0 Rh-positive (33,19%), B Rh-positive (23,14%), A Rh-negative (5, 3.1%), 0 Rh-negative (5,3.1%), AB Rh-positive (4, 2.5%) and AB Rh-negative (1, 0.6%).

Table 1: *Demographic and workplace/duty characteristics of the study population*

	All	Nurse	Doctor	Technician	Non-clinical staff
Age Median (IQR) [min-max]	27 (24-35) [20-60]	25(23-27) [20-43]	38(27-45) [26-60]	29(24-32) [22-49]	28(24-34) [20-56]
Male gender	66 (41)	13 (20)	18 (27.3)	6 (9.1)	29 (48.3)
Workplace					
Emergency room	5 (3.1)	5 (100)	0	0	0
Surgical Units	20 (12.4)	6 (30)	10 (50)	1 (5)	3 (15)
Internal Medicine Units	71 (44.1)	36 (51)	15 (21)	6 (8)	14 (20)
Laboratory	9 (5.6)	0	2(22)	6 (67)	1 (11)
Management	17 (10.6)	0	0	0	17 (100)
Mobile within hospital	25 (15.5)	0	0	3 (12)	22 (88)
Intensive Care Unit	14 (8.7)	10 (72)	1 (7)	0	3 (21)
Total N (%)	161 (100)	57 (35.4)	28 (17.4)	16 (9.9)	60 (37.3)

In a bivariate analysis, those with the A blood group had a higher probability of COVID-19 symptomatic infection (88, 92 %, *p*=0.04). Asymptomatic infection rates were observed to be considerably higher among people with the O blood group (30%, *p*=.002) and those who worked in a surgical unit (30%, p=0.04). Having O blood type is substantially connected to an asymptomatic course of infection (*p*=.001) in the multivariate logistic regression study (Hoshmer-Lemeshov *p*=0.66) that includes age, gender, O blood type, workplace, and duty. Working in a surgical unit is not linked to asymptomatic infection in this model (0.11). Furthermore, neither bivariate nor multivariate analysis found age, sex, or duty to be associated with asymptomatic infection in our study sample.

The rate of hospitalization was 13.5 % (N=22). No one was admitted to the intensive care unit and no deaths were observed during the research period. The most common symptoms were cough (69%), fever (59%), headache (57%), sore throat (39%), loss of taste (37%) and smell (39%), and sputum (13%). 71% of females reported a sore throat, which was significantly greater than males (28%) (*p*=0.01). Ninety percent of participants do not have a co-morbidity (N=146), and there was no relation between comorbidity and hospitalization in our study.

On 118 HCWs, a computerized thorax tomography (CT) was done, with the results falling into four categories: a) negative for pneumonia (N=27, 23%), b) typical for COVID-19 (N=42, 36.2%), c) indeterminate findings (N=7, 6%), and d) atypical appearance (N=40, 34.5%). There was no link between the

CT finding and the need for hospitalization. Five patients were followed up without medication. Hydroxychloroquine was prescribed to 134 patients without hospitalization as monotherapy (N=43, 32%) or combined with azithromycin (N=91, 68%). Hydroxychloroquine was given to all 22 hospitalized patients, azithromycin was combined in 20 of them (91%) and in the case of clinical deterioration, tocilizumab (11, 50%) was used as the subsequent therapy. No HCWs included in this study were deceased because of COVID-19.

During the study period, fifty-two participants declared that they were living alone and 103 HCWs reported living with one or more households (3.2 ± 1.2). Living with a household was found a risk factor for cross-transmission to get the virus from house-contact (OR:1.2; CI: 1.09-1.29) or spread it to them (OR:1.3, CI:1.16-1.43). After this risk was evaluated by duty in hospitals, getting the virus from household contact risk was higher in the non-clinical staff (OR: 1.27, CI:1.09-1.48), and spreading to others in a house risk was higher in doctors (OR:1.4, CI: 1,.005 – 1.850). There is no difference in the risk of transmission to household members between those who are asymptomatic and those who are symptomatic (p=0.6). Due to the limited number of subgroups, we were unable to investigate the association between the number of household members and transmission risk. SARS CoV-2 PCR test has been performed in all cases. Twenty-two HCWs have had no symptoms despite their PCR test results being positive. In symptomatic participants duration between symptom and PCR test median: 0 days (IQR:0-2) (min: – 2 – max: 14). The time between the first positive PCR result and the first negative result: 9 days (IQR: 7-13) (min: 1 max: 28). Figure 1 explains the time of the negative results more clearly.

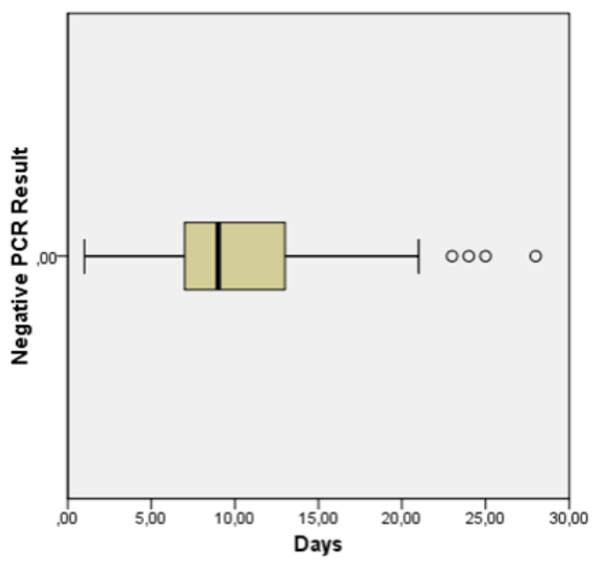

Figure 1. *Positive PCR result and the first negative result.*

There is no significant relationship between negative result duration and sex, age, blood type, smell loss, and taste loss symptoms. Patients with sore throat had a substantially longer (12 ± 4 days) interval for a negative SARS CoV-2 PCR result than those who do not (10±4 days) (***p =.005***). Negative PCR duration took a maximum of 15 days (median: 8, IQR: 7-12) in asymptomatic patients. The following is the questioner's response: (**Q1**) "How might you have gotten the virus?" a multiple-choice question was answered as "*not sure* (*Q1A1*)" by 46% of participants. Other answers were "*might be a family member contact* (*Q1A2*)" (9%), "*might be a co-worker with positive PCR test result contact* (*Q1A3*)" (28%), "*might be a patient with a positive test result contact (Q1A4*)" (17%). Participants, living alone, responded as *Q1A1* higher than ones with household (62% vs 37%, p= .006). HCWs sharing a house someone answered the same question "*Q1A2*" higher than the living alone group (13% vs. 2%, p= .04). There was no difference between living alone and A3, A4. There is a significant association between asymptomatic HCWs and "*Q1A3"* (might get the virus from a co-worker) (p= .05)

(Q2) "Did you have easy access to PPE while working? (*YesQ2=152/NoQ2=9*)" The answers of Q2 didn't show any disparity by occupation type. There is a higher rate of A3 (might got the virus from a co-worker) for Q1 in the HCWs responded in NoQ2 (N=6, 67%, p=001).

(Q3) "Did you know about the isolation precautions before you had COVID-19? (*YesQ3=151/NoQ3=9*)" Nine responded as no and all of them non-clinical staff (15%)(p<0,001) and 5 of them were mobile inside the hospital group (p= .005). The HCWs who were answered Q1 as "might be a family member contact" dominantly responded in the NoQ3 group (N=4, 44%, p=.004).

(Q4) "Were you compliant with the isolation precautions policy of your hospital? (*YesQ4=128/NoQ4=33*"). A vast major of the NoQ4 group (N=22, 67%) answered A3 (transmission might be from a co-worker) and it is higher than the *YesQ4* group (N=18, 19%) (p<0,001). In the *YesQ4* group, the rate of *Q1A2* (transmission might be from a patient) (26, 20%) is significantly higher than the *NoQ4 group (1, 3%) (p = .001).* Analysis of Q4 by workplace and duty, mobile inside hospital group (N=10, 30.3%, p= .01), and non-clinical staff (N=18, 55%, p= .02) responded *NoQ4* answer significantly higher than other groups. There is no significant difference between Q4 and clinical staff (nurse, doctor, technician) in bivariate analysis.

4. DISCUSSION

The spectrum of clinical findings in COVID-19 is wide. A systematic review suggested that up to 33 % of patients may be asymptomatic while having COVID-19 (14). 14% of HCWs were asymptomatic in our cohort. Clinical findings in symptomatic HCWs are similar to the literature (15). Asymptomatic infection in COVID-19 is a concerning problem for transmitting the virus to others in both community and healthcare settings (10, 16) yet we cannot find any association of asymptomatic HCWs with SARS CoV-2 PCR and risk of transmission to/from household members while

significant relationship occurs between asymptomatic ones might get the virus from a co-worker. In our study, participants have a good knowledge and are compliant with isolation precautions tightly while working. We may conclude isolation precautions practice may be loosened during break times and transmission within asymptomatic co-workers may be easier than on duty. There are several articles which are suggesting the probable effects of the blood group on the COVID-19 clinical course (17, 18). Wu et al found that patient with O blood type is related to an asymptomatic infection course, having an A blood group is related to a higher rate of symptomatic one. A study from Turkey aimed to evaluate the effect of blood groups on either transmission risk or clinical course, concluded O blood type is related lower rate of having SARS CoV-2 and there is no relationship between blood type and clinical outcome (19). It is relevant to our finding which is a lower rate of O blood group in SARS CoV-2 PCR, yet we found that HCWs with O blood type have a higher rate of asymptomatic course of COVID-19. Our study focuses on the transmission to HCWs as a high-risk group; thus we will not discuss the clinical outcome and blood group relation, anymore in this paper. In our viewpoint, early prediction of asymptomatic HCWs would be useful to interrupt cross-transmission in a healthcare facility. Our finding supports Triebel et al suggestion that implementing a screening program in a healthcare facility (11) would be beneficial. Rivett et al strongly suggested the implementation of a screening program in healthcare settings particularly after the lockdown was lifted (20). Further studies are required to understand the characteristics of asymptomatic COVID-19 patients leads to implementing a more feasible solution for such a screening program.

Lei et al found that household contact was 10 times higher than other contacts in the community setting (21). A systematic review revealed that household transmission is very important for community spread and has a high secondary attack rate (22). In our study, we found that living with household members is a risk factor for transmission in HCWs although has a lower rate than community-setting. We found that household transmission is particularly high in the non-clinical staff. Moreover, our questionnaire revealed that non-clinical staff has lower knowledge and compliance with isolation measures. It might make household transmission easier in this group. Hospitals should assure non-clinical staff have proper isolation precaution education and audit the compliance frequently.

In our research, we found that the median time from PCR positivity to PCR negativity was 9 days (IQR: 7-13), with HCWs who had a sore throat at the start of COVID-19 having a longer PCR positive period. According to other studies, the median duration of PCR negative for HCWs in Spain, Madrid, is 15 days (IQR: 12–19.5) (23). Another study found that the median duration of PCR negative for HCWs in Japan, Tokyo is 19 days (IQR 6-37) (24). This variance in time to PCR negative between studies may be attributed to changes in the study population, age, gender distribution, and which COVID variant is prevalent at the time of the studies. CDC recommends the implementation of a containment period based on clinical recovery – not PCR test – as a maximum of ten days. To date, the literature suggests prolonged viral shedding after recovery is not significant for the transmission of COVID-19 except for immunocompromised patients (25, 26). We found prolonged viral shedding for up to 15 days (median:8; IQR:7-12). HCWs with sore throat complaints seem to have prolonged viral shedding. Further studies are required to assess its importance in the healthcare setting.

5. CONCLUSION

Comprehending characteristics of asymptomatic infections as blood type may be useful to implement a feasible screening program. Education and audit of the non-clinical staff should be implemented to avoid transmission within both household and hospital settings.

Acknowledgments: The authors acknowledged OH&S specialist Emel Yılmaz who helped to solve technical issues.

Funding: The authors have no funding sources to declare.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

[1] Wilson NM, Norton A, Young FP, Collins DW. Airborne transmission of severe acute respiratory syndrome coronavirus-2 to healthcare workers: a narrative review. Anaesthesia. 2020;75(8):1086-1095.

[2] Ran L, Chen X, Wang Y, Wu W, Zhang L, Tan X. Risk Factors of Healthcare Workers With Coronavirus Disease 2019: A Retrospective Cohort Study in a Designated Hospital of Wuhan in China. Clin Infect Dis. 2020;71(16):2218-2221.

[3] Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention. Zhonghua Liu Xing Bing Xue Za Zhi. 2020;41(2):145-151.

[4] Tran K, Cimon K, Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. PLoS One. 2012;7(4):e35797.

[5] Jain U. Risk of COVID-19 due to Shortage of Personal Protective Equipment. Cureus. 2020;12(6):e8837.

[6] Zhang M, Zhou M, Tang F, Wang Y, Nie H, Zhang L, You G. Knowledge, attitude, and practice regarding COVID-19 among healthcare workers in Henan, China. J Hosp Infect. 2020;105(2):183-187.

[7] Cook T, Kursumovic E, Lennane S. Exclusive: deaths of NHS staff from covid-19 analysed. HSJ 2020;22. Accessed [23 september 2022]. https://www.hsj.co.uk/exclusive-deaths-of-nhs-staff-from-covid-19-analysed/7027471.article

[8] Saqlain M, Munir MM, Rehman SU, Gulzar A, Naz S, Ahmed Z, Tahir AH, Mashhood M. Knowledge, attitude, practice and perceived barriers among healthcare workers regarding COVID-19: a cross-sectional survey from Pakistan. J Hosp Infect. 2020;105(3):419-423.

[9] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):1061-1069.

[10] Shields A, Faustini SE, Perez-Toledo M, Jossi S, Aldera E, Allen JD, Al-Taei S, Backhouse C, Bosworth A, Dunbar LA, Ebanks D, Emmanuel B, Garvey M, Gray J, Kidd IM, McGinnell G, McLoughlin DE, Morley G, O'Neill J, Papakonstantinou D, Pickles O, Poxon C, Richter M, Walker EM, Wanigasooriya K, Watanabe Y, Whalley C, Zielinska AE, Crispin M, Wraith DC, Beggs AD, Cunningham AF, Drayson MT, Richter AG. SARS-CoV-2 seroprevalence and asymptomatic viral carriage in healthcare workers: a cross-sectional study. Thorax. 2020;75(12):1089-1094.

[11] Treibel TA, Manisty C, Burton M, McKnight Á, Lambourne J, Augusto JB, Couto-Parada X, Cutino-Moguel T, Noursadeghi M, Moon JC. COVID-19: PCR screening of asymptomatic health-care workers at London hospital. Lancet. 2020;395(10237):1608-1610.

[12] COVID-19 SCo. COVID-19 Information Page (2021). Accessed [23 september 2022]. https://covid19.saglik.gov.tr/TR-66301/covid-19-rehberi.html

[13] Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, Henry TS, Kanne JP, Kligerman S, Ko JP, Litt H. Radiological Society of North America Expert Consensus Document on Reporting Chest CT Findings Related to COVID-19: Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA. Radiol Cardiothorac Imaging. 2020;2(2):e200152. doi:10.1148/ryct.202.020.0152

[14] Oran DP, Topol EJ. The Proportion of SARS-CoV-2 Infections That Are Asymptomatic : A Systematic Review. Ann Intern Med. 2021;174(5):655-662.

[15] Stokes EK, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, Tie Y, Fullerton KE. Coronavirus Disease 2019 Case Surveillance – United States, January 22-May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(24):759-765.

[16] Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, Ma K. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. 2021;54(1):12-16.

[17] Wu Y, Feng Z, Li P, Yu Q. Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. Clin Chim Acta. 2020;509:220-223.

[18] Li J, Wang X, Chen J, Cai Y, Deng A, Yang M. Association between ABO blood groups and risk of SARS-CoV-2 pneumonia. Br J Haematol. 2020;190(1):24-27.

[19] Göker H, Aladağ Karakulak E, Demiroğlu H, Ayaz Ceylan ÇM, Büyükaşik Y, Inkaya AÇ, Aksu S, Sayinalp N, Haznedaroğlu IC, Uzun Ö, Akova M, Özcebe OI, Ünal S. The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. Turk J Med Sci. 2020;50(4):679-683.

[20] Rivett L, Sridhar S, Sparkes D, Routledge M, Jones NK, Forrest S, Young J, Pereira-Dias J, Hamilton WL, Ferris M, Torok ME, Meredith L; CITIID-NIHR COVID-19 BioResource Collaboration, Curran MD, Fuller S, Chaudhry A, Shaw A, Samworth RJ, Bradley JR, Dougan G, Smith KG, Lehner PJ, Matheson NJ, Wright G, Goodfellow IG, Baker S, Weekes MP. Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. Elife. 2020;9:e58728.

[21] Lei H, Xu X, Xiao S, Wu X, Shu Y. Household transmission of COVID-19-a systematic review and meta-analysis. J Infect. 2020;81(6):979-997.

[22] Qiu X, Nergiz AI, Maraolo AE, Bogoch II, Low N, Cevik M. The role of asymptomatic and pre-symptomatic infection in SARS-CoV-2 transmission-a living systematic review. Clin Microbiol Infect. 2021;27(4):511-519.

[23] Villarreal J, Nieto SV, Vázquez F, Del Campo MT, Mahillo I, de la Hoz RE. Time to a Negative SARS-CoV-2 PCR Predicts Delayed Return to Work After Medical Leave in COVID-19 Infected Health Care Workers. J Occup Environ Med. 2021;63(11):970-974.

[24] Miyamae Y, Hayashi T, Yonezawa H, Fujihara J, Matsumoto Y, Ito T, Tsubota T, Ishii K. Duration of viral shedding in asymptomatic or mild cases of novel coronavirus disease 2019 (COVID-19) from a cruise ship: A single-hospital experience in Tokyo, Japan. Int J Infect Dis. 2020;97:293-295.

How to cite this article: Derin O, Aksoy N, Caydasi O, Yilmaz M, Mert A. A Multi-center Retrospective Analysis of Healthcare Workers after COVID-19: Epidemiological and Clinical Features. Clin Exp Health Sci 2022; 12: 697-701. DOI: 10.33808/clinexphealthsci.1012880

Clinical and Experimental Health Sciences

Effect of Polishing Systems on the Color and Surface Properties of Resin Composites in the Process of Accelerated Artificial Aging

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Received: 24.10.2021 **Accepted:** 22.03.2022

ABSTRACT

Objective: This study aimed to investigate the effects of polishing system on the color stability, surface roughness, and hardness of resin composites in the presence and absence of accelerated artificial aging (AAA).

Methods: Six resin composites (Universal Restorative 200, G-Aenial Anterior, Ceram-X Duo, Admira, IPS Empress Direct, Clearfil Majesty Esthetic) were evaluated. Thirty disc-shaped samples were prepared for each composite group. Resin composite groups were divided into three subgroups: control (Mylar strip), disc (Optidisc), and rubber (Dimanto) (n=10). Color change (ΔE_{00}) was calculated using the CIEDE 2000 formula. Before and after AAA, the surface roughness (Ra, µm) and hardness (VHN) values were measured. Data were analysed using ANOVA, the Bonferroni test, and Pearson correlation ($p<0.05$).

Results: The Mylar strip group showed less color change than the polished groups. Universal Restorative 200 and IPS Empress Direct were associated with less discoloration than other resin composite groups. Before AAA, Mylar strips and Universal Restorative 200 were exhibited smoother values. There was no difference in the surface roughness between Dimanto-treated resin composites those associated with other materials before and after AAA (except Ceram-X Duo and Universal Restorative 200). Universal Restorative 200 yielded higher hardness values than other composites ($p<0.05$). The Mylar strips yielded lower VHN values than the polished groups, but there were no differences among the polishing systems. There was a significant, weak, and positive correlation between color and roughness change.

Conclusion: Composite type, finishing/polishing, and AAA had statistically significant effect on surface roughness and hardness. The Optidisc group exhibited less coloration and smoother surfaces than the Dimanto group. Nanohybrid (IPS Empress Direct) and microhybrid (Universal Restorative 200) resin composites performed better than other resins in terms of color change and roughness. Polishing systems applied to resin composite materials increased hardness.

Keywords: Accelerated artificial aging, color change, polishing, surface properties

1. INTRODUCTION

With developments and improvements of the physical-mechanical properties of resin composites, their clinical use has increased; however, color stability, which affects the life of the restoration, remains a material-specific problem (1). Color change in resin composites is multifactorial, depending on the internal and external coloring of the materials. Internal factors are related to the chemical structure of the resin matrix component of the materials, and external factors are related to the coloring substances (such as the patient's diet, hygiene, smoking habits) (2). Color differences can be assessed visually or with color-measuring instruments. These instruments (colorimeters and spectrophotometers) quantitatively indicate coloration and avoid the pitfalls of subjective visual color comparisons (3). The CIEDE 2000 formula provides better correlations than the CIE L*a*b formula, and the CIEDE 2000 formula determines color differences perceived by the human eye better than the CIE L*a*b formula (4).

The mechanical properties of resin composites in aesthetic restorations are also important factors influencing their clinical longevity (5). Mechanical and physical properties are reflected by differences in filler volume, size, and shape, and improvements in these materials have been implemented via changes in matrix composition and polymerization technology (6). Finishing/polishing can be considered essential steps in restorative procedures that increase the aesthetics and clinical lifespan of restored teeth. Resin matrix and filler particles change in terms of hardness; as a result, they do not wear out to the same plane. Therefore, due to

DOI: 10.33808/clinexphealthsci.1012098

the removal of some particles during the process of finishing/polishing, surface defects may occur, such as microcracks and irregularities in the materials, which reduces the wear resistance of the restorations (7). Clinicians can choose from a variety of polishing tools for clinical use, such as diamond burs, stones, rubber cups, bits, discs, strips, and pastes. Efforts have been made to develop finishing/polishing instruments and one-step systems for resin composites (8). Given that simplified application systems are less time-consuming, it is important for clinicians to know which finishing/polishing systems offer adequate surface quality to increase the longevity of resin composite restorations (9).

A clinically useful lifespan begins immediately after the application of restoration materials. Although it may be possible to immediately assess color and other variables related to aesthetics, it is difficult to predict and compare long-term outcomes due to the rapid development and introduction of new generations in the dental market (2). Artificial aging methods are used to evaluate the effects on the optical and mechanical properties of resin composites (10). Clinical studies must validate the treatment procedures, but such studies are expensive and time-consuming. In vitro studies are designed to simulate clinical conditions as closely as possible within the scope of clinical procedures (11). Accelerated artificial aging (AAA) imitates oral conditions through exposure to 300 h of weathering in a weather-O-meter, which is reportedly equivalent to 1 year of clinical use or intraoral treatments (12). AAA imitates the effects of prolonged exposure to environmental factors, such as differences in light, temperature, and moisture, and it approximates long-term clinical use in short time intervals (13). Studies evaluating AAA and resin composite polishing systems are scarce in the literature.

This study aimed to assess the influence of two polishing methods on color changes, surface roughness, and hardness among six resin composites in the presence and absence of AAA. The following null hypotheses were investigated: (1) different polishing methods would not effect color changes, surface roughness and hardness of resin composites; and (2) AAA would not influence color changes, surface roughness and hardness among resin composites.

2. METHODS

Shade equivalent A2 of six different resin composites (Clearfill Majesty Esthetic, Kuraray, Okayama, Japan; IPS Empress Direct, Ivoclar Vivadent, Schaan, Liechtenstein; Universal Restorative 200, 3M-ESPE, St. Paul, MN, USA; G-Aenial Anterior, GC Corp. Tokyo, Japan; Ceram-X Duo, Dentsply, Konstanz, Germany; Admira, Voco, Cuxhaven, Germany) were used (Table 1). Thirty disc-shaped samples were prepared for each composite, adding up to 180 samples in total. A Teflon mould (8 mm diameter and 2 mm thickness) was used to prepare disc-shaped specimens of the resin composites. The resin composites were placed into holes, and a Mylar strip was placed over the top surfaces. Resin composites were cured with light-emitting diode (LED; Woodpecker LED.E (P), Guilin Woodpeckers Medical Inst. Co., Guilin, China)–curing light at 1200 mW/cm^2 for 40 s directly over Mylar strips. The resin composite groups were randomly divided into three subgroups (n=10). Except for the Mylar strip group, 1200 grit silicon carbide abrasive paper was used with water before application using the polishing systems. Two different polishing systems (Optidisc, KerrHawe, Bioggio, Switzerland; Dimanto, Voco, Cuxhaven, Germany) were used. The polishing systems are shown in Table 1. The four-step OptiDisc system includes four aluminium oxide (Al_2O_3)–embedded discs, and each was used for 15 s in dry conditions. The one-step Dimanto system (rubber cup) includes diamond-embedded discs and was used for 60 s in dry conditions. The polishing systems were applied using a handpiece at a speed of 10,000 rpm. All the specimens were rinsed for 10 s and then stored at 37°C for 24 h in distilled water.

2.1. Color Change Measurements

The initial color measurements were performed using a spectrophotometer (Lovibond RT Series, Tintometer Group, Lovibond House, UK). The spectrophotometer was calibrated according to the manufacturer's instructions. The CIEDE 2000 formula was used to determine color differences (14,15):

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L} \right)^2 + \left(\frac{\Delta C'}{K_C S_C} \right)^2 + \left(\frac{\Delta H'}{K_H S_H} \right)^2 + R_T \left(\frac{\Delta C'}{K_C S_C} \right) \left(\frac{\Delta H'}{K_H S_H} \right) \right]^{\frac{1}{2}}$$

where ΔL', ΔC', and ΔH' are the differences in lightness, chroma, and hue, respectively, between two specimens. The relationship between the variations of chroma and hue in the blue region is defined by the rotation function (R_T). The weighting functions of lightness, chroma, and hue are denoted by S_L, S_C, and S_H, respectively. K_L, K_C, and K_H are the parametric factors of set 1 in this study (16).

2.2. Surface Roughness Measurements

Initial surface roughness was measured using a contact surface roughness device (Mar Surf PS1, Mahr, Göttingen, Germany). The average roughness (Ra, µm) values were recorded, and each specimen was recorded three different times using a profilometer, and the average value was calculated for each.

2.3. Surface Hardness Measurements

Initial surface hardness was measured using a surface hardness device (LHV-1D, Bursam NDT, Bursa, Turkey). A 300-g load with a 10-second dwell period (17) was used on the surface for three measurements, and the average value was calculated for each material.

Table 1. *List of materials used in present study*

Resin Composites	Manufacturer	Type	Composition	wt-vol	Lot No.
Universal Restorative 200	3M Espe, St. Paul, MN, USA	Universal/ Microhybrid	BisGMA, UDMA, Bis-EMA, zirkonium/silica, 0,01-3,5 µm	%82 wt %60 vol	N996478
G Aenial Anterior	GC Corp. Tokyo, Japan	Microhybrid	UDMA, dimethacrylate co-monomers, pre – polymerized organic filler, silica, strontium, lanthanoid fluoride, fumed silica (0,1–17µm)	%73 wt %64 vol	1909091
Ceram.X Duo (Enamel)	Dentsply De Trey GmbH, Konstanz, Germany	Nanoceramic	Bis-GMA, UDMA, TEGDMA, Methacrylate modified ploysiloxane (organically modified ceramic), dimethacylate resin, Bis(4-methyl-phenyl), iodonium hexafluorophosphate, barium-aluminum-borosilicate glass (10 nm), methacrylate functionalised silicon dioxide nano filler	%76 wt %57 vol	180.800.1099
Admira	Voco GmbH Cuxhaven, Germany	Ormocer	Ormocer, BisGMA, UDMA, aromatic and aliphatic dimethacylate, 0.7 µm.	%78 wt. (%56 vol. microfiller)	1914502
IPS Empress Direct (Enamel)	IvoclarVivadent, Schaan, Liechtenstein	Nanohybrid	BisGMA, UDMA, TEGDMA, Barium glass, ytterbium trifluoride, and mixed oxides silicon dioxide, copolymer 0,4 µm-100 nm	%75-79 wt %52-59 vol	Y35243
Clearfil Majesty Esthetic	Kuraray Noritake Dental Inc., Okayama, Japan	Nanohybrid	BisGMA, hydrophobic aromatic dimethacrylate, di-Camhorquinone, silanated barium glass filler, pre – polymerized organic filler, 0,37 µm-1,5 µm	%78 wt %40 vol	4H0173
Finishing/ Polishing materials	**Manufacturer**	**Type**	**Composition**		**Lot No.**
OptiDisc	KerrHawe, Bioggio, Switzerland	Discs	Aluminum impregnated discs, (Coarse-Medium-Fine-Extrafine)		6778506
Dimanto	Voco GmbH Cuxhaven, Germany	Rubber	Diaomond particles impregnated silicon rubber (One-step pre and high gloss polishing)		1915625

Bis-GMA; bisphenol A glycol dimethacrylate; Bis-EMA; bisphenol A ethoxylated dimethacrylate; TEGDMA; triethylene glycol dimethacrylate, UDMA; urethane dimethacrylate.

2.4. AAA

After initial measurements, all specimens were aged for 300 h and 150 kJ/m² (3) in an accelerated ageing chamber (Atlas ci 4000; Atlas Electronic Devices Co, Mount Prospect, Il, USA) (18). The aging procedure was performed as described elsewhere (19). After AAA procedures, color change, surface roughness and hardness measurements were repeated.

2.5. Statistical Analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). First, the normality of the distribution was checked. Paired-samples t-test analysis was used to make before-and-after comparisons to examine the significance of the effects of AAA on the roughness and hardness of the resin composite materials. Independent-samples t-tests were used to compare color changes, surface roughness, and surface hardness of the polishing systems used for the resin composite materials. Color change was analysed using two-way ANOVA. Three-way ANOVA was used to analyse the interaction between composite type, polishing systems, and AAA in the influence of surface roughness and hardness of the composites. Bonferroni tests were used for multiple comparisons. Pearson correlation was used to reveal the relationship between changes in color and surface roughness values. For all statistical tests, the significance level was set at $p<0.05$.

3. RESULTS

3.1. Color Results

Table 2 shows ΔE_{00} values. The analysed factors (composite type and finishing/polishing group) had no significant influence on color changes (Table 3). The ΔE_{00} values of the Ceram-X Duo were indicated to be statistically significantly higher than those of the Universal Restorative 200 and IPS Empress Direct ($p<0.001$). The Optidisc and Dimanto groups were found to have significantly higher mean ΔE_{00} values than the Mylar strip group ($p<0.001$ for each). The Dimanto group had a significantly higher mean ΔE_{00} value than the Optidisc group ($p=0.017$).

Table 2. *ANOVA results for color change (ΔE_{00})*

Interaction factors	Type III Sum of Squares	df	Mean Square	F	p
Group	30.5	2	15.226	24.14	**<0.001**
Composite type	61.6	5	12.321	19.530	**<0.001**
Group ⁜ Composite type	10.5	10	1.051	1.670	0.093

Table 3. *Mean color changes (ΔE_{00}) and standard deviation of the tested materials*

Resin composites	Control	Optidisc	Dimanto	Total
Ceram-X Duo	3.54 ± 0.83	4.21 ± 0.54	4.39 ± 0.47	4.39 ± 0.47 A
Universal Restorative 200	2 ± 0.56	2.9 ± 0.89	3.3 ± 1.13	2.73 ± 1.02 B
G-Aenial Anterior	3.68 ± 0.93	4.28 ± 1.11	4.34 ± 0.61	4.1 ± 0.93 A
IPS Empress Direct	2.52 ± 1.24	2.57 ± 0.57	2.86 ± 0.57	2.65 ± 0.84 B
Clearfil Majesty Esthetic	2.9 ± 0.44	4.16 ± 0.36	4.33 ± 0.75	3.8 ± 0.84 A
Admira	3.01 ± 0.49	3.12 ± 1.29	4.43 ± 0.56	3.52 ± 1.06 A
Total	2.94 ± 0.96 a	3.54 ± 1.08 b	3.94 ± 0.93 c	

Different capital letters represent statistically significant differences in each column (p< 0.05).
Different lower letters represent statistically significant differences in each row (p< 0.05).

3.2. Surface Roughness Results

The analysed factors (composite type, finishing/polishing group, and AAA) had a statistically significant influence on surface roughness (Table 4), and Ra value differences are shown in Table 5. Before AAA, when the compared resin composites in the rubber group were evaluated according to composite type, there were no significant differences (p>0.05). The Ceram-X Duo yielded higher Ra values than the Universal Restorative 200 (p=0.046). The Optidisc with Universal Restorative 200 yielded significantly lower Ra values than the other composites. However, there were no differences between the Ra values associated with IPS Empress Direct and Clearfil Majesty Esthetic. After AAA, Optidisc and Dimanto with Universal Restorative 200 were associated with significantly lower surface roughness values than Ceram-X Duo (p=0.014, p=0.035, respectively). Before and after AAA, the Mylar strip group exhibited lower Ra values than the finishing/polishing groups (p<0.001). Additionally, the Optidisc group exhibited significantly lower roughness values than the Dimanto group (p<0.001).

3.3. Surface Hardness Results

The analysed factors (composite type, finishing/polishing group, and AAA) had a statistically significant influence on surface hardness (Table 4). Vickers hardness number (VHN) differences are shown in Table 6. Among all finishing/polishing groups, the Universal Restorative 200 exhibited the highest VHNs (p<0.05). The polishing groups exhibited significantly higher hardness values than the control group. Before and after AAA, no significant difference was observed between the Optidisc and Dimanto groups in terms of hardness.

Table 4. *Interactions among the three factors using Three-Way ANOVA*

Interaction factors	Roughness	Hardness
	p	p
Composite	**<0.001**	**<0.001**
Group	**<0.001**	**<0.001**
AAA	**<0.001**	**<0.001**
Composite* Group	**<0.001**	**<0.001**
AAA*Composite	0.092	0.239
AAA*Group	**0.001**	**<0.001**
AAA* Composite *Group	**0.020**	**<0.001**

3.4. Pearson Correlation Results

There was a statistically significant, weakly positive relationship between color change and roughness (r=0.168, p=0.024).

Table 5. *Surface roughness values (Ra, µm) (mean±std.deviation) of the resin composites*

	Control			Optidisc			Dimanto		
	Before AAA	After AAA	p	Before AAA	After AAA	p	Before AAA	After AAA	p
C	0.19 ± 0.02 Aa	0.21 ± 0.03 ABC[1]	>0.05	0.25 ± 0.02 ACb	0.28 ± 0.02 A2	<0.05	0.26 ± 0.02 Ab	0.31 ± 0.01 B[2]	<0.05
U	0.15 ± 0.01 Ba	0.16 ± 0.03 B[1]	>0.05	0.15 ± 0.02 Ba	0.23 ± 0.03 C[2]	<0.05	0.24 ± 0.03 Ab	0.27 ± 0.02 A[2]	<0.05
G	0.17 ± 0.02 ABa	0.22 ± 0.04 C[1]	<0.05	0.23 ± 0.03 ACb	0.28 ± 0.02 A[2]	<0.05	0.25 ± 0.03 Ab	0.31 ± 0.03 AB[2]	<0.05
I	0.16 ± 0.04 ABa	0.18 ± 0.02 ABC[1]	>0.05	0.18 ± 0.03 BCa	0.21 ± 0.01 BC[1]	<0.05	0.25 ± 0.02 Ab	0.28 ± 0.01 AB[2]	<0.05
M	0.17 ± 0.03 ABa	0.18 ± 0.02 AB[1]	<0.05	0.19 ± 0.01 BCa	0.25 ± 0.02 AC[2]	<0.05	0.25 ± 0.02 Ab	0.30 ± 0.02 AB[3]	<0.05
A	0.17 ± 0.02 ABa	0.20 ± 0.03 ABC[1]	<0.05	0.21 ± 0.04 Ca	0.27 ± 0.03 AC[2]	<0.05	0.27 ± 0.03 Ab	0.31 ± 0.01 AB[2]	<0.05

C; Ceram-X Duo, U;Universal Restoratif 200, G;G-Aenial Anterior, I;IPS Empress Direct, M;Clearfil Majesty Esthetic, A;Admira, AAA; Accelerated artificial aging, p; represent statistically significant differences in each group of the same resin composites (between before and after AAA of specimens values)
Different capital letters represent statistically significant differences in each column (p<0.05).
Different lower letters (comparisons of before AAA, specimen values between the groups) represent statistically significant differences in each row (p<0.05).
Different superscript numbers (comparisons of after AAA, specimen values between the groups) represent statistically significant differences in each row (p<0.05).

Table 6. *Surface hardness values (VHN) (mean±std.deviation) of the resin composites*

	Control			Optidisc			Dimanto		
	Before AAA	After AAA	p	Before AAA	After AAA	p	Before AAA	After AAA	p
C	52.00 ±1.75 Aa	67.87 ± 1.93 A[1]	<0.05	63.51 ± 2.43 Ab	75.74 ± 1.73 AD[2]	<0.05	60.26 ± 1.72 AEb	73.84 ± 1.51 A[2]	<0.05
U	87.73 ± 2.20 Ba	92.43 ± 2.04 B[1]	<0.05	92.09 ± 2.49 Bb	103.48±3.48 B[2]	<0.05	91.09 ± 2.42 Bab	101.78±4.00 B[2]	<0.05
G	37.70 ± 1.91 Ca	55.32 ± 2.20 C[1]	<0.05	43.28 ± 1.68 Cb	60.97 ± 1.64 C[2]	<0.05	42.83 ± 2.05 Cb	59.60 ± 1.92 C[2]	<0.05
I	50.50 ± 1.61 Aa	75.76 ± 1.84 D[1]	<0.05	61.32 ± 1.01 Ab	78.41 ± 1.97 D[1]	<0.05	58.10 ± 1.36 Ab	77.68 ± 1.99 D[1]	<0.05
M	43.18 ± 3.53 Da	52.40 ± 1.55 CE[1]	<0.05	45.65 ± 1.55 Ca	55.34 ± 0.84 E[1]	<0.05	44.79 ± 1.21 Da	52.55 ± 0.90 E[1]	<0.05
A	57.91 ± 2.15 Ea	68.03 ± 1.24 A[1]	<0.05	63.54 ± 1.90 Ab	73.55 ± 2.06 A[2]	<0.05	61.89 ± 2.05 Eb	71.77 ± 1.50 A[2]	<0.05

C; Ceram-X Duo, U;Universal Restoratif 200, G;G-Aenial Anterior, I;IPS Empress Direct, M;Clearfil Majesty Esthetic, A;Admira, AAA; Accelerated artificial aging, p; represent statistically significant differences in each group of the same resin composites (between before and after AAA of specimens values)
Different capital letters represent statistically significant differences in each column (p<0.05).
Different lower letters (comparisons of before AAA, specimen values between the groups) represent statistically significant differences in each row (p<0.05).
Different superscript numbers (comparisons of after AAA, specimen values between the groups) represent statistically significant differences in each row (p<0.05).

4. DISCUSSION

The interaction between composite type and polishing system was not significant in terms of the influence on color change. However, significant differences were found in the interaction between the composite resin materials and the polishing systems in terms of surface roughness and hardness. Therefore, we failed to fully reject the first null hypothesis. AAA was associated with significant differences in the color, surface roughness, and hardness of the resin composites. Therefore, the second null hypothesis was rejected.

In this study, acceptable perceptibility and acceptability thresholds were 0.81 and 1.77, respectively (15). Color change ΔE_{00} values were all between 2.0–4.43, and the resin composites were associated with clinically unacceptable color changes after AAA. Resin composite color changes are affected by external and internal factors. External factors include the duration and intensity of light emission during light-curing, as well as environmental factors, such as ultraviolet radiation, water, and temperature. Internal factors include the content of the resin matrix, filler loading

and particle size distribution, type of photoinitiators, and remaining C=C bonds (20). During light-curing, initiators and tertiary aromatic amines form products that, under temperature or UV light challenges, cause resin discoloration towards red or yellow (21). A previous study (12) found that L* values decreased and b* values increased in resin composites after AAA. In the present study, L* values decreased and b* values increased in resin composites after AAA. Admira and Ceram-X Duo contain ormocer matrix, which is defined by an interpenetrating network of inorganic-organic polymers (22). In our study, Admira, Ceram-X Duo, G-Aenial Anterior resin composites were associated with more discoloration than the other materials. The staining susceptibility of resin composites may be due to the degree of water sorption and the hydrophilicity of the resin matrix. Resin composites can absorb water and are also able to absorb other fluids with pigments, which results in discoloration (23). Discoloration may be caused by inseparable highly cross-linkable organic networks and inorganic structures (20) and by AAA disrupting the ormocer structure. G-Aenial Anterior consists of a mixture of urethane dimethacrylate (UDMA) and dimethacrylate co-monomers; it is free of Bis-GMA and has been confirmed in previous studies to facilitate discoloration. Bis-GMA and TEGDMA have high water absorption capacity due to their hydrophilic structure. The color stability of UDMA has been demonstrated to be superior to that of Bis-GMA (24); however, our findings were contrary to this observation. G-Aenial Anterior, a microhybrid composite with higher filler volume (64%), did not seem to have advanced color stability compared with Ceram X-Duo and Admira, ormocer-containing materials with lower filler volumes (57% and 56%, respectively). This may indicate that the lower color stability and higher solubility may be associated with monomer structures, for which AAA affects the chemical structures. In a previous study, microhybrid composites were found to be more stain-resistant than nanocomposites and microfilled composites (25). However, in our study, IPS Empress Direct (nanohybrid) exhibited the lowest ΔE_{00} values among the resin composite materials investigated (except for Universal Restorative 200). IPS Empress Direct can resist ageing-related staining. This can be explained by the use of different photoinitiators that remove amine groups, improve polymerization kinetics, and reduce the yellowing effect of curing. Smaller particle size and better dispersion of the resin matrix produces smoother surfaces (24). Although a previous study found that small nanofilled composite resin particles resulted in less discoloration (25), another study found that increased particle size caused a decrease in the organic filler matrix ratio, resulting in less discoloration (26); our study findings with Universal Restorative 200, a microhybrid resin composite, aligned with the latter observation. Use of the Universal Restorative 200 microhybrid and larger particle sizes may be effective for minimizing discoloration.

A previous study investigated color changes, surface morphology, and tooth restoration interface degradation among different resin composites (hybrid, microhybrid, nanoparticle-containing, and silorane) after AAA and found that Filtek Z250 (a microhybrid) was associated with the least color change. It has been stated that physical surface modifications caused by AAA may affect color changes (27) and that color changes caused by AAA are probably due to degradation at the monomer matrix/particle interface (28). An ideal polishing instrument should have abrasive particles harder than the filler contents of the material, thus allowing composites to reduce in terms of both the resin matrix and filler particles during polishing. Soft abrasive particles from the fillers only remove the resin matrix, and the hard aluminium oxide found in most polishing systems is significantly higher harder than most of the fillers in resin composites (29). Lu et al. (30) found that the smoothest surfaces were created using aluminium oxide–coated discs, that can perform an equal amount of abrasion, from both organic resin and inorganic fillers. A previous study evaluated the effects of the aging procedure on the surface roughness of compomer and resin composites (nanohybrid and ormocer). The study found that AAA did not affect the surface roughness, but there were differences between the materials (31). Similarly, another study stated that AAA did not influence the surface roughness of resin composites (32). Increases in color change and surface roughness have been shown to be interrelated (33). Another study (34) reported a lack of statistically significant increases in surface roughness values among resin composites after AAA; the investigators found that there was no correlation between surface roughness and color change. However, in our study, resin composites showed significant increases in surface roughness values after AAA, and a weakly positive correlation was found between roughness change and color change. Surface roughness can influence color change, as surface morphology influences susceptibility to discoloration. Surface roughness evaluation is relevant to the study of composite restoration since surface morphology affects susceptibility to discoloration (23). AAA and polishing produced rougher surfaces, which in turn caused significant color alterations. It has been observed that changes in resin composites are associated with internal factors (34) and external alterations that occur within specimens. A systematic review reported that the surface roughness of nanofiller or submicron composites was not superior to that of conventional microhybrid resin composites (35). In our study, the surface roughness values before and after AAA were found to be between 0.15 and 0.31 µm. Significant differences were observed between the Ceram-X Duo and Universal Restorative 200 control groups before AAA. After AAA, differences were detected between the two microhybrid resin composites (Universal Restorative 200 and G-Aenial Anterior). These differences can be attributed to the different chemical compositions of the materials, especially the filler content ratio. It is well known that the smoothest obtainable surfaces are achieved by curing the material in direct contact with a Mylar strip (23). The higher filler weight and volume of Universal Restorative 200 may have resulted in smoother surfaces in the Mylar strip group. It has been reported that surfaces formed with Mylar strips may have a resin-rich layer and poor physicomechanical properties. After polishing, the surface micromorphology of the composites is

affected by the type, amount, size, and hardness of the filler particles. It is also affected by the flexibility of the polishing materials, the hardness of the abrasive, size, and the method of application (36). In our study, Ceram-X Duo yielded the highest mean Ra value (0.31 µm) after AAA, while the mean Ra value for this material was similar in the rubber and disc application groups. This situation may be caused by the ormocer structure as well as the glass particles in Ceram-X Duo (37). Universal Restorative 200 exhibited a less-rough surface than other materials (except for IPS Empress Direct). Although there is no difference between the materials in the rubber group, disc application may have contributed to the smoothness of the nanohybrid composites. Several studies have found that multi-step systems perform better than one-step systems (9,38). One-step systems can be implemented with a single polishing material, and smooth surfaces are provided in a shorter time (39). One study found that the texture of the final surface depends on the technique and material used (40), but there is no consensus on the materials and techniques that provide the smoothest surfaces for resin composites (41). According to our study, Optidisc (multi-step) created smoother surfaces and, therefore, lower staining susceptibility than Dimanto (one-step). The effectiveness of polishing discs containing aluminium oxide particles to create smooth surfaces tends to diminish gradually (39). The Dimanto polishing system contains particles impregnated with diamonds. Diamond is harder than aluminium. Therefore, diamond abrasive particles may cause deeper scratches on the surface of the composites, which may increase surface roughness. We found that a one-step polishing system did not produce the same surface quality on the resin composites. This is not attributable to the quality of the polishes but entirely to the interaction between the polisher and the composite resin.

In the present study, Mylar strip was associated with lower hardness values than the polishing systems. This finding is similar to other studies (8,42). Alfawaz, (43) investigated two polishing methods (a one-step [PoGo] and a multi-step [Sof-Lex] method) applied to two different composites (Z350 XT and Ceram-X). Mylar strips were chosen as the control group. The control group yielded lower hardness values than the polished groups, but there was no significant difference between the polishing systems. Tornavoi et al. (44) found that AAA did not affect the hardness values of resin composites, but there were significant differences in hardness between the materials. Schulze et al. (45) found that Knoop hardness values of resin composites increased significantly after AAA. In contrast, a previous study (46) reported that microhardness values of resin composites decreased significantly after AAA. In our study, hardness values of resin composites increased significantly after AAA. Factors such as the device used, light, humidity and heat (45) caused differences in the mechanical properties of the materials. Studies have shown that water absorption by the resin matrix and orifice temperature can change the cohesion between the matrix and inorganic particles, reducing the mechanical properties of these materials and causing them to degrade (44,47). The results obtained in our study showed statistically significant differences between dental composites and demonstrated that the type of composites used might also influence the hardness results obtained. In our study, the highest hardness values among all groups were observed in association with Universal Restorative 200, and the lowest hardness values were observed in association with G-Aenial Anterior. This may be attributable to the filler ratio of the composite, as well as the influence of the hardness of the inorganic filler on the general hardness of the material (8). In our study, zirconia particles may have affected the increase in the VHN values of Universal Restorative 200. Previous study (44) reported that, among different resin composites, the composite material (Z250-microhybrid) with silica and zirconia content was harder. The microhardness of resin composites depends on several factors, such as the content of the resin matrix and the type and shape of the particle. Moreover, the hardness of resin composite is directly related to filler particles (48). In this context, the difference in material contents in our study reflects differences in hardness values.

One of the limitations of this study was the evaluation of the color and surface properties of resin composites using an in vitro methodological approach. Within the methodological limitations, we aimed to mimic the effects on resin materials of aging processes that may occur in the oral environment in a short time to estimate the clinical performance of the resin composites. However, various factors in the oral environment, such as saliva, temperature, pH, and brushing, can affect the long-term color stability, surface roughness, and hardness of resin composites. Further studies using different polishing and ageing methods should be conducted on resin composites. It should also be noted that flat sample surfaces were used in the present study, and in clinical practice, restorations created with resin composites consist of convex or concave irregular structures.

5. CONCLUSION

Within the limitations of this in vitro study, it was concluded that:

Composite type, finishing/polishing, and AAA had statistically significant effect on surface roughness and hardness. The Mylar strip groups had smoother surfaces and less discoloration than the polishing groups. The Optidisc (multi-step) group exhibited more stain resistance and smoother surfaces than the Dimanto (one-step) group. Nanohybrid resin composite (IPS Empress Direct) and microhybrid resin composite (Universal Restorative 200) were associated with more favourable color changes and roughness values with Optidisc (multi-step) than with Dimanto (one-step). Polishing systems applied to composite materials increased the hardness values of the materials.

Conflict of interest: Authors declare no scientific and financial interest.

Funding: This study was supported by Scientific Research Projects Coordination Unit of Necmettin Erbakan University. Project number 201924001.

REFERENCES

[1] Roselino Lde M, Cruvinel DR, Chinelatti MA, Pires-de-Souza Fde C. Effect of brushing and accelerated ageing on color stability and surface roughness of composites. J Dent 2013;41 Suppl 5:e54–e61.

[2] Drubi-Filho B, Garcia LFR, Cruvinel DR, Sousa ABR, Pires-de-Souza Fde C. Color stability of modern composites subjected to different periods of accelerated artificial aging. Braz Dent J 2012;23:575–580.

[3] Taşın S, Celik G, İsmatullaev A, Usumez A. The effect of artificial accelerated aging on the color stability, microhardness, and surface roughness of different dental laminate veneer materials. J Esthet Restor Dent (Online publication) 2020. doi:10.1111/jerd.12567

[4] Gómez-Polo C, Portillo Muñoz M, Lorenzo Luengo MC, Vicente P, Galindo P, Martín Casado AM. Comparison of the CIELab and CIEDE2000 color difference formulas. J Prosthet Dent 2016;115:65–70.

[5] Tuncdemir AR, Aykent F. Effects of fibers on the color change and stability of resin composites after accelerated aging. Dent Mater J 2012;31:872–878.

[6] Tekçe N, Pala K, Tuncer S, Demirci M. The effect of surface sealant application and accelerated aging on posterior restorative surfaces: An SEM and AFM study. Dent Mater J 2017;36:182–189.

