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The Middle Black Sea Journal of Health Science, which is international journal, is published by Ordu University Institute of Health Sciences on behalf of the Middle Black Sea Universities Collaboration Platform

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The aim of the journal is to contribute to the international literature with clinical and experimental research articles, case reports, reviews and letters to the editor in the field of health sciences.

The target audience of the journal is all scientists working in the field of health, graduate students and researchers in this field.

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Middle Black Sea Journal of Health Science - adheres to the standards of publication ethics in health science research, Higher Education Council's Scientific Research and Publication Ethics Directive, Committee on Publication Ethics (COPE), Directory of Open Access Journals (DOAJ), Open Access Scholarly Publishers It also adopts the ethical publishing principles published by the Association (OASPA) and the World Association of Medical Editors (WAME).

No fee is charged from the authors for the evaluation and publication of the article.

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• The study is evaluated by sending it to a statistics advisor. After this evaluation, the author may ask for a correction.

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Middle Black Sea Journal of Health Science

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Chapter in Edited Book: Hornbeck P. Assay for antibody production. In: Colign JE. Kruisbeek AM, Marguiles DH, editors. Current Protocols in Immunology. New York: Greene Publishing Associates; 1991. p. 105-32.

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The similarity rate of the articles should be done through iThenticate and should be at most 20% excluding the "References" part.

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Structure

Title

Abstract should be structured with subheadings (Objective, Methods, Results, and Conclusion) (average 200-400 word)

Key words

Introduction

Methods

Results

Discussion

Conclusion

Acknowledgements

References (most 40)

Whole text should not exceed 4500 words except for resources and English summary.

b) Short papers: Prospective, retrospective and all kinds of experimental studies

Structure

Title

Abstract should be structured with subheadings (Objective, Methods, Results, and Conclusion) (average 200-400 word)

Key Words

Introduction

Methods

Results

Discussion

Conclusion

Acknowledgements

References (most 20)

Whole text should not exceed 2700 words except for resources and English summary.

c) Case Report: They are rarely seen articles which differs in diagnosis and treatment. They should be supported by enough photographs and diagrams.

Structure

Title

Abstract (average 100-300 word)

Key words

Introduction

Case report

Discussion

Conclusion

Acknowledgements

References (most 20)

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d) Review articles

Structure

Title

Abstract (average 200-400 word)

Key words

Introduction

The compilation text also including appropriate sub-headings,

Conclusion

Acknowledgements

References (most 50)

Whole text should not exceed 6550 words except for resources and English summary.

MAY 2023 VOLUME 9 ISSUE 2

CONTENTS

EDITORIAL

In our second issue...

We are very proud to present The new issue of our journal to you, our dear readers, which aims to provide the highest benefit to our esteemed physicians, researchers, specialty, doctorate and medical faculty students from all fields, by publishing basic, clinical and community-based original researches made with the appropriate hypothesis, scientific methods, and interdisciplinary cooperation in all fields of medicine.

We would like to express our gratitude to all authors who preferred our journal to publish their scientific research, to our referees who did not turn down our invitation, and to our valuable readers who set their heart on science.

I hope, we can contribute to the spread of professional development and scientific research awareness and contribute to the medical literature, with each new journal we publish, and I wish you all a pleasant reading.

PhD, Assoc. Prof. Ülkü KARAMAN
Editor

Middle Black Sea Journal of Health Science / Mid Blac Sea J Health Sci

May 2023; 9(2): 196-208

RESEARCH ARTICLE

DOI: 10.19127/mbsjohs.1186891

Comparison of Nutritional Status with Serum Vitamin D and B12 Levels in Pregnant Women with Gestational Diabetes

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Abstract

Objective: In this study, it was aimed to evaluate the nutritional adequacy status of pregnant women with gestational diabetes and to examine the relationship between biochemical parameters such as vitamin D, vitamin B12, hemoglobin, hematocrit and fasting glucose.

Methods: In the study, serum vitamin D and B12 levels of 130 pregnant women between 24-28 weeks of gestation were examined. As a result of 75-gram oral glucose tolerance test, 70 pregnant women were diagnosed with gestational diabetes mellitus (GDM). The control group consisted of 60 healthy pregnant women. Nutritional factors were obtained through a questionnaire (anthropometric measurements, micronutrients, 3-day food consumption frequency, use of vitamin supplements) and groups were compared in terms of biochemical parameters (vitamin D, vitamin B12).

Results: Pregnant women with GDM; mean age (30.34 ± 5.28), family history of diabetes (17.1%), pre-pregnancy body mass index percentage (54.3%), rate of skipping meals were higher. The rate of use of vitamin and mineral supplements in pregnant women with GDM was low. According to the analysis of 3-day food consumption records of pregnant women with GDM, it was determined that the intake of micronutrients vitamin D and vitamin B12 was insufficient. Biochemical parameters such as vitamin D, vitamin B12, hemoglobin and hematocrit were found to be lower in pregnant women with GDM.

Conclusion: GDM is the most common endocrinological disorder in pregnancy. The study showed that inadequate nutrient intake negatively affects blood glucose levels and biochemical findings. Individuals with GDM should be referred to a nutritionist, medical nutrition therapy (TBT) appropriate for their individual characteristics should be given and monitored.

Key words: Gestational diabetes, biochemical parameters, vitamin D, vitamin B12, nutrition

Suggested Citation: Çelenk T, Çebi A. Comparison of Nutritional Status with Serum Vitamin D and B12 Levels in Pregnant Women with Gestational Diabetes Mid Blac Sea Journal of Health Sci, 2023;9(2):196-208

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INTRODUCTION

Diabetes Mellitus (DM) is defined as a metabolic disease that has become a global epidemic worldwide, causing abnormalities in carbohydrate (CH), protein and fat metabolism, hyperglycemia, and multiorganal damage due to impaired insulin secretion, insulin action or both (1). In 2017, the number of people with diabetes worldwide was 451 million. It is estimated that by 2045, the number of people with diabetes in the world will reach 693 million (2).

Gestational diabetes mellitus (GDM) is defined as variable carbohydrate intolerance detected during pregnancy. GDM is the most common endocrinological disorder in pregnancy and is found in 7% of all pregnancies (1). Its incidence in Turkey has been reported between 1.2% and 9.2% (3). There is a 20% to 50% risk of developing Type 2 DM in 5-10 years in women with a history of GDM (4). On the other hand, children of women with GDM are also at risk of obesity and diabetes mellitus (DM) in later years.

Since the fetus is fed by the mother during pregnancy, the daily energy and nutritional needs of pregnant women increase. However, many women take insufficient nutrients during this period and cannot eat healthy. It has been reported that increasing the consumption rate of sugary foods and decreasing the consumption rate of fruits and vegetables from fibrous foods cause an increase in fasting glucose (5). Sugar from food crosses the placenta and causes insulin release from the baby's pancreas. This insulin hormone also functions as a growth hormone and leads to macrosomic birth. In addition, uncontrolled GDM causes complications such as preeclampsia, hydroamniosis, hypertension, increased risk of cesarean section in fetuses and infants.

Adequate intake of vitamin D, vitamin B12, zinc, iodine, calcium, iron, folate, and magnesium is particularly important during pregnancy (6). Vitamin D deficiency during pregnancy is associated with adverse complications such as premature birth, increased cesarean delivery, and insulin resistance (7). It has been suggested that low vitamin D levels in diabetic subjects are associated with diabetic control (8). Folic acid and vitamin B12 are involved in the division or proliferation of cells. It is effective in all cells and is a very important vitamin related to the gastrointestinal system (GIS), central nervous system (CNS). According to the data obtained from very few studies on the need for vitamin B12 during pregnancy, serum vitamin B12 values decrease in the following weeks of pregnancy (9).

The aim of this study is to compare pregnant women with gestational diabetes mellitus (GDM) and healthy control group. Anthropometric measurements of pregnant women, nutritional habits and dietary factors causing GDM were determined and the

relationship between biochemical parameters such as vitamin D, vitamin B12, hemoglobin, hematocrit and fasting glucose was tried to be examined

METHODS

The study was carried out between November 2018 and May 2019 in Turkey Ordu University Training and Research Hospital Gynecology Polyclinic, Ordu Private Sevgi Hospital Gynecology Polyclinic, Ordu General Directorate of Public Health. 130 pregnant women between 24-28 weeks diagnosed by doctor (70 gestational diabetes and 60 healthy pregnant women) were examined.

All subjects included their informed consent before participating in the study. Written consent was obtained from the pregnant women for voluntary participation in the study. A questionnaire form was filled in to ask general information about pregnancy to the pregnant women in the case and control groups. Pregnant women who had a pregnancy other than their gestational week and had diabetes complications were excluded from the study.

Ethical aspect of the study

Ethics committee approval was obtained for this study by Giresun University Clinical Research Evaluation Committee with the decision dated 07/11/2018 and numbered 2018/05-06. Before starting the research, written permission was obtained from the hospitals where the research would be conducted.

Dietary evaluation

In the questionnaire applied to pregnant women, their weight, body mass indexes, whether they have chronic diseases in their families, vitamin-mineral supplements, diet program, nutritional habits (meal pattern and why they skip meals, what foods they consume, glycemic index (GI) high food consumption frequency etc.) were questioned. Food consumption was recorded for a total of 24 hours over three days, two on weekdays and one on weekends. The daily energy and nutritional values of the patient were analyzed using the nutrition information system (BEBIS) 8.2 program.

Data on energy and nutritional values obtained from the analysis were compared with the Dietary Reference Intake (DRI) (10) recommendations and ± 30-33 values of the recommended daily consumption for all nutrients were calculated. In the DRI recommendation, the content of macro and micronutrients is considered as insufficient values below 67%, sufficient values 67-133% and values above 133% as excessive consumption.

Biochemical measurements

The biochemical parameters routinely requested by the physician were recorded in the questionnaire and all data were evaluated. GDM diagnosis was made by gynecology and obstetrics clinic physicians according to biochemical blood findings. Blood samples

were taken from the pregnant women who came for routine control at 24-28 weeks of pregnancy after fasting for 10-12 hours in the biochemistry laboratory and measured by photometric method. (Roche Diagnostics GmbH, Mannheim, Germany). At the end of the measurement, 75 g OGTT was performed in patients with FPG 100- ≥126 mg/dl. Because fasting blood glucose is >92 mg/dL according to IADPSG/ADA criteria in pregnant women with GDM, a 2-hour OGTT test with 75 g glucose is recommended. A 2-hour OGTT test with 75 g glucose was performed on pregnant women with GDM (0 hours ≥92 mg/dl, 1 hour ≥180 mg/dl, 2 hours ≥153 mg/dl).

GDM was diagnosed in pregnant women if at least one glucose concentration met or exceeded the criteria as a result of the test. Hemoglobin values were measured by calorimetric method. (Sysmex Corporation, Kobe, Japan). According to the World Health Organization (WHO) criteria, hemoglobin values below 10.5 g/dl in the second trimester of pregnancy are accepted as anemia. In this study, hemoglobin values between 11.2 g/dl and 14.6 g/dL were considered normal, while those <10 g/dl were considered low. The shaped elements were precipitated by centrifugation on Sysmex Xn 1000 (Sysmex Corporation, Kobe, Japan).

The ratio of shaped elements to blood volume was calculated by the device. Hematocrit (Htc) values in women were evaluated according to the reference range of 36-46%. After fasting for 10-12 hours, venous blood samples (Cobas Hitachi E601) were measured by electrochemiluminescence method.

Vitamin B12 was defined as 191-663 pg/ml adequacy level in men and women. In our study, total vitamin D (D2-D3) was measured by electrochemiluminescence method (Cobas Hitachi E601). The lower limit of the electrochemiluminescence method is 3 µg/l and the measuring range is 3-70 µg/l. <20 µg/l vitamin deficiency was defined as 20-29 µg/l deficiency and ≥30 µg/l proficiency level (11).

Statistical Analysis

Descriptive statistics for continuous variables; Expressed as Mean, Standard Deviation, Minimum and Maximum values, while expressed as numbers and percentages for Categorical variables. Student's t-test was used to compare group means in terms of continuous variables. Chi-square test was used to determine the relationship between groups and categorical variables. As a result of the food consumption records of the patients, the data obtained using the BeBIS 8.2 program were transferred to the statistical package program and evaluated. The statistical significance level was taken as 5% in the calculations and the SPSS (ver:23) statistical package program was used for the calculations.

RESULTS

The average age of the groups; GDM cases in the case group were 30.34 ± 5.28 years, and the mean age of healthy individuals in the control group was 28.38 ± 4.85 years. It was determined that 80% of individuals with GDM and 93.3% of healthy individuals used vitamin-mineral supplements. There was a statistically significant difference between usage rates. Looking at the types of vitamin-mineral supplements used; It was determined that individuals with GDM used iron in 15.7%, vitamin D in 28.6%, vitamin B12 in 10%, folic acid in 21.4%, magnesium in 2.9% and probiotic supplements in 1.4%. It was determined that 21.7% of healthy individuals used iron supplements, 38.3% vitamin D, 6.5% vitamin B12, 27% folic acid and 1.4% magnesium supplements.

Healthy pregnant women consumed vitamins and mineral supplements (30.4%) during pregnancy longer than pregnant women with gestational diabetes (29.1%). There were statistically significant differences. It was found that the history of diabetes in the family of pregnant women with GDM (17.1%) was higher than that of healthy pregnant women (11.7%).

When the pre-pregnancy body mass index (BMI) of pregnant women with GDM was evaluated, it was found that the total percentage of mildly obese and obese pregnants (54.3%) was higher than the total percentage of healthy pregnants (50%). The difference between meal skipping rates of GDM and healthy individuals was statistically significant. The highest rate of skipping meals in individuals with GDM was found to be breakfast with 18.6% and lunch with 24.3%. It was determined that 3.3% of healthy pregnant women skipped breakfast and 25% skipped lunch (Table 1). The total fiber ratio of the pregnant women from the foods they consume daily was 27.9±6.6 g in pregnant women with GDM and 44.1±11.7 g in healthy pregnant women. Fiber intake of pregnant women with GDM was found to be significantly lower than the control group ($p<0.05$).

When the nutritional adequacy status of all individuals is evaluated according to DRI recommendations; It was determined that healthy pregnant women took 73.3% more, 25% normal, 1.7% insufficient vitamin D. It was determined that healthy pregnant women took 41.7% more, 45% enough, 13.3% insufficient vitamin B12. It was found that pregnant women with GDM took 7.1% more, 48.6% normal, 44.3% insufficient vitamin D. Pregnant women with GDM took 21.4% more, 55.7% adequate, 22.9% insufficient vitamin D. detected. Vitamin D and vitamin B12 were statistically significant. Vitamin D deficiency (44.3%) was found to be higher in pregnant women with GDM than in healthy pregnant women (1.7%). It was determined that vitamin B12 deficiency was higher in pregnant women

with GDM (22.9%) than in healthy pregnant women (13.3%). Dietary intake of these vitamins in pregnant women with GDM was found to be lower than in healthy pregnant women (Figure 1 and Figure 2).

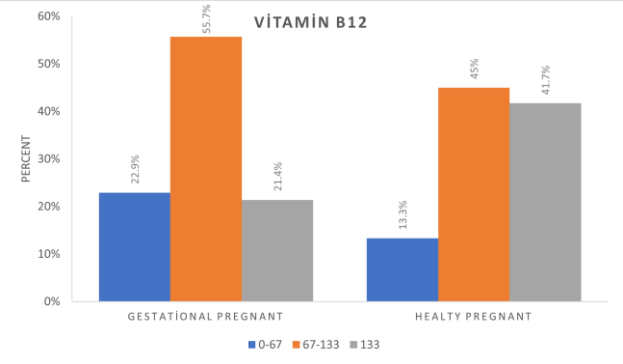

Figure 1. Distribution of adequacy status of vitamin D intakes in daily diets of pregnant women in line with DRI recommendations
(Below 0-67% insufficient, 67-133% sufficient and over 133% excessive consumption)

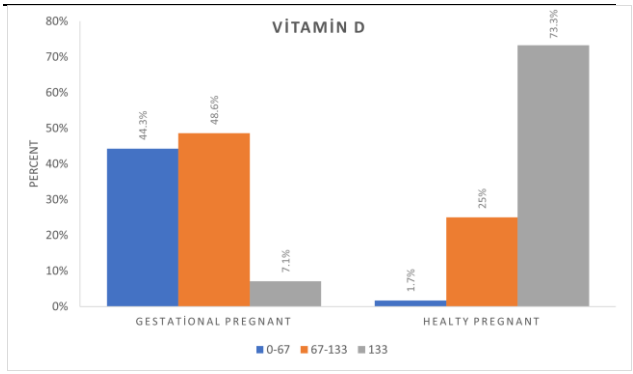

Figure 2. Distribution of adequacy status of vitamin B12 intakes in daily diets of pregnant women in line with DRI recommendations
(Below 0-67% insufficient, 67-133% sufficient and over 133% excessive consumption)

As a result of this study, while vitamin D taken through food was 31.32±7.6 mcg in healthy pregnant women, it was found as 14.1±9.9 mcg in pregnant women with GDM. When the frequency of consumption of milk, cheese and fish products from which we can get vitamin D through food is examined, the daily milk consumption of individuals with GDM is 65.7%, while that of healthy individuals is 75%; Daily cheese consumption of individuals with GDM was 74.3%, and 86.7% of healthy individuals. Percentage values of milk ($p=0.025$) and cheese ($p=0.039$) consumption of the group with GDM were statistically significantly lower than the percent values of healthy pregnant women. 22.9% of individuals with GDM and 8.3% of healthy individuals do not consume fish at all. The percentage of fish consumption of healthy individuals was statistically significantly higher than the group with GDM ($p<0.005$). In addition, iron mineral was found to be very low especially in individuals with GDM compared to healthy individuals (Table 2).

In our study, fasting glucose values of pregnant women with GDM were 99.2 ± 13 mg/dl, and 87.2 ± 9.2 mg/dl in healthy pregnant women. Pregnant women with GDM OGTT 1st hour values were 181.1 ± 2.87 mg/dl and OGTT second hour values were 153.7 ± 3.3 mg/dl. Serum 25 (OH) vitamin D levels of pregnant women with GDM (17.8 ± 10.7 ng/ml) were significantly lower than healthy pregnant women (20.6 ± 8.05 ng/ml).

The other biochemical findings of both groups were compared with those of GDM pregnant women with vitamin B12 (199.9 ± 91.5 pg / ml), hemoglobin (10.8 ± 2.9 g / dL), respectively; It was found that vitamin B12 (234 ± 114.7 pg / ml) and hemoglobin (11.9 ± 3.2 g / dL) values of healthy pregnant women

were significantly lower (p <0.05). Hematocrit values of GDM (30.3 ± 7.4%) and healthy pregnant women (33.01 ± 8%) were close to significant (p = 0.055).

Table 1. General Information About Pregnants

	GDM (n=70)		Control (n=60)	
	n	%	n	%
Age ratio				
Mean	20-44		20-40	
SD	30.34 ± 5.28		28.38 ± 4.85	
BMI				
Lightweight (BMI≥25-29,9 kg/m²)	31	44.3	26	43.3
Obese (BMI≥30,0 kg/m²)	7	10	4	6.7
Does he use vitamin-mineral supplements?				
Yes	56	80	56	93.3
Vitamins and minerals used				
Vitamin B12	7	10	7	6.5
Vitamin D	20	28.6	23	38.3
Iron	11	15.7	13	21.7
Folic acid	15	21.4	12	20
Magnesium	2	2.9	1	1.4
Probiotics	1	1.4	0	0
Vitamin-mineral consumption time				
During pregnancy	16	29.1	17	30.4
Family history of chronic disease				
Diabetes	12	17.1	7	11.7
Most skipped meal				
Breakfast	13	18.6	2	3.3
Lunch	17	24.3	15	25

GDM: gestational diabetes mellitus Control: healthy pregnant, BMI: body mass index, kg: kilogram m²: square meters

Table 2. Distribution of energy and nutrients (vitamin) according to DRI recommendations.