[7] Catelan A, Briso ALF, Sundfeld RH, Dos Santos PH. Effect of artificial aging on the roughness and microhardness of sealed composites. J Esthet Restor Dent 2010;22:324–330.

[8] Erdemir U, Sancakli HS, Yildiz E. The effect of one-step and multi-step polishing systems on the surface roughness and microhardness of novel resin composites. Eur J Dent 2012;6:198-205.

[9] St-Pierre L, Martel C, Crépeau H, Vargas MA. Influence of polishing systems on surface roughness of composite resins: polishability of composite resins. Oper Dent 2019;44:122-132.

[10] Shirinzad M, Rezaei-Soufi L, Mirtorabi MS, Vahdatinia F. Effect of accelerated artificial aging on translucency of methacrylate and silorane-based composite resins. J Dent 2016;3:92–100.

[11] Korkmaz Ceyhan Y, Ontiveros JC, Powers JM, Paravina, RD. Accelerated aging effects on color and translucency of flowable composites. J Esthet Restor Dent 2014;26:272–278.

[12] Paravina RD, Ontiveros JC, Powers JM. Accelerated aging effects on color and translucency of bleaching-shade composites. J Esthet Restor Dent 2004;16(2):117-127.

[13] Pires-de-Souza F, Casemiro LA, Garcia L, Cruvinel DR. Color stability of dental ceramics submitted to artificial accelerated aging after repeated firings. J Prosthet Dent 2009;101:13–18.

[14] Sharma G, Wu W, Dalal EN. The CIEDE2000 color-Difference formula: Implementation notes, supplementary test data, and mathematical observations. Color Res Appl 2005;30:21–30.

[15] Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, Sakaim M, Takahashi H, Tashkandi E, del Mar Perezet M. Color difference thresholds in dentistry. J Esthet Restor Dent 2015;27 Suppl 1:S1–S9.

[16] Lee YK. Comparison of CIELAB DeltaE(*) and CIEDE2000 color-differences after polymerization and thermocycling of resin composites. Dent Mater 2005;21:678–682.

[17] Sanko V, Sezer ÜA, Sezer S, Demirekin ZB, Eroğlu E, Türkaslan S. Investigation of the mechanical and color characteristics of microcrystalline cellulose containing dental composite resins. J Dent Fac Atatürk Uni 2020;30:181–187.

[18] Sagong H, Kim K, Kwon T. Effect of polymerization type and thickness on color stability of resin cements. Korean J Dent Mater 2013;40:297–302.

[19] Lee SM, Choi YS. Effect of ceramic material and resin cement systems on the color stability of laminate veneers after accelerated aging. J Prosthet Dent 2018;120:99–106.

[20] Sirin Karaarslan E, Bulbul M, Yildiz E, Secilmis A, Sari F, Usumez A. Effects of different polishing methods on color stability of resin composites after accelerated aging. Dent Mater J 2013;32:58-67.

[21] Dantas DCB, Mathias IF, Borges AB, Torres CG, Caneppele TMF. Effects of artificial accelerated aging on the optical properties of resin composites. Biosci J 2018;34:1105– 1113.

[22] Kunzelmann KH, Mehl A, Hickel R. Sliding wear of an experimental ormocer and 15 commercial composites, J Dent Res 1998;77:226 Abstr. no. 965.

[23] Sherif R, Abd El-Fattah W, Afifi R. Color stability and surface roughness of an organically modified ceramic (ormocer) and a methacrylate based composite resins (An in-vitro study). Alex Dent J 2020;45:100-105.

[24] Poggio C, Ceci M, Beltrami R, Mirando M, Wassim J, Colombo M. Color stability of esthetic restorative materials: a spectrophotometric analysis. Acta Biomater Odontol Scand 2016;2:95-101.

[25] Nasim I, Neelakantan P, Sujeer R, Subbarao CV. Color stability of microfilled, microhybrid and nanocomposite resins—An in vitro study. J Dent 2010;38 Suppl 2:137-142.

[26] Paravina RD, Roeder L, Lu H, Vogel K, Powers JM. Effect of finishing and polishing procedures on surface roughness, gloss and color of resin-based composites. Am J Dent 2004;17:262-266.

[27] Catelan A, Briso AL, Sundfeld RH, Goiato MC, dos Santos PH. Color stability of sealed composite resin restorative materials after ultraviolet artificial aging and immersion in staining solutions. J Prosthet Dent 2011;105:236–241.

[28] Pires-de-Souza F, Garcia L, Roselino L, Naves LZ. Color stability of silorane-based composites submitted to accelerated artificial ageing—an in situ study. J Dent 2011;39 Suppl 1: e18–e24.

[29] Koh R, Neiva G, Dennison J, Yaman P. Finishing systems on the final surface roughness of composites. J Contemp Dent Pract 2008;9:138–145.

[30] Lu H, Powers JM. Color stability of resin cements after accelerated aging. Am J Dent 2004;17:354–358.

[31] Kucukesmen HC, Kucukesmen C, Usumez A. Effect of aging procedure on surface roughness of different restorative material. J Health Sci (SDU) 2010;1:39-48.

[32] Pucinelli C, Silva R, Mellara T, Patricia G, Pires-de-Souza Fernanda, Lea S. Color stability and surface roughness of composites after artificial accelerated aging. J Dent Indones 2017;24:26–31.

[33] Zanin FR, Garcia Lda F, Casemiro LA, Pires-de-Souza Fde C. Effect ofartificial accelerated aging on color stability and

surface roughness of indirect composites. Eur J Prosthodont Restor Dent 2008;16:10-14.

[34] Rattacaso RM, da Fonseca Roberti Garcia L, Aguilar FG, Consani S, de Carvalho Panzeri Pires-de-Souza F. Bleaching agent action on color stability, surface roughness and microhardness of composites submitted to accelerated artificial aging. Eur J Dent 2011;5:143-149.

[35] Kaizer MR, de Oliveira-Ogliari A, Cenci MS, Opdam NJ, Moraes RR. Do nanofill or submicron composites show improved smoothness and gloss? A systematic review of in vitro studies. Dent Mater 2014;30:e41-e78.

[36] Aytac F, Karaarslan ES, Agaccioglu M, Tastan E, Buldur M, Kuyucu E. Effects of novel finishing and polishing systems on surface roughness and morphology of nanocomposites. J Esthet Restor Dent 2016;28:247-261.

[37] Magdy NM, Kola MZ, Alqahtani HH, Alqahtani MD, Alghmlas AS. evaluation of surface roughness of different direct resin-based composites. J Int Soc Prev Community Dent 2017;7:104-109.

[38] Rodrigues-Junior SA, Chemin P, Piaia PP, Ferracane JL. Surface roughness and gloss of actual composites as polished with different polishing systems. Oper Dent 2015;40:418-429.

[39] Antonson SA, Yazici AR, Kilinc E, Antonson DE, Hardigan PC. Comparison of different finishing/polishing systems on surface roughness and gloss of resin composites. J Dent 2011;39 Suppl 1: e9–e17.

[40] Schmitt VL, Puppin-Rontani RM, Naufel FS, Nahsan FP, Alexandre Coelho Sinhoreti M, Baseggio W. Effect of the polishing procedures on color stability and surface roughness of composite resins. ISRN Dent 2011;2011:617672.

[41] de Carvalho Justo Fernandes ACB, de Assunção IV, Borges BCD, da Costa GdFA. Impact of additional polishing on the roughness and surface morphology of dental composite resins. Rev Port de Estomatol Med Dent Cir Maxilofac 2016;57:74– 81.

[42] Korkmaz Y, Ozel E, Attar N, Aksoy G. The influence of one-step polishing systems on the surface roughness and microhardness of nanocomposites. Oper Dent 2008;33:44-50.

[43] Alfawaz Y. Impact of polishing systems on the surface roughness and microhardness of nanocomposites. J Contemp Dent Pract 2017;18:647-651.

[44] Tornavoi DC, Sato S, Silva LJ, Agnelli JAM, dos Reis AC. Analysis of surface hardness of artificially aged resin composites. Mat Res 2012;15:9–14.

[45] Schulze KA, Marshall SJ, Gansky SA, Marshall GW. Color stability and hardness in dental composites after accelerated aging. Dent Mater 2003;19:612–619.

[46] de A Souza MB, Briso AL, de Oliveira-Reis B, Dos Santos PH, Fagundes TC. Influence of light-curing units on surface microhardness and color change of composite resins after challenge. J Contemp Dent Pract 2019;20:204-210.

[47] Chinelatti MA, Chimello DT, Ramos RP, Palma-Dibb RG. Evaluation of the surface hardness of composite resins before and after polishing at different times. J Appl Oral Sci 2006;14:188-192.

[48] Nithya K, Sridevi K, Keerthi V, Ravishankar P. Evaluation of surface roughness, hardness, and gloss of composites after three different finishing and polishing techniques: An in vitro study. Cureus 2020;12:e7037.

How to cite this article: Fidan M, Dereli Z. Effect of Polishing Systems on the Color and Surface Properties of Resin Composites in the Process of Accelerated Artificial Aging. Clin Exp Health Sci 2022; 12: 702-710. DOI: 10.33808/clinexphealthsci.1012098

Clinical and Experimental Health Sciences

Developing a Diabetes Knowledge Scale for Adults and its Psychometric Properties

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Received: 26.10.2021 **Accepted:** 11.05.2022

ABSTRACT

Objective: This study was conducted to develop and test the psychometric properties of a "Diabetes Knowledge Scale for Adults."

Methods: The sample for this research of methodological design consisted of 500 individuals, ages 18-90, who had presented at a state hospital in Istanbul during the period October 2018-April 2019 with or without a diagnosis of diabetes. Data for the study were collected with a "Sociodemographic Characteristics Descriptive Questionnaire" and the "Diabetes Knowledge Scale for Adults (DKSA)." The validity of the scale was evaluated with the content validity index and construct validity testing (exploratory, confirmatory factor analyses). Its reliability was assessed with KR-20 internal consistency analysis, item-total correlation testing, the item discrimination, item difficulty indexes, and test-retest analysis.

Results: The content validity index for the 28-item scale was found to be 0.92. The exploratory factor analysis revealed five subscales that explained 62.15% of scale variance. The results of the confirmatory factor analysis, GFI= 0.88 CFI= 0.93, AGFI= 0.86, SRMR= 0.01 and $\chi^2/df = 2.43$, confirmed a good and acceptable level of goodness of fit for the scale. The scale's KR-20 reliability coefficient was 0.94, item-total correlations were above .45 and the correlation between the test-retests administered two weeks apart was found to be r=0.99

Conclusion: DKSA is a valid and reliable scale that can be used to determine the knowledge level of between the ages of 18-90 adults about diabetes.

Keywords: Diabetes; knowledge; scale; adult; validity; reliability

1. INTRODUCTON

The rising frequency of diabetes around the world, its prevalence in every age group, its being among the leading five causes of death, and the high cost of lifelong treatment and monitoring makes this disease a global public health issue that has been accepted as an epidemic of the 21st century (1). The International Diabetes Federation (IDF) has reported that there were 463 million individuals with diabetes between the ages of 20-79 around the world in 2019, signifying a prevalence of 9.3%. As in other parts of the world, the prevalence of diabetes is increasing in Turkey as well (2). According to the 2019 Diabetes Atlas, Turkey recorded the highest prevalence of diabetes in Europe, at a rate of 11.1%. Among the 6.6 million people with diabetes in Europe, Turkey has the third highest diabetic population after Germany and the Russian Federation (1). About 60,000 deaths occur as a result of diabetes in Turkey each year, and it is reported that the disease is responsible for approximately one-forth of health expenditure. Additionally, it is asserted that approximately one-third of the country's diabetics are diagnosed with retinopathy and more than half experience at least two diabetes-related complications, meaning that if urgent precautions are not taken, Turkey will be left face-to-face with a diabetes crisis (2-3). All of these data indeed point to a compelling need for urgent measures.

Becoming knowledgeable about the causes of diabetes, the methods of prevention, its signs and symptoms, early diagnosis and treatment options is an effective approach not only for the management of the disease but also for the vital issue of cost efficiency (4-5). About half of individuals with diabetes are not aware of their condition (1,6) The state of having inadequate knowledge about diabetes has an adverse effect on diabetes prevention and self-care behavior (4-5,8). Researchers have reported in various national and international studies that individuals either lack adequate knowledge or possess erroneous or deficient information

DOI: 10.33808/clinexphealthsci.1014888

about diabetes (4-8). It is because of this that there is a need to assess the knowledge of individuals about diabetes.

It was seen in the scan of the literature that Fitzgerald et al. had developed a Diabetes Knowledge Test (DKT) that was revised as the Diabetes Knowledge Test 2 (DKT-2) in 2016 DBT-2 is a tool for assessing the general knowledge of both Type 1 and Type 2 diabetics about diabetes; it consists of 14 items with an additional 9 items evaluating the individual's use of insulin, 23 items in all (9). Various tests assessing diabetes knowledge were discovered in the literature (4,8,10,11-12). It was noted that most of these tests were developed and used for individuals who had received a diagnosis of diabetes.

The Turkish literature also revealed that evaluations had generally been made of the knowledge about diabetes of adults who had been diagnosed with diabetes (13). No instrument was encountered in the literature, however, that assessed the diabetes knowledge of individuals who had not been diagnosed with the illness. It was for this reason that the need was recognized for a valid and reliable measure that would assess the level of adults' diabetes knowledge**.** This study aimed to develop a Diabetes Knowledge Scale for Adults (DKSA) that could be easily used to make an assessment.

Research Questions

1. Is the Diabetes Knowledge Scale for Adults a valid instrument?
2. Is the Diabetes Knowledge Scale for Adults a reliable instrument?

2. METHODS

The study was conducted in methodological research design.

2.1. Ethical Considerations

Permission for the conduct of the study was obtained from a University Ethics Committee (04.06.2018-164). The required permissions were also received from the hospital in which the study would be conducted (11.09.2018.556.07146-604.01.01-E.4857) and from the Istanbul Provincial Health Directorate (12.09.2019 – 16867222-604.01.01-E.2752). The written consent of the study participants was received prior to the start of data collection.

2.2. Participants

The study universe consisted of adults presenting for health services at a state hospital located in Eyüpsultan, Istanbul over the period October 2018 – April 2019. During this period, the individuals applying to the hospital were informed about the nature of the study and invited to participate in the research. It is stated in the literature that in scale development studies, the sample must be 10 times the number of scale items (14). In the development and testing of the scale and its psychometric features in our study, the draft of the scale comprised 49 items. The study universe was made up of individuals who matched the inclusion criteria–198 individuals (Group 1: those diagnosed with diabetes) and 302 individuals (Group 2: those not diagnosed with diabetes), totaling 500 (N=500) adults. Of the 302 adults who were not diagnosed with diabetes, 50 had a chronic disease (hypertension, etc.) other than mental or psychological problems, and 252 did not have any health problems. They were healthy individuals who came to the hospital with the patient as a companion. The inclusion criteria were being age 18 or older, not having any barrier to communication, not have a diagnosed neurological, psychiatric or dementia disease, knowing how to read and write, and being willing to participate in the study.

To test the stability of the scale over time, a retest was administered to 30 individuals two weeks after the initial test.

The mean age of the participants was 44.1±17.06; 68.6% were women, 39.6% were diagnosed with diabetes, 10% were diagnosed with a chronic disease other than diabetes, and 50.4% were healthy. According to Body Mass Index (BMI) classification, 2.4% were underweight, 41.8% were of normal weight, 37.8% were overweight and 18.0% were obese (WHO BMI). Among the participants, 65.3% were married; 39.4% had an education of only five years or less, 23.0% had gone to school for 8-11 years, 37.6% had an education of 15 years or more. There was a diagnosis of diabetes in the families of 31.8%.

2.3. Instruments

The study data were collected with a 20-item closed-ended questionnaire on sociodemographic characteristics and routine habits, and the Diabetes Knowledge Scale for Adults (DKSA).

2.4. Creating the Item Pool

The item pool yielded the creation of a 49-item scale based on the literature (9-12,15) in which basic information about the definition of diabetes, symptom findings of diabetes, blood glucose measurement values (laboratory findings), diabetes risk factors, diabetes complications were questioned. This scale items does not include any detailed information about insulin administration, medications, diet, exercise and foot care in type 1 diabetes. While creating the scale item pool on the opinions and suggestions collected from the President of the Diabetes Nursing Association of Turkey, the diabetes nurses working at the hospitals, academic nurses working at the universities, and Physicians Specialized in Internal Diseases. The 49-item scale was sent to a group of experienced specialists, experts in their field, to test its content validity. The data collected from the specialists were tested by using Polit and Beck's content validity index (16). Lastly, a linguist was asked to make an evaluation, after which the draft instrument was given its final form. The draft of the scale was evaluated in a pilot study conducted with 50 adults.

It was seen that the instrument could be administered in 15-20 minutes. It was decided that the data would be collected by the lead researcher in the patient training room of the hospital.

Since the scale was developed to measure knowledge scoring was based on two sets of items whose responses would be true or false. The options of Yes / No / I don't know were given; those who answered correctly were afforded 1 point, those who responded incorrectly were given 0 points. The third and sixth items were scored in reverse. The maximum possible score on the scale was 49; the minimum was 0.

2.5. Statistical Analysis

The data collected were evaluated on the SPSS 22.0 computer program and with the Amos 16 software. The descriptive analysis of the data used frequencies, percentages, means and standard deviation in the testing. Validity analysis was performed using the Content Validity Index (CVI), Kaiser-Meyer-Olkin and Barlett's tests, Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA). Reliability analysis was performed using Pearson's and Spearman's correlation analyses, Kuder-Richardson-20 (KR-20) analysis and the item difficulty index. The results were found to be in the 95% confidence interval; the level of significance was accepted to be $p<0.05$.

3. RESULTS

3.1. Results of the Validity Analysis of the Scale

After the opinions of 10 experts were reviewed, it was found that the Content Validity Index was .92.

In the Principal Components Analysis, it was found that the Diabetes Knowledge Scale for Adults demonstrated a Kaiser-Meyer-Olkin (KMO) value of .94, indicating that the sample size was suitable for factor analysis. The result of Bartlett's Test indicated statistical significance (p=.00).

3.2. Exploratory Factor Analysis (EFA)

The results of the varimax rotation analysis showed that the scale could be divided into seven subscales, each with an eigenvalue greater than +1. In the calculation of the factor loadings, it was seen that the factor loadings of all items amounted to .41 and above. The factor analysis showed that according to the item factor loadings (±.20), there were 22 overlapping items (3, 4, 5, 6, 7, 15, 16, 18, 19, 21, 22, 25, 26, 27, 28, 30, 31, 38, 39, 40, 43, 44), and therefore these were removed from the scale (Table 2). After these 22 items were removed, the remaining 28 were subjected to an EFA, which showed that the scale had five subscales that explained 62.15% of total variance (Table 1).

Table 1. *Items Deleted according to the exploratory factor analysis of the diabetes knowledge scale for adults*

No	Item	$\bar{x}$	sd	Rjx	α	α^1
3	Insulin is a hormone secreted by the pancreas.	.496	.500	.654	.361	.385
4	Insulin is a hormone that lowers blood glucose.	.528	.500	.586	.390	.508
5	Type 1 diabetics need to take insulin all their lives.	.312	.464	.515	.308	.471
6	Type 2 diabetics do not need to take insulin all their lives.	.264	.441	.475	.410	.493
7	Diabetes can be seen in pregnancy.	.502	.501	.658	.400	
15	Measuring blood glucose at home is important in the management of diabetes.	.642	.480	.670	.392	.406
16	Exercising helps to lower blood glucose.	.624	.485	.687	.351	.486
19	The risk of diabetes is high in those with diabetes in their family or relatives.	.680	.467	.711	.434	
21	The risk of diabetes is high in the overweight.	.686	.465	.664	.544	.330
22	The risk of diabetes is high in people with an excess of blood lipids.	.490	.500	.594	.302	.340
25	The risk of diabetes is high in people under stress.	.520	.500	.587	.516	
26	Smokers are at high risk of diabetes.	.338	.474	.467	.401	.433
27	People who don't eat a healthy diet are at high risk of diabetes.	.658	.475	.678	.348	.491
28	The risk of diabetes is high in people who live sedentary lives.	.656	.476	.667	.432	.465
30	The risk of diabetes is high in people with hypertension.	.290	.454	.488	.445	.423
31	The risk of diabetes is high in people who have low blood sugar a little while after eating.	.356	.479	.567	.431	.502
38	Widespread itching in the body is one of the symptoms of diabetes.	.362	.481	.525	.324	.407
39	Weight gain or loss is one of the symptoms of diabetes.	.540	.499	.658	.319	.330
40	Fatigue and tiredness is one of the symptoms of diabetes.	.550	.498	.654	.488	
43	If diabetes is not managed well, it can lead to hypoglycemia.	.488	.500	.641	.331	.309
44	If diabetes is not managed well, it can lead to wounds in the feet.	.646	.479	.745	.315	.357

$\bar{x}$=Mean item score, sd= Item standard deviation, rjx=Item-total score correlations, α=Item factor loading, α1=Factor loading when item is removed

***Table 2**. Validity and reliability analyses of the diabetes knowledge scale for adults*

Subscales	Old Item No	New Item No.	DKSA İtems	x̄	sd	rjx	α
General Knowledge About Diabetes (KR-20= (KR20=.789)	1	1	Diabetes means a rise in blood glucose.	.680	.466	.622	.436
	2	2	Diabetes is caused by insulin deficiency or inadequacy.	.594	.496	.590	.499
	8	3	Diabetes is congenital, it does not develop afterwards (Y)	.556	.497	.500	.572
	9	4	Diabetes is a lifelong disease.	.498	.500	.498	.552
	10	5	There is no treatment for diabetes, but the disease can be kept under control.	.560	.491	.584	.708
	11	6	Diabetes is a contagious disease. (Y)	.680	.457	.450	.722
Measuring Blood Glucose and Test Results (KR-20=. .851)	12	7	Fasting blood glucose should be between 70-100 mg/dl.	.486	.487	.737	.800
	13	8	Postprandial blood glucose should be below 140 mg/dl.	.388	.500	.721	.828
	14	9	Postprandial blood glucose should be measured 2 hours after the first bite of the meal.	.474	.464	.657	.730
	17	10	The hemoglobin A1c (HbA1c) count provides data on the level of blood glucose in the last 3 months.	.314	.499	.639	.743
	18	11	A diagnosis of diabetes is made on the basis of a blood glucose of 126 mg/dl or over after at least 8 hours of fasting.	.298	.457	.553	.703
Diabetes risk factors (KR-20= (.744)	20	12	Diabetes risk is high at age 40 and above.	.474	.391	.472	.398
	23	13	The risk of diabetes is high in women who deliver babies of 4 kg and over.	.298	.457	.602	.725
	24	14	The risk of diabetes is high in pregnant women with high blood glucose.	.438	.496	.617	.637
	29	15	The risk of diabetes is high in people who have had an infectious (microbial) disease.	.188	.499	.476	.733
Symptoms of Diabetes (KR-20=. .911)	32	16	Extreme thirst, drinking lots of water is one of the symptoms of diabetes.	.686	.496	.747	.754
	33	17	Frequent urination is one of the symptoms of diabetes.	.632	.460	.790	.923
	34	18	Frequent urination during the night is one of the symptoms of diabetes.	.588	.482	.747	.831
	35	19	Increased appetite, overeating is one of the symptoms of diabetes.	.560	.499	.649	.628
	36	20	Blurred vision is one of the symptoms of diabetes.	.470	.470	.595	.507
	37	21	Slow healing of cuts and wounds is one of the symptoms of diabetes.	.640	.480	.719	.577
	40	22	Fatigue and tiredness is one of the symptoms of diabetes.	.550	.497	.782	.569
	41	23	A dry mouth is one of the symptoms of diabetes.	.670	.492	.782	.759
Complications of Diabetes (KR-20=. .901)	45	24	If diabetes is not managed well, it may cause a deterioration of kidney functions.	.640	.499	.783	.690
	46	25	If diabetes is not managed well, it may cause eye diseases that may even lead to the loss of sight.	.670	.495	.786	.600
	47	26	If diabetes is not managed well, it will cause hypertensive diseases.	.472	.470	.658	.803
	48	27	If diabetes is not managed well, it will cause cardiovascular diseases.	.572	.480	.805	.816
	49	28	If diabetes is not managed well, it will cause loss of body parts (particularly hands and feet).	.612	.487	.741	.556

x̄=Mean item score, sd=standard deviation, rjx=Item-total score correlations, α=Item factor loading,

3.3. Results of Factor Item Analysis of the Subscales

General knowledge about diabetes; this subscale assesses how much general

information an individual has about diabetes and consists of six items, of which two are wrong (3 and 6. item) four are right (1, 2, 4 and 5. item). The factor loadings of the items varied between .43-.72.

Blood glucose measurement values; are indicators of an individual's fasting and

postprandial blood glucose. This sub-dimension is made up of five correct statements (items 7, 8, 9, 10, 11) and the factor loadings of the items varied between .70-.82.

Diabetes Risk Factors; measures an individual's knowledge about their risk of

developing diabetes. This sub-dimension is made up of four statements (items 12, 13, 14, 15). The factor loadings of the factor items varied between .39-.73.

Symptoms of Diabetes; This measures the individual's knowledge of the signs and

symptoms of diabetes. The entire subscale is made up of eight correct statements (items 16,17,18,19,20,21,22 and 23) and the factor loadings of the items varied between .50-.92.

Diabetes Complications; this assesses the individual's knowledge about the detrimental

effects of diabetes on the organs and tissues of the body. The entire subscale is made up of five correct statements (items

24, 25, 26, 27, 28) and the factor loadings of the items varied between .55-.81.

For all sub-dimensions of the scale; the higher the score, the greater the individual's knowledge about the diabetes (Table 2).

3.4. Confirmatory Factor Analysis (CFA)

The confirmatory factor analysis showed that the calculated value of the Chi-Square goodness of fit test was 818.998. Dividing the chi-square value by the degree of freedom yielded 2.43. This value was below three and therefore showed excellent fit. When the other fitness indexes were calculated, the Goodness of Fit Index (GFI) was found to be .93. The Adjusted Goodness of Fit Index (AGFI) was calculated as .86. The Comparative Fit Index (CFI) was .93. The Non-normed Fit Index (NNFI) was .90. A Root-Mean-Square Residual (RMR) of 0.05 or below (.01) indicates excellent fit. The RMSA was found to be .54.

A diagram of the model revealed at the end of the CFA can be seen in Figure 1.

According to the results of the CFA, it was found that all of the items in the scale significantly represented the dimensions they were meant to represent (Figure 1).

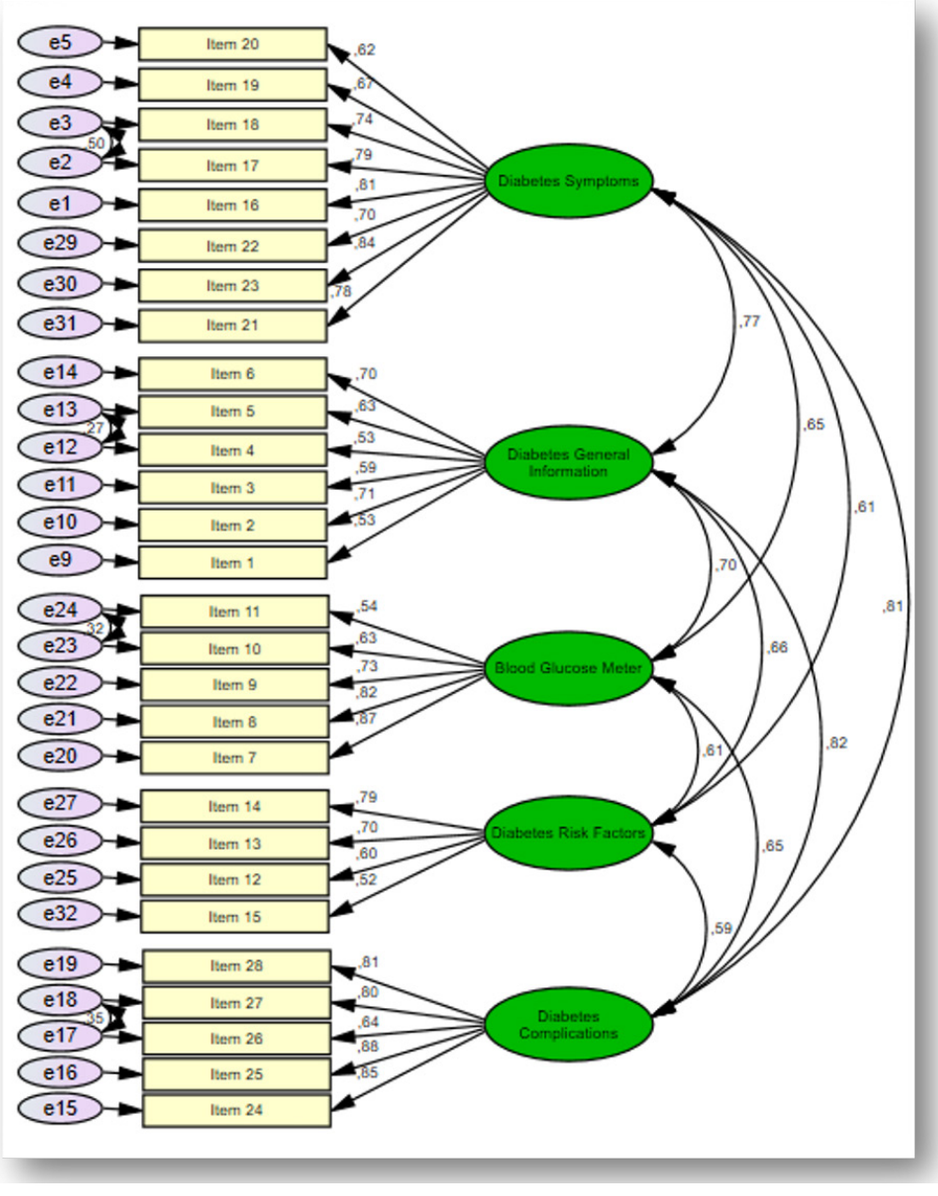

Figure 1. *The Diabetes Knowledge Scale for Adults Path Diagram and Standardized Analysis Results*

3.5. Reliability Analysis Results

A KR-20 reliability coefficient of .94 was found for the overall scale.

In the general diabetes knowledge dimension, the KR-20 alpha value was found to be .78; item-total score correlations varied between .45 and .62.

In the blood glucose measurement dimension, the KR-20 alpha value was .85, item-total correlations varied between .55-.73.

In the diabetes risk factors dimension, the KR-20 alpha value was .74 and item-total correlations varied between .47-.61.

In the diabetes symptoms dimension, the KR-20 alpha value was .91 and item-total correlations varied between .59-.79.

In the diabetes complications dimension, the KR-20 alpha value was .90 and item-total correlations varied between .65-.80 (Table 2).

3.6. Stability over time (test-retest)

Pearson's Correlation Analysis calculated following the retest after two weeks as from the first administration of the test indicated r=.98; p=.00. Statistically, a strong, significant and positive correlation was found between the two measurements.

4. DISCUSSION

At the end of this methodologically designed research, a Diabetes Knowledge Scale for Adults was developed containing 28 items and five subscales for assessing knowledge about diabetes symptoms, general knowledge of diabetes, blood glucose measurements, diabetes risk factors, and diabetes complications. The analyses showed that the scale was valid and reliable.

Content validity and construct validity analyses are the most commonly employed means of testing validity (17). In this study, we tested for content and construct validity (EFA and CFA).

Content Validity Index (CVI): Polit and Beck's (2006) CVI was used in this study (16). The scale items were presented to 10 experts for their views. In this method, the experts are asked to rate each item on the basis of 1-4 with 1 signifying "not appropriate," and 4 meaning "very appropriate." The number of experts rating each item 3 or 4 divided by the total number of raters leads to the calculation of CVI for both the item and the overall scale. For the scale or item to show content validity, CVI must be .80 or above 1(16). Since the scale and its items had CVI values of 0.92, it was concluded that there was a high level of content validity.

Construct validity testing is used to determine what a scale measures and what the scores of the study participants signify. To decide whether the construct validity data are suitable for factor analysis, the Kaiser-Meyer-Olkin (KMO) coefficient is expected to be over .60 and Barlett's test must indicate significance (18). In this study the KMO coefficient was .94, which showed that the sample size was at a very good level for factor analysis (19). Bartlett's test was at a significance level of p=.00, which meant that there was high correlation between the scale variables and the data were of multivariate normal distribution (18,20).

Factor analysis is performed as exploratory and confirmatory factor analysis. Eigenvalues in exploratory factor analysis are used to explain the percentage of factor variance and to decide on the number of subscales (17). In this study, the EFA results showed that the eigenvalue of the scale was greater than +1 and was distributed in seven subscales. It is recommended in exploratory factor analysis that the lower limit on factor loadings is kept at a high level. Because of this, the lower limit for the scale's item factor loadings was kept at .40 and the overlap limit was accepted as – .20 (21). After the factor analysis, 22 items with overlapping factor loadings were removed from the scale and a repeated EFA resulted in 5 subscales and a 28-item scale.

It was seen in the DKSA that was developed that the five factors explained 62.15% of total variance. The high level of variance showed that the scale was able to make a good measurement of the concept.

The factor loading in Exploratory Factor Analysis is represented by a coefficient that explains the relationship of the item with the various factors and is related to sample size; a factor loading of .60 and over is considered a high factor loading regardless of its direction (21). In this study, the item factor loadings were between .39 and .92, pointing to moderate and high factor loading. In this study, the results of the confirmatory factor analysis of DKSA were found to be statistically acceptable.

The data obtained as a result of the confirmatory factor analysis are examined with goodness of fit statistics to find whether or not they acceptably support the model (14, 21).

If dividing one of the goodness of fit statistics, the chi-square value by degree of freedom, as recommended in the literature, results in three or less, this indicates that the model is an excellent fit. Chi-square/degree of freedom in this study came out to 2.43. This indicated an excellent model fit (22).

In terms of other frequently used goodness of fit statistics, a RMSEA value equal or less than .08, a RMR value of less than .10, CFT, NNFI values equal to or greater than .90, an AGFI value equal to or greater than .80 indicates satisfactory fit (23).

The values above the arrows between the factors and items are standardized factor loadings; a statistic of at least .30 or over is recommended (21).

The standardized factor loadings of all of the items in DKSA were found to be in the range of .52-.87 (Figure 1). It was seen accordingly that all of the scale items were related to a subscale and appropriately represented it.

The most commonly used techniques to assess the reliability of a scale are internal consistency (KR 20-21, Cronbach's alpha), item-total score correlations, and test-retest reliability (25).

Internal consistency analysis is performed to assess whether the statements in an instrument are consistent with each other. In internal consistency analysis, the aim is to find out whether the items can measure a specified conceptual construct (14). It is expected that reliable instruments will show a high level of internal consistency. In the literature, the internal consistency coefficient is said to indicate that the scale is not reliable when it is $.00 \leq \alpha < .40$, of low reliability when it is $.40 \leq \alpha < .60$, of satisfactory reliability when it is $.60 \leq \alpha < .80$, and highly reliable when the coefficient is $.80 \leq \alpha < l.0$ (24). The total KR-20 reliability coefficient for DKSA was .94; these values in the subscales varied in the range of .74 – .91 It can be seen that the scale displayed a high degree of reliability. Revised diabetes knowledge scale developed by Colins et al. Cronbach's alpha value is .71. Brief Diabetes Knowledge Test developed by Fitzgerald, et al. (2016) Cronbach's alpha value was found ≥.77. In our study, the Cronbach's alpha value was found to be higher than both studies.

The desired item-total correlation coefficient is a value between .20 – .25 (19). An item-total correlation of .30 and above, it is reported, indicates that the statements can discriminate between individuals (14,18). In this study, the item-total correlation coefficients were above .45 and therefore the analysis indicated that all of the scales were valid tools of measurement.

Item correlations with the total knowledge test score ranged from .23 and .45 on the Revised Diabetes Knowledge Scale. In this study, the item-total correlation (.45) coefficients were above (10).

Assessment of items relative to the item difficulty index: The most frequently used techniques of item analysis are Item Difficulty and Item Discrimination Analyses. The item difficulty index is used when items have more than 1 possible responses (25).

The test-retest method is a way of assessing whether the measurements taken with the same scale are consistent over time (18). Although the variable may change between two measurements according to the particular feature being measured and the sample, it is expected that an interval of two-four weeks is enough to trace a significant correlation between the results; the coefficient should be positive and at least .70 (14). In the present study, there was a statistically positive and strong significant correlation between the two measurements taken of the individuals two weeks apart ($r = .99$; $p = .00$). The strong and positive correlation between the mean scores on the test and retest showed that the scale showed stability over time and reliability was high.

5. CONCLUSION

DKSA is a valid and reliable scale that can be used to determine the knowledge level of between the ages of 18-90 adults about diabetes.

The Adult Diabetes Knowledge Scale, which was developed for the first time in Turkey with this study, and the scale has 28 items and five sub-dimensions. The items of the scale are answered as "Yes", "I don't know", "No". Those who give correct answers receive "1" points, and those who answer incorrectly and "I don't know" receive "0" points. Higher scores indicate higher knowledge about diabetes. DKSA, evaluates the definition of diabetes, basic information about diabetes, diabetes symptom findings, blood glucose measurement values (laboratory findings), diabetes complications.

It might also be suggested that the scale that has been developed can be applied to different samples and in further experimental studies that will shed light on the efficacy of training interventions.

What is the contribution of this article to the application?

- The scale developed in this study was can be used in reducing the physical and moral burden diabetes places on the shoulders of individuals, families and the country, in fighting the global diabetes epidemic, and in increasing awareness in the community about the disease.
- The scale can be applied to healthy individuals at primary care facilities and to those applying to diabetic clinics and can be used as a guide to identify levels of knowledge about diabetes, gaps in knowledge and to facilitate the creation of education programs in this area.
- Scale can be administered before or after training as a pre – and post-test to provide information about the effectiveness of the education.

REFERENCES

[1] Saeedi P, Salpea P, Karuranga S, Petersohn I, Malanda B, Gregg EW, Unvin N, Wild SH, Williams R. Mortality attributable to diabetes in 20–79 years old adults, 2019 estimates: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Clin Pract. 2020; 162, 108086.

[2] Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, Karsidag K, Genc S, Telci A, Canbaz B, Turker F, Yilmaz T, Cakir B, Tuomiletho J. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol. 2013; 28 (2):169–180.

[3] Burki T. Country in Focus: diabetes prevalence on the rise in Turkey. Lancet Diabetes Endocrinol. 2016; 4 (5):391.

[4] Al-Qazaz HK, Sulaiman SA, Hassali MA, Shafie AA, Sundram S, Al-Nuri R, Saleem F. al. Diabetes knowledge, medication adherence and glycemic control among patients with type 2 diabetes. Int J Clin Pharm. 2011; 33 (6):1028–1035.

[5] Zowgar AM, Siddiqui MI, Alattas KM. Level of diabetes knowledge among adult patients with diabetes using diabetes knowledge test. Suadi Med J. 2018; 39(2):161-168

[6] Cosansu G. Diabetes: a global pandemic disease. Okmeydanı Tıp Dergisi, 2015; 31(Additional Issue):1-6.

[7] Trikkalinou A, Papazafiropoulou AK, Melidonis A. Type 2 diabetes and quality of life. World J Diabetes. 2017;8(4): 120-171.

[8] Dussa K, Parımalakrıshnan S, Sahay R. Assessment of diabetes knowledge using diabetes knowledge questionnaire among people with type 2 diabetes mellitus. Asian J Pharm Clin Res. 2015; 8 (2): 254-256

[9] Fitzgerald JT, Funnell MM, Anderson RM, Nwankwo R, Stansfield RB, Piatt GA. Validation of the revised brief diabetes knowledge test (DKT2). Diabetes Educ. 2016; 42(2): 178-187.

[10] Collins GS, Mughal S, Barnett AH, Fitzgerald JT, Lloyd CE. Modification and validation of the Revised Diabetes Knowledge Scale. Diabet Med. 2011; 28: 306-310.

[11] Eigenmann CA, Skinner T, Colagiuri R. Development and validation of a diabetes knowledge questionnare. Practice Diabetes Int. 2011;28(4): 166-170.

[12] Garcia AA, Villagomez ET, Brown SA, Kouzekanani K, Hanis CL. The Starr County Diabetes Education Study: Development of the Spanish-language diabetes knowledge questionnaire. Diabetes care. 2001; 24(1): 16-21.

[13] Olgun N, Yalın H, Demir H. Diagnosis and risk determination of diabetes dealing with diabetes. The Journal of Turkish Family Physician, 2016; 2 (2): 41-49.

[14] Erdogan S, Nahcivan N, Esin MN. Research Process in Nursing, Application and Critical. 2th ed. Nobel Medicine Book Houses; 2014. Data collection methods and tools & reliability and validity of data collection tools; P.195-232

[15] Fitzgerald JT, Funnell MM, Hess GE, Barr PA, Anderson RM, Hiss RG, Davis WK. The reliability and validity of a brief diabetes knowledge test. Diabetes care, 1998; 21(5): 706-710.

[16] Polit DF, Beck CT. The content validity index: are you sure you know what's being reported? critique and recommendations. Res Nurs Health. 2006; (29): 489–497.

[17] Buyukozturk S, Cakmak EK, Akgun OE, Karadeniz S, Demirel F. In education scientific research methods. 30 th ed. Pegem Academy Publishing; 2021.

[18] Buyukozturk S. Data analysis handbook for social sciences, statistics, research design, SPSS applications and interpretation. 28th ed. Pegem Academy Publishing; 2020.

[19] Whitehead D. Nursing and Midwifery Research: Methods and Appraisal for Evidence-Based Practice. 4th ed. Australia: Elseiver; 2013.

[20] Z Schneider, D Whitehead. Nursing and midwifery research: Methods and appraisal for evidence based practice. 5th ed. Australia: Elsevier; 2016.

[21] O Cokluk, G Sekercioğlu, S Buyukozturk. Multivariate statistics for social sciences: SPSS and LISREL applications. 5th ed. Ankara: Pegem Akademi; 2018.

[22] Karakoc FY, Donmez L. Basic principles of scale development. World of medical education, 2014; 40: 39-40.

[23] Ilhan M, Cetin B. Comparing the analysis results of the structural equation models (SEM) conducted using LISREL and AMOS. Journal of measurement and evaluation in education and psychology, 2014; 5(2): 26-42.

[24] Y Karagoz. SPSS – AMOS – META applied istatistical analysis. 2th ed. Ankara: Nobel Academic Publishing; 2019.

[25] Hasancebi B, Terzi Y, Kuçuk Z. Distractor analysis based on item difficulty index and item discrimination index. Journal of Gumushane University Graduate School of Natural and Applied Sciences, 2020;10 (1): 224-240.

How to cite this article: Erenel Yavuz K, Erol S. Developing a Diabetes Knowledge Scale for Adults and its Psychometric Properties. Clin Exp Health Sci 2022; 12: 711-718. DOI: 10.33808/clinexphealthsci.1014888

Clinical and Experimental Health Sciences

Pediatric Open Globe Injury in a University-Based Tertiary Hospital in the Anatolian Region

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Received: 26.10.2021 **Accepted:** 31.01.2022

ABSTRACT

Objective: Evaluation of the epidemiological and clinical features of pediatric open globe injury.

Methods: Medical records of 51 patients under the age of 18 who presented to the emergency department with open globe injury (OGI) between 2009 and 2021 were evaluated retrospectively. Patient demographics, Birmingham Eye Trauma Terminology (BETT) category, pediatric ocular trauma score (POTS), visual acuity (VA), site of injury, and seasonal distribution were evaluated.

Results: Of all patients included, 40 (78.4%) were male, 11 (21.6%) were female, and the mean age was 9.6±5.2 years. Zone I injury was detected in 31 (60.8%) patients. The most common cause of injury was sharp objects such as metal or wood splinters. Of all injuries, 29 (56.9%) occurred outdoors. Ocular trauma occurred more frequently in boys, especially in the summer months. Initial VA was less than 0,1 in 21 (41.2%) of the patients, between 0.1-0.5 in 9 (17.6%) and 0.6 and above in 5 (9.8%) patients. At the final visit, VA was less than 0.1 in 9 (17.6%) patients, 0.1-0.5 in 10 (19.6%) patients, and 0.6 and above in 18 (35.3%) patients. Final VA was related to the Zone and was lower as the Zone increased (p:0.011). VA and POTS scores were positively correlated at the final visit (p=0.001).

Conclusion: It is substantial for emergency physicians to have a comprehensive understanding of pediatric globe injuries so that children with suspected ocular trauma should be evaluated by an ophthalmologist to prevent medicolegal problems.

Keywords: Children, open globe, trauma, prognosis

1. INTRODUCTION

Globe injury is an important health problem causing globe deformities and visual impairment leading to psychological and sociological effects worldwide (1). According to a previous study, approximately 1.6 million people are blind, 2.3 million people have bilateral visual impairment, and 19 million people have unilateral vision loss due to eye trauma (2). Globe injury accounts for 7% of all bodily injuries and 10-15% of all ocular pathologies (3). Ocular trauma is one of the leading causes of unilateral vision loss and non-congenital unilateral blindness in children (4). Poor visual outcome is a result of amblyopia due to prolonged visual deprivation besides the damage to the ocular structures leading to vision loss (5).

Children are more prone to ocular injuries as a result of immature motor skills and limited experience since they are driven by curiosity and may impersonate others neglecting the risks and outcomes of a certain activity (6). The diagnosis and treatment of pediatric ocular injuries have their challenges due to the uncooperative nature of children to the ophthalmic examination and poor compliance to the treatment (1). Population-based studies on pediatric globe injury have revealed that two-thirds of these traumas in children occur at home and are closed globe trauma in boys (7, 8). However, severe visual impairment is mainly due to open globe injuries (9). Traumatic globe injury in children is a significant socioeconomic problem as it requires long-term follow-up and treatment. Furthermore, it affects the social, emotional, and psychological development of the child along with the caused physical disability. Previous studies on pediatric eye injuries suggested that up to 90% of eye injuries can be prevented with better education, supervision of children's activities, and proper protective eyewear (10). Therefore, defining the risk factors and taking preventive measures is beneficial to reduce perilous outcomes of globe injuries in children.

The aim of this study is to describe the epidemiology and characteristics of globe injuries in children over a 12-year period who applied to our clinic. The study also evaluates the frequency and causes of eye injuries in children and the main factors associated with the occurrence of these injuries

DOI: 10.33808/clinexphealthsci.1014872

therefore we aim to offer specific recommendations for the formation of primary prevention measures. The results of the study will provide data in terms of prospective follow-up and prognosis by comparing the type and cause of trauma, surgery, and treatment with the findings of follow-up examination.

2. METHODS

This retrospective study consisted of medical records of 51 patients younger than 18 years of age who were referred to the Department of Ophthalmology, which is located in the central Anatolian region and provides tertiary healthcare services for the surrounding cities, with traumatic globe injury between January 2009 and March 2021. The study was approved by the administration and the local ethics committee of Sivas Cumhuriyet University Faculty of Medicine on 10/03/2021 with the number 2021-03/20 and carried out in accordance with the principles outlined in the Declaration of Helsinki. The medical records of patients were reviewed and the data including epidemiological characteristics such as age and gender, laterality, cause of injury, zone of injury, place of injury, the initial and final best-corrected VA with Snellen, anterior and posterior segment findings, and seasonal distribution were noted. Pediatric penetrating ocular trauma score (POTS) was calculated for each patient (3). The raw points for each variable affecting POTS is presented in Table 1. The following equation: 2 × (age + location of injury) − corresponding pathologies was used to determine POTS for those whose initial VA was absent. The scores were utilized to divide patients into five groups. Group 1 being the poorest and Group 5 the best prognosis. The necessity for additional surgery (e.g. traumatic cataract removal) or any observed secondary complication following primary closure was also noted down. The OGI was classified as globe rupture, penetrating injury, perforating injury, and intraocular foreign body according to Birmingham Eye Trauma Terminology (BETT) (11). Zone of injury was classified according to Ocular Trauma Classification Group as follows: Zone I as wound involvement isolated to the cornea, Zone II as full-thickness wound including the sclera but no more posteriorly than 5 mm from the limbus, and Zone III as full-thickness wound more than 5 mm posterior to the sclera (12). Patients with known systemic diseases, who have a history of any ocular pathology or surgery, who had major head trauma which might result in a negative impact on the visual pathways, and who had a follow-up period of less than 3 months were excluded from the study.

2.1. Statistical Analysis

The data analysis was performed with Statistical Package for the Social Science (SPSS version 20.0. Armonk, NY: IBM Corp.) software for Windows. The normality of the data will be checked with the Kolmogorov-Smirnov test. Independent sample t-test for two independent groups and F-test (ANOVA) for more than two groups were used if parametric conditions were met., Tukey test will be used for those who provide the homogeneity assumption and Tamhane's T2 tests for those who do not provide the homogeneity assumption to determine which group is different from the others when using ANOVA for comparisons with more than two groups. If any or all of the assumptions were not met, the Mann Whitney U test for two independent groups and the Kruskal Wallis test for more than two independent groups were used. Pearson correlation coefficient for parametrics and Spearman correlation coefficient for non-parametrics were used to determine the relationship between variables. In addition, regression analysis was performed to determine the effect. The Chi-square test was used to evaluate the categorical data. A value of $p \leq 0.05$ was considered statistically significant.

Table 1. *Calculating the POTS and raw points for variables*

Variables	Raw Points
Initial visual acuity	
NLP	10
LP/HM	20
CF	30
0.1-0.5	40
0.6-1.0	50
Age (years)	
0-5	10
6-10	15
11-15	25
Wound location	
Zone I	25
Zone II	15
Zone III	10
Concomitant eye pathologies	
Iris prolapse	-5
Hyphema	-5
Organic/unclean injury	-5
Delay of surgery (>48 h)	-5
Traumatic cataract	-10
Vitreous haemorrhage	-20
Retinal detachment	-20
Endopthalmitis	-30

*POTS: Pediatric penetrating ocular trauma score, NLP: No light perception, LP: Light perception, HM: Hand motion, CF: Counting fingers. *POTS = 2 × (age + location of injury) – corresponding pathologies*

3. RESULTS

The mean age of 51 patients was 9.6±5.2 years. The mean age was 10.7±5.0 in boys, and 5.6±4.0 in girls. There was a statistically significant increase in the male to female ratio as the age increased (p=0.02). Of 51 patients, 40 (78.4%) were male and 11 (21.6%) were female. Right eye was involved in 25 of the cases where the remaining 26 injuries were left-sided. There was no statistically significant difference between the genders in terms of the laterality of the affected eye (p=0.8). Evaluation of the location of the injury revealed that 22 (43.1%) were indoors and 29 (56.9%) were outdoors. Outdoor

injury was more common as the age increased. Regarding the cause of the injury, 19 (37.3%) were metal objects such as knives and scissors, 14 (27.5%) were wood, 6 (11.8%) were glass, 6 (11.8%) were stone, 4 (7.8%) were plastic, 1 (2%) was by finger, and 1 (2%) was by a sparkler. The demographics of the study population are summarized in Table 1. The most common zone of injury was Zone I and was observed in 31 (60.8%) patients. Zone II injury occurred in 19 (37.3%) patients, and Zone III injury in only 1 (2%) patient. The distribution of the laterality of the injured eye and the zone of injury are given in Table 2. We observed that ocular trauma occurs more frequently in boys, especially in the summer months. Seasonal distribution of pediatric open globe injuries was given in Figure 1. Immediate primary suturing of the wound was performed under general anesthesia for all patients initially. Prophylactic intravenous cefuroxime sodium, topical moxifloxacine were administered following hospitalization. Postoperative intravenous antibiotic prophylaxis is continued for 3 consecutive days in total and topical antibiotics and and steroid eye drops steroids are tapered based on the findings of the biomicroscopic examination during follow-up. The mean VA was 0.18±0.28 at the initial examination increasing to 0.49±0.40 at the final visit. The increase in VA between the initial and the final ophthalmologic examination was statistically significant (p<0.001). The VA was below 0.05 in 21 (41.2%) of the patients, between 0.1-0.5 in 9 (17.6%), and above 0.6 in 5 (9.8%) patients at the initial examination. However, we were not able to assess VA in 16 (31.4%) patients due to young age, incompliance to examination, or agitation. The initial, postoperative day 1, and final VA of the patients were presented in Table 3. The mean calculation using raw points for POTS was 64.8±19.4 and the mean POTS was 2.7±1.2. The majority of children (66.7%) were in either Group 2 or 3 regarding POTS. The mean follow-up time was 16±13.5 months (3-36 months). A total of 4 (7.8%) patients required vitrectomy. Surgical removal of an intraocular foreign body was performed in 6 patients. Traumatic cataract was present in 13 (25.5%) patients at the initial examination. Additional 6 patients either developed cataract subsequently or were observed to have cataract at the following examinations. A total of 19 (37.3%) patients underwent lensectomy and the mean time interval from primary suturing to lensectomy was 62.6±170 days (1-730 days). Iris prolapse was observed in 8 (15.7%) patients, hyphema in 7 (11.8%), vitreous prolapse in 4 (7.8%), iridodialysis in 1 (2%), and vitreous hemorrhage in 1 (2%) patients at the initial ophthalmic examination. Enophthalmos secondary to orbital floor fracture was observed in 1 (2%) patient. Initial ocular examination findings concomitant with open globe injuries were summarized in Table 5. Following the primary suturing, retinal detachment was observed in 7 (13.7%) patients, vitreous hemorrhage in 6 (11.7%) patients, and hyphema in 3 patients on the postoperative first day. At the final visit, we were not able to assess VA in 14 (27.5%) patients due to young age or incompliance to examination. VA was below 0.05 in 9 (17.6%) patients, between 0.1-0.5 in 10 (19.7%) patients, and above 0.6 in 18 (35.2%) patients. At the last visit, the following pathologies were observed; corneal scarring in 38 (74.5%) patients, retinal detachment in 7 (13.7%) patients, and synechia in 6 (11.8%) patients. A total of 13 (25.4%) patients were pseudophakic, 5 (9.8%) patients were aphakic, and 1 (2%) patient had IOL dislocation. Seven children (13.7%) who had retinal pathologies such as retinal detachment and persistent vitreous hemorrhage were referred for pediatric retinal surgery. The final VA was found to be negatively correlated to the Zone as VA decreased while the Zone increased (p=0.011). Initial VA was positively correlated with the final VA (p=0.02). Also, there was a positive correlation between VA and POTS in children at the last visit (p=0.001). Traumatic cataract was found to be related with poor final VA (p=0.04).

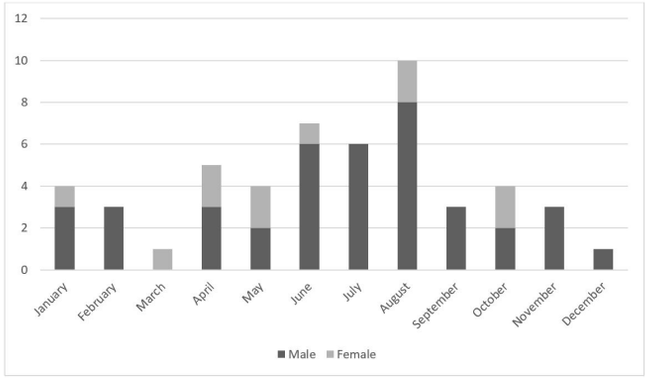

Figure 1. *Seasonal distribution of open globe injuries*

Table 2. *Demographics of the study population (n:51)*

		Mean±SD, n (%)
Age (years)		9.6±5.2
Gender	Male	40 (78.4)
	Female	11 (21.6)
Location of injury	Outdoor	29 (56.9)
	Indoor	22 (43.1)
Cause of injury	**Sharp objects**	44 (86.2)
	Metallic	19 (37.3)
	Wood splinter	14 (27.5)
	Glass	6 (11.8)
	Plastic	4 (7.8)
	Body part (Nail)	1 (2)
	Blunt objects	6 (11.8)
	Stone	6 (11.8)
	Projectiles	1 (2)
	Firework	1 (2)

Table 3. *Distribution of the laterality of the injured eye and the zone of injury*

	n	(%)
Laterality of the globe injury (right/left)	25 / 26	49 / 51
Zone I	31	60.8
Zone II	19	37.3
Zone III	1	2
Total	51	100

Table 4. *The initial, postoperative day 1, and final visual acuity of the patients*

Visual acuity (Snellen)	Initial n (%)	Postoperative day 1 n (%)	Final n (%)
>0.6	5 (9.8)	6 (11.8)	18 (35.2)
0.1-0.5	9 (17.6)	19 (37.2)	10 (19.7)
<0.05	21 (41.2)	19 (37.2)	9 (17.6)
Not specified	16 (31.4)	7 (13.8)	14 (27.5)
Total	51	51	51

Table 5. *Initial ocular examination findings concomitant with open globe injuries*

Examination finding	n*	%
Traumatic cataract	13	25.5
Iris prolapsus	8	15.7
Hyphema	6	11.8
Vitreus prolapsus	4	7.8
Iridodialysis	1	2.0
Synechia	1	2.0
Vitreous hemorrhage	1	2.0

**Total number of patients*

4. DISCUSSION

Open globe injuries may cause visual impairment and at times result in blindness leading to lifelong morbidity. In the present study, the majority of the patients (78.4%) were male. Outdoor injury was more common (56.9%) and metal objects and wood were among the most frequent causes of injury. The most common zone of injury was Zone I and was observed in 31 (60.8%) patients. We also observed that ocular trauma occurs more frequently in boys, especially in the summer months. Presence of traumatic cataract, Zone II and III injury was observed to be in relation with poorer final VA.

In the literature, OGIs were reported to occur mostly in the male gender (13, 14). Our findings were similar with these studies reporting a 3.6:1 ratio. Soylu et al. evaluated the etiology of pediatric perforating eye injuries in southern Turkey and demonstrated a ratio of 2.6:1 (15). This preponderance might be explained by not only the brisk nature of boys but also due to greater physical contact compared to girls (16, 17). Similar to our findings, previous studies have also suggested no significant difference regarding the affected eye (18). Even though the open globe injuries are demonstrated to occur mostly at home (14), studies from developing countries suggest that outdoor injuries are more common (19, 20). Consistent with these studies, we found that most of the open globe injuries in our study occurred outdoors. We also observed that outdoor injury was more common as the age increased given the fact that older children are more likely to engage in outdoor activities without parental supervision compared to younger peers.

Previous studies have reported that injuries with sharp objects such as knives or scissors are the most common cause of globe injury in the pediatric population (21, 22). However, there are studies from Turkey indicating metal objects, pieces of wood or glasses are presumably the cause of injury (23-25). In our study, 86,2% of all injuries were caused by sharp objects in accordance with previous studies(14, 26). In the current study, the injuries due to sharp objects were mostly caused by metal objects, wood splinters, and glass. In addition, one of the 51 injuries was caused by projectiles such as fireworks. To protect children from hazardous injuries at home, objects like knives, scissors, and other sharp utensils should be kept in a drawer with a safety latch. Glass objects should be stored in a high cabinet far from reach. The cause of injury might not be determined at times when parents or caregivers were unable to witness the incident. Studies report various rates of unknown causes of injury yielding a percentage of 2.0%-13.4% (27). However, in our study cause of injury was determined in all patients.

Our findings demonstrated a seasonal alteration regarding OGI. The majority of the OGI occurred in the summer (45.1%) followed by injuries in autumn, spring, and winter (19.6%, 17.7%, 15.7%, respectively). These findings are in accordance with other studies on eye injuries from Turkey (21, 28). Furthermore, studies from other countries including Croatia, China, and Canada have also reported a high percentage of eye injuries during summer months (10, 29, 30). Since the weather is cold, children spend most of their time in school under the supervision of their teachers and occupied with curricular activities such as homework during winter may explain the lower rates of injuries. However, children have the freedom to spend more unsupervised time outside and are thus exposed to potentially hazardous activities during summer.

Similar to previous studies, the majority of the injuries in our study were in Zone I (21, 31, 32). Complete ophthalmic examination and proper assessment may not be possible in the pediatric population especially in the emergency room settings. Therefore, the emergency physicians and ophthalmologists might be unable to determine the VA at the initial examination which is a vital parameter in estimating prognosis. In our study, we were unable to determine the initial VA in 35.2% of the patients. There are studies on OGI reporting various final VA below 0.1, which is described as legal blindness, ranging from 31% to 58% (13, 18, 33). In our study, the final VA was below 0.05 in 17.6% of the patients. This lower percentage might be explained by the unrecorded final VA in 27.5% of the study population.

It is acknowledged that IOP tends to fluctuate following complex ocular surgeries including the globe repair due to penetrating ocular injury. Aldahash et al. documented a final intraocular pressure (IOP) in %74 of the children with OGI (13). However, they did not report the IOP measurements after surgery. We also were unable to obtain IOP measurements after surgery due to lack of patient cooperation in our study population.

There have been controversial studies on the factors affecting the prognosis of OGI in which lens damage is described as one

of the poor prognostic factors yet other studies stated that it does not affect the visual outcome (22, 34). In our study, 13 patients had traumatic cataracts followed by iris prolapsus in 8, and hyphema in 6 patients at the initial examination. In accordance with some of the aforementioned studies, we observed that the presence of lens damage such as traumatic cataract was associated with poor vision. In addition, corneal scars and irregular astigmatism as a consequence of ocular penetrating injury, may be considered as other causes of low final VA since the majority of the pediatric OGI implicate Zone I and II. Also, corresponding retinal pathologies due to ocular trauma may be among potentially sight threatining causes.

We were unable to obtain the initial VA from the medical records in 31.4% of the patients, which is essential for calculating the Ocular Trauma Score (OTS). Kuhn et al. designed the OTS to determine the potential final visual outcome in patients with eye injury (35). Even though it has been a substantial tool to evaluate the prognosis of VA in ocular trauma, some studies revealed its limited predictive efficacy in pediatric ocular injury (36). In addition, OTS requires the evaluation of relative afferent pupillary defect (RAPD), which is difficult to assess especially in children. Therefore, Acar et al. released the POTS which facilitates the challenges of the OTS eliminating the necessity to determine patients' initial VA (3). The POTS is a tool to predict prognosis and visual outcomes regarding eye injuries in the pediatric population (3). Several studies have demonstrated that it is a reliable prognostic scoring system in pediatric globe injury (36, 37). Similar to these aforementioned studies, we found that VA and POTS scores were correlated in children with visual acuity of 0.1 and above at the final visit.

Open globe injury is predominantly more severe and generally accompanies complications, the need for surgical intervention, and poorer prognoses (14). Younger age and lower initial VA were assessed as poor prognostic factors in patients with OGI (38, 39). Younger children are more prone to amblyopia since the visual development continues until the age of 9-10 years (3). Therefore, deprivation amblyopia may cause devastating visual outcomes than the injury itself (3). Due to the nature of pediatric globe injuries and the development of amblyopia, the final VA is worse in children compared to adults even if appropriate medical interventions are provided (16). Educating children to avoid potentially sight-threatening activities, informing parents to take preventive measures, and keeping a safe environment by supervising the child at all times possible may help prevent such injuries.

Difficulties in obtaining data of importance in children such as VA, retrospective setting of the study, variable follow-up times, lack of certain time interval between the onset of ocular trauma and hospital admission, and the timing of surgery following hospital admission were among the limitations of this study. Longer follow-up times may be valuable to observe long-term outcomes of pediatric OGI. Also, we were unable to measure IOP following surgical intervention. Therefore, potential IOP fluctuations following surgery were not documented. Since all patients needed to be operated under general anesthesia, patients that have recently ingested foods could not be operated immediately. Therefore, the timing of the surgery varied to prevent potentially life-threatening complications of general anesthesia. In addition, only patients with OGI applied or referred to our tertiary hospital were included in the study. Therefore, we may have encountered a lower incidence of pediatric OGI.

5. CONCLUSION

Penetrating eye injuries are of great importance among pediatric emergencies. Corneal injuries are more common during childhood in particular. This kind of ocular trauma may cause not only functional but also psychosocial problems ranging from a permanent decrease in visual acuity to eye loss. Child and parent education and taking preventive measures to prepare a safe environment for children are of utmost importance. Since the cause of injury might not be determined at times when parents or caregivers were unable to witness the incident, objects like knives, scissors, and other sharp utensils should be kept in a drawer with a safety latch. Glass objects should be stored in a high cabinet far from reach to protect children from hazardous injuries at home. In addition, it is substantial for emergency physicians to be informed about pediatric penetrating eye injuries and to refer all children with suspected ocular trauma to be evaluated by an ophthalmologist in order to prevent medicolegal problems. Studies with larger sample sizes and longer follow-up times are needed to confirm the long-term outcomes of pediatric penetrating ocular injury.

Acknowledgement: None.

Funding: None.

Conflicts of interest: All authors declare that there is no conflict of interest.

REFERENCES

[1] Al-Mahdi HS, Bener A, Hashim SP. Clinical pattern of pediatric ocular trauma in fast developing country. Int Emerg Nurs. 2011;19(4):186-191.

[2] Négrel AD, Thylefors B. The global impact of eye injuries. Ophthalmic Epidemiol. 1998;5(3):143-169.

[3] Acar U, Tok OY, Acar DE, Burcu A, Ornek F. A new ocular trauma score in pediatric penetrating eye injuries. Eye (London, England). 2011;25(3):370-374.

[4] Jandeck C, Kellner U, Bornfeld N, Foerster MH. Open globe injuries in children. Graefes Arch Clin Exp Ophthalmol. 2000;238(5):420-426.

[5] Saxena R, Sinha R, Purohit A, Dada T, Vajpayee RB, Azad RV. Pattern of pediatric ocular trauma in India. Indian J Pediatr. 2002;69(10):863-867.

[6] Puodžiuvienė E, Jokūbauskienė G, Vieversytė M, Asselineau K. A five-year retrospective study of the epidemiological characteristics and visual outcomes of pediatric ocular trauma. BMC Ophthalmol. 2018;18(1):10.

[7] Armstrong GW, Kim JG, Linakis JG, Mello MJ, Greenberg PB. Pediatric eye injuries presenting to United States emergency departments: 2001-2007. Graefes Arch Clin Exp Ophthalmol. 2013;251(3):629-636.

[8] MacEwen CJ, Baines PS, Desai P. Eye injuries in children: the current picture. Br J Ophthalmol. 1999;83(8):933-936.

[9] El-Sebaity DM, Soliman W, Soliman AM, Fathalla AM. Pediatric eye injuries in upper Egypt. Clin Ophthalmol. 2011;5:1417-1423.

[10] Podbielski DW, Surkont M, Tehrani NN, Ratnapalan S. Pediatric eye injuries in a Canadian emergency department. Can J Ophthalmol. 2009;44(5):519-522.

[11] Kuhn F, Morris R, Witherspoon CD. Birmingham Eye Trauma Terminology (BETT): terminology and classification of mechanical eye injuries. Ophthalmol Clin North Am. 2002;15(2):139-143.

[12] Pieramici DJ, Sternberg P Jr., Aaberg TM Sr., Bridges WZ Jr, Capone A Jr, Cardillo JA, de Juan E Jr, Kuhn F, Meredith TA, Mieler WF, Olsen TW, Rubsamen P, Stout T. A system for classifying mechanical injuries of the eye (globe). The Ocular Trauma Classification Group. Am J Ophthalmol. 1997;123(6):820-831.

[13] AlDahash F, Mousa A, Gikandi PW, Abu El-Asrar AM. Pediatric open-globe injury in a university-based tertiary hospital. Eur J Ophthalmol. 2020;30(2):269-274.

[14] Li X, Zarbin MA, Bhagat N. Pediatric open globe injury: A review of the literature. J Emerg Trauma Shock. 2015;8(4):216-223.

[15] Soylu M, Demircan N, Yalaz M, Işigüzel I. Etiology of pediatric perforating eye injuries in southern Turkey. Ophthalmic Epidemiol. 1998;5(1):7-12.

[16] Cariello AJ, Moraes NS, Mitne S, Oita CS, Fontes BM, Melo LA Jr. Epidemiological findings of ocular trauma in childhood. Arq Bras Oftalmol. 2007;70(2):271-275.

[17] Poon AS, Ng JS, Lam DS, Fan DS, Leung AT. Epidemiology of severe childhood eye injuries that required hospitalisation. Hong Kong Med J. 1998;4(4):371-374.

[18] Thompson CG, Kumar N, Billson FA, Martin F. The aetiology of perforating ocular injuries in children. Br J Ophthalmol. 2002; 86(8):920-922.

[19] Narang S, Gupta V, Simalandhi P, Gupta A, Raj S, Dogra MR. Paediatric open globe injuries. Visual outcome and risk factors for endophthalmitis. Indian J Ophthalmol. 2004;52(1):29-34.

[20] Skiker H, Laghmari M, Boutimzine N, Ibrahimy W, Benharbit M, Ouazani B, Daoudi R. Open globe injuries in children: retrospective study of 62 cases. Bull Soc Belge Ophtalmol. 2007;(306):57-61.

[21] Altıntaş L, Altıntaş O, Yüksel N, Pirhan D, Ozkan B, Cağlar Y. Pattern of open eye injuries in northwest Turkey: a retrospective study. Ulus Travma Acil Cerrahi Derg. 2011;17(4):334-339.

[22] Lee CH, Lee L, Kao LY, Lin KK, Yang ML. Prognostic indicators of open globe injuries in children. Am J Emerg Med. 2009;27(5):530-535.

[23] Tok O, Tok L, Ozkaya D, Eraslan E, Ornek F, Bardak Y. Epidemiological characteristics and visual outcome after open globe injuries in children. J AAPOS. 2011;15(6):556-561.

[24] Çetin EN, Saraç G, Kaşıkçı A, Avunduk AM, Yaylalı V, Yıldırım C. Epidemiologic and clinical features of open-globe injuries in childhood. Turk J Ophthalmol. 2012;42(1):16-19.

[25] Uysal Y, Mutlu FM, Sobaci G. Ocular Trauma Score in childhood open-globe injuries. J Trauma. 2008;65(6):1284-1286.

[26] Qayum S, Anjum R, Rather S. Epidemiological profile of pediatric ocular trauma in a tertiary hospital of northern India. Chin J Traumatol. 2018;21(2):100-103.

[27] Ilhan HD, Bilgin AB, Cetinkaya A, Unal M, Yucel I. Epidemiological and clinical features of paediatric open globe injuries in southwestern Turkey. Int J Ophthalmol. 2013;6(6):855-860.

[28] Cakmak SS, Unlu MK, Olmez G, Caca I, Sakalar YB, Acemoglu H. Penetrating eye injuries from southeastern Anatolia region of Turkey. Public Health. 2004;118(8):570-575.

[29] Bućan K, Matas A, Lovrić JM, Batistić D, Pleština Borjan I, Puljak L, Bućan I. Epidemiology of ocular trauma in children requiring hospital admission: a 16-year retrospective cohort study. J Glob Health. 2017;7(1):010415.

[30] Li C, Lin Y, Xiao H, Lin H, Chen Y, Dai M. Epidemiology of pediatric eye injuries requiring hospitalization in rural areas of Wenzhou and Changsha, China: a 10-year retrospective study. BMC Ophthalmol. 2020;20(1):100.

[31] Acar U, Tok O, Acar DE, Burcu A, Ornek F. Epidemiological and clinical evaluation of open-globe injuries in childhood. Turk J Ophthalmol. 2010;40:62-66.

[32] Acuna OM, Yen KG. Outcome and prognosis of pediatric patients with delayed diagnosis of open-globe injuries. J Pediatr Ophthalmol Strabismus. 2009;46(4):202-207.

[33] Hosseini H, Masoumpour M, Keshavarz-Fazl F, Razeghinejad MR, Salouti R, Nowroozzadeh MH. Clinical and epidemiologic characteristics of severe childhood ocular injuries in southern iran. Middle East Afr J Ophthalmol. 2011;18(2):136-140.

[34] Yalcin Tök O, Tok L, Eraslan E, Ozkaya D, Ornek F, Bardak Y. Prognostic factors influencing final visual acuity in open globe injuries. J Trauma. 2011;71(6):1794-1800.

[35] Kuhn F, Maisiak R, Mann L, Mester V, Morris R, Witherspoon CD. The Ocular Trauma Score (OTS). Ophthalmol Clin North Am. 2002;15(2):163-165.

[36] Xue C, Yang LC, Kong YC. Application of pediatric ocular trauma score in pediatric open globe injuries. Int J Ophthalmol. 2020;13(7):1097-1101.

[37] Awidi A, Kraus CL. A comparison of ocular trauma scores in a pediatric population. BMC Res Notes. 2019;12(1):569.

[38] Baxter RJ, Hodgkins PR, Calder I, Morrell AJ, Vardy S, Elkington AR. Visual outcome of childhood anterior perforating eye injuries: prognostic indicators. Eye (Lond). 1994;8:349-352.

[39] Gupta A, Rahman I, Leatherbarrow B. Open globe injuries in children: factors predictive of a poor final visual acuity. Eye (Lond). 2009;23(3):621-625.

How to cite this article: Bozali E, Yalinbas Yeter D, Apa AS, Vural Ozec A, Erdogan H. Pediatric Open Globe Injury in a University-Based Tertiary Hospital in the Anatolian Region. Clin Exp Health Sci 2022; 12: 719-724. DOI: 10.33808/clinexphealthsci.1014872

Clinical and Experimental Health Sciences

Medication Review in Turkish Older Adults at Community Pharmacy: A Pilot Study by Using Medication Appropriateness Index

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Received: 27.10.2021 **Accepted:** x02.06.2022

ABSTRACT

Objective: The study aimed to evaluate medication review in older adults (≥65 years) at a community pharmacy by identifying the prevalence of potentiality inappropriate medication and calculating medication appropriateness index.

Methods: This descriptive study was carried out in a community pharmacy for six months. The older adults (≥65 years) using one or more medications were included. During clinical pharmacist-led medication review; the medication appropriateness index was calculated for each medication of older adults. Potentially inappropriate medications were evaluated according to the 2019 American Geriatrics Society Beers Criteria®.

Result: Among a hundred older adults, 46.0% were female. The median age of the patients was 75.5 (IQR, 68.0-78.8). The median number of medications was 9.0 (7.0-10.0). Polypharmacy has been detected in 97.0% of the patients. At least one potentially inappropriate medication was detected in 63.0% of them. The median score of medication appropriateness index score was 53.0 (IQR: 38.6-67.9).

Conclusion: To best our knowledge, this is the first study of clinical pharmacist-led medication review by calculating the medication appropriateness index carried out at a community pharmacy in Turkey. There was a high rate of potentially inappropriate medication with a higher score of medication appropriateness in older adults. This study highlights the importance of medication review led by the clinical pharmacist at community pharmacy to optimize medication usage in older adults.

Keywords: Older adults, clinical pharmacist, medication appropriateness index, potentially inappropriate medication

1. INTRODUCTION

The older adult (≥65 years) population is increasing worldwide with higher life expectancy. The ratio of the older adult population in Turkey was 8.2% in 2015, and it was increased to 9.5% in 2020 (https://data.tuik.gov.tr/Bulten/Index?p=Istatistiklerle-Yaslilar-2020-37227, Date accessed: 17.03.2021). In 2020, older adults (individuals aged 60 and over) represented 13.5% of the world's population (https://www.who.int/publications/i/item/978.924.0017900, Date accessed: 17.03.2021). This population is estimated to reach approximately 2.1 billion by 2050 (https://www.who.int/publications/i/item/978.924.0017900, Date accessed: 17.03.2021). In parallel, it is predicted that the probability of problems related to medication use will increase even more in older adults and is resulted in hospitalization. It has been reported that the most common problems associated with medication use in older adults are polypharmacy, adverse drug reactions, drug-drug interactions, poor medication adherence, and potentially inappropriate medication use (PIM) (1, 2).

Medication review is crucial in older adults to identify medication-related issues (including overdose, adverse drug reactions, possible drug-disease, drug-drug interactions, medication adherence) along with pharmacokinetic and pharmacodynamic changes during aging (https://www.uptodate.com/contents/drug-prescribing-for-older-adults, Date accessed: 17.03.2021) (3). Potentially inappropriate medication (PIM) is identified as the use of medications that are predicted to be ineffective in older adults or to pose a high risk if used (4). The prevalence of PIMs has been found to be between 21-63% according to some previous studies (5-11). It was found that one or more medications were used inappropriately in almost half of the older adults who used medications (12). Evaluation criteria such as "2019 American Geriatrics Society (AGS) Beers Criteria®"(13) and/or "STOPP/

DOI: 10.33808/clinexphealthsci.1012114

START criteria" (The STOPP (The Screening Tool of Older Persons' Prescriptions) and START (The Screening Tool to Alert to Right Treatment) criteria) (14) and "Ghent Older People's Prescriptions Community Pharmacy Screening (GheOP^{3}S)-tool" (15) and "Medication Appropriateness Index" (MAI) (16) can be used to identify appropriateness medication used by older patients during clinical pharmacist-led medication review. In recent studies, it was shown that Beers Criteria® had superiority in determining the prevalence of PIMs (17-20). Also, the Beers Criteria® had a more up-to-date version when compared with other tools (13, 14).

MAI is a tool that evaluates the appropriateness of medication with ten criteria: indication, efficacy, dosage, correct instructions, practical instructions, drug-drug interaction, drug-disease interaction, duplication, time, and cost (16). MAI is a useful implicit tool with a simple scoring system while providing a comprehensive medication review (21). However, during the application of MAI criteria, the practitioner should have sufficient clinical experience and knowledge (22). In addition, there are disadvantages of using MAI, such as the inability to evaluate the under-prescribing of current treatment with MAI criteria and the time-consuming evaluation for inappropriate drug use (22). MAI has been used in hospital services, home care services, and community pharmacies to assess patients' medications, and it was determined that the decrease in MAI scores was associated with reducing hospitalization and improving their quality of life (23-26).

In Turkey, to the best of our knowledge, this study was first to evaluate medication appropriateness with MAI score in older adults at community pharmacy setting. Therefore, this pilot study aimed to evaluate clinical pharmacist medication review in older adults in a community pharmacy by determining the prevalence of potentiality inappropriate medication and calculating medication appropriateness index.

2. METHODS

2.1. Participant and Setting

This descriptive study was carried out between July 2018 and December 2018 in a community pharmacy located in Antalya, Turkey. Patients aged 65 and over who visited to a community pharmacy and used at least one medication were included in the study. There were no exclusion criteria. A hundred older adults, who visited the pharmacy, were consecutively included in the study by using convenience sampling method. The analysis of the collected data was carried out in April 2019.

The study was approved by the SBU Antalya Training and Research Hospital Clinical Research Ethics Committee at the meeting dated 05/07/2018 with the decision number 14/5. Informed consent was obtained from all participants.

2.2. Data Collection

Data of the patients, including their age, gender, education level (≥ 8 years and < 8 years), medications, and comorbidities were collected from patient interviews. All medications (including prescription or nonprescription drugs and dietary supplements) were recorded. Polypharmacy was defined as ≥4 concurrent drugs (27-32).

In this pilot study, both the MAI tool and the 2019 AGS Beers Criteria® were used by a single clinical pharmacist (NU) for medication review. Patient data were analyzed using the latest version 2019 AGS Beers Criteria® to assess the presence of PIM (13). MAI, which is used to evaluate the appropriateness of medications, consists of 10 criteria. Each criterion is evaluated as appropriate, neutral inappropriate, or unknown by the pharmacists. The maximum total score of MAI is 18. Higher MAI scores related to the inappropriateness of medication (16). In the study, the MAI score was calculated for each medication separately and total medication regimen.

2.3. Data Analysis

Kolmogorov-Smirnov test was used to analyze the normal distribution of data. Descriptive variables were expressed as median and interquartile range (IQR). The frequency of variables in the data is shown as a percentage (%) and number. The chi-square test was used in the analysis of nominal variables. The Mann-Whitney U-test was used to assess the differences between the two groups (defined as PIM user or not). The results were evaluated within a 95% confidence interval and *P* value of <0.05 was considered statistically significant.

3. RESULTS

The characteristics of the older patients are shown in Table 1. The median age of the patients was 75.5 (IQR, 68.0-78.8). Among them, 46.0% of the patients were female. In addition, 77.0% of all patients had less than eight years of education. The median number of medications were 9.0 (IQR, 7.0-10.0). Polypharmacy was detected in most patients (97.0%).

The MAI score was calculated for a total of 874 drugs in the study. The medication review took about 10 minutes per drug. The median MAI score for total medication regimen was 53.0 (IQR, 38.6-67.9). The median MAI per medication was 6.25 (5.00-8.00). The rate of PIMs was 63.0%. The median MAI score of patients with PIM is statistically higher than the mean MAI score of patients without PIM (*P*<0.05). The MAI scores of the older adults are summarized in Table 2.

The most common medication group with the highest MAI scores and the medication group with the most common PIMs are shown in Table 3. Accordingly, the therapeutic class of medications with the highest MAI score was Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and/or paracetamol (n=28), while the most prevalent PIMs was proton pump inhibitors (n = 42).

Table 1. *Characteristics of older adults*

Characteristic	Total Sample (n=100)	Not PIM users (n=37)	PIM users (n=63)	*P*
Age Median (IQR)	75.5 (68.0-78.8)	72.0 (67.5-80.5)	74.0 (68.0-78.0)	NS
Age (%)				
65-80 years	77 (77.0%)	27 (73.0%)	50 (79.4%)	NS
> 80 years	23 (23.0%)	10 (27.0%)	13(20.6%)	
Gender (%)				
Female	46 (46.0%)	17 (46.0%)	29 (46.0%)	NS
Male	54 (54.0%)	20 (54.0%)	34 (54.0%)	
Education level (%)				
<8 years	77 (77.0%)	29 (78.4%)	48 (76.2%)	NS
≥8 years	15 (15.0%)	5 (13.5%)	10 (15.9%)	
Missing data	8 (8.0%)	3 (8.1%)	5 (7.9%)	
Number of comorbid diseases				
Median (IQR)	5.0 (4.0-5.0)	4.0 (4.0-5.0)	5.0 (4.0-6.0)	NS
Number of medications				
Median (IQR)	9.00 (7.0-10.0)	7.0 (5.0-9.0)	9.0 (8.0-11.0)	<0.001
Polypharmacy (%)				
<4 medications	3 (3.0%)	3 (8.1%)	0 (0.0%)	<0.05
≥4 medications	97 (97.0%)	34 (91.9%)	63 (100.0%)	

PIM: Potentially Inappropriate Medication; NS: Nonsignificant IQR: Interquartile Rate

Table 2. *The MAI scores in older adults*

	Total Sample (n=100)	Not PIM users (n=37)	PIM users (n=63)	*P*
The Median MAI Score (IQR)	53.0 (38.6-67.9)	46.0 (34.8-62.8)	56.0 (39.5-71.5)	<0.05

PIM: Potentially Inappropriate Medication; MAI: Medication Appropriateness Index; IQR: Interquartile Rate

Table 3. *The most common medication group with the highest MAI scores and PIMs*

Medication with higher MAI score	n
NSAIDs and/or Paracetamol	28
Low dose Aspirin and/or dipyridamole	12
CNS-active agent	9
Cardiovascular agent	5
Proton pump inhibitors	5
PIMs	**n**
Proton pump inhibitors	42
NSAIDs	18
CNS-active agent	15
Low dose Aspirin	8
Cardiovascular agent	7

n: Number of Medication; MAI: Medication Appropriateness Index; NSAIDs: Nonsteroidal Anti-Inflammatory Drugs; CNS: Central Nervous System; PIM: Potentially Inappropriate Medication

4. DISCUSSION

To the best of our knowledge, it is the first study conducted in community pharmacy in Turkey to identify PIMs and MAI scores in older adults. In general, our PIMs (63%) result was higher than the studies in the literature. In a retrospective study evaluating the presence of PIMs in Canada, PIMs were detected in 48.3% of older patients (9). In another study conducted retrospectively in the United States, the rate of PIMs was found to be 21% (11). In line with the present study, a multi-center study in Kuwait found 53.1% of PIMs (5). In a research study carried out in Turkey, it was determined 75.3% of PIMs in Turkey using the GheOP³S tool (33).

Line with the studies in the literature, PIMs were associated with the presence of polypharmacy (5, 8, 17). Consistent with the research conducted previously (8), the most common medications related to PIMs, which were coincided with the findings of the present study, were proton pump inhibitors, NSAIDs, and central nervous system medications. In another study conducted with older patients receiving home health care services in Turkey (34), in parallel with the results of our study, the most common presentation of PIMs was proton pump inhibitors and nonsteroidal anti-inflammatory drugs.

In a previous study, the presence of PIMs was 53.1%, while the median MAI score per medication was determined as 0.0 according to the MAI criteria (5). In our study, the presence of PIMs was higher and the median MAI score per medication was higher when compared with this study (5). According to findings of previous studies conducted in Austria (99%) (23) and Denmark (94.3%) (35), the rate of inappropriate medication use the present study was lower. In other studies, MAI score was lower than the calculated scores in the present study (23, 26, 36).

MAI is a useful tool in medication review service involving the collaboration of pharmacists and physicians at primary care in previous studies (23, 36). In the study conducted by Olsson et al. (24), which included patients over 75 years of age and using more than five medications, and performed home medication reviews, the median MAI score was higher than our study (54.0). Since they included patients using 5 or more medications in their study and included patients in a relatively higher risk age group compared to our study, they may be more likely to determine a higher rate of inappropriateness in medication use.

The study includes some limitations. The generalizability of the results has been limited. However, this study is a pilot study to use MAI in older patients at community pharmacy setting. This study is conducted on older patients who visited the pharmacy by using convenience sampling; this can be led to selection bias.

5. CONCLUSION

To best our knowledge, this is the first study of clinical pharmacist-led medication review by calculating MAI score conducted at a community pharmacy in Turkey. There was a high rate of potentially inappropriate medication with a higher score of medication appropriateness in older adults. This study highlights the importance of medication review led by clinical pharmacist at community pharmacy to optimize medication usage in older adults. Our findings show that

MAI is a useful tool in detecting medication appropriateness in the community pharmacy setting and could be used in medication review led by the community pharmacist.

Acknowledgments

The authors confirmed no conflicts of interest concerning the authorship and/or publication of this article.

Funding

The authors received no financial support for the study and/or authorship of this article.

Conflict Of Interest

None

REFERENCES

[1] Fulton MM, Allen ER. Polypharmacy in the elderly: a literature review. J Am Acad Nurse Pract. 2005;17(4):123-32.

[2] Hanlon JT, Schmader KE, Ruby CM, Weinberger M. Suboptimal prescribing in older inpatients and outpatients. J Am Geriatr Soc. 2001;49(2):200-9.

[3] Bahat G, Tufan F, Akin S, Tufan A, Erten N, Karan M. Rational drug use in the elderly. J Gerontol Geriatr Res. 2012;1(1):1-8.

[4] Fick DM, Cooper JW, Wade WE, Waller JL, Maclean JR, Beers MH. Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. Arch Intern Med. 2003;163(22):2716-24.

[5] Awad A, Hanna O. Potentially inappropriate medication use among geriatric patients in primary care setting: A cross-sectional study using the Beers, STOPP, FORTA and MAI criteria. PLoS One. 2019;14(6):e0218174.

[6] Nyborg G, Brekke M, Straand J, Gjelstad S, Romøren M. Potentially inappropriate medication use in nursing homes: an observational study using the NORGEP-NH criteria. BMC Geriatr. 2017;17(1):220.

[7] Morin L, Laroche ML, Texier G, Johnell K. Prevalence of Potentially Inappropriate Medication Use in Older Adults Living in Nursing Homes: A Systematic Review. J Am Med Dir Assoc. 2016;17(9):862.e1-9.

[8] Alhawassi TM, Alatawi W, Alwhaibi M. Prevalence of potentially inappropriate medications use among older adults and risk factors using the 2015 American Geriatrics Society Beers criteria. BMC Geriatr. 2019;19(1):154.

[9] Roux B, Sirois C, Simard M, Gagnon ME, Laroche ML. Potentially inappropriate medications in older adults: a population-based cohort study. Fam Pract. 2020;37(2):173-9.

[10] Gibert P, Cabaret M, Moulis M, Bosson JL, Boivin JE, Chanoine S, Allenet B, Bedouch P, Gavazzi G. Optimizing medication use in elderly people in primary care: Impact of STOPP criteria on inappropriate prescriptions. Arch Gerontol Geriatr. 2018;75:16-9.

[11] Curtis LH, Østbye T, Sendersky V, Hutchison S, Dans PE, Wright A, Woosley RL, Schulman KA. Inappropriate prescribing for elderly Americans in a large outpatient population. Arch Intern Med. 2004;164(15):1621-5.

[12] Maher RL, Hanlon J, Hajjar ER. Clinical consequences of polypharmacy in elderly. Expert Opin Drug Saf. 2014;13(1):57-65.

[13] By the 2019 American Geriatrics Society Beers Criteria® Update Expert Panel. American Geriatrics Society 2019 Updated AGS Beers Criteria® for Potentially Inappropriate Medication Use in Older Adults. J Am Geriatr Soc. 2019;67(4):674-94.

[14] O'Mahony D, O'Sullivan D, Byrne S, O'Connor MN, Ryan C, Gallagher P. STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. Age Ageing. 2015;44(2):213-8.

[15] Tommelein E, Petrovic M, Somers A, Mehuys E, van der Cammen T, Boussery K. Older patients' prescriptions screening in the community pharmacy: development of the Ghent Older People's Prescriptions community Pharmacy Screening (GheOP(3)S) tool. J Public Health (Oxf). 2016;38(2):e158-70.

[16] Hanlon JT, Schmader KE, Samsa GP, Weinberger M, Uttech KM, Lewis IK, Cohen HJ, Feussner JR. A method for assessing drug therapy appropriateness. J Clin Epidemiol. 1992;45(10):1045-51.

[17] Blanco-Reina E, Ariza-Zafra G, Ocaña-Riola R, León-Ortiz M. 2012 American Geriatrics Society Beers criteria: enhanced applicability for detecting potentially inappropriate medications in European older adults? A comparison with the Screening Tool of Older Person's Potentially Inappropriate Prescriptions. J Am Geriatr Soc. 2014;62(7):1217-23.

[18] Brown JD, Hutchison LC, Li C, Painter JT, Martin BC. Predictive Validity of the Beers and Screening Tool of Older Persons' Potentially Inappropriate Prescriptions (STOPP) Criteria to Detect Adverse Drug Events, Hospitalizations, and Emergency Department Visits in the United States. J Am Geriatr Soc. 2016;64(1):22-30.

[19] Demirer Aydemir F, Oncu S, Yakar NM, Utkugun GA, Gokmen N, Comert B, Ucku R, Gelal A. Potentially inappropriate medication use in elderly patients treated in intensive care units: A cross-sectional study using 2019 Beers, STOPP/v2 Criteria and EU(7)-PIM List. Int J Clin Pract. 2021;75(11):e14802.

[20] Oliveira MG, Amorim WW, de Jesus SR, Heine JM, Coqueiro HL, Passos LC. A comparison of the Beers and STOPP criteria for identifying the use of potentially inappropriate medications among elderly patients in primary care. J Eval Clin Pract. 2015;21(2):320-5.

[21] Bulloch MN, Olin JL. Instruments for evaluating medication use and prescribing in older adults. J Am Pharm Assoc (2003). 2014;54(5):530-7.

[22] O'Connor MN, Gallagher P, O'Mahony D. Inappropriate prescribing: criteria, detection and prevention. Drugs Aging. 2012;29(6):437-52.

[23] Castelino RL, Bajorek BV, Chen TF. Retrospective evaluation of home medicines review by pharmacists in older Australian patients using the medication appropriateness index. Ann Pharmacother. 2010;44(12):1922-9.

[24] Olsson IN, Runnamo R, Engfeldt P. Medication quality and quality of life in the elderly, a cohort study. Health Qual Life Outcomes. 2011;9:95.

[25] Grimes TC, Deasy E, Allen A, O'Byrne J, Delaney T, Barragry J, Breslin N, Moloney E, Wall C. Collaborative pharmaceutical care in an Irish hospital: uncontrolled before-after study. BMJ Qual Saf. 2014;23(7):574-83.

[26] Chrischilles EA, Carter BL, Lund BC, Rubenstein LM, Chen-Hardee SS, Voelker MD, Park TR, Kuehl AK. Evaluation of the

Iowa Medicaid pharmaceutical case management program. J Am Pharm Assoc (2003). 2004;44(3):337-49.

[27] Garcia J, Vaz M, Poggi M. Estimated prevalence of contraindicated, severe and moderate interactions in ambulatory patients with polypharmacy in a healthcare provider in Uruguay. Clin Ther. 2015;37(8):e145.

[28] Patton D, Cadogan C, Gormley GJ, Passmore P, Francis J, Kerse N, Hughes C. Using the Theoretical Domains Framework (TDF) to explore barriers and facilitators to adherence to prescribed medicines in community-based older adults. Int J Pharm Pract. 2015;23:11-2.

[29] Ziere G, Dieleman J, Hofman A, Pols HA, Van Der Cammen T, Stricker BC. Polypharmacy and falls in the middle age and elderly population. Br J Clin Pharmacol. 2006;61(2):218-23.

[30] Bahat G, Tufan F, Bahat Z, Tufan A, Aydin Y, Akpinar TS, Nadir S, Erten N, Karan MA. Comorbidities, polypharmacy, functionality and nutritional status in Turkish community-dwelling female elderly. Aging Clin Exp Res. 2014;26(3):255-9.

[31] Rollason V, Vogt N. Reduction of Polypharmacy in the Elderly. Drugs Aging. 2003;20(11):817-32.

[32] Patterson SM, Hughes C, Kerse N, Cardwell CR, Bradley MC. Interventions to improve the appropriate use of polypharmacy for older people. Cochrane Database Syst Rev. 2012(5):CD008165.

[33] Sayın Z, Sancar M, Özen Y, Okuyan B. Polypharmacy, potentially inappropriate prescribing and medication complexity in Turkish older patients in the community pharmacy setting. Acta Clin Belg. 2020:1-7.

[34] Memiş S, Sancar M, Varlıklı O, Akçay B, Varol H, Söylemez SL, Medisoglu MS, Okuyan B. A pilot study of clinical pharmacistled medication review in older adults on polypharmacy and receiving home health care servıces. The Turkish Journal of Geriatrics. 2020;23(4):515-23.

[35] Bregnhøj L, Thirstrup S, Kristensen MB, Bjerrum L, Sonne J. Prevalence of inappropriate prescribing in primary care. Pharm World Sci. 2007;29(3):109-15.

[36] Hanlon JT, Weinberger M, Samsa GP, Schmader KE, Uttech KM, Lewis IK, Cowper PA, Landsman PB, Cohen HJ, Feussner JR. A randomized, controlled trial of a clinical pharmacist intervention to improve inappropriate prescribing in elderly outpatients with polypharmacy. Am J Med. 1996;100(4):428-37.

How to cite this article: Dal S, Ucar N, Altiparmak O, Sancar M, Okuyan B. Medication Review in Turkish Older Adults at Community Pharmacy: A Pilot Study by Using Medication Appropriateness Index. Clin Exp Health Sci 2022; 12: 725-729. DOI: 10.33808/clinexphealthsci.1012114

Clinical and Experimental Health Sciences

The Effect on Birth Pain and Process of the Freedom of Movement in the First Stage of Labor: A Randomized Controlled Study

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Received: 03.11.2021 **Accepted:** 07.07.2022

ABSTRACT

Objective: To determine the effect of freedom of movement implemented in the dilatation stage, which is the first stage of labor, on the labor process in pregnant women who will give their first birth.

Methods: This study was designed as a randomized controlled trial. The study consisted of 70 primiparous women, including 35 in the study group (SG) and 35 in the control group (CG). Freedom of movement was provided to the study group in the first stage of labor. Data were collected using a Personal Information Questionnaire, a Labor Assessment Form, and the Visual Analog Scale (VAS) for perceived pain.

Results: The SG was observed to be walking when dilatations were 4-7 cm and mostly squatting when 8-10 cm (94.2%). The level of effacement, frequency of contraction, and descent of the fetal head were faster ($p<0.01$), and total dilatation, expulsion, placental expulsion, and total labor and delivery time were shorter in pregnant women in the SG ($p<0.001$). After delivery, the SG had shorter initial contact with their babies and first breastfeeding time, and the total 24-hour postpartum hemorrhage was less ($p<0.001$). When there was no freedom of movement during labor, the total dilation time (OR=2.41), expulsion time (OR=1.25), placental expulsion time (OR=1.16), total delivery time (OR=2.76), the VAS score at 6 – 7cm dilatation time (OR=2.48), and first breastfeeding time (OR=1.15) were found to increase ($p<0.05$-0.01).

Conclusion: The freedom of movement allowed at the first stage of labor was observed to reduce perceived labor pain, the process of labor and delivery time, the volume of postpartum hemorrhage, and the time of the first contact with the infant and the first breastfeeding.

Keywords: Labor, delivery, stage, birth process, freedom of movement, effect

1. INTRODUCTION

Labor pain is one of the most severe pain types and is described as a sophisticated experience based on physiological, psychological, and social components (1). While the pain experienced during labor is a normal and important component of the birth process, it may also increase the fear of birth and cause a negative birth experience if not managed well (2, 3). It is stated that women who had a negative birth experience especially talk about the pain that they experienced at birth, in addition to many factors. (4-6). While negative perception about pain causes fear of birth, fear of birth, on the other hand, decreases pain tolerance. This situation may cause many complications, such as prolonged labor, negative birth experience, an increase in reasons to choose epidural anesthesia or cesarean, etc. (6).

Freedom of movement during the labor process means the pregnant can take any position at any time spontaneously according to the normal course of the delivery (7-12). The World Health Organization (WHO) recommends that the labor should be supported in its normal course by maintaining its natural physiology, the woman should be allowed to take any position at any time at her will without any interference with the position she should take at a given stage while the labor is progressing, and that no intervention should be introduced unless necessary (7,11-15).

As the provision of mobility will encourage women to move freely and change positions during labor and birth, supporting the pregnant in this regard is important (10-17). Although there is a belief that all women should be allowed to choose freely and consciously according to labor and birth physiology, a common approach used by health professionals is that the woman should be in bed during labor. Nevertheless, it is not ethical to restrict the freedom of movement of the pregnant especially during labor unless there is a medical obligation (8,18,19).

In birth centers, which provide care with a human rights-based approach, women are in control, and health professionals help

DOI: 10.33808/clinexphealthsci.1016033

and encourage them to take the most comfortable position throughout childbirth (11,16,17,21,22). In the systematic evaluation results on the Cochrane database, it is stated that women should be encouraged and supported for positions that they find suitable, and that free movement should not be restricted unless there is a clinical obstacle (10,22). Since inactivity affects delivery negatively, it is stated that the position should be changed every 30 minutes during labor and that the woman should be encouraged about the position change. It is emphasized that the change or choice of the position should be based on the current instincts and preference, safety, and comfort of the mother, effective progression of the labor, and hemodynamic knowledge (10,22,23). The International Lamaze Organization determined six care practices in 2007 to support normal labor. The second of the six care practices created is "freedom of movement throughout labor." Freedom of movement in labor is stated to make the delivery process easier, increase the sense of control, accelerate the delivery, and increase the comfort of delivery and the possibility of normal vaginal delivery (13).

In the literature, the freedom of movement given to the woman during the first stage of the labor and the positions that she takes are reported to reduce the duration and severity of the uterine contractions that she feels, shorten the delivery time, accelerate the delivery by facilitating the descent of the fetus, bring about positive perineal outcomes, and increase the anatomical and physiological adaptation and the comfort and satisfaction of the mother (10-12, 19-21, 24-27). It is stated that allowing the woman to take any position that she seeks instinctively and providing her with freedom of movement in the first stage of the birth when the dilation occurs supports the normal physiological process but does not interrupt it, and therefore, it is safer and healthier (10,11, 13-17).

In recent years, a considerable number of studies have been conducted about labor positions practiced especially in the second stage of delivery. Yet, research into freedom of movement/positions practiced in the first stage of labor is relatively limited. This study was conducted to determine the effect of freedom of movement encouraged in the first stage of labor on the labor and delivery process in *primiparous women*. In the study, the effect of freedom of movement (instinctively) in labor process on the perceived pain, dilatation, expulsion, placental expulsion, total delivery time, volume of postpartum hemorrhage, newborn, and breastfeeding outcomes of pregnant women was evaluated. It is thought that the results of the study will contribute to the literature on the planning and implementation of care to be given to women who give birth.

2. METHODS

2.1. Study Design

This study was designed as a randomized controlled trial and carried out in the maternity ward of an Education and Research Hospital, a city hospital in Istanbul province between April 2016 and February 2017.

2.2. Study Sample

A G-Power (V3-1.7) analysis was conducted to calculate the number of participants to be taken into groups (The calculation was performed based on an effect size of d= 0.8). In the analysis, it was determined that at least 26 women in the birth process should be included in the groups. The study was carried out with 70 women in the first stage of labor, including 35 in the study group-SG (n=35) and 35 in the control group-CG (n=35).