		GDM (n=70)						Control (n=60)						p value
Vitamins	Reference values	Insufficient (<%67)		Enough (%67-133)		Excess (%133)		Insufficient (<%67)		Enough (%67-133)		Excess (%133)		
		n	%	n	%	n	%	n	%	n	%	n	%	
Vitamin A (mcg)	700	1	1.4	2	2.9	67	95.7	-	-	1	1.7	59	98.3	0.583
Vitamin E (mg)	11	2	2.9	19	27.1	49	70	-	-	17	28.3	43	71.7	0.418
Vitamin B1 (mg)	1.4	29	41.4	40	57.1	1	1.4	-	-	47	78.3	13	21.7	0.001
Vitamin B2 (mg)	1.4	2	2.9	55	78.6	13	18.6	-	-	4	6.7	56	93.3	0.001
Vitamin B6 (mg)	1.9	36	51.4	33	47.1	1	1.4	-	-	51	85	9	15	0.001
Folate (mcg)	600	47	67.1	22	31.4	1	1.4	6	10	51	85	3	5	0.001
Vitamin C (mg)	85	3	4.3	24	34.3	43	61.4	-	-	-	-	60	100	0.001
Vitamin D (mcg)	15	31	44.3	34	48.6	5	7.1	1	1.7	15	25	44	73.3	0.001
Vitamin B12 (mcg)	4.5	16	22.9	39	55.7	15	21.4	8	13.3	27	45	25	41.7	0.037
Minerals														
Potassium (mg)	4.7	45	64.3	25	35.7	-	-	-	-	59	98.3	1	1.7	0.001
Calsiyum (mg)	1000	7	10	57	81.4	6	8.6	-	-	30	50	30	50	0.001
Magnesium (mg)	300	2	2.9	64	91.4	4	5.7	-	-	24	40	36	60	0.001
Phosphorus(mg)	550	-	-	1	1.4	69	98.6	-	-	-	-	60	100	0.353
Iron (mg)	16	45	64.3	24	34.3	1	1.4	-	-	58	96.7	2	3.3	0.001
Zinc (mg)	11	6	8.6	62	88.6	2	2.9	-	-	31	51.7	29	48.3	0.001

mcg: microgram, mg:miligram, n:number of people, p: statistical value, GDM: gestational diabetes mellitus, Control: healthy pregnant women, Dietary Reference Intake (DRI) Dietary Reference Intake

Table 3. Biochemical Findings of Pregnant Women

	GDM (n=70)		Control (n=60)		P value
	Upsıde down	x̄ ±SS	Upsıde down	x̄ ±SS	
Fasting glucose (mg/dl)	72-145	99.2 ± 1	72-112	87.2 ± 9.2	0.001
Hemoglobin (g/dl)	4.2-25	10.8 ± 2.9	7-30	11.9 ± 3.2	0.038
Hematocrit (%)	10.3-45	30.3 ± 7.4	13.9-50	33 ± 8	0.055
Vitamin D (ng/ml)	2-50	17.8 ± 10.7	8.8-52	20.6 ± 8	0.034
Vitamin B12 (pg/ml)	59-626	199.9 ± 91.5	102-627	234 ± 114.7	0.039
Ogtt 1.hour (mg/dl)	114-234	181.1 ± 2.8	-	-	0.424
Ogtt 2.hour (mg/dl)	107-100	145.1 ± 2.5	-	-	0.001

mg: miligram, dl:deciliter, g:gram, ml:millilitre, ng:nanogram, pg:picogram, p:statistic value GDM: gestational diabetes mellitus Control: healthy pregnant

DISCUSSION

Studies have shown that fasting blood sugar increases due to increased consumption of sugary foods such as cakes and pastries, and foods such as margarine and butter, which contain high saturated or trans fats, and decreased consumption of pulpy foods (12).

The ADA (1) recommends that 25 years of age and older be considered as a risk factor for GDM. Our study, along with other studies, supports that advanced gestational age is a risk factor for GDM.

The presence of diabetes in the family history of pregnant women with GDM is a risk factor. Leng et al. (13), it was concluded that 13.4% of pregnant women with GDM had a family history of diabetes and this increased the risk of GDM 1.61 times. In our study, the history of diabetes in the family of pregnant women diagnosed with GDM was found to be 17.1% higher than that of healthy pregnant women, and it was considered as a factor increasing the risk of GDM.

It becomes even more important that pregnant women take enough vitamins during the pregnancy period when their nutritional needs increase. Because the baby needs vitamins to continue its development in a healthy way. In particular, deficiencies of important micronutrients such as iron, iodine, folic acid, vitamin D and vitamin B12 cause various diseases such as megaloblastia, neural tube defects, placental and fetal defects (9). In particular, it was determined that the iron mineral was very low in individuals with GDM compared to healthy individuals. In addition, the use of vitamin and mineral supplements in pregnant women with GDM was found to be lower than in healthy pregnant women. Therefore, vitamins, minerals or both should be used during pregnancy.

BMI is the method used in the measurement of obesity, which is defined as an increase in weight above the desired level as a result of

excessive increase in the ratio of body fat mass to lean mass. Over 20% of body weight increases the risk of hypertension, chronic heart disease, hyperlipidemia, Type 2 diabetes and GDM. On this subject, Shin et al. (14) reported that GDM is 2.78 times more common in individuals with pre-pregnancy body mass index value in the obese category. In a cohort study of 226,958 women in Canada, it was reported that 9.7% of women with GDM were of normal weight before pregnancy and 51.1% were obese (15). In our study, it was determined that pregnant women with GDM started pregnancy as mildly obese and obese, and a finding supporting the literature was obtained.

According to the guidelines, it is recommended to include four snacks in the diet of pregnant women. In countries such as Switzerland and England, the recommended number of snacks is seven (16). Snacking is more important for pregnant women with GDM. Since there is a continuous glucose transfer from mother to baby, it helps to control glucose and insulin in the prevention of hypoglycemia. Especially if the hyperglycemia is more than 15-30 g in the morning, carbohydrate (CH) should be given at breakfast (17). In this study, it was found that the highest rate of skipping meals in individuals with GDM was breakfast. It was evaluated as a risk factor for GDM.

Consumption of fibrous foods should be increased. Since fibers are given to the bloodstream very slowly, they prevent blood sugar from being absorbed by the body and balance the sugar level in the blood (13). Zhang et al. (18) showed that a low-fiber and high-glycemic index diet increased the formation of GDM by 2.15 times. In addition, consumption of simple carbohydrate foods with high sugar and sugar content in women with GDM prevents the beneficial effects of fiber. Our results showed that pregnant women with GDM consumed high glycemic index and low fiber foods in food intake, similar to the study. The reason why the fiber ratios of pregnant women with GDM are low is that they consume less vegetables.

Vitamin D has an important role in glucose balance, insulin secretion and insulin sensitivity (19). Via active oxidative groups, vitamin D has antioxidant effects that can reduce damage and apoptosis of islet β cells (20). Burris et al. (21) in a study conducted with 1314 pregnant women, it was found that a significantly low serum 25 (OH) vitamin D level increased the occurrence of GDM by 3.36 times. It has been determined that GDM is significantly more common in pregnant women with severe serum 25(OH) vitamin D deficiency. (22). In this study, dietary vitamin D levels and serum 25 (OH) vitamin D levels of pregnant women with GDM were found to be significantly lower than

healthy pregnant women. Our findings are compatible with the literature.

Vitamin B12 is a coenzyme and is necessary for protein, fat, carbohydrate metabolism, erythropoiesis. Although its effect is seen in all cells, it is important for bone marrow, gastrointestinal system (GIS), central nervous system (CNS). Krishnaveni et al. (23) found that 43% of pregnant women had low levels of vitamin B12. It shows that vitamin B12 deficiency is associated with increased adiposity, insulin resistance and GDM. Serum B12 and hemoglobin levels of pregnant women with GDM were found to be significantly lower than controls. When the hematocrit levels of GDM and healthy pregnant women were evaluated, the hematocrit levels of the pregnant women with GDM were found to be lower than the healthy pregnant women.

In this study, the effects of foods and high glycemic index distribution on meals were investigated in order to provide glycemic level in pregnant women. Individuals with GDM had high blood sugar levels. It was determined that pregnant women with GDM were undernourished in terms of vitamins, minerals and macronutrients compared to healthy pregnant women.

CONCLUSION

As a result of the study, when the daily food intakes of the individuals were examined in detail, it was determined that the intake of vitamins, minerals and macronutrients was insufficient in pregnant women with gestational diabetes compared to healthy pregnant women. The study showed that insufficient food intake negatively affects blood sugar levels and biochemical parameters, especially vitamin D and B12 values. In order to meet the nutritional needs of the mother and the fetus developing in the mother's womb, it is thought that individual-specific diet should be planned and training should be given.

Considering all the studies in the literature on this subject, well-designed prospective randomized controlled studies with large participation are needed to evaluate the relationship between biochemical parameters and GDM.

Working limitations

This study had some limitations, such as the use of the food consumption frequency questionnaire (BTSA) to assess the possible relationship between food intake and biochemical parameters, based on the assumption that mothers answered all questions and scales correctly. The study relied on participants' ability to recall memories to complete BTSA questions. In line with these limitations, the results obtained from the study can be generalized to this research group.

Ethics Committee Approval: Ethics committee approval was obtained by Giresun University Clinical Research Evaluation Committee with the decision number 2018/05-06 dated

07/11/2018. Written informed consent was obtained from all subjects before participating in the study. The study protocol complies with international agreements.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: T.C, Design: T.C, Literature search: T.C, Data Collection and Processing: H.A, M.A, Analysis or Interpretation: T.C, Writing: T.C, A.C,

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

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The Evolution of Hypothalamus Publications with Bibliometric Analysis During 1980-2020

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Abstract

Objective: The hypothalamus, which is the main control center of the autonomic nervous system has been the subject of many studies around the world with increasing interest. The aim of this study was to make a comprehensive and holistic analysis of the publications on the hypothalamus between 1980 and 2020 with bibliometric approaches.

Methods: The Web of Science database was used to identify publications on the hypothalamus between 1980 and 2020. The identified publications were then analyzed using bibliometric approaches.

Results: As a result of the literature review, a total of 35919 publications were found. The three most active countries in the production of articles on the subject of the hypothalamus were the USA (n=9685), Japan (2636), and the UK (2065). A statistically significant positive correlation was found between the number of articles produced by countries on the hypothalamus and Gross Domestic Product (GDP) and GDP per capita development indicators ($r=0.785$, $p<0.001$; $r=0.511$, $p<0.001$, respectively).

Conclusion: Recent studies on the hypothalamus have focused on the functional connection of the hypothalamus with obesity, stress, and food intake. The findings of this study can be considered to be a comprehensive and useful guide for researchers interested in the topic of the hypothalamus.

Key words: Bibliometric analysis, Hypothalamus, Neuroendocrinology, Obesity, Food intake

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INTRODUCTION

The hypothalamus is a brain structure that is developmentally and functionally linked to the pituitary, which regulates physiological homeostasis and basic behavioral orientations (1). The hypothalamus, located most ventrally in the forebrain, provides the secretion of endocrine hormones and the control of the autonomic nervous system (2,3). The hypothalamus plays a role in vital functions such as circadian rhythm, temperature regulation, and maintaining feeding habits (hunger/satiety) (4-7). The hypothalamus, which is the main control center of the autonomic nervous system provides corticotropin, vasopressin, and oxytocin release, psychological and behavioral regulation, control of circadian rhythm, regulation of body temperature, is the sex control centre, and regulates sweating, food intake, respiratory rate, blood pressure, and heart rate (8). Dysfunction of the hypothalamus has an important place in various health areas, including diabetes insipidus, sleep disorders, and energy imbalance (9-11). The hypothalamus has gained popularity as an important research issue in recent years because of its significant and varied roles in autonomic, homeostatic, and neuroendocrine activities, and has been the subject of many studies around the world with increasing interest.

Bibliometrics is an approach based on mathematical and statistical methods used in the evaluation of various scientific publications such as articles, books, and reviews (12,13). It is a method that allows the identification of the most productive countries and institutions, the most effective authors, and the most qualified publications from a broad perspective by analyzing the publications on a particular field or subject from the past to the present. Bibliometry provides objective, detailed, and comprehensive information on the topic of interest for both researchers and institutions. In addition, the bibliometric analysis provides objective and reliable data about future studies for researchers interested in the subject by revealing past and current research trends related to the subject of interest.

The hypothalamus is a valuable subject of interest in many different disciplines, from anatomy to endocrinology. In the current literature, there is no bibliometric study that provides quantitative and qualitative analysis of scientific outputs related to the hypothalamus from the past to the present. The aim of this study was to conduct a comprehensive and holistic analysis of publications on the hypothalamus between 1980 and 2020 with bibliometric approaches and to identify the research issues and trends that have developed on the hypothalamus over 40 years.

METHODS

In order to reach the articles published on the hypothalamus, the literature search in this study was performed using the keyword

hypothalamus* (hypothalamus, hypothalamic, hypothalamotomy et al.) in the title of articles in the Web of Science (WoS) database (Search codes for repeatability; Title: (hypothalam*) Refined by: Document Types: (Article) Timespan: 1980-2020. Indexes: SCI-Expanded, ESCI, SSCI, CPCI-S, BKCI-SSH, BKCI-S). As a result of this search method, all publications published on the hypothalamus in the WoS database were downloaded (accessed on 30.03.2021; the number of publications may vary according to the search data) and analyzed using bibliometric methods

Statistical analysis

The website (https://app.datawrapper.de) was used for world map drawing. VOSviewer (Version 1.6.13) package program was used for bibliometric network visualizations (14). SPSS package (Version 22.0, SPSS Inc., Chicago, IL, USA, license: Hitit University) was used for statistical data analysis. Assessment of the normal distribution of the data was performed with the Kolmogorov-Smirnov test. Correlation analyses between the number of publications on the hypothalamus and the economic development indicators of countries according to Gross Domestic Product (GDP) per capita were investigated using the Spearman correlation coefficient in accordance with the data distribution (15). Linear regression analysis was used to estimate the number of publications to be made in the coming years. The R^2 value in the regression analysis was used to evaluate the model success. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

A total of 35919 publications were identified as a result of the literature search, of which 25145 (70.005%) were Article, 6899 (19.207%) Meeting Abstract, 1577 (4.390%) Review, 1300 (3.619%) Proceedings Paper, 908 (2.528%) Note, 361 (1.005%) Editorial Material and the remainder were Letter (295), Correction (199), Book Chapter (190), Early Access (36), Correction Addition (21), Book Review (13), News Item (10), Retracted Publication (8), Discussion (7), Retraction (7), Reprint (5), Book (3) and Data Paper (3). For this study, bibliometric analysis was applied to 25145 publications, categorised as articles. Of these, 97.1% (24426) were published in English, and the remaining articles were in Russian (318), French (162), German (81), Spanish (59), Czech (23), Japanese (19), Ukrainian (18), Chinese (14), Italian (10), Polish (6), Portuguese (4), Dutch (2), Hungarian (1), Slovak (1) and Turkish (1).

Development of Publications

The distribution of 25145 articles between 1980 and 2020 is given in Figure 1. The results of the regression analysis for the estimated number of publications in the future are also shown in the figure. From the regression analysis, it was estimated that 712 (CI 95%: 656-768) articles will be published in 2020 and 723 (650-796) articles in 2032 (Figure 1).

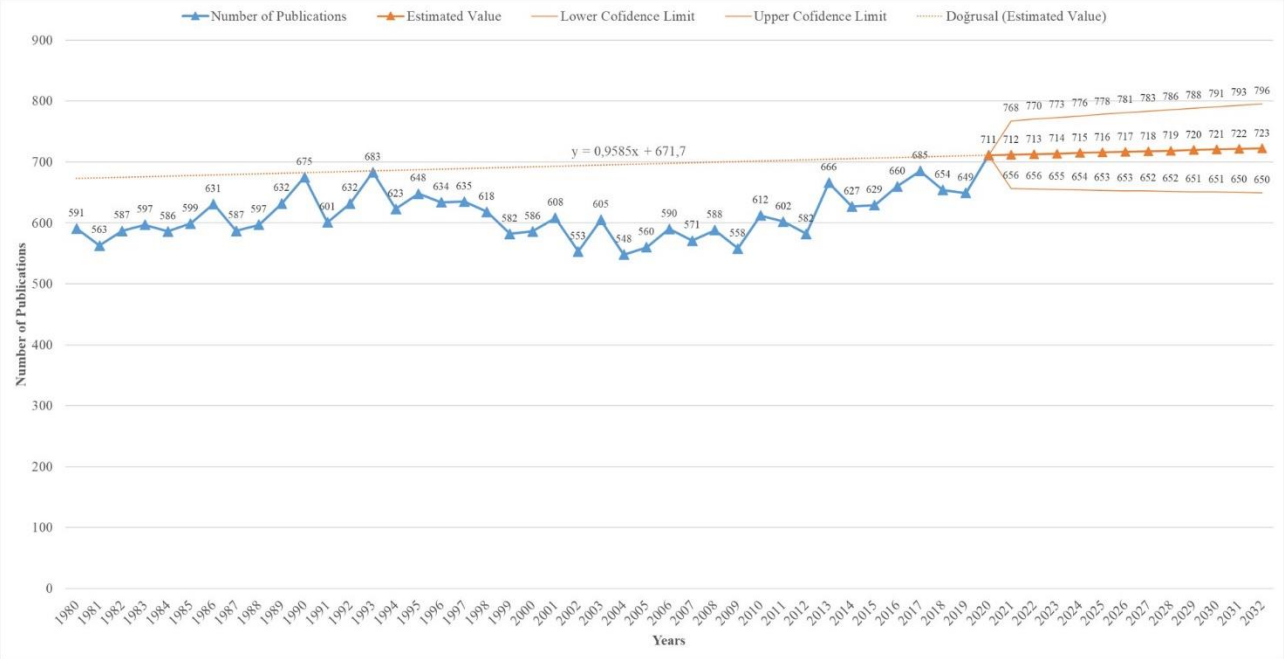

Figure 1. Number of publications by years on the hypothalamus

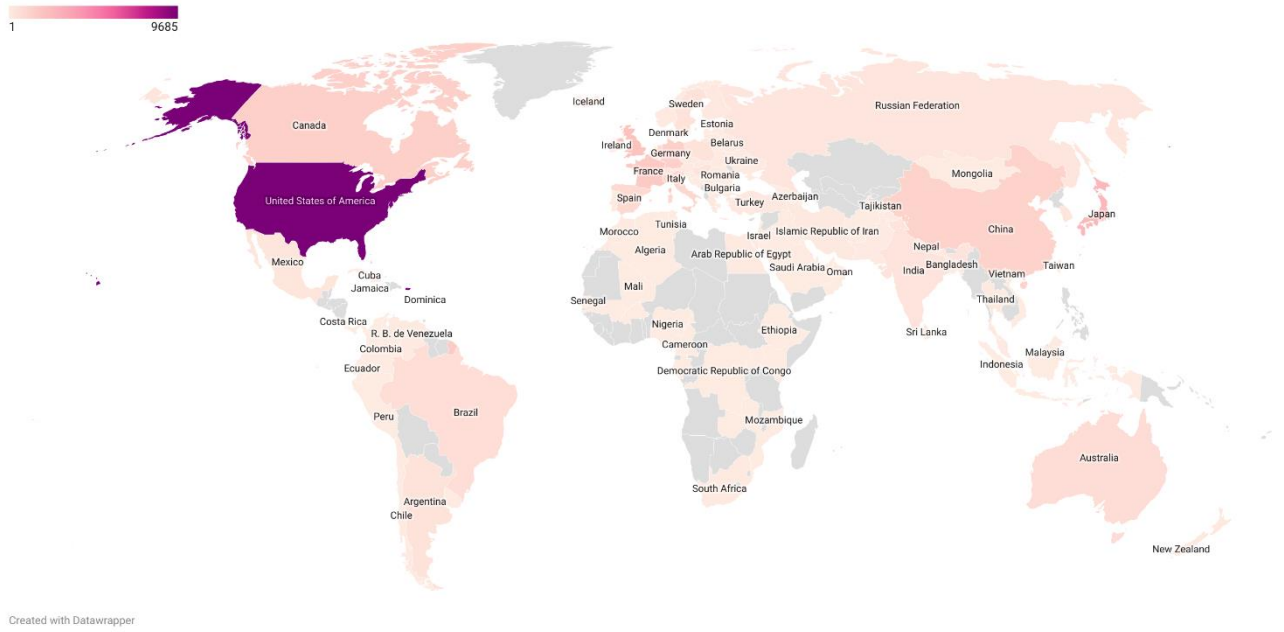

Figure 2. The distribution of the most productive countries on the hypothalamus

Active Countries

The publication distributions of the countries are shown in Figure 2 on the world map. The active countries producing more than 100 articles were seen to be the USA (n=9685), Japan (2636), the UK (2065), France (1608), Germany (1388), Canada (1347), China (1229), Italy (979), Spain (853), Australia (699), the Netherlands (692), Brazil (685), Poland (429), Sweden (426), Argentina (403), Hungary (403), the USSR (387), Switzerland (375), India (341), South Korea (308), Russia (298), Mexico