The pregnant women who were admitted to the hospital for delivery were first evaluated in terms of the inclusion criteria. Sample inclusion criteria were as follows: being primiparous; being at the term/gestation week; having a spontaneous vaginal delivery; a dilatation of ≥4cm; not having cephalopelvic disproportion; the fetus in longitudinal, vertex position; having an alive, single, and healthy fetus; not having maternal and fetal complications and early membrane rupture (EMR); an estimated fetal weight of less than 4 kg; not having language-communication problem. Sample exclusion criteria were as follows: having unexpected maternal-fetal risk conditions during the birth process (bleeding, fetal distress, etc.); having a cesarean section; being unable to adapt to the study; quitting or wanting to leave the study.

Randomization: Women who met the sampling criteria were randomized and divided into groups. The randomization was performed on computer software (http://www.randomizer.org/form.html). The numbering was based on the order of admission to the delivery room. Randomization and stages of the study were given in Figure 1. CONSORT flow diagram.

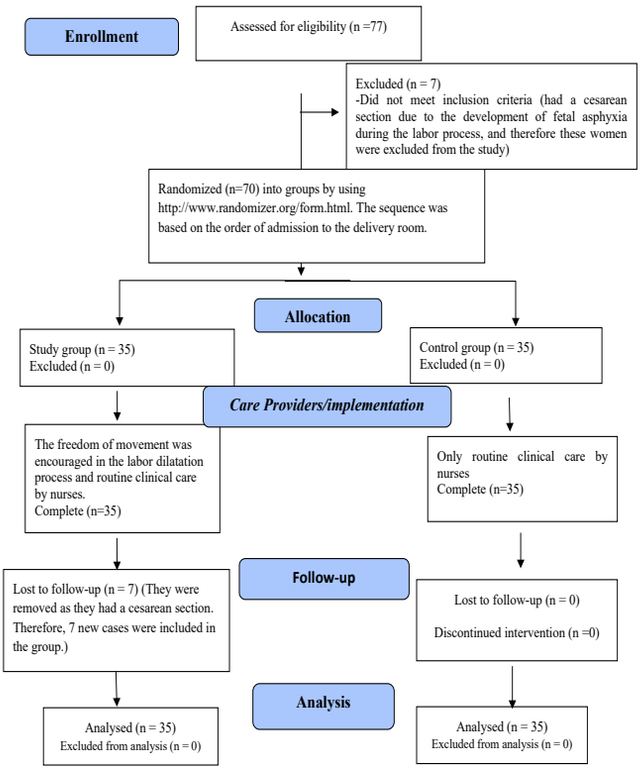

Figure 1. *CONSORT flow chart of the study.*

2.3. Instruments

The study data were collected by using a Personal Information Questionnaire, a Labor Assessment Form, the Visual Analog Scale (VAS), and the Diheng-2000 digital precision scale.

The Personal Information Questionnaire: This questionnaire consists of 13 questions, including 3 open-ended, 4 multiple-choice, and 6 structured items, which were prepared to collect socio demographic and pregnancy-related information about pregnant women.

The Labor Assessment Form: This form was used to evaluate the labor and birth process and the clinical course of the pregnant woman and to assess and record the clinical measurements, observations, and those taken and made by the researchers.

The Visual Analog Scale (VAS): VAS is a scale used to measure perceived pain. Its foundations date back to 1921 when Hayes and Paterson claimed that emotion levels could be shown on a line. The boundaries of these lines are determined by expressions indicating the most extreme states of emotions. VAS is stated to be a more sensitive and reliable measure compared to other one-dimensional scales in the measurement of pain perception. This scale, whose validity and reliability study was conducted, is 10 cm long, and its two ends are named differently on the vertical or horizontal line. This 10-cm long scale reads "no pain" on one end and "the most severe pain possible" on the other end. During pain assessment, individuals are asked to mark the point on the 10-cm line that corresponds to the pain intensity they feel. This scale is used for the assessment of birth pain as well as general pain assessment.[24] In the study, the assessment of birth pain with the VAS was conducted at the dilatation stage of labor, and both groups were evaluated with the VAS three times. To be more specific, evaluations were done in the active phase of dilatation when it reached 4-5 cm, 6-7 cm, and 8-10 cm.

Evaluation of postpartum hemorrhage: A precision weighing scale (Diheng-2000 g) was used to evaluate the 24-hour postpartum hemorrhage. After the birth, all mothers were given hygienic pads (Joly Large Adult Diaper) of the same size and standard. The bleeding findings obtained from the pads weighed with the Diheng-2000 digital precision scale were recorded in the Labor Assessment Form.

2.4. Interventions

This study was carried out during the dilatation process, which is the first stage of labor. In the study, freedom of movement was provided to the study group in the first stage of labor, but no intervention was implemented in the control group other than clinical applications.

The women with pregnancy who came to the clinic for birth were hospitalized in double rooms. In the implementation phase of the study, the women with pregnancy in the study and control groups were placed in different rooms as much as possible to consider ethical issues and to prevent the women with pregnancy from being influenced from each other. The researchers collected all data using the specified data collection tools via the face-to-face interview technique and based on women's self-reports. The intervention procedures of the study for both groups are given in Figure 2.

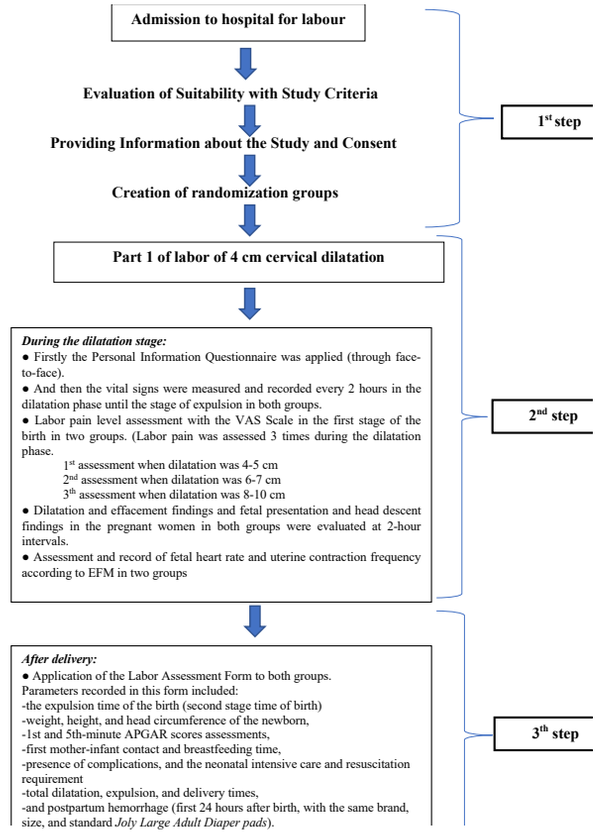

Figure 2. *The intervention procedure stages of the study for both groups*

2.5. Ethical Considerations

At the outset, the ethical committee approval of the non-interventional clinical research ethics committee of Halic University *(27.10.2015, Issue: 55, Decision no: 8)* and the necessary institutional permissions were obtained *(Issue: 150.000.7415).* The study was conducted in compliance with the "Ethical principles for medical research involving human subjects" of the Helsinki Declaration.

2.6. Statistical Analysis

Statistical analysis was performed on the Statistical Package for the Social Sciences software (SPSS, Chicago, IL, USA). Independent t, chi-square, and Fisher chi-square and ANOVA tests were used to compare the findings and the significance level was set at a confidence interval of 95% and a significance level of 0.05. As the values of variables were not normally distributed in the population, the Mann-Whitney U-test was used. The Pearson's correlation test was employed to handle the relationship between parameters of labor and delivery process. The Backward (Conditional) Logistic regression analysis was used to evaluate the effect of no freedom of movement in labor on some labor and delivery process parameters.

2.7. Limitations and Implications

The results cannot be generalized since the study was carried out in a certain place and group.

The freedom of movement that was applied in the study group is not routinely applied in clinics and therefore, there are no institutional or country-based procedures.

The immigrant population is large in the region where the study was conducted, but we could not include them in the study sample due to language problems, so we had difficulty making up the number of cases, and therefore the study took longer than planned (Sometimes, there were no cases at all in the institution on days when the researcher was there.)

Although all the healthcare workers in the institution had been informed about the purpose and process of the study at the outset and they showed approval, some of the staff in the birth service showed negative attitudes towards the freedom of movement of the women with pregnancy during the labor dilatation phase (during labor) (They did not want the women with pregnancy in the service moving around; for this reason, we had to often make explanations and compromise).

The researcher could not attend the institution where the research was carried out every day of the week because she worked in a different institution.

3. RESULTS

The mean age was 22.1 ± 4.0 in the SG and 23.1 ± 3.9 in the CG, and there was no difference between the groups. There was no statistically significant difference in both groups in terms of age, employment status, number of pregnancies, gestational week, the planned status of pregnancy, prenatal education, etc. (p>.05). However, the number of university graduates was significantly higher in the SG (p< .01) (Table 1). All of the pregnant women in both groups had head-first presentation and longitudinal situs. In the first examination findings, there was no statistically significant difference in terms of the fetal position, the status of the amniotic sac membrane, amniotic fluid status, situs, and dilatation (p>.05). Only the percentage of effacement level was significantly higher in the SG (SG: 59.7%; CG: 53.4%) (p< .01) (Table 1).

Table 1. *Characteristics and the first examination findings of women in the two groups*

Characteristics	Study Group (n=35)		Control Group (n=35)		χ^2	p
	n	%	n	%		
Education status						
Illiterate	4	11.4	3	8.6	10.29	**0.01**
Primary education	2	5.7	12	34.3		
High school	17	45.7	15	42.9		
University	12	**37.1**	5	**14.3**		
Number of pregnancies						
Primigravida	29	82.9	30	85.7		
Multigravida (2nd pregnancy)	6	17.1	5	14.3	0.10	0.74
Participation in antenatal classes						
Yes	2	5.7	3	8.6		
No	33	94.3	32	91.4	1.01	0.60
	Average age and gestational week					
	Min-Max	**Mean±SD**	**Min-Max**	**Mean±SD**	**t**	**P**
Age /year	18-35	22.1±4.0	18-34	23.1±3.9	1.08	0.28
Gestational week	38-41	39.3±0.8	37-41	39.3±0.9	0.00	1.00
	The first examination findings of women admitted to the delivery room					
	N	**%**	**n**	**%**	χ^2	**p**
Head presentation	35	100.0	35	100.0		
Situs (longitudinal)	35	100.0	35	100.0		
Fetal Position						
D1*	16	45.7	11	31.4	1.50	0.22
D2**	19	54.3	24	68.6		
Amniotic sac membrane						
Intact	5	14.3	10	28.6	2.12	0.14
Rupture	30	85.7	25	71.4		
Amnion fluid						
Clear	28	85.7	24	80	1.64	0.43
Meconium	2	2.9	1	0.0		
	Effacement and Dilatation					
	Min-Max	**Mean±SD**	**Min-Max**	**Mean±SD**	**t**	**p**
Effacement (%)	50-70	59.7±6.6	40-60	53.4±6.8	13.5	0.004
Dilatation (cm)	1-5	4.6±0.7	4-6	4.6±0.5	2.5	0.45

: D1; The back of the fetus is on the left relative to the mother,: D2; The back of the fetus is on the right relative to the mother.*

The pregnant women in the SG were determined to be generally walking when dilatation reached 4-5 cm, walking around, sitting upright, and leaning against the wall or bed when it reached 6-7 cm, and often squatting when it reached 8-10 cm (p < .01) (Table 2).

Table 2. *The actions of the pregnant women in the study group during the dilatation phase*

Actions	Dilatation			χ^2	p
	4-5 cm n (%)	6-7 cm n* (%)	8-10 cm n* (%)		
Upright sitting	12(34.3)	6(17.1)	-	0.89	0.64
Leaning against the wall / bed	5(11.4)	11(31.4)	15(42.8)	2.90	0.82
Take a walk	19(54.3)	25(71.3)	11(31.4)	2.44	0.87
Squatting	0(0.0)	2(5.7)	33(94.2)	2.15	**0.00**
Total*	36	47	59		

**: Pregnant women were folded because they made more than one movement during the dilatation process.*

Regarding the labor, the means values for total dilatation time / hour (SG: 6.4 ± 0.8; CG: 7.4 ± 1.0), expulsion time/minute (SG: 16.0 ± 5.1; CG: 23.4 ± 6.6), placental expulsion time/minute (SG: 16.5 ± 4.4; CG: 21.0 ± 5.0) and total labor and delivery time / hour (SG: 8.5 ± 0.8; CG: 9.4 ± 0.9) were significantly lower in favor of the pregnant women in the SG (p < .001). Also, the volume of total postpartum bleeding within 24 hours/g (SG: 275.0 ± 33.9; CG: 380.8 ± 47.5) was less among pregnant women in the SG and the mean values for the first contact with baby/minute (SG: 17.2 ± 5.8; CG: 22.7 ± 6.8) and the first breastfeeding time/ minute (SG: 18.9 ± 5.1; CG: 24.4 ± 7.5) was also shorter in the SG (p <0.001). The pain scores in the first VAS assessment done when dilatation was 4-5 cm (SG: 5.1 ± 1.1; CG: 5.8 ± 1.2) and the second VAS assessment performed when it reached 6-7 cm (SG: 6.9 ± 1.0; CG: 7.6 ± 1.1) was significantly lower in the SG (p < .01 – p < .001). On the other hand, the pain scores obtained in the third VAS assessment performed when the dilation was 8-10 cm were found to be similar in the two groups (p> .05). The number of cases developing postpartum complications was very low, and there was no significant difference between the groups in this regard (p> .05). Besides, there was no difference between groups in terms of induction application (SG: 17.1%, CG: 8.60%), non-induction medication (SG: 2.90%, CG: 14.3%), and episiotomy application (SG: 82.9%, CG. 97.1) at birth (p> .05) (Table 3).

Results regarding the Regression Analysis; Regression analysis was based on no freedom of movement in labor. According to the regression analysis conducted for the parameters regarding the effect of no freedom of movement on total dilatation, expulsion, placental expulsion, total labor and delivery times, 1st and 2nd VAS pain scores, and the time of first breastfeeding, the general explanatory coefficient was 100%, sensitivity was 100%, and specificity was 100%. According to the results of the regression analysis, when there was no freedom of movement during labor, there was an increase in the total dilation time (OR=2.41), expulsion time (OR=1.25), placental expulsion time (OR=1.16), total delivery time (OR=2.76), the VAS score at 6-7cm dilatation time (OR=2.48), and first breastfeeding time (OR=1.15) (p<0.05-0.01). Although it increased the 1st VAS score by 1.11, the result was not significant (p>.05) (Table 3).

Results regarding the Correlation Analysis; There was a highly significant negative correlation between the freedom of movement provided to pregnant women in the first stage of labor and 1st VAS and 2nd VAS assessment, total dilatation, expulsion, placental expulsion, total labor, and delivery, first breastfeeding times and the volume of postpartum hemorrhage within 24 hours (p<.01) (Table 4).

Table 3. *The pain levels and birth process data of women according to the groups, and logistic regression analysis results*

Specifications	Study Group (n=35)		Control Group (n=35)		t	p
	Min-Max	Mean±SD	Min-Max	Mean±SD		
Total dilatation time/hrs*	5-8	6.42±0.8	5-9	7.40±1.0	-4.36	**0.00**
Expulsion time/min.	9-30	16.00±5.1	15-40	23.42±6.6	-5.24	**0.00**
Placenta release time/min.	10-25	16.57±4.4	10-35	21.00±5.0	-3.90	**0.00**
The amount of bleeding for 24 hours after birth/g	200-330	275.0±33.9	300-550	380.8±47.5	-10.9	**0.00**
Total duration of labor time/h*	7-10	8.51±0.8	7-12	9.48±0.9	-4.49	**0.00**
First contact time of baby/min.	5-35	17.28±5.8	10-40	22.71±6.8	-3.5	**0.00**
First breastfeeding time/min.	10-35	18.94±5.1	15-45	24.42±7.5	-3.5	**0.00**
Pain levels according to the VAS						
	Min-Max	Mean±SD	Min-Max	Mean±SD	t	p
1st VAS assessment *(dilatation 4-5 cm)*	2-7	5.1±1.1	2-8	5.8± 1.2	-2.48	**0.01**
2nd VAS assessment *(dilatation 6-7 cm)*	4-9	6.9±1.0	3-10	7.6± 1.1	-3.31	**0.00**
3th VAS assessment *(dilatation 8-10 cm)*	8-10	9.4±0.7	9-10	9.7±0.4	-1.56	0.11

Table 3. *Continued*

Multivariate logistic regression analysis results according to the absence of freedom of movement in labor				
Variables	**Multivariate logistic regression**			
	OR	**%95 CI**		**p**
		Lower	**Upper**	
Total dilatation time	2,414	1,083	5,379	**0.031**
Expulsion time	1,256	1,086	1,452	**0.002**
Placenta release time	1,168	1,006	1,370	**0.045**
Total duration of labor	2.767	1.278	5.992	**0.010**
1st VAS assessment *(dilatation 4-5 cm)*	1,116	0,442	2,818	0.816
2nd VAS assessment *(dilatation 6-7 cm)*	2,488	1,220	5,074	**0.012**
First breastfeeding time	1,152	1.052	1,263	**0.002**

** Total dilatation and total delivery times were calculated by accepting the initial dilatation to be 4 cm.*
OR: Odds Ratio, CI: Confidence Interval

Table 4. *Correlation analysis results*

	Variables	1 r	2 r	3 r	4 r	5 r	6 r	7 r	8 r	9 r	10 r	11 r	12 r
1	**Freedom of movement in labor**	1	**-,288****	**-,339****	,229	**-,468****	**-,537****	**-,428****	**-,479****	-,112	,036	**-,798****	**-,396****
2	1st VAS assessment *(D: 4-5 cm)*		1	**,779****	**,275***	,291*	-,007	,060	,205	-,006	,076	,178	**,263***
3	2nd VAS assessment *(D:6-7 cm)*			1	**,443****	,288*	,024	,120	,272*	,020	-,003	,193	,190
4	3th VAS assessment *(D:8-10 cm)*				1	,032	,147	,051	-,068	,083	,042	,072	,184
5	Total dilatation time					1	**,307****	**,270***	**,732****	-,141	-,149	**,320****	**,253***
6	Expulsion time						1	**,427****	**,265***	-,163	-,031	**,484****	**,381****
7	Placenta release time							1	,172	-,084	-,089	**,410****	**,391****
8	Total duration of labor								1	-,083	-,143	**,433****	**,238***
9	First APGAR									1	**,488****	-,186	-,077
10	Second APGAR										1	,006	-,131
11	Total amount of bleeding for 24 hrs.											1	**,415****
12	First breastfeeding time												1

**: p<0,05; **: p<0,01 Spearman correlation analysis was used; r: correlation coefficient*
D: Dilatation

4. DISCUSSION

The vast majority of studies on movement or positions in labor are generally related to the process of expulsion. The discussion in this section will be handled in terms of the data in the literature that is relevant to the results of our study.

Although it is said that keeping pregnant women in bed from the early stages of birth creates a favorable environment for healthcare professionals and constant EFM, most pregnant women would like to walk or change positions frequently, especially in the early stages of delivery (7,29). Since there is not enough evidence that a position is better than another, there is no single position to be recommended or prohibited. In studies, it has been difficult to isolate the independent impact of the position on the progression of labor. Pregnant women cannot keep a single position during a study, and they cannot be expected to do it (21,28,29). As of the active phase, the pregnant women in the SG were determined to be generally walking when dilatation reached 4-5 cm, walking around, sitting upright, and leaning against the wall or bed when it reached 6-7 cm, and often squatting when it reached 8-10 cm (Table 2). In the literature, walking or upright positions are stated to be preferred in the first stage of the birth because they are more comfortable (27,30-40). The results reported in the study of Akin et al. (2017) are similar to the results of the current study related to walking or moving during labor (40).

In our study, the movements that the pregnant women did when the dilatation was 4-5 cm and 6-7 cm can be said to be similar to these data in terms of walking. However, it is noteworthy that pregnant women do not only walk/move but also sit or lean against the wall or bed, and that they squat at the last stage of dilatation to adapt to the labor. These results show us that pregnant women do not perform a fixed pattern of movement in the process of labor and that they shape their movements/positions instinctively according to the labor

process. These findings are considered important in terms of showing that no restriction of movement can be applied or there cannot be a single position for pregnant women in the labor process. The positions are shaped according to the birth process and the pregnant woman's instincts; therefore, support and encouragement about freedom of movement are necessary.

In the study, the dilatation time in the SG participants who were allowed freedom of movement at the first stage of delivery was 1 hour, the expulsion time was 6 minutes, the placental expulsion time was 5 minutes on average, and the total labor and delivery time was 1 hour shorter than that of the pregnant women in the CG (Table 3). Thilagavaty (2012) reported that using an upright position in the active phase in the first stage shortened the second (11 min) and third stage (10 min) of labor (33). Similarly, Miquiletti et al. (2007) stated that taking an upright position in the first stage of labor shortened the time of this phase (36). Also, Mamede et al. (2007) reported that the total delivery time in pregnant women who were allowed to walk in the active phase was between 3 and 14 hours and took an average of 7.66 hours (37). The study results in the literature were found to support our data. The results of our study revealed that the freedom of movement in the first stage had an important role in shortening the first, second, and third stages of labor and the total time of the labor and delivery. Therefore, the findings of our study are thought to make a contribution to the literature in this regard.

In the literature, allowing pregnant women to move freely during labor is reported to make uterine contractions effective and reduce the need for analgesia (17, 22, 39, 43, 44). Miquiletti et al. (2007) and Thilagavaty (2012) reported that VAS pain scores were lower in those who took an upright position in the active phase of the first stage of labor, pregnant women felt more comfortable, and that it increased satisfaction (34). Mamede et al. (2007) reported that when the dilatation was 5 cm, there was a significant correlation between VAS pain scores of walking pregnant women. At the same time, the pain was stated to increase as birth progressed and dilatation increased, also increasing the associated VAS pain scores (37). Jonge et al. (1997) stated that pain scores were less in upright positions in the first stage of labor. In this study, VAS pain scores were significantly lower among pregnant women in the SG who were provided freedom of movement in the first stage of labor when the dilatation was 4-5 cm and 6-7 cm, while no significant difference was found between the groups at 8-10 cm dilatation (30). The literature supporting the study results mentioned above shows that allowing pregnant women to have freedom of movement and to change position as they like during labor and birth process has a positive effect on perceived pain and facilitates pain management until the dilatation reaches 8-10 cm. Besides, these results suggest that the effect of freedom of movement on reducing the perceived pain in the process of labor and birth will contribute positively to the comfort of birth and birth experience.

In the literature, there are many studies investigating the effect of positions implemented in the second stage of birth on postpartum hemorrhage (27,30,33,35,42,43); nevertheless, research into the effect of freedom of movement in the 1st stage of labor on postpartum hemorrhage is limited. In this study, the volume of postpartum hemorrhage within the first 24 hours in pregnant women in the SG who were allowed freedom of movement at the first stage of labor was found to be significantly less than those of the pregnant women who were not provided freedom of movement (SG: 275.0 ± 33.9; CG: 380.8 ± 47.5). Accordingly, the results of the study have been evaluated as an important finding regarding its contribution to the literature. Also, the freedom of movement allowed in the 1st stage of labor may have an essential role in reducing the risk of postpartum hemorrhage, which is one of the most important postpartum complications. In the literature, interventions in labor and prolongation of labor have been reported to possibly delay the first breastfeeding time (20,44). However, there is no study in the literature evaluating the effect of freedom of movement applied in the first stage of labor on breastfeeding and first contact with baby. In this study, the first contact and breastfeeding time of the pregnant women in the SG who were allowed freedom of movement in the first stage of labor was found to be shorter compared to that of the pregnant women in CG. This result suggested that the freedom of movement in the first stage of labor might have shortened the duration of all stages in labor and the total labor process and reduced the perceived pain and that all these results might have been achieved due to the positive effects of the energy of the freely moving mother.

In the correlation analyses of the study, a highly significant relationship was found between the freedom of movement provided in the first stage of labor and birth pain, total dilatation, expulsion, placental expulsion, total delivery, and first breastfeeding times, and the total volume of bleeding. Also, the regression analyses indicated that allowing no freedom of movement in the first stage of labor increased the total dilation time 2.4 times, the expulsion time 1.2 times, the placental expulsion time 1.1 times, the total labor and delivery time 2.7 times, the birth pain 2.4 times, and the first breastfeeding time 1.1 times. All these results are extremely important and are thought to contribute to the literature in terms of showing the positive effects of freedom of movement in the first stage of labor on the process of labor, perceived birth pain, and the onset of first breastfeeding.

5. CONCLUSION

The freedom of movement provided to pregnant women in the first stage of labor was determined to reduce the perceived birth pain, shorten total dilation, expulsion, placental expulsion, and delivery times, decrease the volume of postpartum hemorrhage, and shorten the time of first breastfeeding and contact with baby. Due to the positive birth outcomes, it is thought that this application should be spread in the clinical setting, and it is deemed important that health professionals encourage women who give birth. Also,

conducting training programs on this topic will be beneficial. In addition, it will be beneficial to contribute to and support the literature with randomized controlled trials in which the freedom of movement allowed in the first stage of labor and the effectiveness of each of the different positions that women refer to in this phase in the natural course of the labor are evaluated.

Acknowledgments: The authors would like to express thanks to the pregnant women, colleagues, and the institution for their participation in the study. This paper was created from the doctorate thesis with number 480984.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure statement: The authors report no conflict of interest.

REFERENCES

[1] Ergin A, Mallı P. Aromatherapy in childbirth: a systematic review. KOU Sag Bil Derg. 2019; 5(2):72-80. DOI:10.30934/kusbed.496775. (Turkish)

[2] Moloney S, Gair S. Empathy and spiritual care in midwifery practice: Contributing to women's enhanced birth experiences. Women and Birth. 2015; 28(4): 323–328. DOI:10.1016/j.wombi.2015.04.009

[3] Trainor L, Frickberg-Middleton E, McLemore M, Franck L. Mexican-Born women's experiences of perinatal care in the united states. J Patient Exp. 2020; 7(6): 941-945. DOI: 10.1177/237.437.3520966818

[4] Okumuş F. Midwifery-led models of care for childbearing women: the Netherlands as an example. JACSD. 2016; 7: 120-141. DOI:10.17367/JACSD.201.672.1958. (Turkish)

[5] O'donovan A, Alcorn KL, Patrick JC, Creedy DK, Dawe S, Devilly GJ. Predicting posttraumatic stress disorder after childbirth. Midwifery. 2014; 30(8): 935-941. DOI: 10.1016/j.midw.2014.03.011

[6] Nilsson L, Thorsell T, Hertfeltwahn E, Ekström A. Factors influencing positive birth experiences of first-time mothers. Nurs Res Pract. 2013; 2013: 1-6. DOI:10.1155/2013/349124

[7] Bülbül G. How Birth Has Becomed a Medical Event?. Doğal Doğum.1. baskı. İstanbul: Hayy Kitap, 2016; p.41-59. (Turkish)

[8] Lothian JA. Safe, healthy birth: what every pregnant woman needs to know. J Perinat Educ. 2009; 18(3):48-54. DOI:10.1624/105812409X461225.

[9] Sayıner FD, Özerdoğan N. Natural labor. Maltepe University Journal of Nursing Science and Art, 2009; 2(3):143-148. (Turkish)

[10] Ondeck M. Healthy birth practice #2: walk, move around, and change positions throughout labor. J Perinat Educ. 2014; 23(4):188-93. DOI: 10.1891/1058-1243.23.4.188.

[11] Schiling T, Romano AM, DiFranco JT. Care Practice 2: Freedom of movement throughout labor, J Perinat Educ. 2007; 16(3), 21-24. DOI: 10.1624/105812407X217101.

[12] Karaman ÖE., Yıldız H. Freedom of Movement During Labor: How? What does it provide? What is the role of obstetrics and gynecologic nursing? Turkiye Klinikleri J Nurs Sci. 2018; 10(1):78-87. DOI: 10.5336/nurses.2017-57080 (Turkish)

[13] World Health Organization-WHO. Managing complications in pregnancy and childbirth: A Guide for Midwives and Doctors. Accessed [06 October 2021]. https://apps.who.int/iris/handle/10665/255760

[14] World Health Organization-WHO Recomments for augmentation of labour. 5-26. Accessed [06 October 2021]. https://www.ncbi.nlm.nih.gov/books/NBK258875/pdf/Bookshelf_NBK258875.pdf

[15] World Health Organization WHO recommendations: intrapartum care for a positive childbirth experience. Accessed [06 October 2021]. https://www.who.int/publications/i/item/978.924.1550215

[16] Royal Collage Midwives-RCM (2010), Survey of positions used in labour and birth final report, 3-13. Accessed [06 October 2021]. https://docplayer.net/11994542-The-royal-college-of-midwives-survey-of-positions-used-in-labour-and-birth-final-report.html

[17] Royal Collage Midwives-RCM. Evidence based guidelenes for midwifery-led care in labour: positions for labour and birth, 3-5. Accessed [06 October 2021]. https://waterbirth.org/wp-content/uploads/2021/08/RCM-Guidelines_2012..pdf

[18] Iravani M, Janghorbani M, Zarean E, Bahrami M. An overview of systematic reviews of normal labor and delivery management. Iran J Nurs Midwifery Res. 2015; 20(3):293-303. PMID**:** 26120327

[19] Simkin P, Ancheta R. Labor Progress Handbook, Translate in: Samiye Mete. İstanbul Tıp Bookstore, Third Edition, İstanbul. 2016.p. 277-324.

[20] Ergin AB, Kömürcü N. Pain management in labor: nonpharmacologic methods. KUHEAD. 2009; 6(2):29-35. (Turkish)

[21] ACOG-American College of Obstetricians and Gynecologists, Society for Maternal-Fetal Medicine. (2014). Obstetric care consensus: safe prevention of the primary cesarean delivery. Obstetrics and Gynecology. Accessed [06 October 2021]. https://www.acog.org/clinical/clinical-guidance/obstetric-care-consensus/articles/2014/03/safe-prevention-of-the-primary-cesarean-delivery

[22] Lawrence A, Lewis L, Hofmeyr GJ, Styles C. Maternal positions and mobility during first stage labour. Cochrane Database Syst Rev. 2013; 8: CD003934. https://doi. org/10.1002/14651858.CD003934.pub4

[23] Rathfisch GY, Güngör İ. Evidence based practices in the management of first stage of labor. HEMARGE. 2009; 11(3):53-64. (Turkish)

[24] Leeman L, Fontaine P, King V, Klein MC, Ratcliffe S. The nature and management of labor pain: part I. Nonpharmacologic pain relief Am Fam Physician. 2003; 68(6):1109-12. PMID:14524397

[25] Kadayıfçı O, Çetin-Coşar F. "Labor Positions". Lamaze Philosophy Spiritual and Physical Preparation for Birth, 2013; 1. Baskı. İstanbul: Cross Basım ve Yayıncılık; p.174-7. (Turkish)

[26] Karaçam Z. The effect of positions for delivery to mother and baby health. Journal of Cumhuriyet University School of Nursing. 2001; 5(2):56-61. (Turkish)

[27] Jonge A, Teunissen DA, van Diem MT, Scheepers PL, Lagro-Janssen AL. Women's positions during the second stage of labour: views of primary care midwives. J Adv Nurs. 2008; 63(4):347-356. DOI:10.1111/j.1365-2648.2008.04703.x.

[28] Hofmeyr GJ. Evidence-based intrapartum care. Best Pract Res Clin Obstet Gynaecol. 2005; 19(1):103-115. DOI: 10.1016/j.bpobgyn.2004.10.009.

[29] ACOG American College of Obstetricians and Gynecologists, Approaches to limit intervention during labor and birth (2019). ACOG Committee Opinion No. 766. American College of Obstetricians and Gynecologists. Obstet Gynecol; 133: e164–73. Accessed [06 October 2021]. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2019/02/approaches-to-limit-intervention-during-labor-and-birth

[30] Jonge PR, Johanson RB, Baxen P, Adrians VD, van der Westhuisen S, Jones PW. Randomised trial comparing the upright and supine positions for the second stage of labour. Br J Obstet Gynaecol. 1997; 104(5):567-571. DOI: 10.1111/j.1471-0528.1997.tb11534.x

[31] Jonge A, Van Diem MT, Scheepers PL, Buitendijk SE, Lagro-Janssen AL. Risk of perineal damage is not a reason to discourage a sitting birthing position: a secondary analysis. Int J Clin Pract. 2010; 64(5):611-618. doi:10.1111/j.1742-1241.2009.02316.x.

[32] Bodner-Adler B, Bodner K, Kimberger O, Lozanov P, Husslein P, Mayerhofer K. Women's position during labour: influence on maternal and neonatal outcome. Wien Klin Wochenschr. 2003; 115(19-20):720-723.

[33] Thılagavaty G. Maternal birthing position and outcome of labor, J Fam Welf. 2012; 58(1): 68-73.

[34] Bomfim-Hyppólito S. Influence of the position of the mother at delivery over some maternal and neonatal outcomes. Int J Gynaecol Obstet. 1998; 63(1): 67-73. DOI:10.1016/s0020-7292(98)00186-6.

[35] Terry RR, Westcott J, O'shea L, Kelly F. Postpartum outcomes in supine delivery by physicians vs Nonsupine delivery by midwives, J Am Osteopath Assoc. 2006; 106:199-202.

[36] Miquelutti MA, Cecatti JG, Morais SS, Makuch MY. The vertical position during labor: Pain and satisfaction. RBSMI, 2009; 9(4): 393–398. https://doi.org/10.1590/S1519.382.9200900.040.0002

[37] Mamede, Fabiana Villela, Almeida, Ana Maria de, Souza, Luiz de & Mamede, Marli Villela. Pain during the labor active phase: the effect of walking. Rev Lat Am Enfermagem. 2007; 15(6): 1157-1162. https://dx.doi.org/10.1590/S0104.116.9200700.060.0016

[38] Molina FJ, Solá PA, López E, Pires C. Pain in the first stage of labor: relationship with the patient's position. J Pain Symptom Manage. 1997; 13(2):98-103. DOI:10.1016/s0885-3924(96)00270-9.

[39] Nasir A, Korejo R, Noorani KJ. Childbirth in squatting position. J Pak Med Assoc. 2007; 57(1):19-22.

[40] Akın B, Soğukpınar N, Akman L, Yavuzşen HT. Evaluation The effects of maternal mobilization on mode of birth and newborn health status in the first stage of birth, Gümüşhane University Journal Of Health Sciences. 2017; 6(2): 33 – 41. (Turkish)

[41] Berghella V, Baxter JK, Chauhan SP. Evidence-based labor and delivery management. Am J Obstet Gynecol. 2008; 199(5):445-454. DOI: 10.1016/j.ajog.2008.06.093.

[42] Gupta JK, Sood A, Hofmeyr GJ, Vogel JP. Position in the second stage of labour for women without epidural anaesthesia. Cochrane Database Syst. 2017; Rev. 5, CD002006. https://doi.org/10.1002/14651858.CD002006.pub4.

[43] Souza JP, Miquelutti MA, Cecatti JG, Makuch MY. Maternal position during the first stage of labor: a systematic review. Reprod Health. 2006; 3(10):1-9. DOI:10.1186/1742-4755-3-10.

[44] Tatarlar A, Tokat MA. The effect of birth fear on lactation, infant sucking ability and first breastfeeding results. TAF Prev Med Bull, 2016; 15(2): 83-91. DOI: 10.5455/pmb.1-143.581.9087. (Turkish)

How to cite this article: Karaman OE, Yildiz H. The Effect on Birth Pain and Process of the Freedom of Movement in the First Stage of Labor: A Randomized Controlled Study. Clin Exp Health Sci 2022; 12: 730-738. DOI: 10.33808/clinexphealthsci.1016033

Clinical and Experimental Health Sciences

In Vitro Assessment of Anti-inflammatory Effect of Apigenin on Renal Cell Inflammation

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Received: 03.11.2021 **Accepted:** 24.07.2022

ABSTRACT

Objective: This study aimed to evaluate in vitro effect of apigenin on anti – and pro-inflammatory cytokines including interleukin-6 (IL-6), IL-10, tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) levels in an in vitro model of renal cell inflammation induced with lipopolysaccharide (LPS).

Methods: For the in vitro renal cell inflammation model, the African green monkey kidney cell line (Vero) was used. Four groups as NC (without any treatment), LPS (Vero cells treated with 10 µg/mL of LPS for 4 hours), API (Vero cells treated with 5 µg/mL of apigenin for 12 hours), and LPS+API (Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours) was formed. The non-cytotoxic dose of apigenin in Vero cells was evaluated by a cell count test. IL-6, IL-10, TNF-α, and TGF-β concentrations in the cell culture medium were measured by enzyme-linked immunosorbent assay kits. All analyses were performed in four repetitions.

Results: IL-6, IL-10, TNF-α, and TGF-β concentrations of the LPS group increased compared to NC, API, and LPS+API groups ($p<0.05$). We found that treatment with apigenin led to significant attenuation in the LPS-induced secretion of IL-6, IL-10, TNF-α, and TGF-β in the Vero cell line.

Conclusion: Our findings showed that apigenin significantly reduced LPS-induced IL-6, IL-10, TNF-α, and TGF-β formations in Vero cells. Taken together, these results suggest that apigenin may be a therapeutic candidate for relieving inflammatory renal cell damage. These results need to be supported by in vivo trials and clinical applications.

Keywords: *Apigenin, in vitro, lipopolysaccharide, renal cell damage*

1. INTRODUCTION

Inflammation and immune system activation play role in the etiopathogenesis of acute and chronic kidney diseases (1). Inflammation stimulates cytokines secretion and increases the production and activity of adhesion molecules in renal tissue (2,3). Patients with renal injury have higher serum interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) levels (4-8). IL-6 induces the progression of chronic kidney disease by initiating chronic vascular disease, endothelial injury, and adiponectin expression (9,10). TNF-α stimulates the release of the inflammatory mediator Interleukin 1β (IL-1β), monocyte chemoattractant protein (MCP)-1), and transforming growth factor-beta (TGF-β) (11). TGF-β induces conditions causing chronic progressive kidney disease through regulation of cell proliferation, hypertrophy, apoptosis, and fibrogenesis (12). IL-10 is produced as a growth factor by mesangial cells in the normal adult kidney and induces pathological processes that lead to the progression of renal failure by inducing the synthesis and activity of Cystatin C and TGF-β. Increased TGF-β levels promote fibrosis and glomerulosclerosis with IL-10 (13).

In recent years, scientific studies on the treatment of inflammatory diseases have focused on the use of anti-inflammatory flavonoids. Many flavonoids show medical efficacy as antibacterial, antioxidative activity, free radical scavenging capacity, anti-inflammatory, anticancer and antiviral agents (14). Apigenin, found naturally in fruits and vegetables, has anti-inflammatory effects in various cellular processes (15,16). The prophylactic use of apigenin suppresses cyclooxygenase-2 (COX-2) and nuclear factor **κ**B (NF-**κB**) pathways by decreasing IL-1β, IL-6 and TNF-α concentrations, leukocyte quantity, and neutrophil percentage in bronchoalveolar lavage fluid and thereby alleviates inflammation (16). Apigenin exhibits an anti-inflammatory effect by suppressing chemokine production associated with T helper cell-1 and T-helper cell-2 and modulating mitogen-activated protein kinase (MAPK) in human monocyte cells (17). Apigenin inhibits LPS-induced NF-κB activity in lung tissue, reduces infiltration of inflammatory cells, and accumulation of chemotactic factors (18). It is known that apigenin has a good anti-inflammatory effect but the possible protective effect of apigenin on renal cell inflammation is unknown. The purpose of the present study was to investigate the effect of apigenin on anti-inflammatory cytokines IL-6, IL-10, TNF-α, and TGF-β levels in *vitro* renal cell inflammation model.

DOI: 10.33808/clinexphealthsci.1018335

2. METHODS

2.1. Cell Culture and Treatment

The Vero cell line used in this study was obtained from the Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University. The 10% calf serum, 2 mM l-glutamine, 100 µg/mL streptomycin, 100 U/mL penicillin, and 1 mM pyruvic acid in Minimum Essential Eagle's Medium (MEM, Sigma, Austria) were applied to the Vero cell line. The cells were maintained at 37 °C, 5% CO_2, 95% air, and 100% humidity. Filter cap cell culture flasks were used to prevent possible contamination and the medium was changed every two days. Cell survival and morphological structure were evaluated by an inverted microscope. The inflammation was induced by treatment with LPS (E. coli O111: B4) in Vero cells. In order to induce inflammation, the cells were incubated with culture medium containing different LPS concentrations (0.1 µg/mL, 1 µg/mL, 2 µg/mL, 5 µg/mL and 10 µg/mL) for 2, 4, 8, 16 and 24 hours. The inflammation dose of LPS was selected based on previous scientific studies (19,20). A previous *in vitro* study (21) was taken into consideration to determine the non-cytotoxic dose for apigenin treatment, cells were cultured with different apigenin concentrations (0.1 µg/mL, 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 5 µg/mL, and 10 µg/mL) for 12 hours and the non-cytotoxic dose was determined according to the cell count test result. All experiments were performed in four repetitions.

In the study, four groups NC (negative control group, any application was not done), LPS (treatment with 10 µg/mL of LPS during 4 hours), API (treatment with 5 µg/mL of apigenin during 12 hours), and LPS+API (treatment with 10 µg/mL of LPS during 4 hours+5 µg/mL of apigenin during 12 hours) were formed. After all treatments, cell culture media were centrifuged at 1.550xg for 10 minutes and ELISA analysis was performed in supernatants (22).

2.2. Determination of Non-Cytotoxic Dose of Apigenin

The non-cytotoxic dose of apigenin in Vero cells was assessed by a cell counting kit-8 (96992, Sigma-Aldrich, USA). The test was performed according to the method notified by the manufacturer. For this purpose, 100 µL cell suspension including 1×10^5 Vero cells per milliliter was prepared and then cell suspension was added to the 96-well plate and incubated in a 37 °C incubator. When the cells covered the plate surface at the end of 24 hours, the cell production medium in wells was evacuated and the plates were incubated in a 22 °C incubator by adding stock apigenin from 3 different concentrations, 1 µg/mL, 5 µg/mL, and 10 µg/mL prepared in cell production medium and 10 µg/mL and from each dilution to 2 wells. In addition, 10 µL of cell-producing media was added into the wells of the control cells instead of apigenin. Apigenin in the wells of the plates was taken 12 hours later from the incubator and was evacuated using a pipette and 10 µL of cell counting solution was added to all wells and incubated at 37 °C for 2 hours. At the end of the time, the optical density of the microplate was read in 450 nm wavelength at the microplate reader (Tecan Infinite F50). The test was repeated 4 times. The half-maximal inhibitory concentration (IC50) value for apigenin was calculated and the graph was plotted using the GraphPad Prism 5 software (San Diego, CA, USA).

2.3. Main Outcome Measurements

IL-6 concentration:The monkey-specific ELISA assay (LS-F4822, LifeSpan Biosciences Inc., Seattle Downtown, Washington, USA) was used to measure the concentration of IL-6 in cell culture supernatants. All samples were studied double. ELISA steps were carried out as recommended by the manufacturer. The results were presented as pg/mL.

IL-10 concentration: The concentration of IL-10 in cell culture supernatants was determined using the ELISA kit specific to monkey (LS-F25130, LifeSpan Biosciences Inc., Seattle Downtown, Washington, USA) by the ELISA method. The measuring range of the kit was 0-1000 pg/mL and all samples were studied double. ELISA steps were carried out as recommended by the manufacturer. The results were presented as pg/mL.

TNF-α concentration: The concentration of TNF-α in cell culture supernatants was determined using the ELISA kit specific to monkey (LS-F4818, LifeSpan Biosciences Inc., Seattle Downtown, Washington, USA) by the ELISA method. All samples were studied double. ELISA steps were carried out as recommended by the manufacturer. The results were presented as pg/mL.

TGF-β concentration: The concentration of TGF-β in cell culture supernatants was determined using the ELISA kit specific to monkey (MBS737903, MyBioSource, Inc., San Diego, CA, USA) by the ELISA method. The measuring range of the kit was 0-1000 pg/mL and all samples were studied double. ELISA steps were carried out as recommended by the manufacturer. The results were presented as pg/mL.

2.4. Statistical Analysis

SPSS 22.0 package program was used for statistical evaluation of the findings obtained from the study. Before significance tests, all the data were evaluated using the parametrical test with Shapiro Wilk for normality assumptions and with the Levene test for homogeneity of the variance. Duncan test was used for variables that provide parametric test assumptions as a post-hoc test in cases where the difference between groups is significant. A minimum of $p < 0.05$ value was considered statistically significant for all statistical evaluations.

3. RESULTS

3.1. Non-Cytotoxic Dose of Apigenin on Vero Cells

As determined by the cell viability assay, different concentrations of apigenin resulted in cytotoxicity effects on

the Vero cell line in a dose-dependent manner for 12 hours. The growth of the cell line was strikingly inhibited by different concentrations of apigenin (0.1 µg/mL, 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 5 µg/mL, and 10 µg/mL). In different apigenin concentrations, cell viability was found as 98.0 ± 1.56 % in 0.1 µg/mL, 90.6 ± 4.63 % in 0.25 µg/mL, 83.2 ± 2.92 % in 0.5 µg/mL, 75.8 ± 4.33 % in 1 µg/mL, 50 ± 5.68 % in 5 µg/mL, and 9.88 ± 0.68 % in 10 µg/mL (p<0. 05). Viability assay from four repetitions showed that IC50 of apigenin was 5 µg/mL (Figure 1).

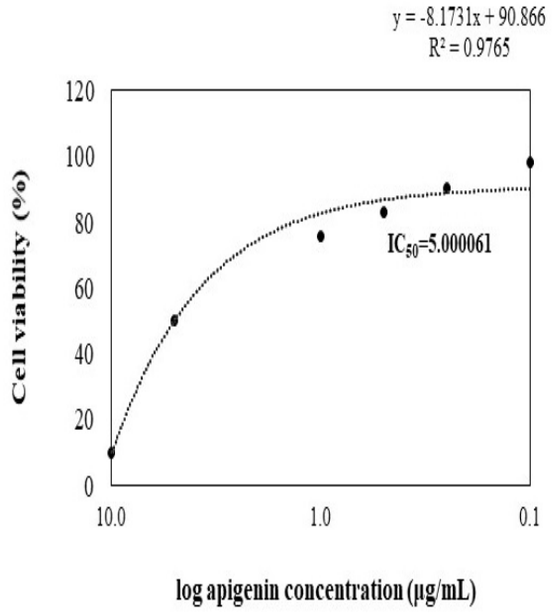


Fig 1. The dose-response curve for the half-maximal inhibitory concentration (IC50) for apigenin. Six different concentrations (ranging from 0.1 to 10 mg/mL) were tested. Data are represented as mean values of at least four independent experiments. The IC50 values were calculated using nonlinear regression analysis of GraphPad Prism software 5 by plotting log inhibitor versus normalized response.

3.2. Effect of Apigenin on IL-6 Concentration in LPS-Treated Vero Cells

The effect of apigenin administration of 5 µg/mL for 12 hours on IL-6 concentration in LPS-treated Vero cells and the differences between groups are presented in Figure 2. The results of the IL-6 levels of the groups were given in Table 1. The IL-6 concentrations were 159.3 ± 6.6 pg/mL in LPS group, 52.8 ± 3.9 pg/mL in NC group , 46.5 ± 1.3 pg/mL in API group and 86.5 ± 5.0 pg/mL in LPS+API group. Treatment of Vero cells with LPS alone notably increased the production of IL-6 compared with the negative control, API, and LPS+API groups. The LPS-treated group showed a 3.0-fold, 3.4-fold, and 1.8-fold increase in IL-6 levels when compared with the negative control group, API group, and LPS+API group, respectively (p<0.05). The secretion of IL-6 in the LPS+API group was significantly suppressed by apigenin compared with the level in the LPS-treated cells (p<0.05).

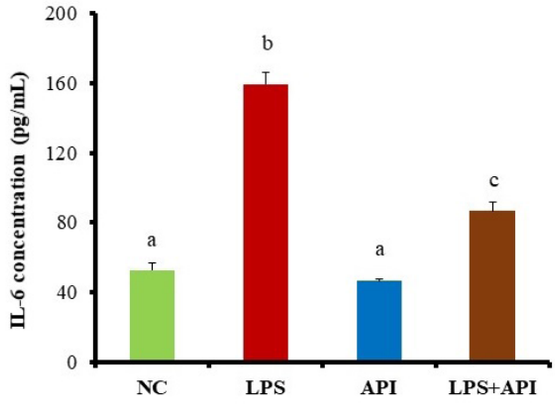


Fig 2. Effect of apigenin on IL-6 concentration in LPS-treated Vero cells. NC: Vero cells received no treatment; LPS: Vero cells treated with 10 µg/mL of LPS for 4 hours; API: Vero cells treated with 5 µg/mL of apigenin for 12 hours; LPS+API: Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours. Data are mean ± SD for four independent experiments. a p<0.05: significantly different from LPS and LPS +API groups; b p<0.05: significantly different from NC, API and LPS+API groups; Duncan test.

Table 1. IL-6, 1L-10, TNF-α, and TGF-β levels of the groups

Cytokine	Groups			
	NC	API	LPS	LPS+API
IL-6 (pg/mL)	52.8 ± 3.9^a	46.5 ± 1.3^a	159.3 ± 6.6^b	86.5 ± 5.0^c
IL-10 (pg/mL)	62.3 ± 8.5^a	58.3 ± 6.4^a	359.0±16.9^b	155.0 ± 9.5^c
TNF-α (pg/mL)	67.3 ± 4.3^a	63.0 ± 4.5^a	292.3 ± 12.5^b	101.0 ± 3.2^c
TGF-β (pg/mL)	27.0 ± 4.2^a	32.3 ± 3.9^a	312.8 ± 10.2^b	150.0 ± 11.0^c

NC: Negative control (Vero cells received no treatment); API: Vero cells treated with 5 µg/mL of apigenin for 12 hours; LPS: Vero cells treated with 10 µg/mL of LPS for 4 hours; LPS+API: Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours. a,b,c Different superscript letters indicate statistically significant differences in the same row (p<0.05; Duncan test)

3.3. Effect of Apigenin on IL-10 Concentration in LPS-Treated Vero Cells

The effect of apigenin administration of 5 µg/mL for 12 hours on IL-10 concentration in LPS-treated Vero cells and the differences between groups are presented in Figure 3. The results of the IL-10 levels of the groups were given in Table 1. The IL-10 concentrations were 359.0±16.9 pg/mL in the LPS group, 62.3 ± 8.5 pg/mL in the NC group, 58.3 ± 6.4 pg/mL in the API group, and 155.0 ± 9.5 pg/mL in the LPS+API

group. The LPS-treated group showed a 5.8-fold, 6.2-fold, and 2.3-fold increase in IL-10 levels when compared with the negative control group, API group, and LPS+API group, respectively ($p<0.05$). The secretion of IL-10 in the LPS+API group was significantly suppressed by apigenin compared with the level in the LPS-treated cells ($p<0.05$).

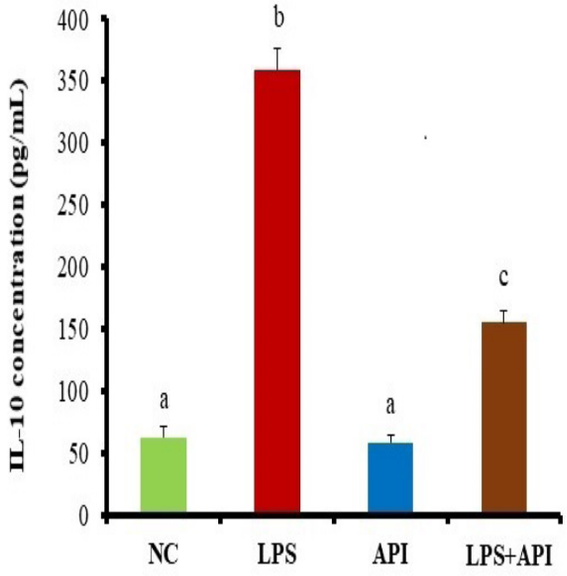
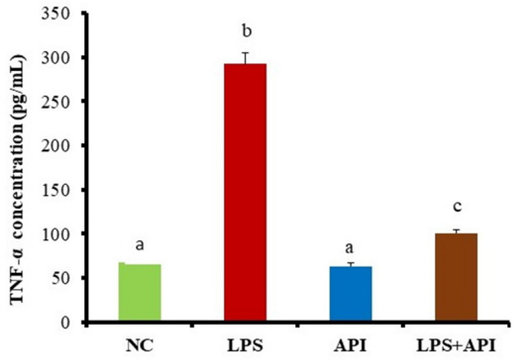


Fig 3. *Effect of apigenin on IL-10 concentration in LPS-treated Vero cells. NC: Vero cells received no treatment; LPS: Vero cells treated with 10 µg/mL of LPS for 4 hours; API: Vero cells treated with 5 µg/mL of apigenin for 12 hours; LPS+API: Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours. Data are mean ± SD for four independent experiments. a p<0.05: significantly different from LPS and LPS+API groups; b p<0.05: significantly different from NC, API and LPS+API groups; Duncan test.*

3.4. Effect of Apigenin on TNF-α Concentration in LPS-Treated Vero Cells

The effect of apigenin administration of 5 µg/mL for 12 hours on TNF-α concentration in LPS-treated Vero cells and the differences between groups are presented in Figure 4. The results of the TNF-**α** levels of the groups were given in Table 1. The TNF-α concentrations were 292.3 ± 12.5 pg/mL in the LPS group, 67.3 ± 4.3 pg/mL in the NC group, 63.0 ± 4.5 pg/mL in the API group, and 101.0 ± 3.2 pg/mL in the LPS+API group. The LPS-treated group showed a 4.3-fold, 4.6-fold, and 2.9-fold increase in TNF-α levels when compared with the negative control group, API group, and LPS+API group, respectively ($p<0.05$). The secretion of TNF-α in the LPS+API group was significantly suppressed by apigenin compared with the level in the LPS-treated cells ($p<0.05$).



Fige 4. *Effect of apigenin on TNF-α concentration in LPS-treated Vero cells. NC: Vero cells received no treatment; LPS: Vero cells treated with 10 µg/mL of LPS for 4 hours; API: Vero cells treated with 5 µg/mL of apigenin for 12 hours; LPS+API: Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours. Data are mean ± SD for four independent experiments a p<0.05: significantly different from LPS and LPS+API groups; b p<0.05: significantly different from NC, API and LPS+API groups; Duncan test.*

3.5. Effect of Apigenin on TGF-β Concentration in LPS-Treated Vero Cells

The effect of apigenin administration of 5 µg/mL for 12 hours on TGF-β concentration in LPS-treated Vero cells and the differences between groups are presented in Figure 5. The results of the TGF-β levels of the groups were given in Table 1. The TGF-β concentration were 312.8 ± 10.2 pg/mL in LPS group, 27.0 ± 4.2 pg/mL in NC group, 32.3 ± 3.9 pg/mL in API group and 150.0 ± 11.0 pg/mL in LPS+API group. The LPS-treated group showed an 11.6-fold, 9.7-fold, and 2.1-fold increase in TGF-β levels when compared with the negative control group, API group, and LPS+API group, respectively ($p<0.05$). The secretion of TGF-β in the LPS+API group was significantly suppressed by apigenin compared with the level in the LPS-treated cells ($p<0.05$).

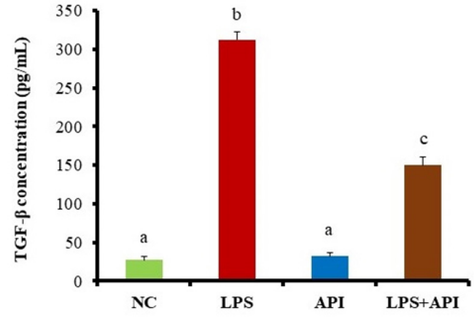


Fig 5. Effect of apigenin on TGF-β concentration in LPS-treated Vero cells. NC: Vero cells received no treatment; LPS: Vero cells treated with 10 µg/mL of LPS for 4 hours; API: Vero cells treated with 5 µg/mL of apigenin for 12 hours; LPS+API: Vero cells treated with 5 µg/mL of apigenin for 12 hours + 10 µg/mL of LPS for 4 hours. Data are mean ± SD for four independent experiments. a p<0.05: significantly different from LPS and LPS+API groups; b p<0.05: significantly different from NC, API and LPS+API groups; Duncan test.

4. DISCUSSION

Inflammation is known to play a role in the pathogenesis of kidney diseases by stimulating the release of cytokines and increasing the production and activity of adhesion molecules (3). Proinflammatory cytokines have a critical role in mediating irreversible tubular injury and nephron failure (23). Scientific reports have suggested that flavonoid supplements exhibit alleviate renal damage by suppressing inflammation (24,25). Apigenin has been shown to exhibit potent anti-inflammatory activity (15-18). Our present study focused on the anti-inflammatory activity of apigenin in renal cell inflammation. For this purpose, *in vitro* effect of apigenin on anti – and pro-inflammatory cytokines including IL-6, IL-10, TNF-α, and TGF-β in renal cell inflammation induced with LPS was evaluated by ELISA.

The prophylactic use of apigenin has been reported that alleviates inflammation by suppressing COX-2 and NF-κB pathways by decreasing IL-1β, IL-6, and TNF-α concentrations, leukocyte count, and neutrophil percentage in bronchoalveolar lavage fluid (16). In human monocyte cells, apigenin has been shown to exert an anti-inflammatory effect by suppressing T helper cell-1 and T helper cell-2-related chemokine production and modulating MAPK (17). It has also been stated that apigenin inhibits LPS-induced NF-κB activity in lung tissue, reduces the infiltration of inflammatory cells, and the accumulation of chemotactic factors (18). Hesperidin has been reported to alleviate the acetaminophen-induced inflammation in renal tubular cells (26). Consumption of isoflavone-rich soybean foods can alleviate systemic inflammation through inflammatory mediators in hemodialysis patients has been reported (27). Chrysin at different doses has been shown to alleviate kidney damage in adenine-induced experimental chronic kidney disease in rats (28). A previous study has reported that epicatechin supplementation with diet alleviated the renal cortex inflammation in high-fructose-diet-fed rats (29). Quercetin protects the kidney against lead-induced kidney injury by affecting the MAPK and NF-κB signaling pathways (30). Naringin treatment in cisplatin-induced nephrotoxicity has been reported to reduce TNF-α concentration in kidney tissue (31). Genistein has been shown to significantly inhibit urinary MCP-1 excretion and renal intercellular adhesion molecule – 1 expression in diabetic mice (32). Baicalin, which was used to ameliorate tubulointerstitial fibrosis in mice with unilateral ureteral obstruction, has been reported to inhibit the inflammatory process by inactivating NF-κB and MAPK signaling pathways (33). Malik, *et al.* reported that nobiletin alleviates acute kidney injury induced by cisplatin in a dose-dependent manner (34).

Markedly increased TNF-α concentration in HK-2 cells which were exposed to 10 μg/ml LPS for 3 hours has been reported (35). Similarly, in our study, the TNF-α level in the group treated with 10 μg/mL for 4 hours of LPS was significantly increased when compared to the negative control group, API group, and LPS+API group ($p<0.05$). TNF-α secretion in the LPS+API group was determined to be significantly inhibited by apigenin compared to the level in cells treated with LPS ($p<0.05$). In another study, it has been shown that apigenin inhibits the production of IL-1β, IL-8, and TNF-α, by suppressing NF-κB activity and modulating immune responses by suppressing inflammation in LPS-induced mouse macrophages (15). The researchers have stated that apigenin at the doses of 1 μg/mL and 10 μg/mL suppresses the LPS-induced IL-6 concentration, however, did not affect TNF-α concentration. Similar to these findings, we also determined that the IL-6 level was significantly increased when Vero cells were exposed to 10 mg/mL LPS for 4 hours compared to the negative control, API, and LPS+API groups. However, we determined that the increased IL-6 level due to LPS exposure was significantly suppressed by apigenin at a dose of 5 mg/ml administered for 12 hours. In addition, administration of LPS to Vero cells resulted in a significant increase in IL-10 levels. IL-10 level in the LPS+API group was determined to be significantly suppressed by apigenin compared to the level in cells treated with LPS ($p<0.05$). Apigenin inhibits LPS-induced IL-6 and/or TNF-α production in murine macrophage cells has been reported (21). It has also been reported that TNF-α-induced NF-kB transcriptional activation is significantly inhibited by apigenin (36). Apigenin administration significantly reduced the levels of TNF-α, IL-1β, and TGF-β in the kidneys in mice with cisplatin-induced kidney injury in a dose-dependent manner (37). Apigenin-rich diet has been reported to exert anti-inflammatory activity by reducing miR-155 and TNF-α expressions in the lungs of LPS-treated mice (38). In our study, the LPS-treated Vero cell line showed a significant increase in TGF-β levels when compared with the negative control group, API group, and LPS+API group ($p<0.05$). However, the elevated TGF-β level in LPS-treated cells was significantly suppressed by the administration of 5 mg/mL apigenin for 12 hours ($p<0.05$).

5. CONCLUSIONS

Our findings showed that apigenin significantly reduced LPS-induced IL-6, IL-10, TNF-α, and TGF-β formations in Vero cells. These findings indicate that apigenin has potent anti-inflammatory effects in LPS-induced *in vitro* renal cell damage by modulating pro-inflammatory and anti-inflammatory cytokine responses which are inflammatory mediators. These results need to be supported by *in vivo* trials and clinical applications.

Acknowledgments This research article was summarized from the first author's master's thesis. We would like to thank Prof. Dr. Semra Gümüşova and Res. Assist. Bahadır Müftüoğlu from the Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University for cell culture experiments.

Funding This study was supported by the Scientific Project Management Office of the Ondokuz Mayis University, Turkey (PYO.VET.1904.17.014).

Conflict of Interest The authors declared that there is no conflict of interest.

REFERENCES

[1] Imig J, Ryan M. Immune and inflammatory role in renal disease. Compr Physiol 2013;3(2):957-976.

[2] Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. Int J Mol Sci 2019;21(1):263.

[3] Tbahriti FH, Meknassi D, Moussaoui R, Messaoudi A, Zemour L, Kaddous A, Bouchenak M, Mekki K Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines World J Nephrol 2013;6:31-37.

[4] Al-Eisa AA, Al Rushood M, Al-Attiyah RJ Urinary excretion of IL-1β, IL-6 and IL-8 cytokines during relapse and remission of idiopathic nephrotic syndrome. J Inflamm Res 2017;23:1-5.

[5] Nechemia-Arbely Y, Barkan D, Pizov G, Shriki A, Rose-John S, Galun E, Axelrod JH. IL-6/IL-6R axis plays a critical role in acute kidney injury. J Am Soc Nephrol 2008;6:1106-1115.

[6] Sonkar GK, Singh RG. Evaluation of serum tumor necrosis factor alpha and its correlation with histology in chronic kidney disease, stable renal transplant and rejection cases. Saudi J Kidney Dis Transpl 2009;20:1000-1004.

[7] Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimbürger O, Cederholm T, Girndt M. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia-the good, the bad, and the ugly. Kidney Int 2005;67:1216-1233.

[8] Yeo ES, Hwang JY, Park JE, Choi YJ, Huh KB, Kim WY. Tumor necrosis factor (TNF-alpha) and C-reactive protein (CRP) are positively associated with the risk of chronic kidney disease in patients with type 2 diabetes. Yonsei Med J 2010;51:519-525.

[9] Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: *in vivo* and *in vitro* investigations in humans. Am J Physiol Endocrinol Metab 2003;285:E527-533.

[10] Zhang W, Wang W, Yu H, Zhang Y, Dai Y, Ning C, Tao L, Sun H, Kellems RE, Blackburn MR, Xia Y. Interleukin 6 underlies angiotensin II-induced hypertension and chronic renal damage. Hypertension 2012;59:136-144.

[11] Idasiak-Piechocka I, Oko A, Pawliczak E, Kaczmarek E, Czekalski S. Urinary excretion of soluble tumour necrosis factor receptor 1 as a marker of increased risk of progressive kidney function deterioration in patients with primary chronic glomerulonephritis. Nephrol Dial Transplant 2010;12:3948-3956.

[12] Schnaper HW, Jandeska S, Runyan CE, Hubchak SC, Basu RK, Curley JF, Smith RD, Hayashida T. TGF-beta signal transduction in chronic kidney disease. Front Biosci (Landmark Ed). 2009;1;14:2448-2465.

[13] Sinuani I, Beberashvili I, Averbukh Z, Sandbank J. Role of IL-10 in the progression of kidney disease. World J Transplant 2013;3(4):91-98.

[14] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal 2013;2013:162750.

[15] Nicholas C, Batra S, Vargo MA, Voss OH, Gavrilin MA, Wewers MD, Guttridge DC, Grotewold E, Doseff AI. Apigenin blocks lipopolysaccharide-induced lethality *in vivo* and proinflammatory cytokines expression by inactivating NF-kappaB through the suppression of p65 phosphorylation. J Immunol 2007;179:7121-7127.

[16] Wang J, Liu YT, Xiao L, Zhu L, Wang Q, Yan T. Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway. Inflammation 2014;37:2085-2090.

[17] Huang CH, Kuo PL, Hsu YL, Chang TT, Tseng HI, Chu YT, Kuo CH, Chen HN, Hung CH. The natural flavonoid apigenin suppresses Th1 – and Th2-related chemokine production by human monocyte THP-1 cells through mitogen-activated protein kinase pathways. J Med Food 2010;13:391-398.

[18] Cardenas H, Arango D, Nicholas C, Duarte S, Nuovo GJ, He W, Voss OH, Gonzalez-Mejia ME, Guttridge DC, Grotewold E, Doseff AI. Dietary apigenin exerts immune-regulatory activity *in vivo* by reducing NF-**κB** Activity, Halting Leukocyte Infiltration and Restoring Normal Metabolic Function. Int J Mol Sci 2016;1:323.

[19] Huang MY, Chaturvedi LS, Koul S, Koul HK. Oxalate stimulates IL-6 production in HK-2 cells, a line of human renal proximal tubular epithelial cells. Kidney Int 2005;68:497-503.

[20] Zager RA1, Johnson AC, Geballe A. Gentamicin suppresses endotoxin-driven TNF-alpha production in human and mouse proximal tubule cells. Am J Physiol Renal Physiol 2007;293:F1373-1380.

[21] Smolinski AT, Pestka JJ. Modulation of lipopolysaccharide-induced proinflammatory cytokine production *in vitro* and *in vivo* by the herbal constituents apigenin (chamomile), ginsenoside Rb(1) (ginseng) and parthenolide (feverfew). Food Chem Toxicol 2003;41:1381-1390.

[22] Li B, Hu Y, Zhao Y, Cheng M, Qin H, Cheng T, Wang Q, Peng X, Zhang X. Curcumin attenuates titanium particle-induced inflammation by regulating macrophage polarization *in vitro* and *in vivo*. Front Immunol 2017;31;8:55.

[23] Qian Q. Inflammation: A Key Contributor to the Genesis and Progression of Chronic Kidney Disease. Contrib Nephrol 2017;191:72-83.

[24] Impellizzeri D, Bruschetta G, Ahmad A, Crupi R, Siracusa R, Di Paola R, Paterniti I, Prosdocimi M, Esposito E, Cuzzocrea S. Effects of palmitoylethanolamide and silymarin combination treatment in an animal model of kidney ischemia and reperfusion. Eur J Pharmacol 2015;762:136-149.

[25] Kandemir FM, Kucukler S, Eldutar E, Caglayan C, Gulcin I. Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: A Multi-Biomarker Approach. Sci Pharm 2017;85:4.

[26] Ahmad ST, Arjumand W, Nafees S, Seth A, Ali N, Rashid S, Sultana S. Hesperidin alleviates acetaminophen induced toxicity in Wistar rat by abrogation of oxidative stress, apoptosis and inflammation. Toxicol Lett 2012;208:149-161.

[27] Fanti P, Asmis R, Stephenson TJ, Sawaya BP, Franke AA. Positive effect of dietary soy in ESRD patients with systemic inflammation—correlation between blood levels of the soy isoflavones and the acute-phase reactants. Nephrol Dial Transplant 2006;21:2239-2246.

[28] Ali BH, Adham SA, Al Za'abi M, Waly MI, Yasin J, Nemmar A, Schupp N. Ameliorative effect of chrysin on adenine-induced chronic kidney disease in rats. PLoS One 2015;10:e0125285.

[29] Prince PD, Lanzi CR, Toblli JE, Elesgaray R, Oteiza PI, Fraga CG, Galleano M. Dietary (-)-epicatechin mitigates oxidative stress, NO metabolism alterations, and inflammation in renal cortex from fructose-fed rats. Free Radic Biol Med 2016;90:35-46.

[30] Liu CM, Sun YZ, Sun JM, Ma JQ, Cheng C. Protective role of quercetin against lead-induced inflammatory response in rat kidney through the ROS-mediated MAPKs and NF-**κB** pathway. Biochim Biophys Acta 2012;1820:1693-1703.

[31] Chtourou Y, Aouey B, Aroui S, Kebieche M, Fetoui H. Anti-apoptotic and anti-inflammatory effects of naringin on

cisplatin-induced renal injury in the rat. Chem Biol Interact 2016;243:1-9.

[32] Elmarakby AA, Ibrahim AS, Faulkner J, Mozaffari MS, Liou GI, Abdelsayed R. Tyrosine kinase inhibitor, genistein, reduces renal inflammation and injury in streptozotocin-induced diabetic mice. Vascul Pharmacol 2011;55:149-156.

[33] Wang W, Zhou PH, Xu CG, Zhou XJ, Hu W, Zhang J. Baicalein attenuates renal fibrosis by inhibiting inflammation via down-regulating NF-**κB** and MAPK signal pathways. J Mol Histol 2015;46:283-290.

[34] Malik S, Bhatia J, Suchal K, Gamad N, Dinda AK, Gupta YK, Arya DS. Nobiletin ameliorates cisplatin-induced acute kidney injury due to its anti-oxidant, anti-inflammatory and anti-apoptotic effects. Exp Toxicol Pathol 2015;67:427-433.

[35] Zager RA, Johnson AC, Geballe A. Gentamicin suppresses endotoxin-driven TNF-alpha production in human and mouse proximal tubule cells. Am J Physiol Renal Physiol 2007;293:1373-1380.

[36] Funakoshi-Tago M, Nakamura K, Tago K, Mashino T, Kasahara T Anti-inflammatory activity of structurally related flavonoids, Apigenin, luteolin and fisetin. Int Immunopharmacol 2011;11:1150-1159.

[37] He X, Li C, Wei Z, Wang J, Kou J, Liu W, Shi M, Yang Z, Fu Y. Protective role of apigenin in cisplatin-induced renal injury. Eur J Pharmacol 2016;789:215-221.

[38] Arango D, Diosa-Toro M, Rojas-Hernandez LS, Cooperstone JL, Schwartz SJ, Mo X, Jiang J, Schmittgen TD, Doseff AI. Dietary apigenin reduces LPS-induced expression of miR-155 restoring immune balance during inflammation. Mol Nutr Food Res 2015;59:763-772.

How to cite this article: Ozsoy S, Yarim GF. In Vitro Assessment of Anti-inflammatory Effect of Apigenin on Renal Cell Inflammation. Clin Exp Health Sci 2022; 12: 739-745. DOI: 10.33808/clinexphealthsci.1018335

Clinical and Experimental Health Sciences

The Effects of Therapeutic Intermittent Hypoxia Implementation on Complete Blood Count Parameters: An Experimental Animal Model

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Received: 04.11.2021 **Accepted:** 18.01.2022

ABSTRACT

Objective: Intermittent hypoxia (IH) implementation is a method performed by intermittently decreasing oxygen concentration in inhaled air at specific rate. This method varies between studies in terms of its application. This study aims to examine the changes in Complete Blood Count (CBC) parameters caused by IH implementation at therapeutic dose ranges with a single model.

Methods: Ten Sprague Dawley type adult male rats were divided into two groups. In the study group, FiO_2 level of inhaled air, was reduced to 10% in hypoxic cycle. 5 minutes normoxia-hypoxia cycle was used in each 30 minutes experiment period for study group. Control group remained in normoxic air for 30 minutes. 1 cc of blood was taken from mandibular vein from all rats at the end of 6th day. CBC analyzes were performed and differences between two groups were investigated.

Results: Significant differences were detected in some CBC parameters between the two groups. It was determined that significant increase in MONO ($p<0.001$), MONO% ($p<0.001$), MCH ($p=0.03$), PLT ($p=0.013$) and PCT ($p=0.007$) parameters and significant decrease in MPV ($p=0.02$) parameters, in favor of study group.

Conclusion: IH implementation was caused significant changes in MONO, MONO%, MCH, PCT, PLT and MPV parameters in the CBC analysis of rats. Considering the study results, therapeutic IH implementation may thought to have important effects in terms of lung protection and regeneration. Further research may focus on this point for precising and supporting of this study' results.

Keywords: Intermittent hypoxia, complete blood count tests, animal model

1. INTRODUCTION

Using the complete blood count test (CBC) is spread for predicting respiratory system status in the last years (1). This method is also started using in studies on intermittent hypoxia (IH) implementation.

IH implementations are classified as chronic and acute implementations. IH occurs after the decrease of arterial partial pressure of oxygen (PaO_2) in the blood of the living organism when the inspired oxygen level reached to the range of 35-60 mmHg (2). Although knowledge on the dosage and method of implementation for IH is not clear, it is generally implemented with varying episodes of lower oxygen levels for 30 minutes. It has been reported that the principle of "moderate hypoxia, few episodes" could increase the effectiveness of IH and could help avoid harmful effects (3).

Many useful effects of intermittent hypoxia implementation have been investigated until today. These studies include that phrenic long-term facilitation, hypoglossal long-term facilitation, ventilatory long-term facilitation, brain-derived neurotrophic factor (BDNF) and serotonin release, erythropoietin, hemoglobin levels and monocyte counts (4,5,6,7,8,9).

CBC is a group of tests that analyse cells circulating in the blood along with white blood cells (WBC), red blood cells (RBC) and platelets (PLT). The methods of implementation of intermittent hypoxia varies according to studies in the literature. In addition, differences are observed between studies for CBC analysis. This study aims to examine the changes in CBC parameters caused by IH implementation at therapeutic dose ranges with a single model. In this way, we think that results will shed light on the expected positive or negative changes in CBC parameters for future studies on IH.

DOI: 10.33808/clinexphealthsci.1018541

2. MATERIALS AND METHODS

2.1. Experimental Animals

Sprague Dawley type 7 – 10 weeks old male rats constituting the sample of our study constituted the study sample. Their weight was between 321 and 408 grams. Rats were randomly selected into groups. The sample size of our study was calculated using the PS Power and Sample Size version 3.1.2 Software Program (United States). The data of Linnarsson et al. were used for the sample size calculation (10). When the power analysis of the study was determined to be "p" less than 0.05 ($p<0.05$) and 80% reliability, the sample size was determined as 10.

The experimental animals were divided into two groups.

(1) Study group (n = 5): Intermittent Hypoxia group

(2) Control group (n = 5): Normoxic atmosphere group

During the experiment, rats were kept under controlled environmental conditions (12 hr light/dark cycle, temperature 23°C) and standard laboratory food and water were provided ad libitum until the end of the study. Rats were kept in different cages until the end of the study.

2.2. Ethical Approval

The research was conducted in accordance with the Declaration of Helsinki. Ethical approval for animal experiments was obtained from the Local Ethics Committee of Bezmialem Vakif University (Date: 27.09.2019, No: 2019/227).

2.3. Experimental Procedure

To implement the hypothesis, two completely identical cabinets of 20x30x40 cm were used. The oxygen level, pressure, temperature and humidity sensors were placed that inaccessible to the rats, in the study cabin and insulated cables were taken out of the box and computer connection was established. Arduino UNO (Ivrea, Italy) was used as an intermediate module and sensors were operated on this platform. The oxygen level of the cabin was determined with the Winsen electrochemical cell (Zhengzhou Winsen Electronics Technology Co., Ltd. Zhengzhou, China), placed on the Grove brand sensor card (Seeed Technology Co.,Ltd. Shenzhen, China). The highly sensitive sensor was working in the range of 0 to 25% oxygen concentration. The voltage information sent from the sensor was transformed into its real value via the microcontroller. During the experiments, this information was continuously displayed on a screen. At the same time, the information recorded in the serial port was put into the prepared report.

The control of oxygen ratio in the cabin was provided by nitrogen gas released into the cabin. When the oxygen ratio wants to be reduced to 10%, a 1.5 bar pressure was given from the nitrogen tank compressed into the cabin. The Grove sensor was continuously controlled and the gas flow was stopped when it reached its real value. When there was a decrease in the oxygen rate, air flow was provided from the environment through a cover that could be opened from the outside and so the oxygen value was kept at the desired value. All ports, opening to the outside, were covered with silicone for preventing air passage. Thus, the cables were prevented from being damaged by rats, and the rats are prevented from harming themselves through cables and devices. During the normoxia period, the door of the cabinet was opened and the environment in which the rats were located was provided to atmospheric conditions. It was shown that atmospheric conditions were formed in line with the data from the oxygen sensor as shown in Figure 1. Holes were drilled in the cabin of the control group in order not to disturb the atmospheric weather conditions. In this way, the atmospheric conditions were protected in their environment.

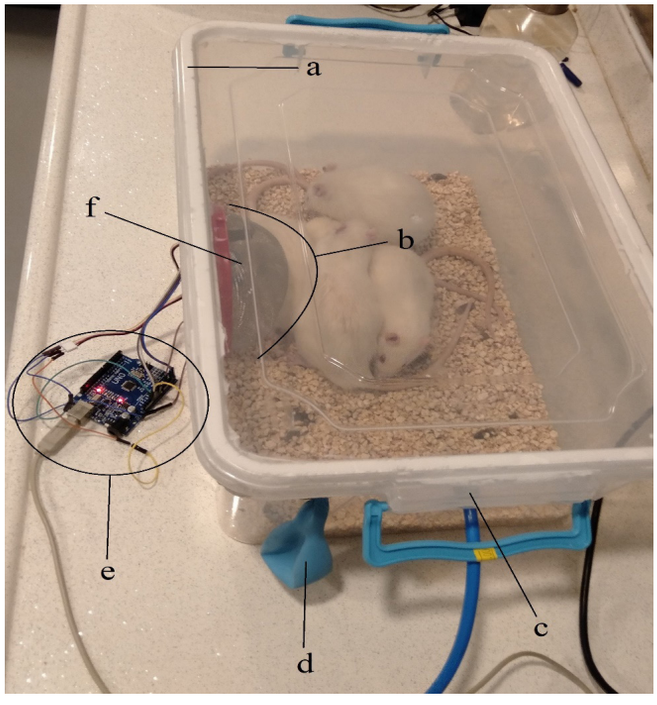

Figure 1. *Cabinet environment of study group rats (a; Insulating material to prevent air passage, b; Protection of sensors, c; Nitrogen gas inlet, d; Pressure balancing balloon, e; Data transfer module, f; Sensors).*

During the experiment, five rats were placed in each cabin. The experiment ended six days and the rats were put in the cabin at the same time each day. Sensors transferred data to the computer every five seconds. The experiment was implemented for 30 minutes, once a day. During the experiment, both rat groups were kept in the box for the same time. The experimental procedure was continued by the same researcher. At the end of the experiment on 6th day, rats were anesthetized with isoflurane in 50% O_2 (balance N_2) for CBC measurement followed by 1 cc blood drawn from the jugular vein. The experimental procedure is shown in Figure 2..

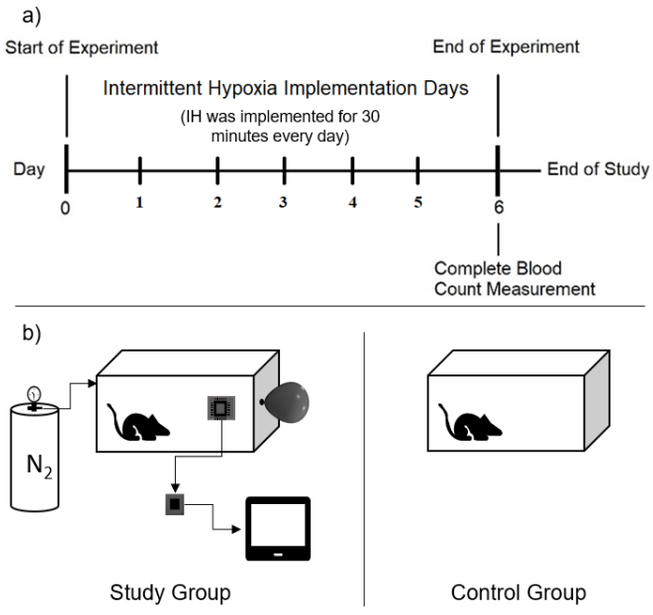

Figure 2. *(a) Flow chart of the experiment; (b) Illustration of the study and control groups.*

2.4. IH Implementation

The study group was exposed to hypoxia every five minutes. Hypoxia was provided by supplying nitrogen gas into the box till oxygen level reaches to 10% (11). The balloon was fixed to the box to balance the pressure of the compressed air and the pressure inside was kept constant. Following each period of hypoxia, the study box was restored to a five-minutes 21% oxygen level, completing the cycle. 15 minutes of hypoxia and 15 minutes of normoxia were implemented to the rats every day during the experiment as shown in Figure 3.

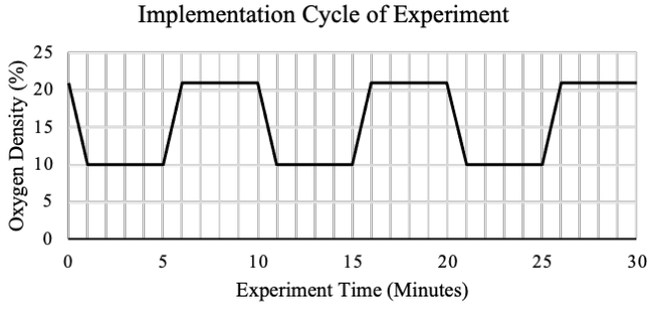

Figure 3. *Graphical form of hypoxia episodes.*

The rats in the control group were provided with a box same to the one in the study group. During the experiment, the rats in the control group were also taken into the experimental boxes. It was aimed not to originate an environment difference between the two groups. While hypoxic time periods were established in the study group box, the control group box was kept in normoxic state for 6 days. The subjects in this group were taken from their cages in the laboratory as in the study group and placed in a box equivalent to that of the study group for 30 minutes. No other procedure was implemented to the rats in this group as shown in Figure 3.

CBC measurements: CBC measurements were made with the same device located in the same laboratory (Abacus Junior Vet 5, Seico Scientific ltd. Punjab, Pakistan). Performed CBC measurements included WBC count, RBC count, lymphocyte (LYM), lymphocyte percentage (LY%), monocytes count (MONO), monocyte percentage (MONO%), granulocytes count (GRA), granulocyte percentage (GR%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDWc), platelets count (PLT), plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc) (1,12,13,14).

2.5. Statistical Analysis

Descriptive statistical analyzes were used to present the obtained data. The Mann Whitney U test was used to evaluate the statistical significance of the CBC measurement values of the study and control groups. Analyzes were performed using Instat Statistical Package Program (GraphPad Prism Version 8 Software Program San Diego, CA) and $p<0.05$ was considered as statistically significant.

3. RESULTS

One cc of blood was drawn from the rats three hours after the end of the experiment and placed in tubes to use in CBC analysis. The results were listed as expressed as shown in Table 1.

Table 1. *Complete blood count analysis results.*

Parameters CBC Measurement	Study Group Mean ± SD	Control Group Mean ± SD	p
WBC (10^9/L)	14.856 ± 0.69	14.302 ± 0.76	>0.05
LYM (10^9/L)	10.438 ± 1.05	10.628 ± 1.02	>0.05
MONO (10^9/L)	0.99 ± 0.8887	0.204 ± 0.06989	<0.001***
GRA (10^9/L)	3.574 ± 0.832	3.474 ± 0.36	=0.13
LY%	69.38 ± 7.516	74.18 ± 3.473	=0.16
MONO%	6.66 ± 5.953	1.4 ± 0.4637	<0.001***
GR%	23.96 ± 4.862	24.4 ± 3.564	>0.05
RBC (10^9/L)	8.356 ± 0.318	8.592 ± 0.124	=0.09
HGB (g/dL)	15.38 ± 0.497	15.36 ± 0.2074	=0.12
HCT (%)	46.15 ± 1.738	46.76 ± 0.7592	=0.14
MCV (fL)	55.2 ± 0.836	54.4 ± 0.894	=0.18
MCH (pg)	18.4 ± 0.3317	17.88 ± 0.2864	=0.03*
MCHC (g/dL)	33.34 ± 0.61	32.84 ± 0.492	=0.19
RDWc (%)	15.98 ± 0.216	15.82 ± 0.37	>0.05
PLT (10^9/L)	908.4 ± 106.2	872.6 ± 23.64	=0.013*
PCT (%)	0.586 ± 0.0789	0.584 ± 0.01517	=0.007**
MPV (fL)	6,46 ± 0,114	6,66 ± 0,114	=0.02*
PDWc (fL)	33.1 ± 1.072	33.32 ± 0.96	>0.05

(, p<0.05; **, p<0.01; ***, p<0.001, CBC: complete blood count, WBC: white blood cells, LYM: lymphocyte, MONO: monocyte count, GRA: granulocytes count, LY%: lymphocyte percentage, MONO%: percentage of monocytes, GR%: granulocyte percentage, RBC: red blood cells, HGB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDWc: red cell distribution width, PLT: platelets count, PCT: plateletcrit, MPV: mean platelet volume, PDWc: platelet distribution width)*

3.1. MONO and MONO% Values

In the CBC analyzes of the rats, it was observed that the MONO ($p<0.001$) and MONO% ($p<0.001$) values increased significantly in the study group compared to the control group as shown in Figure 4a, 4b.

The mean MONO value was determined as 0.99x10^9/L (±0.88887) in the study group, and 0.204 x10^9/L (±0.06989) in the control group, respectively. In addition, the minimum MONO value was measured as 0.09 x10^9/L and the maximum MONO value was measured as 1.95 x10^9/L. In the control group, the minimum MONO value was measured as 0.0 9 x10^9/L and the maximum MONO value was measured as 0.28 x10^9/L. For the MONO% parameter, the mean value detected in the study group was 6.66% (±5.953), and the determined value in the control group was 1.4% (±0.4637). The MONO% value measured in the study group was determined as minimum 0.6% and maximum 13.3%. In the control group, the measured minimum MONO% value was 0.6% and the maximum MONO% value was 1.8%.

3.2. MCH Values

According to the analysis, the MCH value in the study group increased significantly compared to the control group ($p=0.03$). In the study group, the mean MCH value was determined as 18.4 pg (±0.3317), the minimum was 18.1 pg, and the maximum was 18.8 pg as shown in Figure 4c. In the control group, the mean MCH value was determined as 17.88 pg (±0.2864), the minimum was 17.5 pg, and the maximum was 18.3 pg as shown in Figure 4c.

3.3. PCT Values

It was found that PCT values increased significantly in the study group compared to the control group ($p=0.007$). The mean PCT values were determined as 0.586% (±0.07893) in the study group and determined as 0.584% (±0.01517) in the control group. On the other hand, the minimum PCT value was measured as 0.49% and the maximum PCT value was measured as 0.7% in the study group, while the minimum PCT value was measured as 0.57% and the maximum PCT value was measured as 0.61% in the control group.

3.4. MPV Values

There was a significant decrease in MPV values in the study group ($p=0.02$). The mean MPV values was determined as 6.46 fL (±0.114) and 6.66 fL (±0.114) in the study and control groups, respectively. The minimum MPV value was measured as 6.3 fL and the maximum MPV value was 6.6 fL in the study group. In the control group, the minimum MPV value was detected as 6.5 fL and the maximum MPV value was 6.8 fL as shown in Figure 4d.

3.5. PLT Values

PLT value in the study group increased significantly compared to the control group ($p=0.013$). While the mean PLT value in the study group was determined as 908.4 x10^9/L (±106.2), it was determined as 872.6 x10^9/L (±23.64) in the control group. In the study group, the minimum PLT value was detected as 786 x10^9/L and the maximum PLT value was 1066 x10^9/L. In the control group, the minimum PLT value was measured as 844 x10^9/L and the maximum PLT value was determined as 901 x10^9/L as shown in Figure 4e.

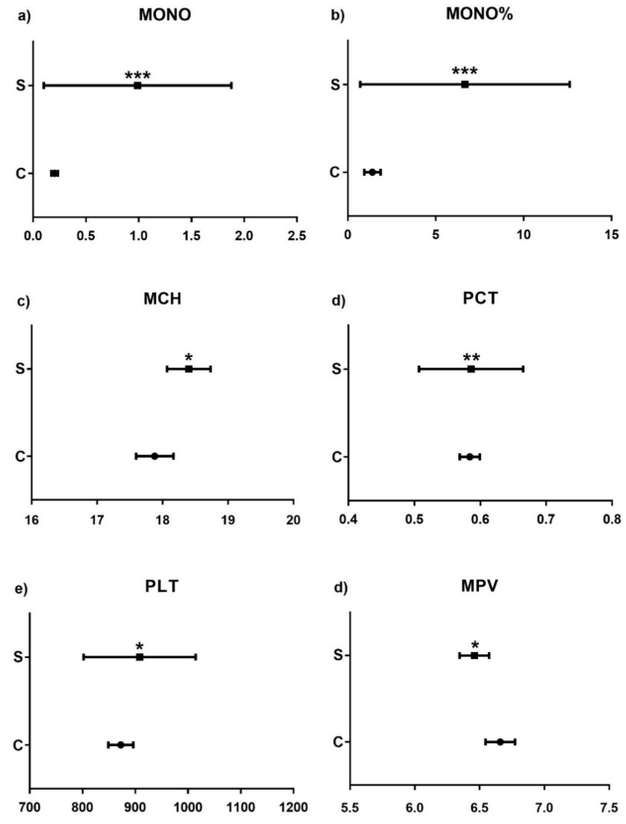

Figure 4. *(a) Monocyte count (MONO) ($p<0.001$); (b) Percentage of monocytes (MONO%) ($p<0.001$); (c) Mean corpuscular hemoglobin (MCH) ($p=0.03$); (d) Plateletcrit (PCT) ($p=0.007$); (e) Platelet (PLT) count ($p=0.013$); (f) Mean platelet volume (MPV) ($p=0.02$); S, study group; C, control group.*

3.6. HGB and HCT Values

There was no significant difference in HGB and HCT values between the control and study groups. The mean HGB values were found as 15.38 g/dL (±0.497) and 15.36 g/dL (±0.2074) in study and control groups, respectively. In addition, the mean HCT values were determined as 46.15% (±1.738) and 46.76% (±0.7592) in study and control groups, respectively.

No significant difference was found between the two groups' WBC, LYM, GRA, LY%, GR%, RBC, MCV, MCHC, RDWc and PDWc parameters as shown in Table 1.

4. DISCUSSION

The purpose of this study is to observe results that IH implementation causes in CBC analysis. Evaluations were made after 10% FiO_2, 5-minutes normoxia-hypoxia cycles,

daily 30-minutes implementations for 6 days. As a result of CBC analysis, changes were found in MONO, MONO%, MCH, PCT, PLT and MPV parameters. While there was a significant increase in the values of MONO, MONO%, MCH, PCT and PLT parameters in the study group, the opposite was found in the MPV value.

In the study, the IH protocol was implemented using a special cabinet. There is no consensus in the literature regarding the IH implementation method in rats. In some studies, this point is not clearly stated in the methodology; In some publications, the use of masks is stated. Since we wanted to eliminate the risk of leakage in the use of masks in the rat, IH was implemented using a special cabin in this study. The cabin used in the research was specially designed by the research team during the preparation phase. With special sensors used in the cabin design, the oxygen density in the cabin was checked and verified before each implementation.

IH implementation does not have a standard protocol. According to literature, FiO_2, normoxia-hypoxia cycles, daily implementation times and total implementation time differ. Considering these differences, it has been observed that beneficial effects can be obtained from IH without pathological effects when moderate hypoxia (9-16% inspired FiO_2) and low cycle number (3-15 episodes per day) are used. On the other hand, severe hypoxia (2-8% inspired FiO_2) and high cycle number (48-2,400 episodes per day) were found causing pathological effects (3). In addition to achieve standardization, it is necessary to reduce the PaO_2 value in the blood from 90 (± 5,5) mmHg to 35 – 60 mmHg (2).

In line with this information, we evaluated the responses of IH implemented in CBC analyzes without causing pathological effects in the study.

Alvarez-Martins et al. reported that chronic IH implementation caused an increase in MONO numbers in rats with obstructive sleep apnea (9). However, chronic IH implementations consist of severe hypoxia (5% inspired FiO_2) and a prolonged implementation period (10.5 h / day). In addition to this study, an increase in monocyte was found in both acute and chronic IH implementations. The increase in MONO, which occurs without any infection, may correlate improvement of the lungs. The reason for this situation is thought to be the physiological response of the body, it is thought to be an infectious response due to hypoxia inducible factor (HIF) synthesis due to IH administration (15,16). The reason of the increase in MONO% ($p<0.001$) parameter was due to the increase in MONO ($p<0.001$).

MCH refers to the amount of hemoglobin per erythrocyte, and low-level MCH has been found to be strongly associated with intensive care admission due to myocardial infarction (17). In a study, low level MCH was found to be highly correlated with mortality rate, and a significant relationship was pointed out between very high level of MCH and mortality (18). In our study, in terms of the CBC analysis, there was no significant difference in hemoglobin and hematocrit levels between the two groups, but it was found that MCH increased significantly in the study group ($p=0.03$). In other words, IH implemented for 30 minutes for 6 days increased the amount of hemoglobin per erythrocyte. The increase of the MCH levels, can be interpreted as increasing the hemoglobin number in the erythrocyte for to meet the oxygen demand of the tissue against the decrease in PaO_2 levels, and supporting the efficient transport and use of oxygen.

McDonald et al. showed that increased PLT levels were demonstrated and related to the hypoxia (19). Berg et al. reported that platelets play an important role in the repair of the lung parenchyma (20). In their studies, it was observed that with the increase of alveolar hypoxia, platelet-derived growth factor-B production increased to protect the lung tissue. Other studies, high levels of PCT were reported in pediatric cases with chronic lung disease, and the PCT value has been reported to show a positive correlation with C – reactive protein (CRP). In addition, in the pediatric mortality studies in the hospital, they were reported that the survival rate was higher in cases with high PCT values (21,22,23). According to our study, increased PCT ($p=0.007$) values can be interpreted as a reaction developed against hypoxia. Recently literature has reported that individuals' who was affected from COVID-19, the MCH levels have diminished (24). Additionally, it was found that the decrease continued until the end of the treatment and continued after the therapy. In our study, we found that the MCH levels increased significantly after IH. We think that alternative IH implementations may provide positive respiratory effects in patients with critically pulmonary diseases. Comprehensive studies associated with IH implementation are needed on this area.

MPV, which indicates the mean size of the platelets in the bloodstream, is one of the indicators of platelet activity (25). There are many studies investigating the change of MPV levels in non-communicable diseases. Slavka et al. defended the relationship between MPV and thromboembolic complications and cardiovascular events (26). Kodiatte et al. have suggested a high-level MPV relationship with diseases such as diabetes mellitus and hypertension, while Przygodzki et al. defended the opposite view (27,28). Kanbay et al. reported that the high MPV values in obstructive sleep apnea associated with the severe hypoxemic periods experienced by these cases (29). In the current study, a decrease was observed in MPV value in rats treated with IH as shown in Figure 4f. This result suggests that MPV may change according to period, duration and severity of hypoxic episodes. There are require to further research on this subject.

Brito et al. were found that there was an increase in HGB and HCT values in adults who were exposed to chronic IH (30). According to the study conducted by Zhang et al. it was observed that there was a significant increase in HGB, HCT and RBC levels in IH implemented rats compared to the control group (31). The experiment period in this study lasted 28 days (31). In our study, there was an insignificant decrease in HCT ($p=0.14$) and RBC ($p=0.09$) parameters compared to the control group. On the other hand, there was

an insignificant increase in the HGB parameter (p=0.12). No significant difference was found in other values measured in CBC analysis. Our experiment completed in 6 days. However, if the duration of IH implementation is extended, we think that HGB, HCT and RCB values may change.

This study contains some limitations. Since saturation monitoring can be performed under anesthesia in rats, saturation measurements could not be performed in our study due to the direct effect of anesthesia. The lack of measurement of blood gas parameters was another limitation. The distance between the evaluation and test laboratories on our campus has prevented the preservation of the samples taken. An additional device could not be purchased due to high costs. On the other hand, since the rats should not enter into hypovolemia, the location of the rats was not changed and this measurement could not be made.

5. CONCLUSION

It has been observed that the parameters of the CBC were evaluated a very limited level in the studies on IH. The target of this study is based on what kind of changes will be discussed in the CBC analysis with IH implementation at therapeutic levels. In this study, changes in CBC parameters were detected with IH, which was applied systematically. Each rat in the study group was exposed to a uniform IH pattern. As a result of IH implementation, significant changes were observed in MONO, MONO%, MCH, PCT, PLT and MPV parameters in the CBC analysis of rats. We think that the effects of therapeutic IH implementation on MONO and MONO% levels may be effective in lung regeneration and lung protection. In addition, our results support that the PLT activity is inhibited by the decrease in the MPV level despite the increase in the PLT level. In this way, we can say that the risk of thrombosis is reduced. The increase in PCT expected due to the decrease in MPV appeared as a normal result. However, the expected reduction in PDWc in correlation with the decrease in MPV was not statistically significant.

The intermittent hypoxia method used in this study, which was defined as “Therapeutic dose” and consisted of moderate hypoxia (9-16% FiO2) – low cycles (3-15 episodes per day), did not cause a pathological effect and gave positive results on CBC. The changes revealed by IH implementation on CBC parameters suggested that this dose protocol used could provide minimum negative effect and maximum gain. This supports the applicability of the IH model in our study in clinical trials under controlled conditions. In future studies, the effects of therapeutic IH implementation on lung tissue healing, increasing blood oxygen transport efficiency and immune system can be investigated in appropriate patient population and clinical conditions.

Considering all these conditions, therapeutic IH implementation may thought to have important effects in terms of lung protection and regeneration. Further research may focus on this point for precising and supporting of this study’ results.

Authors Contributions

Conception and design of the experiments: T.K., M.S. and A.Y.O. Collection, analysis and interpretation of data for the experiments: T.K., M.S., S.U., A.K., H.D. and A.Y.O. Drafting and revising the article critically for important intellectual content: T.K. and A.Y.O. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Competing Interests

None

Funding

There is no funding support for this study.

REFERENCES

[1] Guang Y, Jie Z, Feng D, Hui L. Surrogate scale for evaluating respiratory function based on complete blood count parameters. J Clin Lab Anal 2018;32(5):e22385.

[2] Xing T, Pilowsky PM. Acute intermittent hypoxia in rat in vivo elicits a robust increase in tonic sympathetic nerve activity that is independent of respiratory drive. J Physiol 2010;588(Pt 16):3075-3088.

[3] Navarrete-Opazo A, Mitchell GS. Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol 2014;307(10):R1181-1197.

[4] Baker TL, Mitchell GS. Episodic but not continuous hypoxia elicits long-term facilitation of phrenic motor output in rats. J Physiol 2000;529 Pt 1(Pt 1):215-219.

[5] Ryan S, Nolan P. Episodic hypoxia induces long-term facilitation of upper airway muscle activity in spontaneously breathing anaesthetized rats. J Physiol 2009;587(Pt 13):3329-3342.

[6] Nakamura A, Olson EB Jr, Terada J, Wenninger JM, Bisgard GE, Mitchell GS. Sleep state dependence of ventilatory long-term facilitation following acute intermittent hypoxia in Lewis rats. J Appl Physiol 2010;109(2):323-331.

[7] Lovett-Barr MR, Satriotomo I, Muir GD, Wilkerson JE, Hoffman MS, Vinit S, Mitchell GS. Repetitive intermittent hypoxia induces respiratory and somatic motor recovery after chronic cervical spinal injury. J Neurosci 2012;32(11):3591-3600.

[8] Nagel MJ, Jarrard CP, Lalande S. Effect of a single session of intermittent hypoxia on erythropoietin and oxygen-carrying capacity. Int J Environ Res Public Health 2020;17(19):7257.

[9] Alvarez-Martins I, Remédio L, Matias I, Diogo LN, Monteiro EC, Dias S. The impact of chronic intermittent hypoxia on hematopoiesis and the bone marrow microenvironment. Pflugers Arch 2016;468(5):919-932.

[10] Linnarsson S, Björklund A, Ernfors P. Learning deficit in BDNF mutant mice. Eur J Neurosci 1997;9(12):2581-2587.

[11] Wen MH, Wu MJ, Vinit S, Lee KZ. Modulation of serotonin and adenosine 2A receptors on intermittent hypoxia-induced respiratory recovery following mid-cervical contusion in the rat. J Neurotrauma 2019;36(21):2991-3004.