(257), Israel (238), Belgium (199), Denmark (194), Taiwan (168), Turkey (158), Finland (137), New Zealand (135), Iran (123), Austria (118) and Greece (109). In addition, an international collaboration network visualization map and density map between 71 different countries producing at least 15 publications from these countries are presented in Figures 3.a and 3.b, respectively

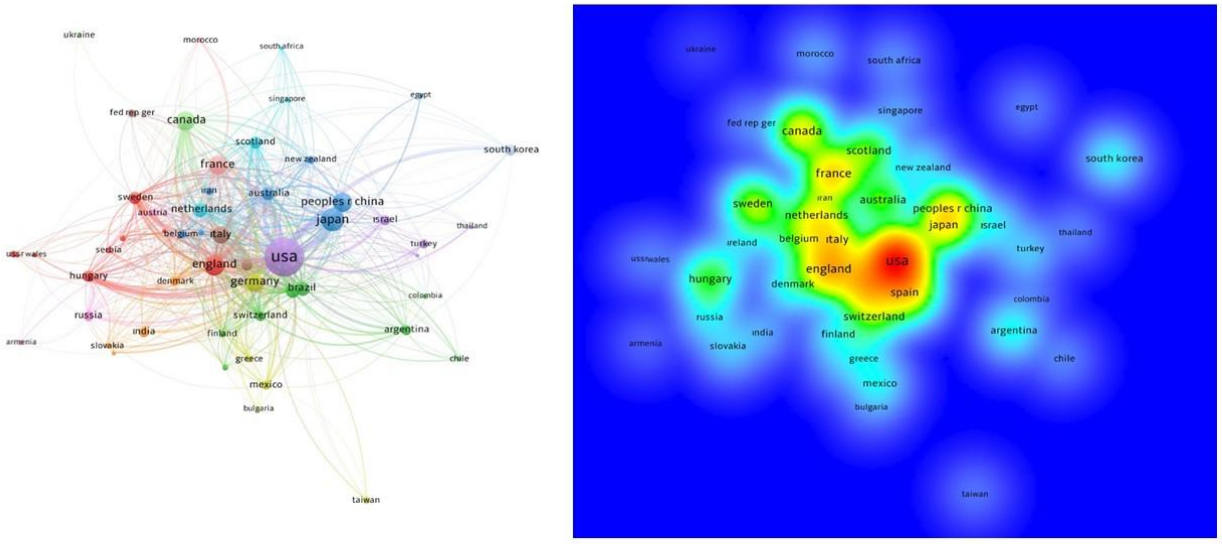

Figure 3. Network visualization map showing the international collaboration of countries on hypothalamus. Footnote: The size of the circle shows a large number of publications; the colours indicate the cluster of collaborations.

Correlation Analysis

A statistically significant positive correlation was found between the number of hypothalamic articles produced by countries and the development indicators of GDP and GDP per capita, ($r=0.785$, $p<0.001$; $r=0.511$, $p<0.001$ respectively).

Active Authors

The top 10 authors who produced the most articles on the hypothalamus were Pfaff DW (n=122), Leibowitz SF (98), Chrousos GP (93), Swaab DF (93), Lightman SL (92), Clarke IJ (85), Bloom SR. (83), Dieguez C (78), Kalra SP (77), and Ueta Y (77).

Active Institutions

The 13 most productive universities that have produced more than 200 publications on the hypothalamus are the University of Texas (n=350), Rockefeller University (338), the University of Sao Paulo (312), the University of California- Los Angeles (296), Harvard University (274), Yale University. (267), the University of Michigan (259), Oregon Health & Science University (254), the University of Toronto (254), the Institut National de la Sante

et de la Recherche Medicale (INSERM) (247), the National Institute of Mental Health (NIMH) (244), the Karolinska Institutet (204), and the Salk Institute for Biological Studies (203).

Active Research Areas

The top 10 research fields with the most studies on the hypothalamus were found to be Neurosciences (10305; 40.9%), Endocrinology Metabolism (6985; 27.7%), Pharmacology Pharmacy (2081; 8.2%), Physiology (2018; 8%), Biochemistry Molecular Biology (1760; 6.9%), Behavioral Sciences (1154; 4.5%), Clinical Neurology (1073; 4.2%), Multidisciplinary Sciences (1012; 4%), Cell Biology (943; 3.7%), and Psychiatry (939; 3.7%).

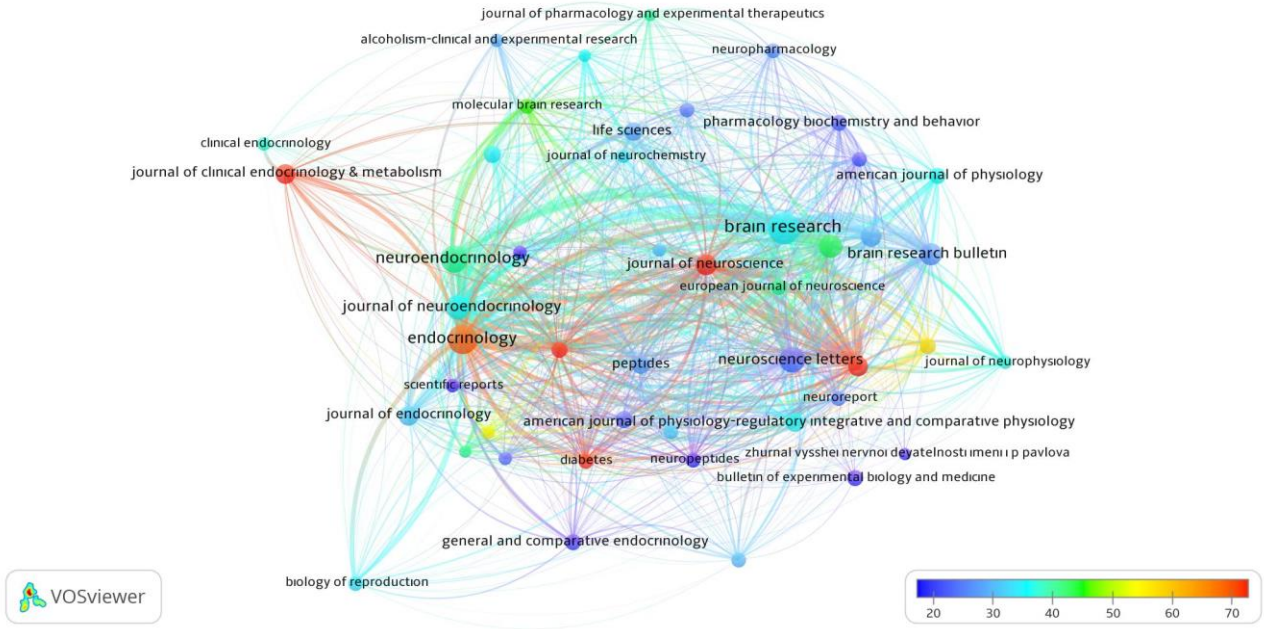

Figure 4. Network visualization map for citation analysis of active journals in hypothalamus Footnote: The number of average citations increases from blue to red (blue-green-yellow-red).

Active Journals

There have been 25145 publications published on the hypothalamus in 2257 different journals, with over 90 articles appearing in 47 of the journals. Table 1 shows the total number of citations attained by these journals, the articles in the journals, and the average number of citations per article. The visualization map of the citation network between these publications is illustrated in Figure 4.

Citation Analysis

In Table 2, which includes the average number of citations per year in the last column, the 18 publications with the greatest amount of citations from 1000 of the 25145 articles published on the hypothalamus between 1980 and 2020 are included

Co-citation Analysis

There were references to 400,314 unique publications among 25145 articles. These publications contain the first 11 publications with more than 400 citations seen to be in descending order, Lowry OH (1951) (Number of co-citation: 888), Paxinos G (1986) (736), Swanson LW (1980) (700), Schwartz MW (2000) (677), Swanson LW (1983) (562), Bradford MM (1976) (474), Paxinos G (1982) (464), Paxinos G (1998) (451), Sakurai T (1998) (443) , Vale W (1981) (434), and Swanson LW (1980) (421) (16-26).

Keyword Analysis and Trend Topics

In the 25145 articles published on the hypothalamus, were 21432 different keywords used, 71 of which appeared in at least 100 several publications. These words are given in Table 3To determine the usage association of the keywords used in the studies, a map of network visualization for cluster analysis was drawn and the cluster network visualization map between these keywords is given in Figure 5. The trend visualization network map showing the distribution of keywords used in studies conducted between 1980-2020 by years is given in Figure 6 and the citation network visualization map made to identify effective topics that received more citations is given in Figure 7.