[12] Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H, Hohlfeld R. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med 1999;189(5):865-870.

[13] Lommatzsch M, Niewerth A, Klotz J, Schulte-Herbrüggen O, Zingler C, Schuff-Werner P, Virchow JC. Platelet and plasma BDNF in lower respiratory tract infections of the adult. Respir Med 2007;101(7):1493-1499.

[14] Yamamoto H, Gurney ME. Human platelets contain brain-derived neurotrophic factor. J Neurosci 1990;10(11):3469-3478.

[15] Scott MKD, Quinn K, Li Q, Carroll R, Warsinske H, Vallania F, Chen S, Carns MA, Aren K, Sun J, Koloms K, Lee J, Baral J, Kropski J, Zhao H, Herzog E, Martinez FJ, Moore BB, Hinchcliff M, Denny J, Kaminski N, Herazo-Maya JD, Shah NH, Khatri P. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. Lancet Respir Med 2019;7(6):497-508.

[16] Colgan SP, Furuta GT, Taylor CT. Hypoxia and innate immunity: keeping up with the HIFsters. Annu Rev Immunol 2020;38:341-363.

[17] Huang YL, Hu ZD. Lower mean corpuscular hemoglobin concentration is associated with poorer outcomes in intensive care unit admitted patients with acute myocardial infarction. Ann Transl Med 2016;4(10):190.

[18] Honda H, Kimachi M, Kurita N, Joki N, Nangaku M. Low rather than high mean corpuscular volume is associated with mortality in Japanese patients under hemodialysis. Sci Rep 2020;10(1):15663.

[19] McDonald TP. Platelet production in hypoxic and RBC-transfused mice. Scand J Haematol 1978;20(3):213-220.

[20] Berg JT, Breen EC, Fu Z, Mathieu-Costello O, West JB. Alveolar hypoxia increases gene expression of extracellular matrix proteins and platelet-derived growth factor-B in lung parenchyma. Am J Respir Crit Care Med 1998;158(6):1920-1928.

[21] Draz IH, Shaheen IA, Youssef EA. Platelets count and platelets indices; mean platelet volume and plateletcrit in pediatric chronic lung disease. Egypt Pediatric Association Gaz 2020;68:69.

[22] Sayed SZ, Mahmoud MM, Moness HM, Mousa SO. Admission platelet count and indices as predictors of outcome in children with severe sepsis: a prospective hospital-based study. BMC Pediatr 2020;20(1):387.

[23] Golwala ZM, Shah H, Gupta N, Sreenivas V, Puliyel JM. Mean platelet volume (MPV), platelet distribution width (PDW), platelet count and plateletcrit (PCT) as predictors of in-hospital paediatric mortality: a case-control study. Afr Health Sci 2016;16(2):356-362.

[24] Zhao G, Su Y, Sun X, Cui X, Dang L, Zhao L, Tan X, Wang H, Yang M. A comparative study of the laboratory features of COVID-19 and other viral pneumonias in the recovery stage. J Clin Lab Anal 2020;34(10):e23483.

[25] Tsiara S, Elisaf M, Jagroop IA, Mikhailidis DP. Platelets as predictors of vascular risk: is there a practical index of platelet activity? Clin Appl Thromb Hemost 2003;9(3):177-190.

[26] Slavka G, Perkmann T, Haslacher H, Greisenegger S, Marsik C, Wagner OF, Endler G. Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease. Arterioscler Thromb Vasc Biol 2011;31(5):1215-1218.

[27] Kodiatte TA, Manikyam UK, Rao SB, Jagadish TM, Reddy M, Lingaiah HK, Lakshmaiah V. Mean platelet volume in type 2 diabetes mellitus. J Lab Physicians 2012;4(1):5-9.

[28] Przygodzki T, Luzak B, Kassassir H, Mnich E, Boncler M, Siewiera K, Kosmalski M, Szymanski J, Watala C. Diabetes and hyperglycemia affect platelet GPIIIa expression. Effects on adhesion potential of blood platelets from diabetic patients under in vitro flow conditions. Int J Mol Sci 2020;21(9):3222.

[29] Kanbay A, Tutar N, Kaya E, Buyukoglan H, Ozdogan N, Oymak FS, Gulmez I, Demir R. Mean platelet volume in patients with obstructive sleep apnea syndrome and its relationship with cardiovascular diseases. Blood Coagul Fibrinolysis 2013;24(5):532-536.

[30] Brito J, Siqués P, León-Velarde F, De La Cruz JJ, López V, Herruzo R. Chronic intermittent hypoxia at high altitude exposure for over 12 years: assessment of hematological, cardiovascular, and renal effects. High altitude medicine & biology 2007;8(3):236–244.

[31] Zhang CY, Zhang JX, Lü XT, Li BY. Effects of intermittent hypoxic exposure on the parameter of erythrocyte and serum hypoxia inducible factor-1 alpha and erythropoietin levels. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2009;25(10):932-934.

How to cite this article: Kilic T, Sengor M, Ustunova S, Kilic A, Daskaya H, Ozer AY. The Effects of Therapeutic Intermittent Hypoxia Implementation on Complete Blood Count Parameters: An Experimental Animal Model. Clin Exp Health Sci 2022; 12: 746-752. DOI: 10.33808/clinexphealthsci.1018541

Clinical and Experimental Health Sciences

Investigation of Neurogenesis in Kindled Wistar and Genetic Absence Epilepsy Rats

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Received: 09.11.2021 **Accepted:** 30.11.2021

ABSTRACT

Objective: The most common type of epilepsy affecting about 50 million people worldwide is temporal lobe epilepsy (TLE). Chemical and electrical kindling methods in animals can be used to form TLE model. In the present study, it was aimed to investigate neurogenesis in the hippocampus of adult kindled Wistar rats and genetic absence epilepsy rats from Strasbourg (GAERS) rats by immunofluorescence methods.

Methods: Adult Wistar and GAERS albino rats weighing 250-300 gr were injected pentylenetetrazole (PTZ) (35 mg/kg, s.c.) every other day to produce chemical kindling. Animals having 5 times grade 5 seizures were considered to be kindled. Intracardiac perfusion was performed under deep anesthesia on the 7th and 14th days after the last grade 5 seizure. Immunofluorescence methods were used to demonstrate newly formed neurons, astroglial cells, and mature neurons, by using anti-doublecortin (DCX), anti-glial fibrillary acidic protein (GFAP), and anti-neuronal nuclear antigen (NeuN) primary antibodies, respectively. Sections were then examined under a fluorescence microscope.

Results: DCX (+) cells were found to be increased in GAERS control groups compared to the Wistar control groups; and in Wistar PTZ groups compared to the Wistar control groups. DCX (+) cells were decreased in GAERS PTZ groups compared to their controls and to Wistar PTZ groups.

Conclusion: The findings of the present study suggest that the resistance to electrical kindling of GAERS reported in previous studies might be related to the increased neurogenesis in this strain.

Keywords: Doublecortin, hippocampus, kindling, neurogenesis

1. INTRODUCTION

Genetic absence epilepsy rats from Strasbourg (GAERS) is a well known animal model of absence epilepsy. In the beginnig of 1980's, researchers found out that 30% of control Wistar rats exhibited spike and wave discharges on EEG. They inbred these animals and named as GAERS (1).

Epilepsy affects 50 million people worldwide and the most common form of this disease is temporal lobe epilepsy (TLE) (2). Chemical and electrical kindling in experimental animals allow to mimic this disease (3). Pentylenetetrazole (PTZ) is one of the agents to generate TLE model.

Idiopathic generalized absence epilepsy and TLE in the same patient are rarely seen and the reason is not well understood (4, 5). GAERS strain was shown to have only stage 2 seizure, although Wistar controls had stage 5 seizure (5). The underlying mechanisms of absence epilepsy were suggested to be responsible for the resistance to secondary generalization of limbic seizures.

Neurogenesis occurs in two regions in the adult brain: Subventricular zone and the subgranular zone of hippocampal dentate gyrus. (6). Studies have shown that migrating neurons differentiate into mature, functional neurons and astrocytes (7, 8, 9). Recent studies have reported that abnormal hippocampal neurogenesis had a role in the pathophysiology of TLE (10, 11, 12).

Epileptic seizures affect neurogenesis. Acute seizures or limbic epileptogenesis stimulates neurogenesis (9, 13).

DOI: 10.33808/clinexphealthsci.1021171

Seizures cause some neurogenic cells to migrate (9). These neurons trigger epileptogenic hippocampal circuit (10).

Doublecortin (DCX) is a microtubule-associated protein and widely expressed during embryonic and early postnatal development in the developing neurons (14). It is widely investigated in neurogenesis studies.

In the present study, neurogenesis in the kindled Wistar and GAERS rats was investigated by using immunofluorescence methods.

2. METHODS

2.1. Animals

Adult male Wiatar albino and GAERS rats weighing 250-300 g were used in the present study. Animals were obtained from Marmara University, The Experimental Animal Implementation and Research Center. All experiments were done according to the national guidelines on animal experimentation and were approved by the Marmara University Local Ethical Committee for Experimental Animals (70.2017.mar, 09.10.2017). The animals were housed with free access to water and food in a 12-h light/dark cycle and humidity controlled room (21±2°C and 65-70% humidity).

2.2. Experimental Groups

Groups were as follows:

1. Sham-operated Wistar group 7 days (n=6)
2. Sham-operated Wistar group 14 days (n=6)
3. PTZ Wistar group 7 days (n=6)
4. PTZ Wistar group 14 days (n=6)
5. Sham-operated GAERS group 7 days (n=3)
6. Sham-operated GAERS group 14 days (n=3)
7. PTZ GAERS group 7 days (n=4)
8. PTZ GAERS group 14 days (n=3)

2.3. Chemical Kindling

Animals were injected 35 mg/kg PTZ (s.c.) every other day to induce chemical kindling. After the injection, animals were observed for 30 min and seizure development stages were recorded according to Racine's scale (15, 16). Animals having 5 times stage 5 seizures were accepted as kindled. Sham-operated groups were injected saline and observed for 30 min.

2.4. Perfusion Fixation

Animals in the PTZ group were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine hydrochloride) and perfused 7 and 14 days after the last stage 5 seizure. Then the animals were perfused with a fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3. After decapitation, brain tissues were obtained and incubated in the same fixative solution overnight at 4°C. Sham-operated animals were also perfused with the same method on the same days as PTZ group.

2.5. Immunofluorescence Method

After fixation, tissues were cryoprotected by incubating in sucrose solution (0.1 M phosphate buffer, pH 7.3). Then the tissues were frozen at –80ºC and 5-micron-thick sections were obtained by a cryotome. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide. Protein block was applied to prevent nonspecific staining (Skytek Superblock Solution). Sections were then incubated in anti-DCX antibody (1:4000, ab18723) to show newly formed progenitor neurons, for 1 h at 37ºC. Sections were then incubated either in anti-glial fibrillary acidic protein (anti-GFAP, 1:500, MAB3402) to show astrocytes, or in anti-neuronal nuclear antigen (anti-NeuN, 1:5000, MAB377) antibody to show mature neurons, for 1 h at 37ºC. Dylight 550 conjugated goat anti-rabbit secondary antibody (1:600, Thermo Pierce 84541) was used for anti-DCX antibody 1 h at room temperature at dark. After washing in phosphate buffer, Dylight 488 conjugated goat anti-mouse secondary antibody (1:400, Thermo Pierce 35502) was applied for anti-GFAP or anti-NeuN antibodies for 1 h at room temperature at dark. Sections were then mounted on glass slides with a mounting medium containing diamidino-2-phenylindole dihydrochloride (DAPI). Hippocampal regions were examined under a DP72 Olympus CCD camera attached BX51 photomicroscope (Japan).

3. RESULTS

3.1. Wistar Sham-Operated Groups

DCX (+) cells were seen after applying DCX-GFAP and DCX-NeuN immunofluorescence methods. DCX (+) cells were observed in subgranular zone (Figure 1a, b). NeuN (+) cells were observed in hilus and granular layer in 7 days (Figure 2a) and 14 days (Figure 2b) groups.

3.2. Wistar PTZ Groups

DCX (+) cells were observed in Wistar PTZ 7 days (Figure 1c) and 14 days (Figure 1d) groups in subgranular zone. These cells were increased both in Wistar PTZ 7 days and 14 days groups compared to the sham-operated control groups. DCX and NeuN (+) cells were observed in subgranular zone in 7 days (Figure 2c) and 14 days (Figure 2d) groups.

3.3. GAERS sham-operated groups

DCX (+) cells were observed in 7 days (Figure 1e) and 14 days (Figure 1 h) groups in subgranular zone. These cells

were observed to be increased compared to Wistar sham-operated groups. DCX and GFAP (+) cells were observed in dentate gyrus both in 7 days and 14 days groups. DCX-NeuN (+) cells were observed in subgranular zone in 7 days group (Figure 2e). Some cells in hilus showed both NeuN and DCX positivity in 14 days groups (Figure 2f).

3.4. GAERS PTZ Groups

DCX (+) cells were observed in subgranular zone in 7 days and 14 days groups and some DCX (+) cells were observed to form clusters (Figure 1g). DCX (+) cells in 14 days group (Figure 1h) were less compared to Wistar PTZ 14 days group (Figure 1d). DCX and NeuN (+) cells were observed in hilus in 7 days group (Figure 2g) and in subgranular zone in 14 days group (Figure 2h).

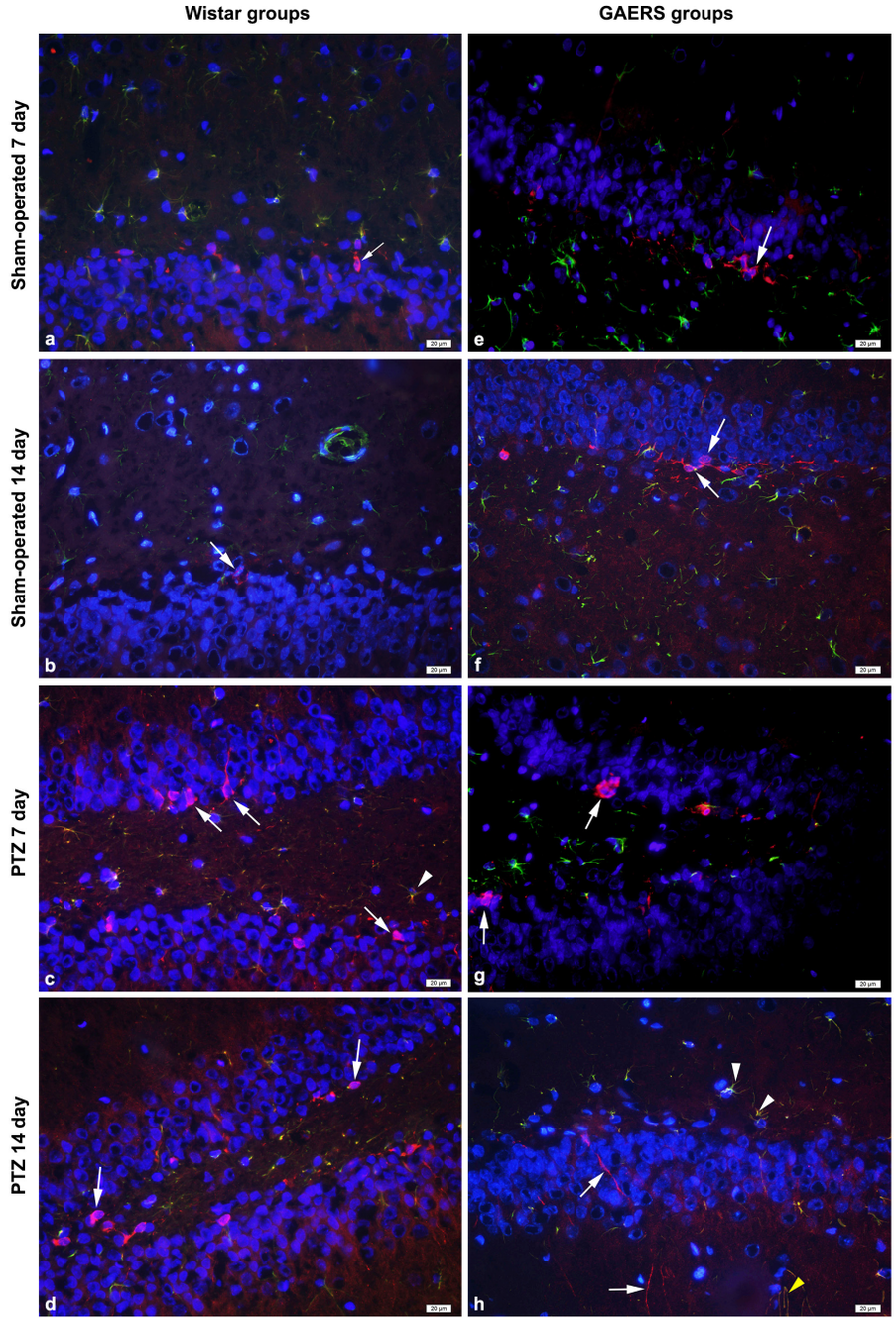

Figure 1. *DCX-GFAP-DAPI triple immunofluorescence staining. Green: GFAP, red: DCX, blue: DAPI. (a) Wistar sham-operated 7 days group. Arrow: DCX (+) cell. (b) Wistar sham-operated 14 days group. Arrow: DCX (+) cell. (c) Wistar PTZ 7 days group. Arrows: DCX (+) cells. Arrowhead: DCX-GFAP (+) cell. (d) Wistar PTZ 14 days group. Arrow: DCX (+) cells in the subgranular layer. (e) GAERS sham-operated 7 days group. Arrow: DCX (+) cell in the subgranular layer. (f) GAERS sham-operated 14 days group. Arrows: DCX (+) cells. (g) GAERS PTZ 7 days group. Arrows: DCX (+) cells. (h) GAERS PTZ 14 days group. Arrows: DCX (+) cell projections. White arrowhead: GFAP (+) cells. Yellow arrowhead: DCX-GFAP double (+) cell projections.*

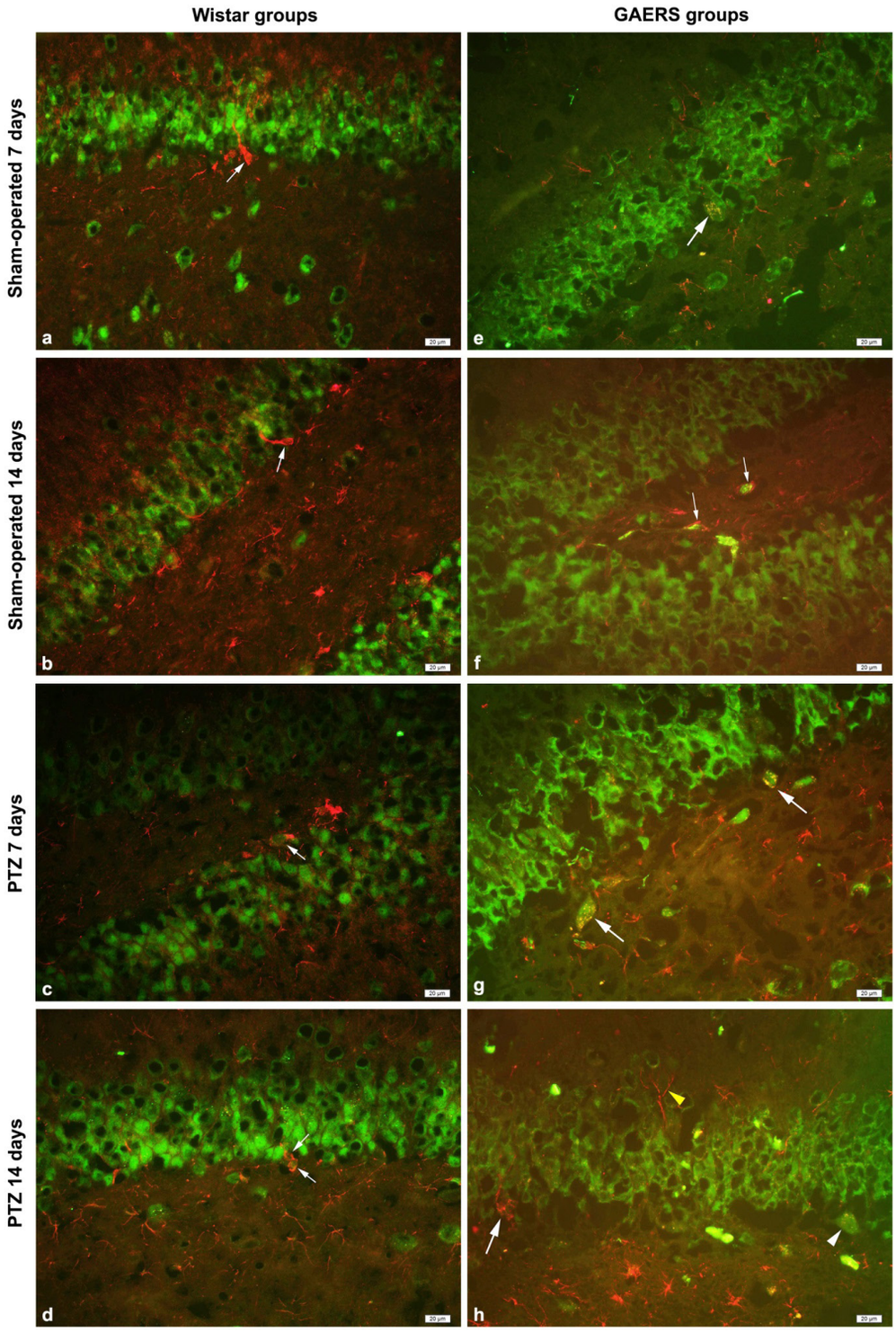

Figure 2. *DCX-NeuN double immunofluorescence staining. Green: NeuN, red: DCX. (a) Wistar sham-operated 7 days group. Arrow: DCX (+) cell. (b) Wistar sham-operated 14 days group. Arrow: DCX (+) cell. (c) Wistar PTZ 7 days group. Arrow: DCX-NeuN (+) cell. (d) Wistar PTZ 14 days group. Arrows: DCX-NeuN (+) cells. (e) GAERS sham-operated 7 days group. Arrow: DCX-NeuN (+) cell. (f) GAERS sham-operated 14 days group. Arrows: DCX-NeuN (+) cells. (g) GAERS PTZ 7 days group. Arrows: DCX-NeuN (+) cells. (h) GAERS PTZ 14 days group. Arrow: DCX (+) cell. Yellow arrowhead: DCX (+) cell projection. White arrowhead: DCX-NeuN (+) cell.*

4. DISCUSSION

In the present study, control Wistar albino and GAERS rats were injected 35 mg/kg (s.c.) PTZ and chemical kindling model was generated. Neurogenesis was found to be increased in Wistar PTZ groups compared to Wistar sham-operated groups and

in GAERS control groups compared to Wistar control groups. It was decreased in GAERS PTZ groups compared to their controls and to Wistar PTZ groups.

Neurogenesis is observed in subgranular zone of the hippocampal dentate gyrus and in subventricular zone in the adult brain (6). In our study, in parallel with the previous studies, DCX (+) cells were observed in the dentate gyrus in all groups and these were suggested to be the newly born neural progenitor cells.

GAERS strain rats were shown to resist electrical kindling previously (5). However, in the present study, we observed that GAERS rats were not resistant but more susceptible to PTZ kindling compared to Wistar rats. PTZ is a GABA-A receptor agonist and is used to generate epileptic seizures in experimental animals (17, 18). In GAERS rats, extracellular GABA was shown to be increased by microdialysis method in the thalamus (19, 20). Because there is an increased GABAergic mechanisms in this strain, GABAergic system may have been supressed and seizure susceptibility might have been increased. In a previous study, GAERS were found to be susceptible to GABA agonists, and that they needed less dose compared the controls (21). Our findings were in line with this study and GAERS rats needed less number of PTZ injections and had grade 5 seizure. Similarly, in GAERS, GABA withdrawal syndrome was generated and it was reported that convulsive seizures induced by PTZ injections were more in GAERS (22). Researchers related this cortical vulnerability induced by GABA withdrawal and PTZ to spike and wave discharges.

TLE models induced by kainic acid and pilocarpine showed cell proliferation in hippocampal dentate gyrus (8, 23). However, neurogenesis is decreased in chronic TLE (24). In animal models of absence epilepsy, including gamma-hydroxybutyrate, AY-9944 and WAG/Rij, different results were obtained (25, 26). The reason might be the strain differences. Scott et al. observed no difference between absence epilepsy group and control group in terms of neurogenesis; however, they observed increased neurogenesis in kindling group, which is a TLE model (25). In that study, it was concluded that spike and wave discharge frequency in absence epilepsy might not be sufficient for stimulating increased neurogenesis. Cell increase in kindling model in the same study was related to cell death. However, cell death was not observed in absence epilepsy. In our study, we used GAERS rats as an absence epilepsy model and we observed more DCX (+) cells compared to Wistar controls and concluded that neurogenesis was increased in this model. The reason for the different findings in the present study compared to that of Scott et al. might be that different absence epilepsy models and histological methods were used in these two different studies. Scott et al. used BrdU to show neurogenesis and TUC-4 as a marker for neurogenesis (25).

In the present study, it was observed that Wistar PTZ groups had more DCX (+) cells compared to their control groups. However, in PTZ GAERS groups, when compared to their sham-operated control groups and to PTZ Wistar groups, less DCX (+) cells were observed. The reason for this might be that GAERS reached stage 5 seizure in less time compared to Wistar rats and there may not be sufficient time for neurogenesis. Our findings suggest that seizure type may also be a factor affecting neurogenesis. Our study, showing the relation between GAERS and neurogenesis, fills a gap in the literature. To our knowledge, there is no study showing neurogenesis in GAERS.

In a previous study, it was shown that mature astrocytes also express DCX in human neocortex in some disease conditions (27). This dual presence of GFAP-DCX in astrocytes was suggested to play a role in glia-neuron communication. In the same study, it was also reported that DCX was expressed as punctate-like structures in the progenitor cells. In line with these previous studies, we observed DCX and GFAP (+) astrocyte like cells in all groups. The function of GFAP in multipotent neural progenitor cells in the subgranular zone is not known. In our study, we observed DCX and GFAP (+) cells in the Wistar PTZ 14 days group and that these cells showed bipolar morphology. Similarly, Garcia et al. reported that adult multipotent neural progenitor cells expressing GFAP were the major source of *in vivo* adult neurogenesis and they showed bipolar or unipolar morphology, and had less projections compared to non-neurogenic multipolar astrocytes (28). These findings suggest that GFAP expressing neural progenitor cells are different from astrocytes. In the present study, we observed that both neural progenitor cells and mature astrocytes showed the dual presence of DCX and GFAP.

In a previous study, DCX (+) cell clusters were demonstrated in the subgranular zone of dentate gyrus in the first days after BrdU injection (29). Similarly, we observed DCX (+) cells in clusters in GAERS PTZ group. Brown at al. reported that these cells had no distinct cell projections and the others resembled neuroblasts and their projections were parallel to the granular layer. In the same study, it was observed that DCX (+) cells were integrated into the granular layer 7 days after BrdU injection and they projected towards molecular layer. Similarly, we observed that DCX (+) cell projections projected towards molecular layer by passing through the granular layer. These dendrite-like DCX (+) projections were more distinct in the PTZ groups. This finding suggests that newly formed neurons in PTZ groups contribute to the neural plasticity.

DCX-NeuN coexistence was reported in previous studies in adult brain of the rodents (29). Brown et al. reported that NeuN (+) cells started expressing DCX 10 days after BrdU injection and this expression lasted until 14th day, and double expression was not observed after 14th day. Another study reported that 8% of newly born neurons also expressed NeuN (30). Liu et al. did not observe coexistence of DCX-NeuN in normal human brain; however, in the epileptic temporal lobe cortex, 40% of DCX (+) cells were also positive for NeuN (31). Based on these findings, they concluded that neurogenesis was increased in epileptic brain and newly formed neurons were more mature than that of in the normal brain. In our

study, we observed DCX-NeuN dual presence in the same cell both in kindling and control groups in GAERS strain. We concluded that these cells might be in a more mature stage in their development, and DCX-NeuN (+) cells in the hilus might have migrated to hilus after being formed in SGZ. Moreover, Scott et al. observed dual presence of TUC-4, a marker for progenitor neurons, and NeuN (25).

5. CONCLUSION

In conclusion, our findings showed increased neurogenesis in the sham-operated genetic absence epilepsy rats compared to sham-operated Wistar controls, and this result suggested to be related to their resistance to electrical kindling. Besides, DCX presence both in mature astrocytes and in neural progenitors, suggests that marker for this protein is not specific to neurogenesis. We suggest that when used in neurogenesis studies, marker for this protein should be accompanied by the use of other newly born neuron markers.

FUNDING

This study was supported by Marmara University Research Fund (SAG-C-YLP-131.217.0654).

REFERENCES

[1] Kuruba R, Hattiangady B, Shetty A.K. Hippocampal neurogenesis and neural stem cells in temporal lobe epilepsy. Epilepsy Behav 2009;14(Suppl 1):65-73.

[2] Engel J Jr. Intractable epilepsy: Definition and neurobiology. Epilepsia 2001;42(Suppl 6):3.

[3] Geller EB, Lancman ME, Van Ness P, Dinner DS. Coexistence of generalized and partial epilepsies. Electroencephalogr Clin Neurophysiol 1995;95:17P.

[4] Koutroumanidis M, Hennessy M, Elwes RD, Binnie CD, Polkey CE. Coexistence of temporal lobe and idiopathic generalized epilepsies. Neurol 1999;53(3):490-495.

[5] Eskazan E, Onat FY, Aker R, Oner G. Resistance to propagation of amygdaloid kindling seizures in rats with genetic absence epilepsy. Epilepsia 2002;43(10):1115-1119.

[6] Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 1969;137(4):433-457.

[7] Praag HV, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature 2002;415(6875):1030-1034.

[8] Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci 1997;17(10):3727-3728.

[9] Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 2001;435(4):406-417.

[10] Avanzini G, Franceshetti S. Cellular biology of epileptogenesis. Lancet Neurol 2003;2(1):33-42.

[11] Scharfman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. J Neurosci 2002;20(16):6144-6158.

[12] Bengzon J, Kokaia Z, Elmér E, Nanobashvili A, Kokaia M, Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc Natl Acad Sci USA 1997;94(19):10432-10437.

[13] Clark PJ, Kohman RA, Miller DS, Bhattacharya TK, Haferkamp EH, Rhodes JS. Adult hippocampal neurogenesis and c-fos induction during escalation of voluntary wheel running in C57BL/6J mice. Behav Brain Res 2010;213(2):246-252.

[14] Francis N, Farinas I, Brennan C, Rivas-Plata K, Backus C, Reichardt L, Landis S. NT-3, like NGF, is required for survival of sympathetic neurons, but not their precursors. Dev Biol 1999;210(2):411-427.

[15] Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. Electroencephalogr Clin Neurophysiol 1972a;32(3):2269-2279.

[16] Racine R.J. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972b;32(3):281-294.

[17] Park JH, Cho H, Kim H, Kim K. Repeated brief epileptic seizures by pentylenetetrazole cause neurodegeneration and promote neurogenesis in discrete brain regions of freely moving adult rats. Neuroscience 2006;140(2):673-684.

[18] Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. Nat Rev Neurosci 2004;5(7):553-564.

[19] Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult neural stem cells bridge their niche. Stem Cell 2012;10(6):698-708.

[20] Richards DA, Lemos T, Whitton PS Bowery NG. Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. J Neurochem 1995;65(4):1674-1680.

[21] Vergnes M, Boehrer A, Reibel S, Simler S, Marescaux C. Selective susceptibility to inhibitors of GABA synthesis and antagonists of GABA(A) receptor in rats with genetic absence epilepsy. Exp Neurol 2000;161(2):714-723.

[22] Brailowsky S, Montiel T, Boehrer A, Marescaux C, Vergnes M. Susceptibility to focal and generalized seizures in Wistar rats with genetic absence-like epilepsy. Neuroscience 1999;93(3):1173-1177.

[23] Gray WP, Sundstrom LE. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. Brain Res 1998;790(1-2):52-59.

[24] Fahrner A, Kann G, Flubacher A, Heinrich C, Freiman TM, Zentner J, Frotscher M, Haas CA. Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. Exp Neurol 2007;203(2):320-332.

[25] Scott BW, Chan KF, Wong G, Ahmed M, Chieverton L, Liu RR, Wood J, Burnham WM. Cytogenesis in the adult rat dentate gyrus is increased following kindled seizures but is unaltered in pharmacological models of absence seizures. Epilepsy Behav 2010;18(3): 179-185.

[26] Russo E, Citraro R, Donato G, Camastra C, Iuliano R, Cuzzocrea S, Constanti A, De Sarro G. mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy. Neuropharmacol 2013;69: 25-36.

[27] Verwer RW, Sluiter AA, Balesar RA, Baayen JC, Noske DP, Dirven CM, Wouda J, van Dam AM, Lucassen PJ, Swaab DF. Mature

astrocytes in the adult human neocortex express the early neuronal marker doublecortin. Brain 2007;130(Pt12):3321-3335.

[28] Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MW. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nat Neurosci 2004;7(11):1233-1241.

[29] Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 2003;467(1):1-10.

[30] Yang HK, Sundholm-Peters NL, Goings GE, Walker AS, Hyland K, Szele FG. Distribution of doublecortin expressing cells near the lateral ventricles in the adult mouse brain. J Neurosci Res 2004;76(3):282-295.

[31] Liu MA, Curtis HM, Gibbons EW, Mee PS, Bergin HH, Teoh B, Connor M, Dragunow M, Faull RL. Doublecortin expression in the normal and epileptic adult human brain. Eur J Neurosci 2008;28(11):2254-2265.

How to cite this article: Kandemir C, Yavuz M, Karakaya FB, Cilingir Kaya OT, Onat F, Sirvanci S. Investigation of Neurogenesis in Kindled Wistar and Genetic Absence Epilepsy Rats. Clin Exp Health Sci 2022; 12: 753-759. DOI: 10.33808/clinexphealthsci.1021171

Clinical and Experimental Health Sciences

The Relationship Between Gluten Entheropathy and Nail Capileroscopy Findings and Disease Activation

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Received: 12.11.2021 **Accepted:** 28.03.2022

ABSTRACT

Objective: Nailfold Videocapilloroscopy (NVC) is an examination method that is used as an aid in the diagnosis, follow-up, and treatment strategy of rheumatic diseases such as systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and gives an idea about microcirculation by examining the vascular bed. It is a cheap, easily applicable, and quickly accessible method. Because of these features, we aimed to use the NVC method in patients with Gluten Enteropathy (GE) to determine whether this method will be a helpful technique in the diagnosis, activation decision, remission follow-up, and treatment strategy in patients with GE.

Methods: In this study, 67 patients diagnosed with GE (n=35 disease-active group (AGE), n=32 disease-related remission group (RGE), and control group (CG)-27 healthy people whose diagnosis of GE was ruled out were included in this study. Group and CG were divided into ten parameters in capillary pathologies (capillary density loss, dilated capillary, giant capillary, microhemorrhage, avascular area, tortuosity, branched capillary, disorganization, extravasation, angiogenesis). They were divided into two groups as RGE and compared with the results obtained from NVC measurements.

Results: When patients diagnosed with GE and CG were evaluated in terms of capillary disorder with NVC, While all of the patients with capillary disorders were in the GE group, no capillary disorders were found in the control group ($p<0.01$). When patients diagnosed with GE were divided into two groups (AGE and RGE), NVC measurements were compared; All patients with capillary disorders were found in the AGE group ($p<0.01$). Capillary density loss and/or avascular area were detected in 80.9% of patients with capillary disorders.

Conclucion: Our study found a statistically significant difference in NVC measurements between GE patients and CG ($p<0.01$). The fact that all patients with capillary disorders were in the active group in terms of the disease and no capillary disorders were detected in any patients in remission showed that this method could be used as an auxiliary technique in the diagnosis of GE, making the decision of activation or remission, monitoring the disease and determining treatment strategies.

1. INTRODUCTION

Gluten enteropathy (GE) is a disease that affects the small intestines. It progresses with chronic damage due to the inflammatory T cell response against the storage proteins called “gluten” in wheat, rye, barley, and oat (1). The clinical manifestations of this disease, which is also defined as celiac disease, are recurrent episodes of diarrhea and constipation, abdominal distention, abdominal pain, gastroesophageal reflux, weight loss, fatigue, anemia, osteoporosis, and malnutrition (2). The diagnosis combines clinical features, specific serological markers, endoscopic appearance, and biopsy results (3).

Nailfold Videocapilloroscopy (NVC) is an examination method used to diagnose rheumatic diseases such as systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and gives an idea in terms of microcirculation by examining the vascular bed. With NVC, the capillaries in the nail bed are enlarged approximately 200 times, and parameters such as capillary density loss, hemorrhage, avascular area, neogenesis, tortuosity, and disorganization are evaluated.

DOI: 10.33808/clinexphealthsci.1022573

This method is the best non-invasive technique used to evaluate microcirculation in vivo. It is an inexpensive, easily applicable method that guides the diagnosis, activation, remission, and even response to treatment of autoimmune diseases (4).

The pathogenesis of the extraintestinal manifestations of celiac disease is unknown. For example, some patients are faced with a clinic that leads to cirrhosis due to severe hepatotoxicity. The proposed mechanism in these patients was to find more hepatotoxins in the portal circulation. The presence of anti-tyroglobulin autoantibodies in liver biopsies supports this mechanism and suggests vascular-mediated organ damage (5). It has also been shown that celiac disease is associated with autoimmune diseases that cause microvascular damage, such as Sjögren's syndrome and systemic lupus erythematosus (6). In conclusion, although there is no study showing that celiac disease causes microvascular damage, it has suggested the possibility that this disease itself may cause damage to the microvascular bed by an unknown mechanism. This study was planned because capillaroscopy is the easiest method of examining the microvascular bed.

In this study, we aimed to determine whether there is a relationship between nail bed capillaroscopic findings of Celiac Disease, a systemic disease. We desired to see whether a Celiac disease helps decide on activation or remission with our results.

2. METHODS

2.1. Inclusion Criteria

Sixty-seven patients diagnosed with GE were included in the study. GE anti-tissue transglutaminase [a-TTG] sought positivity in these patients. Afterward, the small intestine biopsy taken by the endoscopic method was retrospectively scanned and evaluated histologically with the March-OberHuber classification. Patients in the Type 3 class with a high probability of GE biopsy findings based on intraepithelial lymphocyte increase (IEL), crypt hyperplasia, and villus shortening findings were included in the study (7,8).

Two groups were formed, the patient group with a diagnosis of GE, 35 patients with active disease (AGE), and 32 patients who met the criteria for remission (RGE). AGE's inclusion criteria were active clinical symptoms, positive a-TTG, and MOH type 3 class on endoscopic biopsy. All of the patients in this group were recruited from newly diagnosed patients. The patients in the RGE group were previously clinically positive, a-TTG positive, endoscopically in the MOH type 3 class, diagnosed with GE, had no clinical findings with a gluten-free diet, had negative autoantibodies, and lost typical pathological findings in endoscopic biopsy (MOH type 0, 1 and 2) patients are included. On the other hand, 27 non-smokers whose CG was admitted to the outpatient clinic of our hospital with nonspecific dyspeptic complaints, GE diagnosis was ruled out by serology and biopsy, without any systemic disease and pregnancy, were included.

2.2. Exclusion Criteria

In our study, patients younger than 16 years of age and older than 65 years, with any autoimmune disease such as systemic sclerosis, systemic sclerosis, systemic diseases such as hypertension, diabetes mellitus, chronic kidney failure, acute kidney failure, congestive heart failure, chronic lung diseases, malignancy, dilated cardiomyopathy. Patients with conditions or habits such as smoking and alcohol were not included in the study. Persons with congenital nail anomaly and anatomical defect or who lost a part of their finger in any way were also excluded from the study.

2.3. Methodology

NVC examination in all patients was performed by a VideoCap 3.0 videocapillaroscopy device and an experienced capillaroscopy specialist. The study was planned to be single-blind from the point of view of the capillaroscopist. Control capillaroscopy was designed one month later to demonstrate the reproducibility of the analysis. However, it could not be performed due to the possibility of changing the capillaroscopy of the patients under treatment and affecting the results. The biggest shortcoming of our study is the lack of reproducibility. NVC measurements were divided into ten parameters: dilated capillary, giant capillary, loss of capillary density, avascular area, disorganization, tortuosity, extravasation, microhemorrhage, branched capillary, and angiogenesis. Each individual to be examined for NVC was left in the test room at a temperature of 20-22˚C for at least 15 minutes before the procedure. The thickness of the standard capillary wall is 0.5 micrometer (µm), and its diameter is 4-9 µm (9). The diameter of the arterial column is 5-15 µm, and the diameter of the venular column is 7-18 µm. Dilated capillaries were defined as the arterial column diameter of the capillary larger than 15 µm or the venular column diameter more significant than 20 µm in NVC measurements. Capillaries with an arterial or venous column larger than 50 µm were defined as giant capillaries. Capillary density; is defined as the number of capillaries with a length of 1 millimeter (mm) in the distal row at each nail base (10). The presence of 9 to 13 capillaries per 1 mm in length was taken as a reference, and fewer than nine capillaries were accepted as a decrease in capillary density (11). The avascular area was considered two or more capillary reductions in different regions of the nail fold (10). Normal capillaries are straight like a hair or inverted u-shaped like a hairpin, and the deterioration of this architectural structure was considered disorganization. The term tortiotized was used for capillary folding. A hazy appearance on capillaroscopy due to extravasation of plasma from the damaged capillaries was accepted as extravasation. Bleeding outside the capillary due to capillary damage was considered a microhemorrhage. Capillaries with bush-like branches were deemed to be branched capillaries. Tortuosity in the capillary bed, branching, bush-type capillaries,

and anastomoses between capillaries were accepted as angiogenesis (11).

AGE, RGE, and C.G. were measured with our NVC device, and the relationship between the groups was investigated.

2.4. Statistical Analysis

AGE, RGE, and CG were measured with our NVC device, and all the patients investigated age, gender, and the relationship between them and NVC. The analysis tested compliance of numerical data with normal distribution with the ShaphiroWilk test. The Mann-Whitney U test was used to compare the non-normally distributed variables in the two groups. The relationship between categorical variables was tested with Chi-square. Descriptive statistics are given as mean ± standard deviation and median (25%-75%) for numerical variables and numbers (%) for categorical variables. Analytical measurements used SPSS 22.0 package program in the analysis, and $p<0.05$ was considered significant.

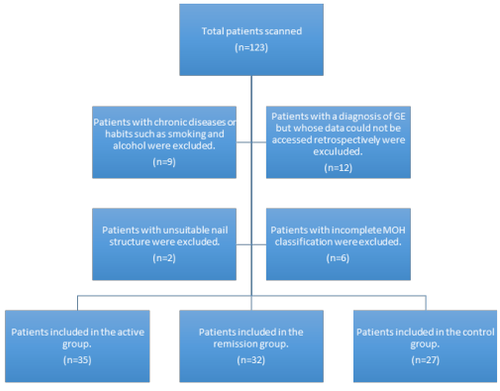
2.5. Ethics Approval

This study was carried out by T.C. It has been prepared with the approval of Gaziantep University Faculty of Medicine Ethics Committee with the number 300/2017.

3. RESULTS

The summary of the population included in our study is in table 1. The people included in the study consisted of 71.3% (n=67) patients with a diagnosis of GE, 28.7% (n=27) from CG, and a total of 94 people were included in the study. When GE and CG were compared in gender, 70.4% (n=19) of CG was female, and 70.1% (n=47) of GE was female. When GE and CG were compared in terms of gender and age, no statistically significant difference was found (female 70.1% vs. 70.4%, p=0.983, 27.64±10.06 years vs. 28.81±3.82 years, p=0.275).

Table 1. *Flowchart of the study population.*



Distribution of GE patients participating in the study according to the March-OberHuber classification; type 0: 40.3% (27), type 1: 4.5% (3), type 2: 3% (2), type 3a: 3% (2), type 3b: 6% (4), type 3c: 43.3% (29) was found (Figure 1). The patients with Type 0, 1 and 2 were in the RGE class, and the patients in Type 3 were in the AGE class.

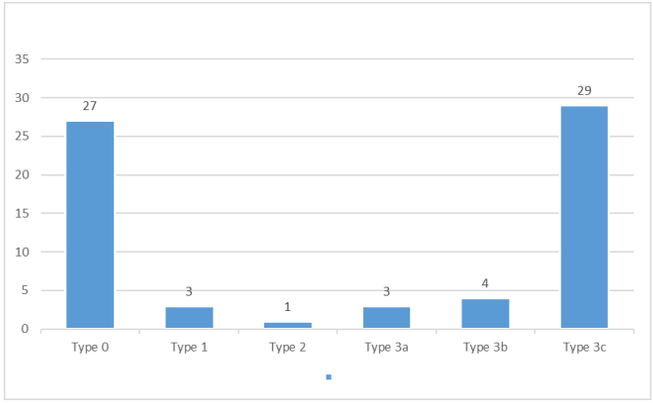


Figure 1. *Distribution of patients according to March-OberHuber classification*

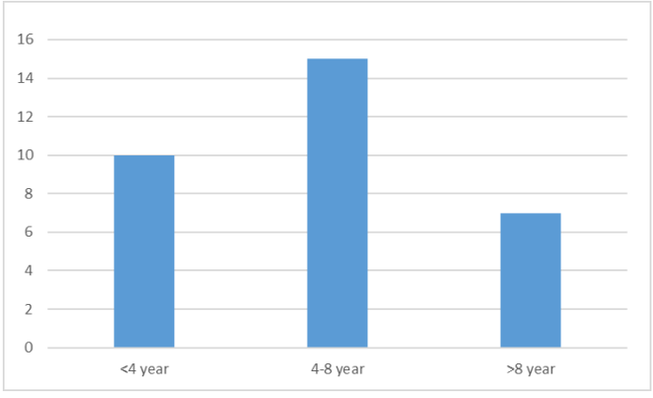


Figure 2. *Disease duration of the RGE group*

The distribution of the group in remission according to the years of follow-up; was determined as 31% (10) under four years, 47% (15 between 4 and 8 years), 22% (7) at eight years and above (Figure 2).

When the NVC measurement results of GE and CG were compared, the capillary disorder was found in 31.3% (21) of GE, and no pathology was detected in CG (p=0.001) (Table 2).

Table 2. *Capillary disorder comparison between GE and CG.*

PATIENT/CONTROL GROUP						
		GE (n=67)		CG (n=27)		p
Capillary Disorder	Negative	46	68,70%	27	100,00%	*0,001*
	Positive	21	31,30%	0	0,00%	

When GE was evaluated within itself, it was divided into two groups as AGE and RGE. No significant difference was found between AGE and RGE in evaluating gender and age (respectively; 71.4% female vs. 68.8% female, p=0.81, 28.80±11.05 years vs. 26.37±8, 85 years, p=0.355).

In NVC measurements made between AGE and RGE, the capillary disorder was detected in 60% (n=21) of AGE, and no capillary disease was detected in NVC measurements made in RGE. This result was statistically significant (Table 3).

Table 3. *Capillary disorder comparison between AGE and RGE.*

		GROUP				
		AGE (n=35)		RGE (n=32)		p
Capillary Disorder	Negative	14	40,00%	32	100,00%	*0,001*
	Positive	21	60,00%	0	0,00%	

Considering the subclasses of NVC measurements in AGE, capillary density loss and avascular area coexistence in 7 patients, density loss, dilated capillary and avascular area coexistence in 4 patients, density loss and dilated capillary coexistence in 2 patients, density loss, avascular area, and microhemorrhage coexistence in 1 patient were found. Also, loss of density in 2 patients, tortuosity in 2 patients, microhemorrhage in 1 patient, dilated capillary area in 1 patient, avascular location in 1 patient, and capillary disorder were detected in 21 patients in total. Giant capillaries, branching, disorganization, extravasation, and neogenesis were not seen in any patients.

4. DISCUSSION

During the development of GE disease, the duration of exposure to gluten and the onset and development of autoimmune disease is directly proportional (12). In the measurements we made in our study, all patients with capillary disorders were newly diagnosed and active in terms of the disease. This result shows that there is a significant relationship between capillary disorder and GE. While no capillary disorders were detected in our 32 patients in clinical, pathological, and complete laboratory remission, the detection of all patients with capillary disorders in the active patient group supports that gluten exposure is directly related to microvascular damage.

With the ingestion of gluten in GE, immunological events in which clinical findings occur due to the combination of gliadin peptides found in the small intestinal mucosa and HLA class II molecules begin. The tissue groups that show this reaction the most are HLA-DQ2 and HLA-DQ8 (13). It has been demonstrated that the 33-mer peptide in the structure of the gliadin molecule is the precursor molecule that initiates the inflammatory response in genetically predisposed individuals (14). This inflammatory response may also be causing damage at the capillary level. It is known that the level of serum a-TTG is related to the degree of villous atrophy (15). In the evaluation of these patients, it was determined that all 21 patients with capillary disorders were in the active AGE group, and antibody positivity was shown in all active patients. These results lead us to a significant relationship between the activity of the disease and the capillary disorder. The absence of capillary damage in any patient in remission supports this finding. As a result, it has been suggested that this method is a non-invasive and easy-to-apply helpful method that can be used in diagnosing GE, distinguishing active or remission disease, and monitoring the effectiveness of treatment.

In the NVC measurements we made in our study; We have classified capillary disorders into ten subclasses as capillary density loss, dilated capillary, giant capillary, microhemorrhage, tortuosity, avascular area, extravasation, branched capillary, disorganization, and neogenesis. In our measurements, capillary density loss in 76.2% (16) of patients with capillary disorders, an avascular area in 61.9% (13), dilated capillaries in 33.3% (7), and 9.5% (2) we detected microhemorrhage and tortuosities. In our measurements, capillary density loss in 76.2% (16) of patients with capillary disorders, the avascular area in 61.9% (13), dilated capillaries in 33.3% (7), and 9.5% (2) we detected microhemorrhage and tortuosities. In particular, loss of capillary density is seen in Raynaud's phenomenon and scleroderma. In contrast, the avascular area is seen in Wegener's disease, systemic lupus erythematosus, and scleroderma, and these two pathologies show direct tissue hypoxia (10,11). In our study, these two pathologies were detected together in 12 of our patients. When evaluated in total, we found that at least one of these two pathologies was present in 17 of 21 patients with capillary disorders (80.9% of patients with capillary disorders). This result suggested that the capillary disorder developing in the nail bed in GE disease was predominantly secondary to tissue hypoxia. These findings are similar to NVC findings seen in scleroderma. In a single-case publication presented by R.Thonhofer et al., it was found that an active scleroderma pattern was detected in the capillaroscopy measurement performed in a patient diagnosed with GE, and NVC measurements were found to improve with the removal of gluten from the diet (15). This single case presentation on this subject, which can be found in the literature review, supports the results of our study.

5. CONCLUSION

As a result of our study, it has been seen that NVC measurements are a proper method in diagnosing GE, predicting disease activation, and monitoring the response to treatment. Disruption of nail microarchitecture in active GE suggested that the disease may have systemic effects through immunological mechanisms. This finding may indicate that there are new pathways in the pathogenesis of the disease. These findings may contribute to elucidating the immunology of the disease.

Acknowledgment: All authors declare that there is no conflict of interest between them. Informed consent was obtained from the patient for this article. No financial support has been received for this article.

REFERENCES

[1] Mearin ML. Celiac disease among children and adolescents. Curr Probl Pediatr Adolesc Health Care 2007;37:86-105.

[2] Rodrigo L. Celiac disease. World J Gastroenterol. 2006 Nov 7;12(41):6585-93. doi: 10.3748/wjg.v12.i41.6585.

[3] Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for standardized report schema for pathologists. Eur J Gastroenterol Hepatol 1999; 11: 1185-94.

[4] Guyton H. Basic physiology book (Trans. Ed. Çavuşoğlu). Nobel Medical Bookstores, Tenth Edition, 162-182.

[5] Korponay-Szabó I.R., Halttunen T., Szalai Z., Laurila K., Király R., Kovács J.B., Fésüs L., Mäki M. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004;53:641–648.

[6] Iltanen S, Collin P, Korpela M, Holm K. Celiac disease and markers of celiac disease latency in patients with primary Sjogren's syndrome. *Am. J. Gastroenterol.* 1999;94:1042–1046.

[7] Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for standardized report schema for pathologists. Eur J Gastroenterol Hepatol 1999; 11: 1185-1194.

[8] Ensari A. Gluten-sensitive enteropathy (Celiac Disease) controversies in diagnosis and classification. Arch Pathol Lab Med 2010; 134: 826-836.

[9] Tavakol ME, Fatemi A, Karbalaie A, Emrani Z, Erlandsson BE. Naillfold capillaroscopy in rheumatic diseases: Which parameters should be evalutes? Hindawi Publishing Corporation BioMed Research International 2015;974530.

[10] Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008;22(6):1093-108.

[11] Ventura A, Magazzù G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. Gastroenterology 1999; 117: 297-303.

[12] Maki M, Lohi O. Celiac Disease. In: Walker WA, Goulet O, Kleinman RE, Sherman PM, Shneider BL, Sanderson IR (eds). Pediatric Gastrointestinal Disease. 4th ed. Ontario: B.C. Decker, 2004: 932-43.

[13] Molberg O, McAdam S, Lundin KE, Kristiansen C, Arentz-Hansen H, Kett K, Sollid LM. T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tis – sue transglutaminase. Eur J Immunol 2001; 31: 1317-1323.

[14] Cataldo F, Marino V, Ventura A, Bottaro G, Corazza GR. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP) and "Club del Tenue" Working Groups on Coeliac Disease. Gut 1998;42:362-365.

[15] Thonhofer R, Trummer M, Siegel C. Capillaroscopy shows an active pattern of scleroderma in coeliac disease. Scand J Rheumatol. 2010;39(5):438-439.

How to cite this article: Okyar B, Yildirim AE, Barutcu S. The Relationship Between Gluten Entheropathy and Nail Capileroscopy Findings and Disease Activation. Clin Exp Health Sci 2022; 12: 760-764. DOI: 10.33808/clinexphealthsci.1022573

Clinical and Experimental Health Sciences

The Relationship Between Nurses' Professional Values and Their Attitudes Towards Care Giving Roles: A Structural Equation Model

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Received: 14.11.2021 **Accepted:** 07.02.2022

ABSTRACT

Objective: The study was carried out to examine the relationship between nurses' professional values and caregiver roles.

Methods: This study is based on a descriptive correlational research design using a structural equation model. 366 nurses participated in the study. It was gathered using Nurse Promotion Form the Professional Values Scale of Nurses and the Attitude Scale for Their Caregiver Roles.

Results: The total mean score of the Nurses' Professional Values Scale was determined as 94.73±17.87. Nurses' Perceptions of Caregiving Roles Attitude Scale total score mean was determined as 62.41±9.22. According to the Structural Equation model, professional values of nurses explain 39% of the change in the value of the attitude towards caregiver roles (R^2 =0.39).

Conclusion: Nurses' professional values and attitudes towards caregiver roles are at a good level and professional values of nurses partially affect the attitude towards caregiver roles.

Keywords: Care, care giving role, nursing values, structural equation model

1. INTRODUCTION

Values are goals and beliefs that affect a person's decisions, behaviors, choices and the resulting lifestyle (1). Each individual has its own values and these values are gained as a result of education, experience, culture and interpersonal relations (2). Values representing basic beliefs about what is right, good or desired affect both personal and professional behavior (3). Professional values are defined as common beliefs about the value or quality of common concepts and behaviors over a particular discipline. Professional values as common beliefs bring together individuals with different cultural backgrounds and are considered the basis for creating a standard professional identity and ideology (4,5). Professional values that form the basis of nursing practices (6) are one of the most important factors that guide nurses' thoughts, actions, practices and interactions with patients (2,7).

Nurses are guided by professional values in the decision-making process regarding care actions to be implemented and in resolving ethical problems. Nurses who internalize professional values gain competence in resolving conflicts and determining priority actions and so they contribute to maintaining a quality, safe and ethical care (8). It is extremely important for nurses to be aware of professional values such as accuracy, honesty, altruism, autonomy, and equality in order to offer a qualified and humanistic patient care (9). Studies examining the professional values of nurses have shown that nurses adopt values such as human dignity, social justice, autonomy, honesty, responsibility, professional commitment and trust (4,10,11).

Nursing care is based on a series of universal humane and altruistic values that will bring meaning and satisfaction to people's lives in their relationships with others (5). Conditions such as insufficient or undeveloped nurses 'professional values affect nurses' goals, strategies, actions and especially caregiver roles (1). The care giving role emerges as an ancient role that forms the core of the traditional practice of nurses. And other roles of modern nursing are thought to derive from the caregiver role, while nurses are most independent in practicing their caregiver roles, and this role is the role of nursing profession (12). In their study, Pang et al. (13) have determined that nurses' problem solving and critical thinking skills, determining patient care needs and resolving patient and family concerns are affected

DOI: 10.33808/clinexphealthsci.1019658

by values. In addition, similar studies have reported that nurses' attitudes towards caregiver roles are positive (14,15).

American Nurses Association (ANA) have stated that professional values improve the quality of nursing care (16). The relevant literature reports that nurses 'professional values affect nurses' caregiver roles (1). However, no study explaining this relationship has been found. Therefore, in this study, it was aimed to examine the extent to which professional values affect nurses' caregiver roles by using the structural equation model. Nurses' awareness of their values and how these values affect their care behavior are the basis of humanistic nursing care. Therefore, it is important to demonstrate care roles developed by nurses based on their professional values.

1.1. Purpose of the Research

This study examines the relationship between the professional values of nurses working in a university hospital in Central Anatolia Region of Turkey and their attitudes towards caregiver roles.

1.2. Hypotheses

H_1 hypothesis assumed that providing care positively affects the attitude towards nurses' caregiver roles.

H_2 hypothesis assumes that professionalism positively affects the attitude towards nurses' caregiver roles.

H_3 hypothesis assumes that activism positively affects the attitude towards nurses' caregiver roles.

H_4 hypothesis assumes that justice positively affects the attitude of nurses' caregiver roles.

H_5 hypothesis assumed that the professional values of loyalty positively affect the attitude towards nurses' caregiver roles.

2. METHODS

2.1. Design

In this study, a model was predicted to examine the relationship between nurses' professional values and attitudes towards caregiver roles. The predicted model based on the literature was tested with a descriptive research design using structural equation modeling. In the predicted model, it was assumed that the professional values of nurses will positively affect their attitudes towards caregiver roles (Figure 1).

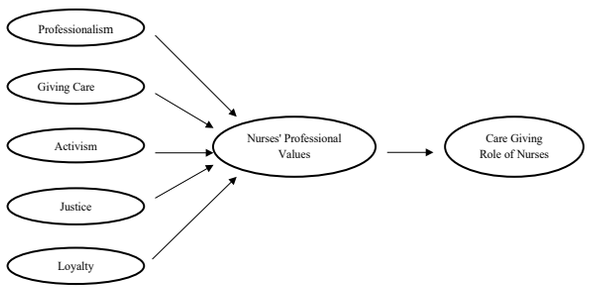

Figure 1. *Predicted model.*

2.2. Sample and Data Collection

The descriptive correlational study was conducted in the second largest university hospital in Konya, between July and September 2018. The hospital, with a total bed capacity of 934, also serves patients from nearby provinces. A total of 501 nurses work in the hospital. Working hours are carried out in two shifts, 08: 00-16: 00, 16: 00-08: 00. Due on the service, a nurse provides care to an average of 15 patients. The hospital does not have an in-service training program on nursing care and nursing values.

The entire population was created without sample selection. All the nurses working in this hospital (N:501) form the universe, the sample of the study consisted of 366 (72%) nurses meeting the inclusion criteria. The inclusion criteria were determined as follows: working as a nurse in internal diseases, surgical and intensive care clinics, providing one-to-one patient care, volunteering to participate in the research and not being on leave/have report between the dates of the research (Figure 2).

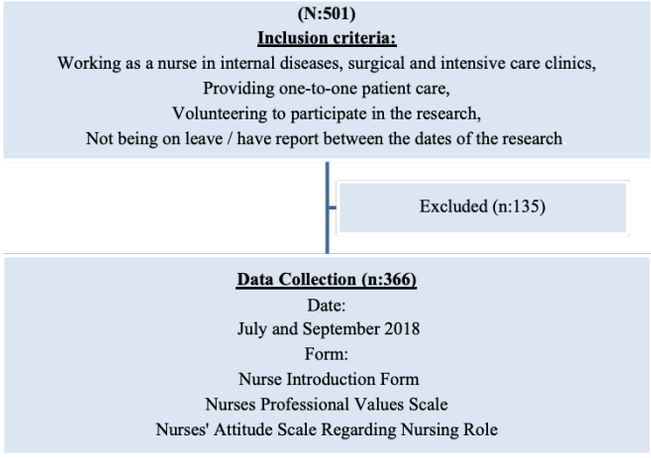

Figure 2. *The characteristics of study population.*

Nurse Promotion Form, Nurses Professional Values Scale, Nurses' Attitude Scale Regarding Nursing Role were used to collect data. Before starting the study, pilot study was performed with 10 nurses who were not included in the sample. After the pre-application, it was determined that all the questions in the data collection forms were understandable. The nurses included in the pre-application were not sampled. Verbal consent was obtained from the nurses by explaining the purpose of the study, and then a questionnaire was given. The questionnaire forms were taken back from the nurses one day later in order to be filled in during the hours when the workload was low. Forms filled in about 15 minutes.

2.2.1. Nurse Promotion Form

In this form, which was created in line with the literature (4,17,18) by the researchers, there were 14 questions that questioned the socio-demographic and working characteristics of nurses.

2.2.2. Nurses' Professional Values Scale

Nurses Professional Values Scale was developed by Weis and Schank, to determine the level of perception of nurses and nursing students' professional values (19). The Professional Values Scale of the Nurses whose Turkish version was made is a 5-point Likert type with 26 items (Not important= 1, Somewhat important= 2, Important= 3, Very Important 4, Very Important 5). The scale includes five factors: caregiving, professionalism, activism, justice and loyalty. The lowest score that can be received from the scale is 26 and the highest score that can be received is 130. The high score indicates that professional values are high. The Turkish version of the scale was considered valid and reliable based on the study by Geçkil et al., (2) (Cronbach's alpha=0.92). In this study, the Cronbach's alpha value for the whole scale was 0.96; for the subscales, it ranged between 0.74 and 0.90.

2.2.3. Attitude Scale of Care Giving Role of Nurses

Nurses' Attitude Scale Regarding Caregiving Roles (NARRCR) was developed by (Koçak et al., 2014) to determine the attitudes of nurses towards caregiver roles. And this scale is a 5-point Likert type scale consisting of 16 items whose validity and reliability have been made by the same researchers. NARRCR consists of three sub-dimensions, namely, Elimination of the patient's self-care needs and attitude towards the nurse's counseling role, Attitude towards the nurse's role in protecting the individual and respect for their rights, and Attitude towards the nurse's role in the treatment process. The items of the scale are evaluated by the participants in line with the answers I definitely disagree, I disagree, I partially agree, I agree, I totally agree. And these evaluations are scored between 1-5 points. The lowest score received from the scale is 16, and the highest score is 80. Accordingly, as the score obtained from the scale increases, the attitudes of nurses towards caregiver roles are interpreted positively. As the score received from the scale decreases, it is interpreted as negative. The Cronbach Alpha value found in the work of Koçak et al. 0.91 was found (12). The Cronbach's alpha value for the whole scale was 0.96; for the subscales, it ranged between 0.72 and 0.83.

2.3. Ethical Dimension

Ethics committee approval was obtained from a university's Drug and Non-Medical Device Research Ethics Committee (2018/1446), institutional permission was obtained from the hospital where the study was conducted. Participants were informed on the front cover of the questionnaire, and those who gave consent were included in the research. Necessary use permit was reached from author to use the Nurses 'Occupational Values Scale and from author to use the Attitude Scale of the Nurses' Care Giving Roles.

2.4. Evaluation of the Data

The reliability analysis of the scales used in the study were determined by the Cronbach's Alpha coefficient and the relationship between the scales by Pearson correlation coefficient. Student t test, One-Way Variance Analysis and Kruskal Wallis analysis and structural equation model were used in the statistical evaluation of scale scores according to demographic characteristics.

3. RESULTS

3.1. Results Related to Participants' Socio-Demographic and Working Characteristics

When the distribution of 366 nurses included in the study according to their socio-demographic and study characteristics is examined the following have been identified: 60.7% of these nurses are women, 60.7% of the nurses were trained at the Health Vocational High School/Associate Degree, and the average age was 28.66 ± 6.63, 34.2% of the nurses work in internal clinics, 53.8% of them work in the form of day and night shifts. In addition, the average of the nurses' weekly working hours was found to be high (43.89 ± 4.67) and it was found that the nurses had just started the profession (7.80 ± 5.52) (Table 1).

Table 1. *Distribution of nurses by socio-demographic and working features (n=366)*

Socio-demographic Features	Number	%
Gender		
Female	**222**	**60.7**
Male	144	39.3
Educational status		
HVS / Associate Degree	**222**	**60.7**
License	122	33.3
Postgraduate	22	6.0
Average Age (x̄±SS)	**(28.66±6.63)**	**(Min= 19 Max= 49)**
Clinics		
Internal Clinics	**125**	**34.1**
Surgical Clinics	98	26.8
Intensive care	105	28.7
Pediatrics	38	10.4
Working Method		
Permanent Night	47	12.8
Permanent Day	122	33.3
Day and night	**197**	**53.9**
Weekly Working Hours Average. (x̄±SS)	**(43.89±4.67)**	**(Min= 35 Max= 76)**
Occupational Work. Year Average (x̄±SS)	**(7.80±5.52)**	**(Min= 1 Max= 49)**

3.2. Results Regarding the Relationship between Nurses' Professional Values and Caregiver Roles

The NARRCR score average of the nurses is 94.73 ± 17.87. The mean scores achieved from the sub-dimensions were 30.86 ± 5.89 in the Giving Care sub-dimension, 24.78 ± 5.18 in the Professionalism sub-dimension, 17.84 ± 4.00 in the Activism sub-dimension, 11.02 ± 2.35 in the Justice sub-dimension and 10.23 ± 2.59 in the Loyalty sub-dimension (Table 2).

Table 2. *Nurses' professional values scale scores and caregiving role attitude scale scores*

Sub Dimensions and Scale Total	Min.	Max.	x̄	SS	Crobach Alfa
NARRCR (Total Score)	28.00	130	94.73	17.87	0.96
Giving Care	8.00	40.00	30.86	5.89	0.90
Professionalism	7.00	35.00	24.78	5.18	0.90
Activism	5.00	25.00	17.84	4.00	0.88
Justice	3.00	15.00	11.02	2.35	0.77
Loyalty	3.00	15.00	10.23	2.59	0.74
NARRCR (Total Score)	30.00	80.00	62.41	9.22	0.90
OGGDRT[a]	11.00	35.00	26.91	4.51	0.83
BKHSORT[b]	7.00	20.00	16.39	2.48	0.73
TSRT[c]	7.00	25.00	19.10	3.21	0.72

a: Meeting the self-care needs of the patient and attitude towards the counseling role of the nurse
b: Attitude towards the nurse's role to protect the individual and to respect their rights
c: Attitude towards the role of the nurse in the treatment process

It was determined that the mean item point average of the Nurses' total score was 62.41 ± 9.22. When the sub-dimensions were analyzed, it was determined that the item score averages were meeting the self-care needs of the patient and attitude towards the counseling role of the nurse (OGGDRT) 26.91 ± 4.51, attitude towards the role of the nurse in the treatment process (TSRT) 19.10 ± 3.21, attitude towards the nurse's role to protect the individual and to respect their rights (BKHSORT) 16.39 ± 2.48 respectively (Table 2).

Figure 3 shows the path analysis model drawn with SPSS-AMOS. β values shown in the figure are standardized values. Hypothesis test results are as shown in Table 3.

Table 3. *Hypothesis test results*

Hypothesis Relationships	Standard β	p	Acceptance / Rejection
H1: Care Giving → Attitude Towards Nurses' Care Giving Roles	-0,020	0,901	Rejection
H2: Professionalism → Attitude towards Nurses' Care Giving Roles	-0,028	0,897	Rejection
H3: Activism → Attitude towards Nurses' Care Giving Roles	0,053	0,764	Rejection
H4: Justice → Attitude Towards Nurses' Care Giving Roles	0,004	0,980	Rejection
H5: Loyalty → Attitude Towards Nurses' Care Giving Roles	0,617	0,002	Acceptance
X2/df= 2,481 CFI=0,87 NFI= 0,80 IFI= 0,87 RMSEA=0,06			

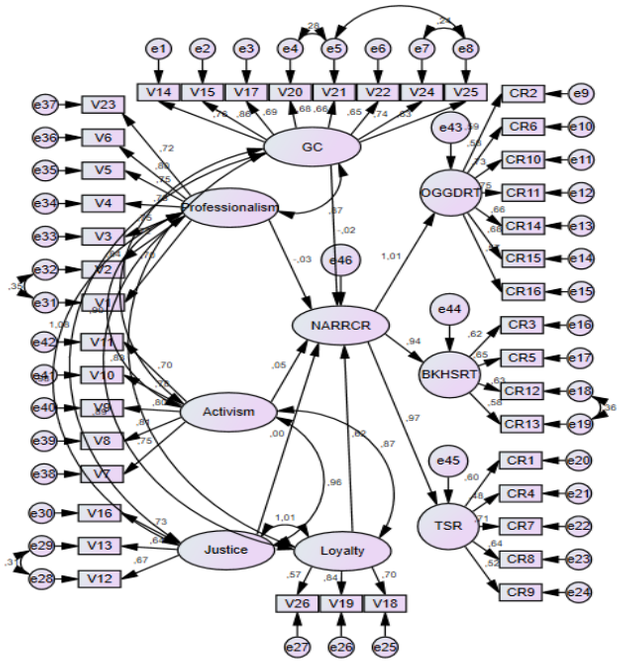

Figure 3. *Structural equation results regarding the research model.*

V: Value, CR: Caregiving Role, GC: Giving Care, NARRCR: Attitude Scale of Care Giving Role of Nurses, OGGDRT: Meeting the self-care needs of the patient and attitude towards the counseling role of the nurse, BKHSRT: Attitude towards the nurse's role to protect the individual and to respect their rights, TSR: Attitude towards the role of the nurse in the treatment process.

Fit indices were used to test the suitability of the established structural equation model. Fit indices X^2=2.481; Comparative fit index (CFI) = 0.87, incremental fit index (IFI) =0.87 and the mean square root of the approximate errors (Root Mean Square Error of Approximation, RMSEA) =0.06. These results show that the established model is suitable.

H_1 hypothesis assumed that providing care positively affects the attitude towards nurses' roles. Standard β coefficient obtained as a result of path analysis this hypothesis was rejected at 0.05 significance level with a value of – 0.020.

H_2 hypothesis assumes that professionalism positively affects the attitude towards nurses' caregiver roles. Standard β coefficient obtained as a result of path analysis this hypothesis was rejected at 0.05 significance level with a value of – 0.028.

H_3 hypothesis assumes that activism positively affects the attitude towards nurses' caregiver roles. Standard β coefficient obtained as a result of path analysis this hypothesis was rejected at the 0.05 significance level with a value of 0.053.

H_4 hypothesis assumes that justice positively affects the attitude of nurses' caregiver roles, standard coefficient β obtained as a result of path analysis this hypothesis was rejected at the significance level of 0.004 with 0.05.

H_5 hypothesis assumed that the professional values of loyalty positively affect the attitude towards nurses' caregiver roles.

Standard β coefficient obtained as a result of path analysis this hypothesis was accepted at the level of 0.05 and a significance level of 0.05.

Care giving, professionalism, activism, justice, loyalty variables explain 39% of the change in the value of attitudes towards nurses' caregiver roles ($R^2 = 0.39$) (Figure 3) (Table 3).

4. DISCUSSION

This study was determined nurses' perception of professional value and attitudes towards caregiver roles a good. It was found that the professional values of the nurses explain 39% of the change in the value of attitudes towards nurses' caregiver roles. Hypothesis that professional values of loyalty positively affect the attitude towards nurses' caregiver roles was accepted in the path analysis.

The reason for the existence of nursing is the nursing philosophy, which guides the behaviors and practices towards care. However, the most important building block of this philosophy is nursing values (5). Professional values gained by nurses through their education and practice throughout their lives are essential for professional nursing practices (1,7).

In our study, the mean NARRCR total score of the nurses was 94.73 ± 17.87. Considering that the highest score that can be obtained from NARRCR is 130, it can be stated that nurses' perception of professional value is at a good level. Similar to our study finding, there are studies showing that nurses' perception of professional value is good (2,4,17,20-22).

NARRCR total score of the nurses was found as 62.41 ± 9.22. When the highest score that can be obtained from NARRCR is considered to be 80, According to these results, it can be said that nurses' attitudes towards caregiver roles are at a good level (24), it was found that there were similar findings with our research result. It was found that nurses have a good level of caregiver roles. Similar results were obtained in other studies on this subject (25-27).

According to the models developed in this study, we found that care giving, professionalism; activism and justice values do not directly affect the attitude towards the caregiver role. Although care is not specific to nursing, it is unique for nursing and is the main responsibility of nurses all around the world (28). It is a factor which contributes significantly to the relationship between the nurse and the patient and to the treatment and cure of the patient (29). Professionalism is defined as expertise, knowledge, skills and behaviour in a given field (30). Nurses who embrace the role of professional caregivers take more responsibility in care and thus quality of care is improved (31,32). Activism, another value, is an action which is more than awareness, participation and advocacy and refers to a conscious, strong effort to achieve change. Activism is a component of the nursing's social contract and contract with people. Nurses prefer activism as an intervention to address inequalities in healthcare and so nursing transforms from a passive but supportive role to a role that takes action to create change (33). Justice includes equal treatment and fair distribution of resources in healthcare services (34). Justice which plays a role in developing a professional identity is necessary to protect people's health and survival just like healthcare. For nurses justice includes participating in social actions, taking action for vulnerable people and addressing weaknesses of the healthcare system (35). Values described according to the information found in the literature are estimated to have a positive effect on the attitude towards the caregiver role (24). American Association of Nurses have reported that the professional values of nurses improve the quality of nursing care and are directly related to their caregiver roles (16). Geyer et al. (36) have reported that care behavior and work performance are positively influenced by professional values. Another study have reported that nurses with positive professional values take more responsibility and provide better patient care compared to their colleagues (5). However although nurses received high scores in these set of values, these were not found to have any effect on the caregiver role. In addition, the fact that the explanation rate of all values is 39% also supports this result. The reason for this finding could be that although nurses have professional values at cognitive and emotional level, they have trouble in reflecting these in behaviour and attitude. Because attitude is an emotional, cognitive state which is developed based on education and experience and has the power to orientate and affect one's behaviour. Attitude is for the long term and includes the process of cognitive, emotional and behavioural development (37). We believe that the nurses included in this study did not have sufficient care attitude due to their low education level and work experience.

In the hypothesis direct effect of loyalty on the attitude towards the caregiver role is supported. Loyalty refers to being loyal and keeping promises. Loyalty principle includes the commitment in the patient-nurse relationship and keeping promises based on this principle ensures to maintain an environment of trust (38). This finding can be associated with strong loyalty value which is among the personal values of nurses.

4.1. Strengths and Limitations

This study was conducted on 501 nurses and achieved the desired power to find significant relationships. In addition, this study is the first to examine the relationship between nurses' attitudes towards care giving roles and their professional values. This study has some limitations. First, the results cannot be generalized since the study was carried out only in a single hospital. Secondly, results may have been affected by Turkish healthcare system and cultural characteristics of the nurses. Another limitation is that the study is based on self-reporting. The tendency to respond as expected in such survey studies may affect the results of the study.

4.2. Implications for Practice and Research

Professional values ensure improvement of care outcomes and preservation of professional identity. It is important for the professional values to be addressed and improved as the driving force behind the care behaviors of the nurses. Further studies that evaluate how professional values of nurses are reflected in behaviour and on-the-job training about values for nurses who are newly recruited and for nurses with high school/associate degree are recommended.

5. CONCLUSION

As a result of these data obtained, it was found that nurses' professional values and attitudes towards caregiver roles are at a good level. It was seen that the professional values of the nurses partially affect the attitude towards the caregiver roles. Care giving, professionalism, activism, justice, loyalty variables were found to explain 39% of the change in the value of attitudes towards care giving roles of nurses.

Acknowledgements

We are grateful to the nurses who participated in this study.

Funding

No financial support was obtained for this manuscript.

Conflict of interest

We declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions:

Study design: HB, SS

Data collection: HB, SS

Data analysis: HB, SS

Manuscript writing: HB, SS

This study was presented as an oral presentation at the 5th National 1st International Essential Nursing Care Congress held in Antalya, Turkey, 06-08 December 2019.

REFERENCES

[1] Kaya A, Boz İ. The development of the professional values model in nursing. Nurs Ethics 2019;26(3):914–923.

[2] Geçkil E, Ege E, Akın B, Göz F. Turkish version of the revised nursing professional values scale: Validity and reliability assessment. Japan J Nurs Sci 2012;9(2):195–200.

[3] Kaya H, Işık B, Şenyuva E. Kaya N. Personal and professional values held by baccalaureate nursing students. Nurs Ethics 2017;24(6):716–731.

[4] Poorchangizi B, Farokhzadian J, Abbaszadeh A. Mirzaee M, Borhani F. The importance of professional values from clinical nurses' perspective in hospitals of a medical university in Iran. BMC Med Ethics 2017;18(1):1–7.

[5] Torabizadeh C, Darari F, Yektatalab S. Operating room nurses' perception of professional values. Nurs Ethics 2019;26(6):1765–1776.

[6] Arries EJ. Professional values and ethical ideology: Perceptions of nursing students. Nurs Ethics 2019;27(3):726–740

[7] Çöplü M, Tekinsoy Kartın P. Professional self-concept and professional values of senior students of the nursing department. Nurs Ethics 2019;26(5):1387–1397.

[8] Acaroğlu R. Revize edilen hemşirelerin mesleki değerleri ölçeği Türkçe formunun güvenirlik ve geçerliği [Reliability and validity of Turkish version of the nurses professional values scale–revised]. Florence Nightingale Hemsire Derg 2014;22(1):8–16. (Turkish)

[9] Dehghani A, Mosalanejad L, Dehghan-Nayeri N. Factors affecting professional ethics in nursing practice in Iran: a qualitative study. BMC Med Ethics 2015;16(1):1–7.

[10] Shahriari M, Mohammadi E, Abbaszadeh A. Bahrami M, Marjaneh M Fooladi MM. Perceived ethical values by Iranian nurses. Nurs Ethics 2012;19(1):30–44.

[11] Al-Banna DA. Core professional and personal values of nurses about nursing in Erbil city hospitals: a profession, not just career. Nurse Care Open Acces J 2017;2(6):169–173.

[12] Koçak C, Albayrak SA, Büyükkayacı DN. Developing an attitude scale for nurses in caregiving roles: Validity and reliability tests. J Educ Res Nurs 2014;11(3):16–21.

[13] Pang D, Senaratana W, Kunaviktikul W, Klunklin A, McElmurry BJ. Nursing values in China: The expectations of registered nurses. Nurs Health Sci 2009;11(3):312–317.

[14] Altınbaş Y, İster DE. Hemşirelerin bakım verici rollerine ilişkin tutumları ve bireyselleştirilmiş bakım algıları [Nurses' attitudes towards caregiver roles and perceptions of ındividualized care]. STED 2020;29(4):246-254.

[15] Tuna R, Şahin, S. The effect of professional values of nurses on their attitudes towards caregiving roles. Int J Nurs Pract 2020;27(1):1-10.

[16] American Nurses Association (ANA). Code of Ethics for Nurses with Interpretive Statements. 2nd ed. Silver Spring, MD: ANA; 2015.

[17] Çetinkaya-Uslusoy E, Paslı-Gürdoğan E, Aydınlı A. Professional values of Turkish nurses: A descriptive study. Nurs Ethics 2017;24(4):493–501.

[18] Erkus G, Dinc L. Turkish nurses' perceptions of professional values. J Prof Nurs 2018;34(3):226–32.

[19] Weis D, Schank MJ. Development and psychometric evaluation of the Nurses Professional Values Scale-Revised. J Nurs Meas 2009;17(3):221–231.

[20] Gallegos C, Sortedahl C. An exploration of professional values held by nurses at a large freestanding pediatric hospital. Pediatr Nurs 2015;41(4):187-195.

[21] Kim K, Han Y, Kim JS. Korean nurses' ethical dilemmas, professional values and professional quality of life. Nurs Ethics 2015;22(4):467–478.

[22] Işık G, Egelioğlu Cetişli N, Tokem Y. Yılmaz D, İlhan A. Hemşirelerin bireysel ve profesyonel değerlerinde kuşaklararası farklılıklar [Personal and professional difference between the generation of nurses]. HSP 2018;5(1):72–80. (Turkish)

[23] Çulha Y, Acaroğlu R. The relationship amongst student nurses' values, emotional intelligence and individualised care perceptions. Nurs Ethics 2018;26(7-8):2373–2383.

[24] Sabancıoğlu S. Doğan S. Profesyonel kimlik gelişimi ve hemşirelik. [Professional identity development and nursing]

Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi 2012;15(4):275-282. (Turkish)

[25] Charalambous A, Katajisto J, Välimäki M, Leino-Kilpi H, Suhonen R. Individualised care and the professional practice environment: Nurses' perceptions. Int Nurs Rev 2010;57(4):500–507.

[26] Suhonen R, Gustafsson ML, Katajisto J. Välimäki M, Leino-Kilpi H. Nurses' perceptions of individualized care. J Adv Nurs 2010; 66(5):1035–1046.

[27] Shonen R, Papastavrou E, Efstathiou G. Lemonidou C, Kalafati M, Antunes da Luz D, Idvall E, Berg A, Racaroğlu R, Sendir M, Kanan N, Sousa VD, Katajisto J, Välimäki M, Leino-Kilpi H. Nurses' perceptions of individualized care: An international comparison. J Adv Nurs 2011;67(9):1895–1907.

[28] Gül Ş, Dinç L. Hastaların ve hemşirelerin hemşirelik bakımına yönelik algılarının incelenmesi. HUHEMFAD 2018;5(3):192-208. (Turkish)

[29] İnce S, Bingöl S. Acil serviste çalışan hemşirelerin hemşirelik bakım algıları. HUHEMFAD 2020;7(1):23-30. (Turkish)

[30] Björkström MN, Athlin EA, Johansson IS. Nurses' development of professional self–from being a nursing student in a baccalaureate programme to an experienced nurse. J Clin Nurs 2008;17(10):1380-1391.

[31] Karadaş A, Kaynak S, Duran S, Ergün S. Hemşirelerin profesyonellik düzeylerinin değerlendirilmesi: Balıkesir örneği. Balıkesir Sağlık Bil Derg 2018;7(3):74-80. (Turkish)

[32] Hisar KM, Oflaz A. Ameliyathane hemşirelerinde iş doyumu ve mesleki profesyonellik. Genel Tıp Derg 2019;29(4):175-182. (Turkish)

[33] Florell MC. Concept analysis of nursing activism. Nurs Forum 2021;56(1):134-140.

[34] Schmidt BJ, McArthur EC. Professional nursing values: A concept analysis. Nurs Forum 2018;53(1):69-75.

[35] Johnstone MJ. Nursing and justice as a basic human need. Nurs Philos 2011;12(1):34-44.

[36] Geyer NM, Coetzee SK, Ellis SM. Uys LR. Relationship of nurses' intrapersonal characteristics with work performance and caring behaviors: A cross-sectional study. Nurs Health Sci 2018;20:370-379.

[37] Karadağ A, Hisar F, Baykara ZG, Çalışkan N, Karabulut H, Öztürk D. A longitudınal study on the effect of tailored training and counseling on the professional attitude of nursing students. J Prof Nurs 2015;31(3):262-270.

[38] Karadağlı F. Profesyonel hemşirelikte etik. DUSBED 2016;6(3):197-200. (Turkish)

How to cite this article: Bakır H, Su S. The Relationship Between Nurses' Professional Values and Their Attitudes Towards Care Giving Roles: A Structural Equation Model. Clin Exp Health Sci 2022; 12: 765-771. DOI: 10.33808/clinexphealthsci.1019658

Clinical and Experimental Health Sciences

Interventions Preventing Osteoporosis in Primary Care: A Systematic Review

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Received: 05.11.2021 **Accepted:** 25.05.2022

ABSTRACT

Objective: The aim of this study was to conduct a systematic review to find interventions in primary health care that would increase osteoporosis prevention behaviors.

Methods: Systematic searches of CINAHL, Eric, Medline complete, PsycInfo, Ovid, Web of Science, Cochrane Library (N=1270). A total of 17 published articles met our inclusion criteria. English-language articles published between January 2000-May 2019, in primary care settings, participants with healthy or risk of osteoporosis, investigated osteoporosis preventing behaviors. The data extracted included population characteristics, diagnosis, mean age and setting, intervention and control groups, and outcomes of significance to the review question and specific objectives. In this review, a meta-analysis could not be performed due to the heterogeneity of the data.

Results: The majority of studies have been found to use multiple strategies to prevent osteoporosis. Eight studies focused only on the female gender and seven studies focused only on older adults. Compared to the control groups, it was found that the participants' osteoporosis knowledge increased significantly ($p<0.01$) with four interventions including prevention and self-management courses, counseling session, group discussion, various exercises, educational booklets, and motivational messages. Studies that assessed outcomes found that osteoporosis knowledge, calcium and vitamin D intake, bone mineral density, exercise, factors underlying behavior, quality of life, risk management, respectively.

Conclusion: Using educational interventions can improve osteoporosis knowledge, calcium-vitamin D intake, and bone mineral density among healthy or at-risk populations about osteoporosis in primary care. Behavior change provide also health promotion. Handling an individual's health beliefs, attitudes, and self-efficacy can facilitate behavior change.

Keywords: Intervention, osteoporosis, prevention, systematic review, primary care

1. INTRODUCTION

Osteoporosis (OP) is a systemic bone disease marked by decreased bone mass, degradation of bone tissue, disturbance of bone microarchitecture, and increased bone fragility (1). Osteoporosis is characterized only by a T score of less than 2.5 standard deviations below the mean Bone Mineral Density (BMD) of the young adult reference population (2).

It is estimated that OP affects millions of people around worldwide and fractures related to OP more than 8.9 million osteoporotic fractures occur yearly (3). OP and related health concerns are also important in Turkey. Although Turkey has a relatively lower incidence of osteoporosis compared to some European countries, the prevalence of osteoporosis increases with aging (4). According to Tüzün et al., the number of hip fractures will more than double in the next 25 years without modifying the age and gender risk variables (4). Furthermore, the prevalence of OP was shown to be higher in women than in men in various situations (4–6).

OP and the resultant fragility fractures have an impact in terms of mortality and morbidity on individuals, health care systems, and communities (7). According to National Osteoporosis Foundation, OP is responsible for billions of bone fractures and billions of economic burdens each year (8). In addition, the economic burden of OP-related fracture is significant, costing approximately $17.9 per annum in the USA (9). OP is a preventable and treatable disease, but we need to improve the awareness about the disease in general and the risk factors that lead to the problem (10–12).

Researches indicate that there are many modifiable and non-modifiable risk factors for OP and osteoporotic fractures. Major modifiable risk factors include poor nutrition, vitamin D deficiency, eating disorders, excessive alcohol consumption (>2 units daily), smoking, estrogen deficiency, insufficient exercise, fall risk, low body mass index (BMI<20 Kg/m^2), cigarette smoking, frequent falls. Major non-modifiable risk factors include history

DOI: 10.33808/clinexphealthsci.1019617

of falls, older age, women, white ethnicity, prior fracture, hormonal factors (13,14). The main goal of OP treatment is to prevent fractures. Pharmacological interventions for OP include calcium and vitamin D supplementation, hormone replacement therapy and bisphosphonates (9). In addition, robust screening programs are required (7).

OP and osteoporotic fractures can result permanent physical disability, decreased self-sufficiency, hospitalization, and an increased risk of death (15). Therefore, finding OP preventive behaviors require the need to decrease the risk of this disease. OP intervention is important to promoting bone health and improving OP preventive health behaviors (10). Consuming a healthy amount of calcium and engaging in weight-bearing exercise are two typical recommendations for lowering one's risk of developing OP (16). Education of people risk of OP and on various non-pharmacologic approaches to prevention and treatment that include nutrition, exercise, fall prevention and awareness of bone health, and would be vital for women of menopausal age and older (11).

Several interventions to minimize the risk of OP and hip fractures associated with OP have been described. The educational and counseling interventions aimed to promote OP knowledge and awareness, as well as with OP illness prevention. Calcium consumption, awareness, and self-efficacy are all improved through OP education initiatives.

OP education programs improve calcium intake, knowledge and self-efficacy (10,16,17). A multifaceted community-based intervention improved management of OP in high risk patients. This intervention includes bone mineral density testing, patient education and patient-tailored recommendations for OP treatment (18). Fall prevention exercise programs can reduce fall incidence and can decrease osteoporotic fracture (19). Providing hip protectors increases acceptance and adherence with hip protector use and giving education increases hip protector use in people living in the community (20). Using hip protectors lead to a reduction in hip fracture (21).

The aim of this study was to conduct a systematic review in order to find interventions in primary health care that would increase OP prevention behaviors.

2. METHODS

2.1. Search Strategy

A preliminary search was conducted in the following databases: CINAHL, Eric, Medline complete, PsycINFO, Ovid, Web of Science, Cochrane Library. The search was limited to original 18 years and older age, English articles that appeared in publications from January 2000 to May 2019. Search terms were as follows: [Osteoporosis OR "osteoporosis preventing behaviors" OR "bone mineral density" OR "dual energy x-ray absorptiometry"] AND [intervention OR program OR "health education"] AND ["primary care" OR "general practice" OR "family medicine" OR "primary care clinic" OR "community settings"].

All citations were imported into an Endnote version X9 and duplicates removed. Titles and abstracts were screened by search terms. Two researchers evaluated the results independently of each other, considering the inclusion and exclusion criteria.

2.2. Study Selection and Data Extraction

This review includes individuals aged 18 years and older who are healthy or at risk of OP.

The educational programs were defined as interventions, planned to improve knowledge, self-efficacy, health beliefs, self-monitoring, awareness of adults with OP screening, compliance of taking calcium and D vitamin, adherence with use of hip protectors, risk management, exercise, nutrition, prevention of the disease. Primary outcome reported OP preventing behaviors.

Studies were included if they (a) performed with adults and older adults at risk of OP (b) were recruited from primary care settings (a general practice, family practice, or primary care clinic, community settings) (c) were either randomized controlled trials (RCTs), quasi-experimental, or pretest-posttest studies, (d) investigated OP preventing behaviors (e) in English language (f) conducted with January 2000-May 2019. Studies were excluded if they existed a confirmed diagnosis of OP or osteopenia or taking medication related to OP. The result of the search and selection process are shown in Figure 1.

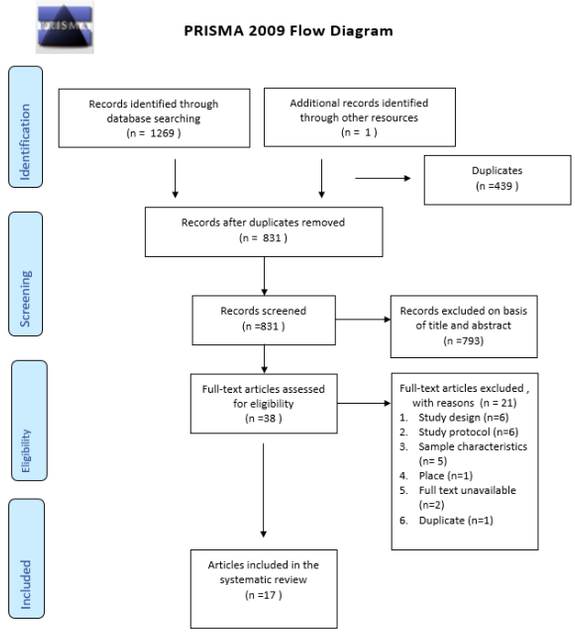

Figure 1. *Flow the diagram for study selection according to PRISMA*[22]

One researcher extracted data from each article by the Joanna Briggs Institute (JBI) SUMARI data extraction instrument. The data extracted included about population characteristic, diagnosis, mean age and setting, intervention and control groups, and outcomes about review question (see Table 1).

Table 1. *Characteristics of included studies*

References	Population	Groups	Follow-up	Outcome	Result
Randomized Controlled Trial					
Babatunde et al.	**Diagnosis:** at risk of OP **Mean age:** 70.2 **Male/Female:** 11/99 **Setting:** churches and community-senior centers	**Intervention group:** Osteoporosis education program n=59 6 weekly education program sessions. Short presentations/lectures, hands on activities and demonstrations involving the participants. Intervention guided by Revised Health Belief Model. **Control group:** Waiting list n=51	None	1.Calcium intake 2.Knowledge 3.Health belief 4.Self-efficacy	1.+p<0.001 2.+p<0.001 3.-p>0.05 4.+p<0.001
Birks et al.	**Diagnosis:** women with risk hip fracture **Mean age:** 78 **Male/Female:** 0/4168 **Setting:** primary care	**Intervention group**: Hip protectors sending mail three pairs of hip protectors along with instructions on how to use them and receiving a leaflet about other methods of reducing their fracture risk. n=1387 **Control group:** Sending mail leaflet n=2781	12 months 18 months 24 months	1.Hip fractures 2.Falling 3.Fear of falling	1: – p=0.40 2:+ p<0.001 (12 months) 2:+ p<0.01 (24 months) 3:+ p=0.003 (12 months) 3: – p=0.07 (18 months) 3:+p=0.04 (24 months)
Blaloc et al.	**Diagnosis:** women with menopause **Mean age:** 47.0±4.40 **Male/Female:** 0/547 **Setting:** twelve counties	**Intervention group I:** Tailored educational intervention includes two packet of written materials and one brief telephone counseling session**.** n=114 Intervention guided by Precaution Adoption Process Model. **Intervention group II:** Community-based intervention includes establishing an Osteoporosis Resource Center, conducting a workshop on osteoporosis prevention, and offering free bone density screening. n=159 **Control group (I):** n=105 **Individual level,** Nontailored education, received two packets of informational materials. No information specific to the individual participant. **Control group (II):** n=169 Community level, nontailored education.	3 months 6 months 12 months	1.Calcium intake 2.Exercise level Stage of change was assessed as a moderating variable. (unengaged, engaged action)	**Tailored intervention** 1: – p>0.05 **unengaged** 1: – p>0.05 **engaged** (baseline) 1: – p<0.10 (3 months) 1: + p<0.01 (6 months) 1: – p>0.05 (12 months) 1: – p>0.05 **action** (baseline) 1:+ p<0.05 (3 months) 1: + p<0.05 (6 months) 1: + p<0.05 (12 months) **Community intervention** 1: – p>0.05 **unengaged**(baseline) 1:+p<0.05 **(**3 months) 1: – p<0.10 (6 months) 1: – p>0.05 (12 months) 1: + p<0.01 **engaged** (baseline) 1: – p>0.05 (3-6-12 months) 1: – p>0.05 **action** (baseline-3-6 months) 1: – p<0.10 (12 months) **Tailored intervention** 2: – p>0.05 **unengaged** 2: – p>0.05 **engaged** 2: – p<0.10 **action** (baseline) 2: – p>0.05 (3-6-12 months) **Community intervention** 2: – p<0.10 **unengaged**(baseline) 2: – p>0.05 (3-6 months) 2: – p<0.10 (12 month) 2:+p<0.05 **engaged**(baseline) 2: – p>0.05 (3-6-12 months) 2: – p>0.05 **action** (baseline-3-6-12 months)

Table 1. *(Continued)*

Cameron et al.	**Diagnosis:** People at high risk of hip fracture **Age:**75 years or more **Sex:** Unclear **Setting:** Community and hospital trial	**Intervention group I:** The no cost group-who were fitted with free hip protectors (Community) n=58 **Intervention group II:** Community combined group-received free hip protectors and educational sessions about their use n=60 Intervention group III: The no cost group-who were fitted with free hip protectors (hospital) n=106 **Intervention group IV:** Hospital combined group-received free hip protectors and educational sessions about their use. n=99 **Control group I:** Provided a brochure about hip protectors (Community) n=53 **Control group II:** Provided a brochure about hip protectors (Hospital) n=103	3 months 6 months	1.Adherence with use of hip protectors(mean percentage) 2.Number of Falls: (mean per participant) 3.Number of fractures: hospital 4. Number of fractures :community 5.Number of hospitalizations: hospital 6. Number of hospitalizations: community	1: + p<0.001 (3-6 months) (comparison within hospital) 1: + p=0.000 (3-6 months) (comparison within community) 1:+p<0.001 (3-6 months) (comparison between hospital and community) 2:+p=0.010 (6 months) (comparison within hospital) 2:-p=0.356 (comparison within community) 2:-p=0.26 (comparison between hospital and community) 3: 1 (hospital control) 3: 5 (hospital no cost) 3:1 (hospital combined) 4: 0 (community control) 4: 2 (community no cost) 4: 0 (community combined) 5: 0 (hospital control) 5: 3 (hospital no cost) 5: 2(hospital combined) 6: 0 (community control) 6: 1 (community no cost) 6: 0 (community combined)
Ciaschini et al.	**Diagnosis:** Patients at risk for osteoporosis and fractures **Mean age:** 71.9±7.2 **Male/Female:** 12/188 **Setting:** Community dwelling	**Intervention group:** A multifaceted community-based care program. This group received personalized counseling about osteoporosis, provided written OS management plan and received educational materials on calcium and vitamin D, risk factors for OS and their BMD results. n=101 **Control group:** Usual care n=100	12 months	1.OS risk management 2.OS management 3.Quality of life	1:-p>0.05 2:-p=0.48 3: I: – p=0.58 C= – p=0.26
Cleghorn et al.	**Diagnosis:** women who are within five years of the menopause **Mean age:** Group 1: 52.2 Group 2: 51.8 **Male/Female:** 0/115 **Setting:** Community	**Intervention group I:** Received a supplement of 3L of calcium-fortified milk weekly in the first year In the second year, reverted to their usual diets. n=115 **Intervention group II:** Followed their usual diets in the first year. In the second year, received the milk supplement.	2 years	1.BMD at spine 2.BMD at forearm 3.Fasting urine levels	1:+p=0.006 2.-p>0.05 3. +p=0.03
Duckham et al.	**Diagnosis:** older adult **Mean age:** 72±5 **Male/ Female:**128/191 **Setting:** primary care	**Intervention group I:** Otago exercise program (OEP) consist of three 30-min home exercise session and at least two 30-min session of walking each week. Each participant received an instruction booklet, followed up two home visits and eight telephone calls. n=88 **Intervention group II:** Falls exercise management (FAME) consist of one 60-min exercise class, two 30-min home exercise sessions and two 30-min sessions of walking each week. n=105. **Control group:** Usual care n=126	6 months	1.BMD at femoral neck 2.BMD at other skeletal sites	1:-p=0.44 (OEP) 1:-p=0.53 (FAME) 2:-p>0.05

Table 1. *(Continued)*

Francis et al.	**Diagnosis:** people with risk factors **Mean age:** 63 **Sex:** 92% female **Setting:** Community	**Intervention group:** The Osteoporosis Prevention and Self-Management Course (OPSMC) consist of four weekly sessions which run for 2h and are led by two facilitators. n=103 Control group : Wait list n=95	6 weeks	1.Knowledge 2.Self-efficacy: exercise 3.Self-efficacy:Ca 4.Self-monitoring 5.Health-directed behavior	1:+p<0.001 2:-p=0.583 3:-p=0.711 4:-p=0.057 5:+p=0.020
Kalkim and Daghan	**Diagnosis:** risk of osteoporosis **Age range:**30-45 years **Male/Female:** 0/73 **Setting:** Family health center	**Intervention group:** An osteoporosis prevention program consists of a 4-week education program and a 24-week counseling program. Intervention guided by Health Belief Model. n=37 **Control group:** Usual care n=36	3 months 6 months	1.Knowledge 2.Health belief 3.Self efficacy 4.Calcium intake 5.Exercise	1: +p<0.001 2: +p<0.001 3: +p<0.001 4: +p<0.001 5: +p<0.001
Kloseck et al.	**Diagnosis:** older adults and risk of osteoporosis **Mean age:** 80.5±6.9 **Sex:** 88.6% female **Setting:** retirement community	**Intervention group:** Peer-led osteoporosis education and mentorship program. n=53 **Control group:** Usual care n=52	6 months	1.Behavior-BMD 2.Behavior-Vitamin D 3.Knowledge	1: +p<0.001 2: +p=0.02 3: – p=0.21
Kukuljan et al.	**Diagnosis:** healthy older man **Age range:**50-79 **Male/Female:** 180/0 **Setting:** community dwelling	**Intervention group I:** Exercise + milk group n=45 **Intervention group II:** Exercise group n=46 **Intervention group III:** Milk group n=45 **Control group :** Usual care n=44 **Exercise program:** warm-up and cool down, cycling and stretching, resistance training exercise, weight-bearing exercise **Milk group**: Calcium-vitamin D fortified milk	12 months 18 months	1.BMD 2.Take calcium and Vitamin D 3.Serum PTH	1:+p<0.01 (Lumbar spine) 2: +p<0.001 (greater in fortified group compared with nonfortified group) 3:-p>0.05 (12 months) (exercise-fortified milk group) 3: +p<0.05 (18 months) (a significant decrease exercise group compared to non-exercise group)
Levy et al.	**Diagnosis:** 65 years and older women **Mean age:**74.0 **Male/Female:**0/204 **Setting:** family physician	**Intervention group I:** Chart reminder n=102 **Intervention group II:** Patient education + chart reminder n=62 **Control group :** Usual care n=31	14 months	1.the rate of BMD	1:+p<0.029
Piaseu et al.	**Diagnosis:** students **Mean age:** 18,5 **Male/Female:**0/100 **Setting:** Nursing school	**Intervention group I:** Osteoporosis educational program (3 hours) This program consists of instructional materials and slide presentation n=50 **Control group :** wait list n=50	2 weeks	1.Knowledge 2.Attitude 3.Self-efficacy	1: + p<0.01 2: + p<0.01 3: + p<0.01
Hien et al.	**Diagnosis:** postmenopausal women **Age range:** 55-65 years **Male/Female:** 0/108 **Setting:** rural communes	**Intervention group:** Nutrition education (provided with visual education materials such as posters, leaflets, booklet and video tape) n= 57 **Control group :** Usual diet (provided education at the end of data collection period) n= 51	18 months	1.Calcium intake 2.Bone mass 3.Serum PTH	1: + p<0.01 2: + p<0.01 3: + p<0.01
Woo et al.	**Diagnosis:** elderly people **Age range:** 65-74 **Male/Female:**90/90 **Setting:** Community	**Intervention group I**: Tai Chi group n=60 **Intervention group II:** Resistance exercise group n=60 **Control group:** No exercise n=60	12 months	1.BMD 2.Muscle strength 3.Balance and flexibility 4.Falls	1: +p<0.05 (women-hip) 1:-p>0.05 (men-hip) 1:+p<0.05 (comparison between intervention and control group) 2:-p>0.05 3:-p>0.05 4:-p>0.05

Table 1. *(Continued)*

Yuksel et al.	**Diagnosis**: ≥ 65 or 50-64 years with one major risk factor **Mean age:** 62 **Male/Female:** 2/3 female **Setting:** community pharmacy	**Intervention group :** Multifaceted intervention (screening and patient education) printed materials, education and quantitative ultrasound n=129 **Control group:** Usual care n=133	16 weeks	1.BMD 2.Medication 3.Calcium intake 4.Vitamin D 5.Knowledge 6.Quality of life	1:+p=0.011 2:-p=0.30 3:+p=0.011 4:-p=0.66 5:-p>0.05 6:-p>0.05
Quasi Experimental Study					
Law and Shapiro	**Diagnosis:** frail elderly **Mean age:** 63.9±12.1 **Male/Female:** 0/116 **Setting:** community pharmacy	**Intervention group:** Screening and awareness program. Educational brochure, providing Ca supplements (Citracal), follow-up phone call. n=116 **Control group:** none	A week	1.Awareness 2.Self-rated risk	1:+p<0.001 2:-p>0.348

OP: Osteoporosis
BMD: Bone mineral density

2.3. Data Syntesis

Due to the variability of the individual's socio-demographics, intervention duration, and outcome assessments, meta-analysis was not possible. Using the JBI's established critical appraisal instruments, two researchers independently assessed papers for methodological quality for Randomized Controlled Trials (RCTs) and Quasi-Experimental studies (22) (Table 2 and Table 3). Scores on the critical appraisal instruments were determined maximum 13 points for RCTs and 9 points for quasi-experimental studies.