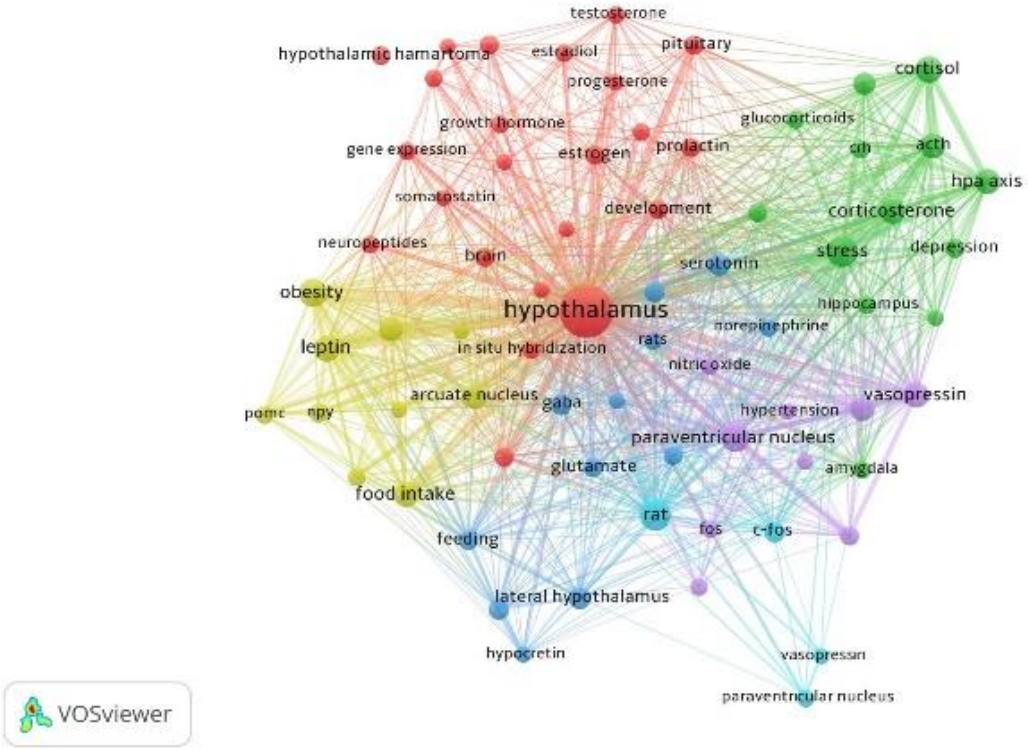

Figure 5. Network visualization map showing cluster analysis results based on keyword analysis on hypothalamus Footnote: Colours indicate clustering.

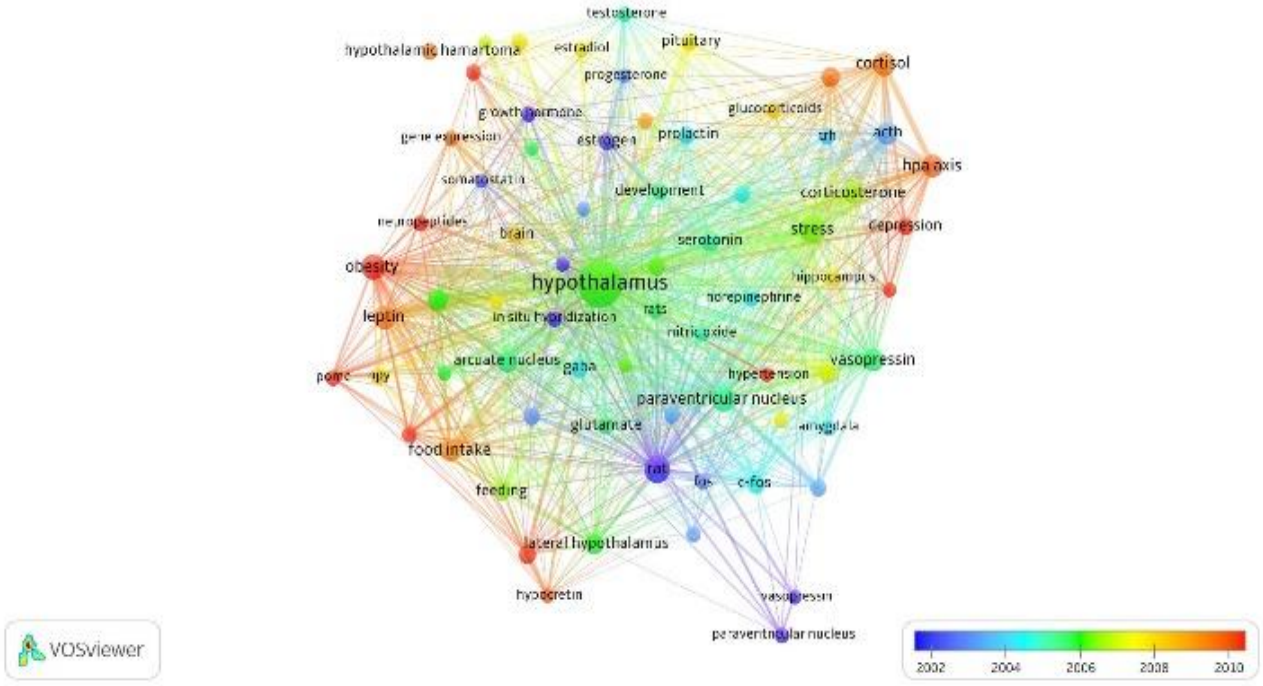

Figure 6. Network visualization map for trends based on keyword analysis on hypothalamus. Indicator shows current publications from blue to red.

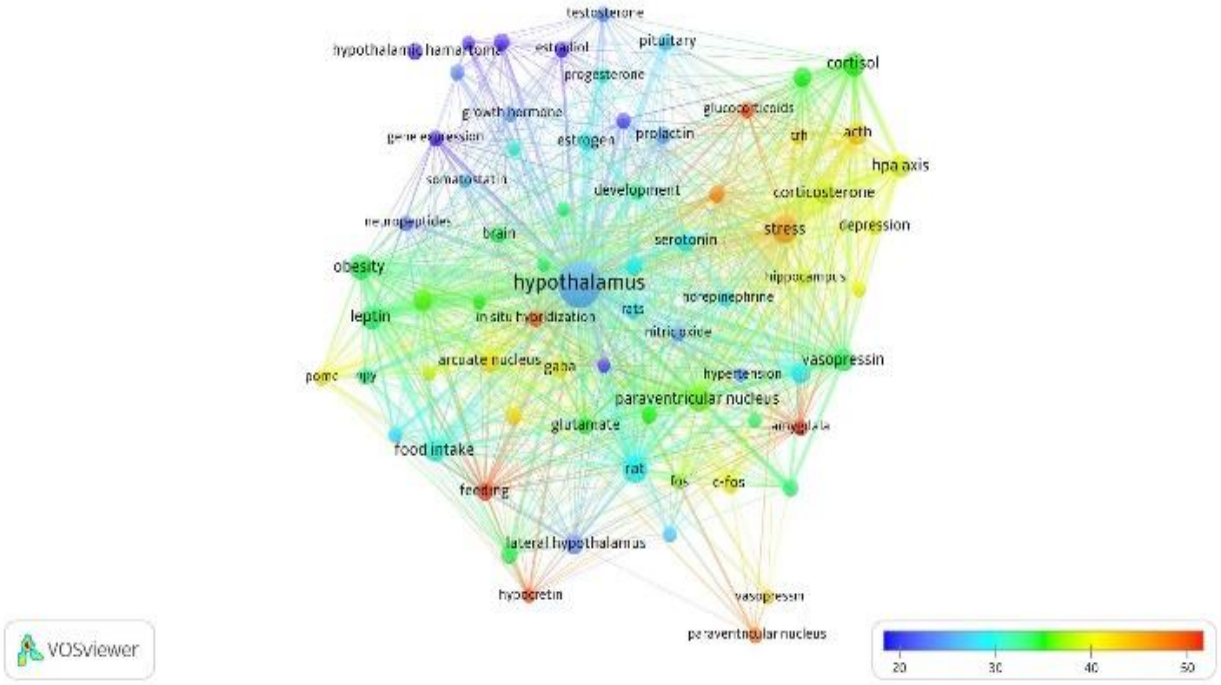

Figure 7. Network visualization map for citations based on keyword analysis on hypothalamus. The number of citations increases from blue to red (blue-green-yellow- red).

Table 1. Active journals on hypothalamus

Journals	RC	C	AC	Journals	RC	C	AC
Brain Research	1431	51118	35.72	Behavioural Brain Research	159	3615	22.74
Endocrinology	1167	78125	66.95	Diabetes	159	14688	92.38
Neuroendocrinology	872	35332	40.52	Biology of Reproduction	152	5226	34.38
Neuroscience Letters	710	16947	23.87	Regulatory Peptides	149	4737	31.79
Journal of Neuroendocrinology	682	24983	36.63	Neuropeptides	147	2470	16.80
Neuroscience	526	22495	42.77	Cell and Tissue Research	140	4246	30.33
Brain Research Bulletin	464	12784	27.55	European Journal of Pharmacology	139	3571	25.69
Physiology & Behavior	399	11926	29.89	Molecular Brain Research	137	6294	45.94
Journal of Neuroscience	380	34562	90.95	Neuroreport	136	3515	25.85
Journal of Clinical Endocrinology & Metabolism	341	24963	73.21	Neuropharmacology	135	3637	26.94
Journal of Comparative Neurology	324	29379	90.68	Biochemical and Biophysical Research Communications	125	6670	53.36
Journal of Endocrinology	307	9728	31.69	Clinical Endocrinology	123	4719	38.37
Peptides	282	7941	28.16	Journal of Neurochemistry	123	4169	33.89
American Journal of Physiology-Regulatory İntegrative and Comparative Physiology	264	9690	36.70	Journal of Neurophysiology	110	4158	37.80
Life Sciences	257	7430	28.91	Neuroendocrinology Letters	110	639	5.81
Psychoneuroendocrinology	220	7804	35.47	Developmental Brain Research	103	3186	30.93
Plos One	212	5049	23.82	Molecular and Cellular Endocrinology	102	2425	23.77
American Journal of Physiology	187	6944	37.13	Alcoholism-Clinical and Experimental Research	101	2987	29.57
Pharmacology Biochemistry and Behavior	187	4466	23.88	Scientific Reports	101	1081	10.70
General and Comparative Endocrinology	183	3812	20.83	Journal of Pharmacology and Experimental Therapeutics	97	3886	40.06
Proceedings of The National Academy of Sciences of the United States of America	175	21801	124.58	American Journal of Physiology-Endocrinology and Metabolism	96	3940	41.04
Bulletin of Experimental Biology and Medicine	171	149	0.87	Zhurnal Vysshei Nervnoi Deyatelnosti Imeni I P Pavlova	94	183	1.95
European Journal of Neuroscience	170	6952	40.89	Hormones and Behavior	93	3383	36.38
Journal of Physiology-London	167	9414	56.37				

RC: Record Count, C: Number of Citation, AC: Average Citation Per Document

Table 2. The 18 most cited articles on hypothalamus

No	Article	Author	Journal	PY	TC	AC
1	Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin	Vale, W. *et al.*	Science	1981	4160	101.46
2	Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior	Sakurai, T. *et al.*	Cell	1998	3691	153.79
3	The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity	De Lecea, L. *et al.*	Proceedings of the National Academy of Sciences of the United States of America	1998	2695	112.29
4	Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress	Liu, D. *et al.*	Science	1997	2175	87
5	Isolation of a novel-38 residue-hypothalamic polypeptide which stimulates adenylate-cyclase in pituitary-cells	Miyata, A. *et al.*	Biochemical and Biophysical Research Communications	1989	1653	50.09
6	A receptor in pituitary and hypothalamus that functions in growth hormone release	Howard, AD. *et al.*	Science	1996	1585	60.96
7	Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass	Ducy, P. *et al.*	Cell	2000	1504	68.36
8	Cholinergic innervation of cortex by the basal forebrain - cyto-chemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus-monkey	Mesulam, MM. *et al.*	Journal of Comparative Neurology	1983	1455	37.31
9	Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor	Sapolsky, R. *et al.*	Science	1987	1440	41.14
10	Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress	Tsigos, C. *et al.*	Journal of Psychosomatic Research	2002	1428	71.4
11	Identification of targets of leptin action in rat hypothalamus	Schwartz, MW. *et al.*	Journal of Clinical Investigation	1996	1261	48.5
12	Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) messenger-rna, median-eminence CRF content and stress-induced release in adult-rats	Plotsky, PM. *et al.*	Molecular Brain Research	1993	1248	43.03
13	The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion.	Wren, AM. *et al.*	Endocrinology	2000	1233	56.05
14	Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis	Kirschbaum, C. *et al.*	Psychosomatic Medicine	1999	1228	53.39
15	The paraventricular nucleus of the hypothalamus - cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal-cord as demonstrated by retrograde fluorescence double-labeling methods	Swanson, LW. *et al.*	Journal of Comparative Neurology	1980	1207	28.74
16	AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus	Minokoshi, Y. *et al.*	Nature	2004	1156	64.22
17	The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis	Cowley, MA. *et al.*	Neuron	2003	1139	59.95
18	Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice	Sudo, N. *et al.*	Journal of Physiology-London	2004	1089	60.5

PY: Publication year, TC: Total citation, AC: Average citations per year

Table 3. The most used trend keywords on hypothalamus

Keyword	O	Keyword	O	Keyword	O
hypothalamus	3736	development	194	blood pressure	137
stress	698	depression	192	glucocorticoids	136
rat	676	corticotropin-releasing hormone	189	crh	135
obesity	573	brain	187	rats	132
paraventricular nucleus	543	glutamate	183	kisspeptin	131
cortisol	469	gnrh	177	estradiol	129
leptin	450	pituitary	177	hippocampus	129
food intake	415	supraoptic nucleus	177	growth hormone	125
corticosterone	413	prolactin	176	fos	123
hpa axis	397	estrogen	174	ventromedial hypothalamus	122
vasopressin	381	microdialysis	172	insulin	116
acth	369	immunohistochemistry	170	puberty	116
arcuate nucleus	365	npy	163	vasopressın	116
oxytocin	343	gene expression	155	preoptic area	112
neuropeptide y	302	testosterone	154	immunocytochemistry	108
lateral hypothalamus	294	in situ hybridization	153	hypothalamic paraventricular nucleus	106
hypothalamic-pituitary-adrenal axis	291	norepinephrine	153	anxiety	105
serotonin	247	ghrelin	148	gonadotropin-releasing hormone	105
dopamine	229	amygdala	147	proopiomelanocortin	104
orexin	217	nitric oxide	147	somatostatin	102
c-fos	213	pomc	146	hypertension	101
gaba	208	aging	142	hypocretin	101
feeding	196	neuropeptides	139	progesterone	101
hypothalamic hamartoma	195	paraventrıcular nucleus	139		

O: Number of occurrences

DISCUSSION

The number of articles published on the hypothalamus was 591 in 1980 and 711 in 2020. From the regression analysis results was projected that interest in the hypothalamus will continue, and accordingly, the number of articles on this subject will increase to reach an estimated 723 by the end of 2032.

Of the 10 countries that produce articles about the hypothalamus the most, 9 were the USA, Japan, the UK, France, Germany, Canada, Italy, Spain, and Australia, all of which are developed countries with strong economies. The effectiveness of developed countries in the productivity of publications shows that the productivity of publications on the subject of the hypothalamus is directly

related to economic power. Although classified as a developing country, China has managed to become one of the top ten most active countries as a result of its growing economy and support for scientific studies. The statistically significant correlation analysis results also confirmed the hypothesis of the association between article productivity and some development indicators.

When the cooperation between the countries was evaluated, it was seen that although there was some cooperation based on geographical proximity (Brazil, Argentina, Chile, Colombia), the geographical region did not have a great effect on cooperation. These findings are in contrast to findings in the literature on this issue. Previous bibliometric studies in the literature, on the other hand, have generally focused on specific diseases such as carpal tunnel syndrome, celiac disease, or diabetic retinopathy, and so a possible reason for the difference in these findings could be that the hypothalamus is a relatively more universal subject for which there is a constant worldwide demand for research, independent of geographical cooperation.

The journals with the most published articles were determined to be Brain Research, Endocrinology, Neuroendocrinology, Neuroscience Letters, Journal of Neuroendocrinology, Neuroscience, Brain Research Bulletin, Physiology & Behavior, Journal of Neuroscience, Journal of Clinical Endocrinology & Metabolism, Journal of Comparative Neurology, and Journal of Endocrinology, respectively. Authors who want to publish on the hypothalamus can prioritize these journals. The Proceedings of the National Academy of Sciences of the United States, Diabetes, Journal of Neuroscience, Journal of Comparative Neurology, and the Journal of Clinical Endocrinology and Metabolism were found to be the most productive journals when the results of the citation network visualization map analysis of the journals were evaluated. Therefore, it can be suggested that researchers who want their articles to be read more and be cited more should first consider these journals.

The most cited study, according to the total number of citations received, was published in the journal Science by Vale et al. and was titled "Characterization of a 41-residue ovine hypothalamic peptide that stimulates the secretion of corticotropin and beta-endorphin" (25). In this study, a peptide with 41 residues that induces the release of immunoactivities similar to corticotropin and 1-endorphin in both vitro and in vivo was purified, its sequence was analyzed, and its whole synthesis was described. According to the authors, this peptide may play an important role in mediating and integrating an organism's endocrine, visceral, and behavioral responses to stress (25). The second most cited study was by Sakurai et al. published in Cell journal,

entitled “Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior” (24). In this study, the authors discovered two new neuropeptides, Orexin-A and -B, that are derived from the same precursor via proteolytic processing and bind to and activate two previously unknown orphan G protein-coupled receptors. Following these two studies, the most cited studies were De Lecea et al. 'The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity' published in the Proceedings of the National Academy of Sciences of the United States of America and a study by Liu et al. published in the journal Science, entitled “Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress” (27,28). This study examined the behavior of mothers during the first ten days of life is "critical" for scratching effect on HPA (hypothalamic-pituitary-adrenal) development. It was stated that neonatal programming occurs in response to changes in maternal behavior by differentiating the glucocorticoid receptor system in forebrain neurons that govern HPA axis (loop) activity. When the studies were evaluated based on the average number of citations per year, Sakurai et al., De Lecea et al., and Vale et al. had the top three most influential cited articles (24,25,27). Influential articles with the most average citations ranked fourth, fifth, and sixth were by Thaler et al. entitled “Obesity is associated with hypothalamic injury in rodents and humans” published in the Journal of Clinical Investigation, by Liu et al., and by Tsigos et al. published in the Journal of Psychosomatic Research entitled “Hypothalamic-pituitary-adrenal axis, neuroendocrine factors, and stress” (28-30). According to the co-citation numbers of all the analyzed articles, Lowry (1951), Paxinos (1986), Swanson (1980), Schwartz (2000), Swanson (1983), Bradford (1976), Paxinos (1982), Paxinos (1998), Sakurai (1998), Vale (1981), and Swanson (1980) were identified as the most influential (16-26). For researchers interested in the subject of the hypothalamus or planning new research, evaluating the above-mentioned studies and other studies in the table first may be beneficial in terms of producing higher quality, more effective studies.

When the results of the keyword analysis were analyzed, it was discovered that the cluster analysis had produced 6 distinct colored clusters. The hypothalamus studies were conducted in 6 different clusters, with the keywords rat, lateral hypothalamus, obesity, aging, stress, and paraventricular nucleus at the center, according to the results of the keyword clustering analysis. The paraventricular nucleus, obesity, and stress-centered clusters stand out as major study clusters.

When the results of the analysis made to state the trend topics of the hypothalamus articles from the past to the present were evaluated, the most used keywords before and after 2002 were determined to be rat, vasopressin, fos, paraventricular nucleus, growth hormone, estrogen, somatostatin, progesterone, and in situ hybridization. Before and after 2004, paraventricular nucleus, c-fos, GABA, prolactin, norepinephrine, prolactin, and testosterone were the most used keywords, and before and after 2006, these were seen to change to the arcuate nucleus, rats, stress, vasopressin, glutamate, lateral hypothalamus, and feeding. The most used keywords before and after 2008 were pituitary, estradiol, neuropeptide Y (npy), cortisol, HPA axis, leptin, food intake hypothalamic hamartoma, gene expression, and cortisol. In 2010 and after, the most preferred keywords were obesity, depression, proopiomelanocortin (POMC), and neuropeptides. When the keywords used in hypothalamus studies conducted after 2010 were evaluated, it was seen that the subject of research was the role of the hypothalamus in mood disorders, eating habits, and sexual behaviors. From the analysis of the keywords used in the hypothalamus articles, it can be seen that they were the subject of many different disciplines in terms of subject and content in the fields of the neuroendocrine system, physiology, neurology, psychiatry, behavioral sciences, nutrition and dietetics, and anatomical morphology.