3. RESULTS

In all, 1270 articles were retrieved and appraised from our systematic review. A total of 17 published articles met our inclusion criteria (Figure 1) (23). Sixteen interventions were RCTs and one was quasi-experimental design (Table 1).

3.1. Methodological Quality

Assessment of methodological quality was carried out by two independent reviewers for the 16 RCTs and one Quasi-Experimental studies. Only two studies adopted allocation concealment (20,24). Six studies were high, with scores ranging from nine (18,21,25) to ten (20,24,26) in RCTs, one study score was eight (11) in quasi-experimental study. The methods of randomization were reported in ten studies. These studies were used block randomization (24,26), computer-randomization system (21,25,27), concealed number envelopes with the randomization sequence from a random numbers table (20,21), drawing lots (28), a random number generator (12,29). The three trials (10,17,18) did not report how they achieved randomization. The three trails (19,30,31) randomization process were unclear (Table 2 and Table 3).

Table 2. *Critical appraisal results for randomized controlled trials*

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Score
Babatunde et al.	Y	U	Y	U	U	U	Y	N	Y	U	Y	Y	Y	7/13
Birks et al.	Y	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	9/13
Blalock et al.	Y	U	Y	U	U	U	Y	Y	N	Y	Y	Y	Y	8/13
Cameron et al.	Y	Y	N	U	U	Y	Y	Y	Y	Y	Y	Y	Y	10/13
Ciascihinni et al.	Y	N	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	9/13
Cleghorn et al.	U	U	N	U	U	U	Y	Y	N	Y	Y	Y	Y	6/13
Ducham et al.	U	N	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	8/13
Francis et al.	Y	U	Y	U	U	U	Y	Y	N	Y	Y	Y	Y	8/13
Hien et al.	U	N	Y	N	N	N	Y	Y	N	Y	Y	Y	Y	7/13
Kalkim&Daghan	Y	N	Y	N	N	N	Y	Y	N	Y	Y	Y	Y	8/13
Kloseck et al.	Y	U	Y	N	U	U	Y	Y	N	Y	Y	Y	Y	8/13
Kukuljan et al.	Y	U	Y	U	U	U	Y	Y	Y	Y	Y	Y	Y	9/13
Levy et al.	Y	U	Y	U	U	U	Y	Y	N	Y	Y	Y	Y	8/13
Piaseu et al.	Y	U	N	N	N	N	Y	NA	N	Y	Y	Y	Y	6/13
Woo et al.	Y	Y	N	Y	Y	U	Y	Y	N	Y	Y	Y	Y	10/13
Yuksel et al.	Y	N	Y	U	U	Y	Y	Y	Y	Y	Y	Y	Y	10/13

Y=Yes, N=No, U=Unclear, NA=Not applicable

JBI critical appraisal checklist for randomized controlled trials: Q1: Was true randomization used for assignment of participants to treatment groups? Q2:Was allocation to treatment groups concealed? Q3: Were treatment groups similar at baseline? Q4: Were participants blind to treatment assignment? Q5: Were those delivering treatment blind to treatment assignment? Q6:Were outcome assessors blind to treatment assignment? Q7: Were treatment groups treated identically other than the intervention of interest? Q8: Was follow-up complete, and if not, were strategies to address incomplete follow up utilized? Q9: Were participants analyzed in the groups to which they were randomized? Q10:Were outcome measured in the same way for treatment groups? Q11: Were outcomes measured in a reliable way? Q12: Was appropriate statistical analysis used? Q13: Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?

Table 3. *Critical appraisal results for quasi-experimental study*

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Score
Law& Shapiro	Y	Y	Y	N	Y	Y	Y	Y	Y	8/9

Y=Yes, N=No, U=Unclear, NA=Not applicable

JBI critical appraisal checklist for quasi-experimental studies (non-randomized experimental studies): Q1: Is it clear in the study what is the "cause" and what is the "effect" (i.e., there is no confusion about which variable comes first?) Q2: Were the participants included in any comparisons similar? Q3: Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? Q4: Was there a control group? Q5: Were there multiple measurements of the outcome both pre and post the intervention/exposure? Q6: Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed? Q7: Were the outcomes of participants included in any comparisons measured in the same way? Q8: Were the outcomes of participants included in any comparisons measured in the same way? Q9: Was appropriate statistical analysis used?

3.2. Intervention Participants

The mean age of the participants ranged from 18.5 (12) to 87 years (32). Of the 17 intervention studies, seven included both gender adult (10,18,19,24,26,27,32) eight included female-only samples participants (11,12,17,21,28–31) one included unclear and one included male-only samples adult participants (25). The sample sized ranged from 73 (28) to 4168 (21). Of the included studies, seven studies involved people with risk of OP or/and with risk of fracture related to OP (10,18,20,21,27,28,32), three studies involved people with menopausal women (17,30,31), seven studies involved people with older adult (11,19,24–26,29,32) one study involved people with students (12), one study involved people with healthy older man (25).

3.3. Intervention Settings

The included studies were conducted in Australia (20,25,27,30), Canada (18,26,32), China (24), Thailand (12), Turkey (28), UK (19,21), USA (10,11,17,29), Vietnam (31). Of the included studies, two included community-dwelling (18,25) one included nursing school (12), two included community pharmacies (11,26), eight included community (10,17,20,24,27,30–32), four included primary care (19,21,28,29).

3.4. Application of Theories or Models

Of the included studies, model was used in four studies and theory was not used in any study. These were two studies using Health Belief Model (10,28), one study using Precaution Adoption Process Model (17), one study using peer-support model (32).

3.5. Characteristics of Interventions

In all studies were implemented educational approaches improve preventing behaviors for OP. These approaches included short slide presentations/lectures, hands-on activities (e.g. pamphlet, brochure), demonstrations involving the participants (e.g. exercise), group discussion, provided printed materials and management plan (10,12,18,25,28,30).

3.6. Evidence of Intervention Effect on Outcome

3.6.1. Change Knowledge

Six studies examined OP knowledge after education interventions (10,12,26–28,32). There was a statistically significant difference in OP knowledge between in intervention group and the control group in four studies (10,12,27,28). These educational programs included slide presentation (10,12,28), group discussion, demonstration about exercise (12), hands on activities (10,28), CD about exercise, magnet board, educational booklet and motivational message (28). Length of the intervention ranged from 6 weeks or less (13, 26, 29) to 24 weeks (28). Other two studies did not find any difference in OP knowledge in groups ($p>0.05$) (25, 32). One of these studies included peer-led community education and mentorship program in six months follow up period (32).

3.6.2. Ca and Vitamin D Intake

After the interventions, seven studies focused at calcium and/or vitamin D intake (10,17,25,28,31,32). Six study found a statistically significant difference in calcium consumption between the intervention group and the control group in six studies (10,25,26,28,31). Calcium intake in the intervention group improved significantly ($p<0.001$) in two studies (10,28). In this trials, the intervention group received instruction regarding the benefits of calcium intake (10,28), the effect on bone, the calcium content of foods given (28), and the barriers to lowering risk factors related to calcium and vitamin D intake (10). The intervention group demonstrated statistically significant progress in calcium intake ($p<0.01$) after the 18-month nutrition education intervention compared to the control group (31). Visual education materials were distributed to the participants (e.g. posters, leaflet, booklet and video tape) (31). In addition, the same study reported that serum Parathyroid hormone (PTH) levels decreased by 12% in intervention group ($p<0.01$) (31). Kukuljan et al. (2011) reported a significant improvement calcium and vitamin D intake in the fortified group compared with non-fortified group at 12-18 months follow-up ($p<0.001$) (25). Yuksel et al. (2010) study reported that compare with control group, calcium intake in intervention group significantly improved ($p=0.011$), however there was no statistical significantly change vitamin D intake ($p=0.66$) (26).

3.6.3. Bone Mineral Density (BMD) or Bone Mass

After education interventions, eight studies examined bone mineral density or bone mass (18,19,24–26,29–32). Seven studies found a statistically significant difference between the intervention group and control group (e.g. printed materials,

education and ultrasounography or routine care) in BMD behavior, BMD test, bone mass, and BMD rate (24–26,29–32). The speed of sound (SOS (m/s) was measured using QUS instrument, and it was shown to be lowered by 0.5 percent in controls after 18 months (p<0.01) (31). Also, Cleghorn et al (2001) study measured bone mineral density by an XR-36 Quickson dual-energy x-ray densitometer at the lumbar spine and forearm at the first appointment and one and two years later. Using supplementing diet with calcium-fortified milk early in postmenopausal period delays bone loss at the spine (p=0.0006) (30). Another study measured femoral neck or other skeletal sites BMD results however no such effects were found for BMD (p>0.05) (19). Kloseck et al. (2017) reported a significant difference with regard to change in OP behavior (defined as obtaining BMD assessment, returning to their family physician and obtaining BMD results) compared with intervention group and control group (p<0.001) (32). The study to include weight bearing activities or resistance training by Kukuljan et al. (2011) was reported a significant net gains in femoral neck BMD compared the groups following the intervention period. In addition, increased BMD was reported in all intervention groups (exercise + milk or exercise or milk group) (p<0.01) (25). In Levy et al. study with only women older adults were of four groups that chart reminder (CR) alone, chart reminder plus mailed patient education (CR+ PtEd) and one to usual care. After the interventions, the rate of BMD testing were 45.2% in CR+ PtEd, 31.4% in CR and 9.7% in the usual care. The effect of CR+ PtEd group increased the rates of BMD (p<0.029) (29). The study to include Tai Chi or resistance exercise by Woo et al. (2007) was reported a significant change percentage change of BMD at the hip and spine comparison between the exercise and control groups (p<0.05) (24). Yuksel et al. (2010) reported that increase BMD testing in the multifaceted intervention group for 16 weeks (p=0.011) (26).

3.6.4. Exercise

Exercise was measured in three studies (17,24,28). Only one study reported a significant progress in exercise group compared to usual care group. This study provided CD about exercise for participants aged 30-45 (28). Other studies found no statistically significant effect on exercise (17), muscle strength, balance and flexibility (p>0.05) (24). Interventions in both studies consisted of behavioral exercise card (17), tai chi and resistance exercise (24).

3.6.5. Factors Underlying Behavior

In the study of Piaseu et al consisting of student participants health belief, attitudes and confidence in exercise and calcium intake had significant increase in the intervention group compared control group (p<0.01) (12). Babatunde et al. (2011) reported that a significant difference with regard to OP self-efficacy between the two groups for calcium intake (p<0.001) (10). In addition to the same study found no statistically significant effect on health belief with regard to OP prevention (p>0.05) (10). Kalkim and Dağhan (2017) reported that combined health belief and self-efficacy about OP was a significant increase (p<0.001) (28). One study reported that the OP screening and awareness program (e.g. educational brochure, providing Ca supplements, follow up phone calls) attendance improved OP awareness (p<0.001) however no such effects were found self-rated risk about OP (p>0.348) (11). Calcium self-efficacy were measured by Francis et al. (2009), using intervention about nutrition focus on calcium and vitamin D at 6 weeks, this study found no statistically significant effect on calcium self-efficacy (27). During follow-up, the same study found significant improvements in health-directed behavior, positive and active life engagement, skill and technique acquisition, and social integration and support (27).

3.6.6. Quality of Life

Only two studies assessed quality of life (18,26). One study intervention consists of personalized counseling, a management plan and educational materials on calcium, vitamin D intake, and OP risk factors. The immediate intervention protocol group (p=0.58) and delayed intervention protocol group/control group (p=0.26) had similar quality of life (18). Another study found no statistically significant (p>0.05) effect on quality of life (26).

3.6.7. Risk Management

Ciaschini et al. (2010) study conducted in community dwelling and 94% of the participants in this study were women. Participants in the intervention group increased pharmacological treatment, calcium and vitamin D intake. However, this study found no statistically significant difference in OP management (p=0.48) and risk management (p>0.05) (18). Cameron et al. (2011) study performed with 75 years or more individuals that having high risk of hip fracture in community and hospital settings. The study included into three groups. Control group received a brochure on hip protectors, intervention group-1 was given free hip protectors, intervention group-2 received free hip protectors and education about their use. The study reported that using of hip protectors was higher in the community setting at the time of the 3-month follow-up visit (p<0.001) (20). One study found no statistically significant difference on falls between either intervention or controls after 12 months (p>0.05) (24). Birks et al. (2004) study conducted in primary care and all of the participants in this study were women. Participants in the intervention group received three pairs of hip protectors along with instructions on how to use them and also a leaflet about fracture risk decrease methods. During follow-up period hip fracture status was similar between intervention and control groups (p=0.40) (21).

4. DISCUSSION

Osteoporosis and its related fractures are gradually becoming a global epidemic because of increase aging population. The

present review provides a first attempt to systematically review to preventive interventions for OP in primary care. While several risk factors cannot be modified, nutrition, calcium and vitamin D intake, exercises play a key role in bone health promotion and prevention of OP (33). Therefore, bone health education programs are an essential measure to prevent OP. Most of the interventions included our review were multifaceted, targeted participants at healthy people or at-risk/high risk patients and provided by physicians, nurses or community pharmacist.

Osteoporosis prevention programs that have been published varied greatly, but only four studies used model in this review. The usage of a model is known as useful in guiding interventions, understanding behavior change (10) and factors related to this behavior (10,28). Similarly, theories guide the techniques used to understand the conditions that affect behavior change and to achieve the expected goals of the interventions which will provide this change (34). It may be beneficial to increase the model or theory based interventions that improved attitude and self-efficacy in changing behaviors such as screening and nutrition.

The mean age of the participants, sample sizes, interventions variety and duration of follow up changed. Most studies focused only female participants (11,17,21,28–31) and older adults (11,19,24–26,29,32). The prevelance of osteoporosis is known to be higher in women than in men (4,5). However, this does not mean that OP is an insignificant disease in men. Rinonapoli et al. review (2021) explains why researchers and physicians should care about OP in men. It has indicates that secondary osteoporosis is more common in men, as OP is perceived as a female disease (35), and men thus benefit less from preventive approaches (36). In addition, being women and having older age are among the important risk factors in OP and its related fractures (13,14). It seems that this trend also continues in most of the studies included in this systematic review. Studies on younger participants have determined having insufficient knowledge and awareness about osteoporosis risk factors (12,28). In future studies, it should be considered that an increase in interventional studies focusing on the male gender and younger population will be beneficial in the prevention of osteoporosis.

The current review showed positive effects OP knowledge for the intervention group in four studies (10,12,27,28), although there were no significant differences between intervention and control groups in two studies (26,32). On the other hand, Kloseck et al. (2017) study reported statistically significant change behavior BMD and vitamin D intake (32). If a peer educator or mentoring programs is planned, criteria such as peer educators or mentors' education level, age, willingness to volunteer should be taken into consideration. Sava et al. (2020) study found that female community leaders had knowledge of OP and its risk factors, increase levels of health motivation and decrease levels of perceived barriers toward behavioral change (37). Knowing OP and its associated risk factors plays an important role in revealing behavioral change. In addition, factors underlying behavior affect OP knowledge. Bordes et al. (2020) study reported that participants had inadequate knowledge about OP, its risk factors and prevention. In addition des Bordes et al. (2020) study found that many misconceptions and concerns about medication side effects or fear of dependence (35). Nurses and physicians can play a leading role OP prevention and awareness.

Pinar et al (2017) had conducted a cross-sectional study (n=1792) to determine the prevalence of OP and associated risk factors in Turkish women aged 18-49 years. Pinar's study reported that most participants were at low risk of developing OP, %6.9 were at medium to high risk of developing the disease. From BMD levels measure by DXA 33.3% were osteopenia, %4.0 were osteoporotic (38). Current review found that seven studies reported that there was a statistically significant difference in the BMD behavior, BMD test, bone mass and the rate of BMD between the intervention group and the control group (e.g. printed materials, education and ultrasound or usual care) (24–26,29–32). Similarly, in the literature showed that interventions consisting in education and follow-up significantly improved BMD testing (39,40). These results indicated that necessary public health strategies might be beneficial not only for the risk group but also healthy individuals in protecting and improving bone health.

Weaver et al (2016) review reported the evidence of a positive effect of physical activity on bone mass and density as strong (41). Shoja et al (2020) review confirmed the significant positive effects of dynamic resistance exercise on BMD in post-menopausal women (42). In this systematic review found that exercise was measured only three studies (17,24,28). One study reported a significant improvement in exercise in the intervention group compared to control group (28). These studies used exercise CD for participants (28), behavioral exercise card (17), tai chi and resistance exercise (24).

5. CONCLUSION

Our systematic review contributes to the current knowledge of educational and multifaceted interventions for OP preventing behaviors. Using educational interventions can improve osteoporosis knowledge, calcium-vitamin D intake and bone mineral density among healthy or at risk populations about osteoporosis in primary care. Behavior change provide also health promotion. Handling individual's health beliefs, attitudes, and self-efficacy can facilitate behavior change. The results inform primary care physicians, nurses and community pharmacist assist in deciding on early interventions to prevent OP and its related fractures. Future studies may be focus on younger, male gender and healthy adults to improve bone health. Furthermore, it may be beneficial to increase the model or theory based interventions that improved attitude and self-efficacy in changing behaviors such as screening and nutrition. Exercise, quality of life and risk management are included in a few several interventions in this review. Therefore, researchers should take into consideration also with these issues.

This systematic review has some limitations. Meta-analysis could not be performed due to heterogeneity of the participants' socio-demographics factors, duration of intervention and outcome measurements. Despite this limitations, we believe this review provides brief and informative interventions that can be adapted in practical life.

Ethical approval

Not applicable.

Funding

The authors received no financial support of this article.

Conflict of interest

None

REFERENCES

[1] NIH consensus development panel on osteoporosis prevention, diagnosis and therapy. JAMA 2001;285(6):785–95.

[2] Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9(8):1137–1141.

[3] Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med 1993;94(6):646–650.

[4] Tuzun S, Eskiyurt N, Akarirmak U, Saridogan M, Senocak M, Johansson H, Kanis JA. Incidence of hip fracture and prevalence of osteoporosis in Turkey: the FRACTURK study. Osteoporos Int 2012;23(3):949–955.

[5] Keskin Y, Çekin MD, Gündüz H, Lüleci NE, Giray E, Sur H, Akyüz G. The prevalence of osteoporosis in the thrace region of Turkey: A community-based study. Turk J Phys Med Rehabil 2014;60(4):335–340.

[6] Kucukler FK, Simsek Y, Turk AÇ, Arduc A, Guler S. Osteoporosis and silent vertebral fractures in nursing home resident elderly men in Turkey. J Clin Densitom 2017; 20(2):188–195.

[7] Clynes MA, Harvey NC, Curtis EM, Fuggle NR, Dennison EM, Cooper C. The epidemiology of osteoporosis. Br Med Bull 2020;133(1):105–117.

[8] NOF. Learn What Osteoporosis Is and What It's Caused by. National Osteoporosis Foundation 2018 May. Available from URL: https://www.nof.org/patients/what-is-osteoporosis/

[9] Hernlund E, Svedbom A, Ivergård M, Compston J, Cooper C, Stenmark J, McCloskey EV, Jönsson B, Kanis JA. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos 2013;8(1):136.

[10] Babatunde OT, Himburg SP, Newman FL, Campa A, Dixon Z. Theory-driven intervention improves calcium intake, osteoporosis knowledge, and self-efficacy in community-dwelling older Black adults. J Nutr Educ Behav 2011;43(6):434–440.

[11] Law A V., Shapiro K. Impact of a community pharmacist-directed clinic in improving screening and awareness of osteoporosis. J Eval Clin Pract 2005;11(3):247–255.

[12] Piaseu N, Belza B, Mitchell P. Testing the effectiveness of an osteoporosis educational program for nursing students in Thailand. Arthritis Care Res 2001;45(3):246–251.

[13] Dontas IA, Yiannakopoulos CK. Risk factors and prevention of osteoporosis-related fractures. J Musculoskelet Neuronal Interact 2007;7(3):268–272.

[14] Pouresmaeili F, Kamalidehghan B, Kamarehei M, Goh YM.A comprehensive overview on osteoporosis and its risk factors. Ther Clin Risk Manag 2018;6(14):2029–2049.

[15] Leboime A, Confavreux CB, Mehsen N, Paccou J, David C, Roux C. Osteoporosis and mortality. Jt bone spine 2010;77 (2):107-112.

[16] Pai M V. Osteoporosis prevention and management. J Obstet Gynecol India 2017;67(4):237–242.

[17] Blalock SJ, DeVellis BM, Patterson CC, Campbell MK, Orenstein DR, Dooley MA. Effects of an osteoporosis prevention program incorporating tailored educational materials. Am J Health Promot 2002;16(3):146–156.

[18] Ciaschini PM, Straus SE, Dolovich LR, Goeree RA, Leung KM, Woods CR, Zimmerman GM, Majumdar SR, Spadafora S, Fera LA, Lee HN. Community based intervention to optimize osteoporosis management: Randomized controlled trial. BMC Geriatr 2010;10(1):60.

[19] Duckham RL, Masud T, Taylor R, Kendrick D, Carpenter H, Iliffe S, Morris R, Gage H, Skelton DA, Dinan-Young S, Brooke-Wavell K. Randomised controlled trial of the effectiveness of community group and home-based falls prevention exercise programmes on bone health in older people: The ProAct65+ bone study. Age Ageing 2015;44(4):573–579.

[20] Cameron ID, Kurrle S, Quine S, Sambrook P, March L, Chan D, Stocks J, Lockwood K, Cook B, Schaafsma. Increasing adherence with the use of hip protectors for older people living in the community. Osteoporos Int 2011;22(2):617–626.

[21] Birks YF, Porthouse J, Addie C, Loughney K, Saxon L, Baverstock M, Francis RM, Reid DM, Watt I, Torgerson DJ, Primary Care Hip Protector Trial Group. Randomized controlled trial of hip protectors among women living in the community. Osteoporos Int 2004;15(9):701–706.

[22] Tufanaru C, Munn Z, Aromataris E, Campbell J, Hopp L. Systematic Reviews of Effectiveness. In: JBI Manual for Evidence Synthesis. JBI 2020.

[23] Moher D, Liberati A, Tetzlaff J, Altman DG, The Prisma Group. Preferred reporting ıtems for systematic reviews and meta-analyses: The PRISMA statement. Ann Intern Med 2009;151(4):264-269.

[24] Woo J, Hong A, Lau E, Lynn H. A randomised controlled trial of Tai Chi and resistance exercise on bone health, muscle strength and balance in community-living elderly people. Age Ageing 2007;36(3):262–268.

[25] Kukuljan S, Nowson CA, Sanders KM, Nicholson GC, Seibel MJ, Salmon J, Daly RM. Independent and combined effects of calcium-vitamin d3 and exercise on bone structure and strength in older men: An 18-month factorial design randomized controlled trial. J Clin Endocrinol Metab 2011;96(4):955–963.

[26] Yuksel N, Majumdar SR, Biggs C, Tsuyuki RT. Community pharmacist-initiated screening program for osteoporosis: Randomized controlled trial. Osteoporos Int 2010;21(3):391–8.

[27] Francis KL, Matthews BL, Van Mechelen W, Bennell KL, Osborne RH. Effectiveness of a community-based osteoporosis education and self-management course: A wait list controlled trial. Osteoporos Int 2009;20(9):1563–1570.

[28] Kalkım A, Dağhan Ş. Theory-based osteoporosis prevention education and counseling program for women: A randomized controlled trial. Asian Nurs Res 2017;11(2):119–127.

[29] Levy BT, Hartz A, Woodworth G, Xu Y, Sinift S. Interventions to improving osteoporosis screening: An Iowa Research Network (IRENE) study. J Am Board Fam Med 2009;22(4):360–367.

[30] Cleghorn D, O'Loughlin PD, Schroeder BJ, Nordin BE. An open, crossover trial of calcium-fortified milk in prevention of early postmenopausal bone loss. Med J Aus 2001;175(5):242–245.

[31] Hien VTT, Khan NC, Mai LB, Lam NT, Phuong TM, Nhung BT, Nhien NV, Nakamori M, Yamamoto S. Effect of community-based nutrition education intervention on calcium intake and bone mass in postmenopausal Vietnamese women. Public Health Nutr 2009;12(5):674–679.

[32] Kloseck M, Fitzsimmons DA, Speechley M, Savundranayagam MY, Crilly RG. Improving the diagnosis and treatment of osteoporosis using a senior-friendly peer-led community education and mentoring model: A randomized controlled trial. Clin Interv Aging 2017;12: 823–833.

[33] Plawecki K, Chapman-Novakofski K. Effectiveness of community intervention in improving bone health behaviors in older adults. J Nutr Gerontol Geriatr 2013;32(2): 145–160.

[34] Bohlen LC, Michie S, Bruin M De, Rothman AJ, Kelly MP, Groarke HNK, Carey RN, Hale J, Johnston M. Do combinations of behavior change techniques that occur frequently in ınterventions reflect underlying theory ? Ann Behav Med 2020 54(11):827–842.

[35] des Bordes J, Prasad S, Pratt G, Suarez-Almazor ME, Lopez-Olivo MA. Knowledge, beliefs, and concerns about bone health from a systematic review and metasynthesis of qualitative studies. PLoS One 2020;15(1):827-842.

[36] Rinonapoli G, Ruggiero C, Meccariello L, Bisaccia M, Ceccarini P, Caraffa A. Osteoporosis in men: A review of an underestimated bone condition. Int J Mol Sci 2021;22(4):2105.

[37] Sava M, Tseng LY, Valderrama M, Mabey D, García PJ, Hsieh E. Osteoporosis knowledge and health beliefs among female community leaders in Peru. Women's Heal Reports 2020;1(1):47–54.

[38] Pinar G, Kaplan S, Pinar T, Akalin A, Abay H, Akyol M, Sezer N, Akkuş S, Sarıyıldız S, Dinç S. The prevalence and risk factors for osteoporosis among 18 – to 49-year-old Turkish women. Women Heal 2017;57(9):1080–1097.

[39] Kastner M, Perrier L, Munce SEP, Adhihetty CC, Lau A, Hamid J, Treister V, Chan J, Lai Y, Straus SE. Complex interventions can increase osteoporosis investigations and treatment: a systematic review and meta-analysis. Osteoporos Int 2018;29(1):5–17.

[40] Martin J, Viprey M, Castagne B, Merle B, Giroudon C, Chapurlat R, Schoot AM. Interventions to improve osteoporosis care: a systematic review and meta-analysis Osteoporosis Int 2020;31(3):429–446.

[41] Weaver CM, Gordon CM, Janz KF, Kalkwarf HJ, Lappe JM, Lewis R, Karma O, Wallace TC, Zemel BS. The National Osteoporosis Foundation's position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. Osteoporos Int 2016;27(4):1281–1386.

[42] Shojaa M, von Stengel S, Kohl M, Schoene D, Kemmler W. Effects of dynamic resistance exercise on bone mineral density in postmenopausal women: a systematic review and meta-analysis with special emphasis on exercise parameters. Osteoporosis Int 2020;31(8):1427–1444.

How to cite this article: Baz S, Toraman AU. Interventions Preventing Osteoporosis in Primary Care: A Systematic Review. Clin Exp Health Sci 2022; 12: 772-782. DOI: 10.33808/clinexphealthsci.1019617

Clinical and Experimental Health Sciences

Case Report: Motor and Sensory Development of a Case Followed with Suspicion of Neonatal Thiamine Metabolism Dysfunction Syndrome

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Received: 30.11.2020 **Accepted:** 24.01.2022

ABSTRACT

The aim of this study was to follow early motor and sensory development of the infant with Thiamine Metabolism Dysfunction Syndrom (TMDS). Newborn with 38 weeks gestational age, 2600 kilograms weight admitted to neonatal intensive care unit (NICU) due to respiratory distress, absence of suction reflex, and floppy appearance. Case had respiratory support during 5 weeks. Infant was referred to SANKO University Physiotherapy unit on postterm 12th week due to hypotonia after discharge. Prechtl's Generel Movements (GMs) and Hammersmith Infant Neurological Evaluation (HINE) was performed at 3rd and 4th months. Sensory processing parameters were evaluated with the Newborn Sensory Profile-2 (NSP-2). Case had no Fidgety movements (FMs). The HINE score was 37-45 in the 3th and 4th month respectively. Total score in NSP-2 was 33 in the 3th month (general = 12, auditory = 7, visual = 8, tactile = 2, movement = 2, oral sensory processing = 1). While the case's Newborn Sensory Profile-2 (NSP-2) total score was in newborn norms, visual, tactile, movement but intraoral sensory parameters and auditory parameters were in low limits. The low motor performance was associated with low NSP-2 score and showed interaction with motor-sensory development. It is concluded that early physiotherapy program can be effective.

Keywords: Thiamine Metabolism Dysfunction Syndrome, Sensory Profile, Prechtl's Generel Movements (GMs)

1. INTRODUCTION

Thiamine; is a complex B vitamin that helps to support growth, is necessary for the healthy function of the nervous system, heart and other tissues. Neurological findings such as motor function, cognitive, speech and communication in patients with thiamine metabolism dysfunction syndrome (TMDS), also there may be developmental disorders, cardiological, orthopedic retardation with a high mortality risk [1, 2].

Thiamine metabolism dysfunction (TMDS) is an autosomal recessive neurometabolic condition [3, 4]. TMDS is a rare disorder. This disorder has several acronyms since it was first described by Ozand et al, in 1998 [4]. These names include solute carrier family 19 (thiamine transporter), member 3 (SCL19A3) gene defect, biotin-responsive basal ganglia disease (BBGD), biotin-thiamine–responsive basal ganglia disease (BTBGD), and thiamine metabolism dysfunction syndrome 2 [5]. The worldwide incidence and prevalence of SLC19A3 gene defect disorders are unknown. It is most commonly reported in Saudi Arabians, who contributed 70 (52%) of the 134 known cases. However, this gene defect is pan ethnic [4, 5].

Patients have episodes of acute encephalopathy with symmetrical lesions in the cortex, basal ganglia, thalamus, or periaqueductal gray matter, biotin or thiamine deficiency [6, 7]. It may lead to coma and death, with progressive neurodegeneration such as confusion, seizures, and dysphagia [4]. Early signs of thiamine deficiency include fussiness and irritability in infants. Weakness, nystagmus, ophthalmoplegia, ataxia, and cognitive impairment accompany progression of the disease. Infants may be noted to have a lack of tone. Neurologic symptoms are often reversed quickly with treatment, but lasting effects may be seen in severe cases or with delayed treatment. As thiamine deficiency progresses, extreme loss of muscle mass can be observed. Early reports of the phenomenon particularly indicate severe wasting of the gastrocnemii. Adults demonstrate considerable weakness related to muscle loss and peripheral neuropathy [7]. But there is no assessments about motor and sensory development in infants with TMDS in the literature. Long term stay in NICU can cause sensorial deficits in TMDS infants, too. So there can be asssosciation between motor and sensory

DOI: 10.33808/clinexphealthsci.833672

develeopment of these infants. Therefore, the aim of this study was determine the deviations in motor and sensory development of a newborn who is followed up with the TMDS. In addition, to determine the improvements in motor development with early physiotherapy program.

2. CASE PRESENTATION

The infant was followed up due to lack of movement and intrauterine growth retardation in prenatal period, was admitted to the SANKO University Neonatal Intensive Care Unit (NICU) (Table 1). Case had respiratory distress, lack of sucking, floppy appearance. Demographic information was taken from hospital records (Table 1). Cephalic hematoma was observed at Magnetic Resonance Imaging (MRI) in the right frontoparietal area. SMN1-2, SLC19A3 gene analysis and neonatal metabolic screening were performed. Hypotonia and delay in motor development were observed in the postterm 12th week after discharge, then referred to the Physiotherapy and Rehabilitation Unit of SANKO University, followed up with home physiotherapy program by monthly visits. Observations of the case before the Physical Therapy program was shown in Table 1.

Table 1. *Demographic Characteristics, motor and sensory assessments of the case*

<table>
<tr><th colspan="2">Demographic Characteristics of the Case</th><th colspan="2">12th week (3 months) observations of the case (BPT)</th><th colspan="2">16 th week (4 months) observations of the case (APT)</th></tr>
<tr><td>Birth weight (kg)</td><td>2.600</td><td colspan="2">– Excessive hypotonic posture
– Exaggerated external rotation in the hips and frog posture
– Bilateral lower extremities kicking were low
– Hands were not in the midline and could not touch knees.
– Antigravity movements were too limited</td><td colspan="2">– Midline orientation
– Normal tone at lower and upper extremities
– Hands were in midline and can touch to the knees
– More antigravity movements observed.
– Weight shifting were observed in pelvis and hips.
– Achieved hand to hand/ hand to mouth
– Hands can reach to the objects in midline.</td></tr>
<tr><td>Birth length (cm)</td><td>52</td><td colspan="2" rowspan="5"></td><td colspan="2" rowspan="5"></td></tr>
<tr><td>Gestational Age (w)</td><td>38</td></tr>
<tr><td>Hopitalization duration(d)</td><td>90</td></tr>
<tr><td>Apgar score (1 - minute)</td><td>5</td></tr>
<tr><td>Live birth of mother</td><td>3</td></tr>
<tr><td>Mother age</td><td>32</td><td colspan="2">Newborn Sensory Profile (NSP-2) Row Scores</td><td colspan="2" rowspan="11"></td></tr>
<tr><td>MV Support (w)</td><td>10</td><td>General processing</td><td>32</td></tr>
<tr><td>Intra-Incubator oxygenation (w)</td><td>5</td><td>Auditory processing
Visual processing</td><td>7
8</td></tr>
<tr><td>Discharge of NICU (d)</td><td>90</td><td>Tacdil processing</td><td>2</td></tr>
<tr><td colspan="2" rowspan="7"></td><td>Movement processing</td><td>2</td></tr>
<tr><td>Oral sensorial processing</td><td>1</td></tr>
<tr><td colspan="2">Neonatal Sensory Profile (NSP-2) age compliance code quadrants</td></tr>
<tr><td>Sensorial Seeker</td><td>4</td></tr>
<tr><td>Sensorial Avoidant</td><td>7</td></tr>
<tr><td>Sensorial Sensitivity</td><td>7</td></tr>
<tr><td>Sensory Recorder</td><td>5</td></tr>
<tr><td colspan="2" rowspan="2">Motor assessments of the case</td><td>FMs
(3 months)</td><td>F-</td><td>FMs
(4 months)</td><td>F-</td></tr>
<tr><td>HINE
(3 months)</td><td>37</td><td>HINE
(4 months)</td><td>45</td></tr>
<tr><td colspan="6">Neonatal Sensory Profile of the Case NSP-2 age-matched quadrant at 3 months</td></tr>
<tr><td colspan="2" rowspan="2">Neurological Threshold Process</td><td colspan="4">Behavior Response Process</td></tr>
<tr><td colspan="2">Acting in harmony with the threshold</td><td colspan="2">Acting on the threshold</td></tr>
<tr><td colspan="2"></td><td colspan="2">High</td><td colspan="2">Low</td></tr>
<tr><td colspan="2">Poor recording ability</td><td colspan="2">+</td><td colspan="2"></td></tr>
<tr><td colspan="2">Seeking stimuli</td><td colspan="2"></td><td colspan="2">+</td></tr>
<tr><td colspan="2">Sensitivity to stimulus</td><td colspan="2"></td><td colspan="2">+</td></tr>
<tr><td colspan="2">Avoiding the stimulus</td><td colspan="2"></td><td colspan="2">+</td></tr>
</table>

Kg: kilograms, cm: centimeter, w: week, d: day, BPT: before physical therapy, APT: after physical therapy, NICU: Neonatal Intensive Care Unit, FMs: Fidgety movements, HINE: Hammersmith Infant Neurological Examination, F-: No Fidgety movements
NSP-2: Neonatal Sensory Profile

Prechtl's General Movements (GMs) assessment shows quality of spontaneous motor movements without any stimulus [8]. GMs analysis has high reliability and validity (98% sensitivity) in predicting developmental disorders such as CP. GMs shows Fidgety character during 9-20 weeks [8]. Fidgety movements (FMs) was assessed by the 5 minute video records, 2 times until the end of the 4th month. Fidgety movements (FMs) of the case were evaluated. There was no Fidgety (F-) in the 14 and 16 weeks (Table 1). The posture of the case at 14th and 16th week during GMs assessments was shown in Figure 1.

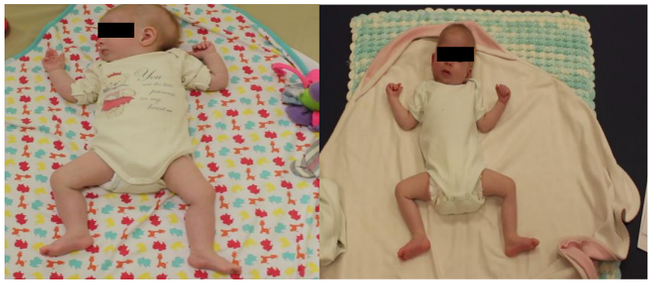

Figure 1. *The posture of the case at 14th and 16th week during GMs assessments*

Hammersmith Infant Neurological Evaluation (HINE) is one of the neuromotor evaluation methods of infants between 2 and 24 months old. It can be used in different high and low risk populations for preterm and term infants. Total score range from 0 to 78. 67-70> score indicates normal range, <57 shows that there is a 96 % risk for CP between 3-6 months. The HINE test has a 90 % predictive value for CP risk in infants aged 2-24 months [9]. HINE was performed at 3th and 4th months. HINE score of the case was 37 and 45, respectively (Table 1).

The physiotherapy program included training of parents about therapeutic holding and carrying principles, facilitation of voluntary head, trunk-hip extension against gravity in prone position, ensuring elongation on the weight-bearing with unilateral reaching in prone position with 'hands on' pelvic stabilization, weight bearing of the forearms with keeeping scapular adduction, active head movements with the support of physiological flexion in supine, supportied sitting or sidelying position, midline orientation in sidelying with various supports, reaching out of hands to feet with active or passive movements in various supine positions, facilitation of head and trunk movements with using wrighting reactions, passive 'hands on' rotation facilitations from supine to prone or opposite, facilitation of the use of hands during high lyied, supported or independent sitting position and development of voluntary hand-eye coordination, head control, grasping, and trunk control. Family trained according to the goals like head control, holding feet, middline orientation, rotations of two side, reaching objects, devoloping righting reactions, weight bearings and weight shiftings set within the framework of the motor movements that the infants could achieve. Also feeding or functional playing postures were explained for the writhing and balance reactions.

Sensory processing parameters were evaluated with the Newborn Sensory Profile-2 (NSP-2) questionnaire filled by the mother at 3 months. It bases on individual family responses, adapted from the Infant Toddler Sensory Profile (ITDP) [10, 11, 12]. The NSP-2 has 24-question with consisting of sensory profile, general sensory, auditory, visual, tactile, motion processing, oral sensory processing sub-scores. The total raw score of NSP is calculated with the sum of sub scores (Table 1). In addition, age compliance codes quadrants of the baby such as seeking, avoiding, sensitivity and recording are defined. NSP-2 raw total score of the case was 32 (overall:12, auditory:7, visual:8, tactile:2, movement: 2, oral sensory processing: 1) at 3 months (Table 1). NSP-2 total score of our case had lower range than her peers. Accordingly, when looking at the compatibility of the infant's general sensory processing, infant seemed 4 % sensorial seeker, 7% sensorial avoidant and 7% sensorial sensitivity, and 5 % sensory recorder. Also it was observed that the case had poor recording ability, lower threshold for seeking stimuli, sensitivity to stimulus and avoiding the stimulus acoording to their healty peers quadrant scores.

3. DISCUSSION

It has been shown that, sensory processing and motor problems can be detected at an early stage of life, and improvement with the motor development can be with early physiotherapy program with this case report. Also the interaction of motor-sensory development was revealed. Sensory processing has been conceptualized by Dunn as the emergence of appropriate responses and behaviors in neurological processes where messages from visual, auditory, tactile, oral, olfactory, vestibular, proprioceptive and kinesthetic inputs are regulated [13]. The interactions between the individual's neurological thresholds, emotional and behavioral responses or self-regulation strategies are permanent. Dunn developed four different response categories based on the interaction between the individual's neurological threshold and behavioral responses [13]. These are called sensory seeking, avoidance, low registration, and increased sensory sensitivity [13]. These processes develop in accordance with natural stimuli from infancy. However, sometimes there may be deviations in development from early infancy. One of them is NICU, which is required to support vital functions. Reduced spontaneous movements for any reason and exposure to excessive sensory stimuli may cause negative consequences in the normal sensory and motor development of the infant. Newborn preterm infants receive less tactile and vestibular stimulation in the NICU than prenatal period. However, there are negative stimulations such as bright lights, high noise levels, excessive use and frequent painful interventions. This condition can have permanent effects on the developing brain and affect the natural development of sensory systems [14, 15]. Machadoa et all. and Celik et all. found a significant difference between

term infants and preterm infants in terms of sensory profile scores [14, 15]. Although our case was term, some deficiencies in sensory parameters revealed. It is thought that, this may be due to the relationship between motor development and sensory development, or to the difference in hospitalization time compared to other term infants.

Studies examining the relationship between sensory processing parameters and motor development in infants are limited in the literature from the neonatal period [15]. Preterm infants are thought to be exposed to these stimuli for a longer period of time. However, the fact that the term infant, who was exposed to many stimuli in the long-term NICU in this study, was less sensitive in terms of sensory processing total scores showed that sensory processing problems could also occur in term infants in the early period of life. Although cognitive and behavioral problems are detected in hypotonic infants, studies examining sensory processing and motor problems in early infancy are limited [15]. Excessive inactivity and low motor performance suggested that low NSP-2 total scores might be related in our case. However, the fact that the sensory profile test was not repeated with the increase in motor development scores constituted a limitation of our study in terms of determining its relationship with the increase in motor parameters. It is recommended to conduct long-term studies with large samples that examine the relationship of motor-sensory development in early infancy in further studies.

4. CONCLUSION

It is concluded that sensory processing and motor problems can determine at an early period of life, also motor development can be improved by early physiotherapy and rehabilitation program.

Conflict of Interest

The authors have no conflict of interest to declare.

REFERENCES

[1] Kwong A, Shin YV, Who CJ. SLC19A3 (solute carrier family 19 (thiamine transporter), member 3. Atlas Genet Cytogenet Oncol Haematol. 2015;19(6):401-403.

[2] Mimouni A, Mimouni B, Goldberg H, Goldberg S, Strausberg R, Brezner A, Heyman E, Inbar D, Kivity S, Zvulunov A, Sztarkier I, Fogelman R, Fattal A, Fattal V. Thiamine deficiency in infancy: long-term follow-up. Pediatric Neurology. 2014;51(3):311-316.

[3] Pérez B, Pérez D, Serrano M, Rebollo M, Muchart J, Gargallo E, Dupuits C, Artuch R. Reversible lactic acidosis in a newborn with thiamine transporter-2 deficiency. Pediatrics. 2013;131 (5):1670-1674.

[4] Ozand PT, Gascon GG, Al Essa M, Al Essa M, Joshi S, Al Jishi E, Bakheet S, Al Watban J, Al-Kawi MZ, Dabbagh O. Biotin-responsive basal ganglia disease: a novel entity. Brain. 1998;121(7):1267–1279.

[5] Alfadhel M, Tabarki B. SLC19A3 Gene defects sorting the phenotype and acronyms: review. Neuropediatrics. 2017;9 (02):083-092.

[6] Pérez B, Pérez D, Serrano M, Rebollo M, Muchart J, Gargallo E, Dupuits C, Artuch R. Reversible lactic acidosis in a newborn with thiamine transporter-2 deficiency. Pediatrics. 2013;131(5):e1670–e1675.

[7] Smith TJ, Johnson CR, Koshy R, Hess SY, Qureshi UA, Mynak ML, Fische PR. Thiamine deficiency disorders: a clinical perspective. Ann. N.Y. Acad. Sci. 2021;1498(1):9–28.

[8] Einspieler C, Marschik P B, Bos AF, Ferrari F, Cioni G, Prechtl PRH. Early markers for cerebral palsy: Insights from the assessment of general movements. Future Neurol. 2012;7(6):709-71.

[9] Novak I, Morgan C, Adde L, Blackman J, Boyd N, Brunstrom-Hernandez J, Cioni G, Damiano D, Darrah J, Eliasson AC, Vrie LS, Einspieler C, Fahey M, Fehlings D, Ferriero DM, Fetters L, Fiori S, Forssberg H, Gordon AM, Greaves S, Guzzetta A, Hadders-Algra M, Harbourne R, Kakooza-Mwesige A, Karlsson P, Krumlinde-Sundholm L, Latal B, Loughran-Fowlds A, Maitre N, McIntyre S, Noritz G, Pennington L, Romeo MD, Shepherd R, Spittle JA, Thornton M, Valentine J, Walker K, White R, Badawi N. Early, accurate diagnosis and early intervention in cerebral palsy, advances in diagnosis and treatment. Jama Pediatrics. 2017;171(9):897-907.

[10] Germani T, Zwaigenbaum L, Bryson S, Brian J, Smith I. Roberts W, Szatmari P, Roncadin C, Sacrey RA, Garon N, Vaillancourt T. Brief Report: Assessment of early sensory processing in infants at high-risk of autism spectrum disorder. J Autism Dev Disord. 2014;44(12):3264–3270.

[11] Eeles L, Spittle JA, Anderson JP, Brown N, Lee JK, Boyd NR, Doyle WL. Assessments of sensory processing in infants: A systematic review. Abbey Developmental Medicine&Child Neurology. 2013;55(4):314-326.

[12] Metz AE, Boling D, DeVore A, Holladay H, Liao FS, Vlutch KV. Dunn's Model of Sensory Processing: An investigation of the axes of the four-quadrant model in healthy adults. Rief Report. Brain Sci. 2019;9(2):35.

[13] Backhouse M, Harding L, Rodger S, Hindman N. Investigating sensory processing pattern in boys with duchenne muscular dystrophy using the sensory profile. British Journal of Occupational Therapy. 2012;75(6):271-280.

[14] Machadoa PVCA, Oliveiraa RS, Magalhãesb CL, Mirandaa MD, Bouzada FCM. Sensory processing during childhood in preterm infants: A systematic review. Rev Paul Pediatr. 2017; 35(1):92-101.

[15] Celik HI, Elbasan B, Gucuyener K, Kayihan H, Huri M. Investigation of the relationship between sensory processing and motor development in preterm infants. AJOT, 2018;72(1): 720.119.5020p1-720.119.5020p7.

How to cite this article: Adiguzel H, Kayihan H, Sarikabadayi YU, Elbasan B. Case Report: Motor and Sensory Development of a Case Followed with Suspicion of Neonatal Thiamine Metabolism Dysfunction Syndrome. Clin Exp Health Sci 2022; 12: 783-786. DOI: 10.33808/clinexphealthsci.833672