Obesity, kisspeptin, neuropeptides, anxiety, depression, orexin, ghrelin, POMC, hypertension, and hypocretin were the trend keywords used most recently when the most commonly investigated (more than 100) terms on the hypothalamus were assessed according to publication years. Feeding, amygdala, hypocretin, glucocorticoids, in situ hybridization, and paraventricular nucleus were the search terms most frequently used in cited papers.

A limitation of this study could be said to be that the literature search was only made using the WoS database, and the Pubmed, Scopus EBSCO, ProQuest, Google Scholar, Crossref, Index Copernicus and Publons databases etc. were not searched. It was decided not to use the Pubmed database because citation analysis could not be performed, and the Scopus database was not selected because there were journals with low impact levels in the database. Since journals with high impact factors are indexed in the WoS database, WoS is a more reliable database in terms of citation compared to other databases (31-38). In addition, the use of more than one database in studies in which a large number of articles are analyzed causes the same article to be included in the analysis more than once, which affects the reliability of the results. Therefore, the WoS database has

been used in many bibliometric studies conducted in recent years (31-38).

Ethics Committee Approval: Ethical approval is not required for this study as the present study was related to statistical analyzes of the publications in WoS database.

Author Contributions: Concept: F O, Design: F O, G A, Literature search: G A, Data collection and Processing: F O, G A, Analyses and Interpretation: F O, G A, Writing: F O, G A.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: There is no funding to the study.

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Determining the Use of Peripheral Intravenous Catheter in Installed Patients with Some Markers

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Abstract

Objective: The aim of this study is to evaluate the medical conditions of patients who are inpatient with a peripheral intravenous catheter inserted in a private hospital in Northern Cyprus and to determine the frequency of use and risk factors that have occurred or may occur.

Methods: The research is descriptive and cross-sectional. All patients who are hospitalized in a private hospital in Northern Cyprus formed the universe. The data were collected using a form of with 18 items. Data collection was provided face-to-face with the patients and from their own patient files. Written informed consent was obtained from the university ethics committee, the university administration and the patients for the study.

Results: It was determined that most of the patients were male (49.09%) and peripheral intravenous use was more used (92.73%) in patients older than 18 years of age. It was determined that 98.18% of the patients had the date of the catheter documented, and 54.55% did not specify the insertion time. The anterior part of the arm was found to be the most common catheter application (36.36%). In 85.45% of the patients, a catheter-related problem(s) did not develop and 61.82% of them were evaluated in the last 24 hours.

Conclusion: The latest status of peripheral intravenous catheter applications and compliance and focus on internationally published guidelines in peripheral intravenous catheter applications and management bring about a serious improvement in surveillance, evaluation, decision-making, minimizing application errors, reducing the risk of complications, and documentation.

Key words: Catheter, intravenous, patient, peripheral

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INTRODUCTION

Peripheral intravenous catheter (PIC) applications are frequently used to provide vascular Access (1, 2). Thanks to PIC, intravenous (IV) fluid and electrolyte treatments, total parenteral nutrition, drug treatments, administration of blood and blood products, laboratory techniques, hemodialysis treatment, hemodynamic monitoring can be done easily (2, 3). Peripheral venous catheters, central venous catheters, pulmonary artery catheters and peripheral artery catheters are widely used in hospitals and other healthcare institutions (3 - 5).

PIC applications can be life-saving especially when done correctly. However, complications may develop due to inadequate diagnosis, erroneous application and some problems that may occur while giving care. These complications are important causes of mortality and morbidity (1). Complications that may develop from PIC include thrombophlebitis, infection (catheter entry site, catheter colonization, septic thrombophlebitis, endarteritis, pocket and/or tunnel infection, bloodstream and metastatic infections), extravasation, occlusion, embolism, fistulation, cardiac arrhythmias, pneumothorax, hemothorax (3, 6 - 8).

PICs are often used. It is stated that approximately 300 million intravenous catheters are used annually in the United States (USA) and 1.2 billion in the world (9 - 11). Alexandrou et al. (2018) conducted a study on PIC involving 51 countries. 40.620 PICs were evaluated in the study. It has been stated that PICs are mostly worn in inpatient clinics or general health centers, nurses perform the application most often, PIC is administered for IV drug therapy, 20-22G intraketes are used most often, and the application area is mostly hands (12).

Regulations are made with published guidelines for the proper use of PICs, which are so common and cause serious complications. According to this, The size of the catheter should be chosen according to the age of the patient, the condition of the vein, the treatment to be applied, the diagnosis and the activity status of the individual. According to the recommendation of the Center for Disease Control and Prevention, catheters can be used up to 72 - 96 hours if they do not pose a risk in terms of infection and phlebitis. The patient should be constantly monitored for complications (1, 13).

Aim of the Study

The aim of this study is to evaluate the medical conditions of PIC patients who are hospitalized in a university hospital in Northern Cyprus, and to determine the frequency of use and risk factors that may occur**.**

Questions of The Study

1. What is the status of inpatients regarding PIC?

2. Do inpatients have any problems with PIC?

METHODS

Type and Population of the Study:

The population of this cross-sectional and descriptive study consisted of patients who were hospitalized in the inpatient wards of a university hospital in Northern Cyprus and voluntarily accepted to participate in the study.

Inclusion Criteria for Research:

Among the criteria for inclusion in the research; The patient had PIC, agreed to participate in the study voluntarily, and did not have any communication problems. All patients who were being treated at the hospital on the day the data were collected were included in the study. The data of the study were collected in a single day. No specific sampling method was applied.

Data Collection:

The data were collected by means of a data collection form (10,12) containing 18 items (consisting of questions and statements) and prepared in line with the literature. The application of the data collection form for each patient took between 15-20 minutes. During the data collection phase, information was collected and verified by face-to-face interviews with patients and from patient files (written or computerized). During the implementation phase, the patients were given verbal information about the research and their written consents stating that they participated in the study were obtained.

Statistical analysis

Data were analyzed in the Statistical Package for Social Sciences (SPSS) (version 21.0) (IBM Corp.; Armonk, NY, USA) program. In the analysis of the data, the frequency distributions of the patients and PIC characteristics were evaluated. The resulting frequency results are given in the tables as numbers (n) and percentages (%) and interpreted.

Ethical Approach:

In order to carry out the study; Institutional permission from the head of the university, approval of the university ethics committee (YDU/2020/83-1158), and written consent from the patients who accepted the study were obtained.

RESULTS

The presence of PIC according to the descriptive characteristics of the hospitalized patients is given in Table 1. According to this; PIC was found to be higher in male patients (49.09%) (n=27) and in patients older than 18 years (92.73%) (n=51) (Table 1).

Table 2 gives the first part of some descriptive characteristics related to PIC. PIC insertion date was documented in 98.18% (n=54) of the patients, but PIC insertion time was not specified in 54.55% (n=30) patients. PIC was inserted in 72.73% (n=40) of the patients due to IV drug therapy. It was

determined that the nurses applied to patients with PIC (96.36%, n=53), the majority of the catheters (81.82%, n=45) were inserted in the patient's room, and 36.36% (n=20) of these catheters were placed in the antecubital/forearm region of the arm (Table 2).

Table 1. PIC distribution according to the descriptive characteristics of the patients (n=55)

Introductory Feature	Number(n)	Percentage(%)
Gender		
Female	27	47.27
Male	26	49.09
Not reported	2	3.64
Age		
<18 years	3	5.45
>18 years	51	92.73
Not reported	1	1.82
Total	55	100

Table 2. Distributions related to PIC (1) (n=55)

	Number (n)	Percentage (%)
The day of insertion of the catheter		
Documented	54	98.18
Undocumented	1	1.82
Catheter insertion time		
Documented	25	45.45
Undocumented	30	54.55
Catheter insertion reason		
IV fluid	13	23.64
IV medication/drug	40	72.73
Unstability/Resuscitation	1	1.82
Unknown	1	1.82
The person applying the catheter		
Nurse	53	96.36
Technician	2	3.64
Department where the catheter is inserted		
Operating room	3	5.45
Intensive care unit	7	1.73
Inpatient clinics	45	81.82
Where the catheter is located		
Back of hand	15	27.27
Wrist	13	23.64
Forearm	20	36.36
Upper arm	6	10.91
Foot	1	1.82

Table 3 shows the distributions related to PIC. 74.55% (n=41) of the patients had a 20G (pink color) catheter. No catheter-related symptoms or complications developed in 85.45% (n=47) of the patients. Most of the patients were evaluated within the last 24 hours (61.82%, n=34). When the sites with the catheter were examined, it was determined that 92.73% (n=51) were clean, dry and intact. It was determined that 38.18% (n=21) of the catheters used stepcock/3-way taps (Table 3).

Table 3. Distributions related to PIC (continued) (n=55)

	Number (n)	Percentage (%)
Catheter size		
16G (grey)	1	1.82
18G (green)	3	5.45
20G (pink)	41	74.55
22G (blue)	9	16.36
24G (yellow)	1	1.82
Catheter site assessment		
No clinical symptoms	47	85.45
Redness >1 cm from the catheter site	2	3.64
Swelling >1 cm from the catheter site	2	3.64
Bruising, tearing of the skin	2	3.64
Hardening of the vein	1	1.82
Blood in the catheter line	1	1.82
Is the catheter evaluation documented?		
Yes	34	61.82
No	20	36.36
Newly applied	1	1.82
Catheter dressing assessment		
Clean, dry, solid	51	92.73
Dry, dirty and runny	2	3.64
Other	2	3.64
IV connectors		
Stepcock/3-way faucet	21	38.18
IV end cup	12	21.82
Direct connection	20	36.36
None	2	3.64

Table 4 shows the distributions related to PIC. The patients were given more crystalloid fluids (69.09%, n=38) by IV catheter, the number of patients who received continuous infusion was high (47.27%, n=26), the catheters

were flushed with 0.9% Sodium Chloride as 100% (n=55), however, 98.18% (n=54) of these washes were not documented, and 27.47% (n=15) of the patients received analgesia on the day of data collection (Table 4).

Tablo 4. Distributions related to PIC (continued) (n=55)

	Number (n)	Percentage (%)
Treatment administered during the day		
Crystalloid (e.g. normal saline, 5% dextrose)	38	69.09
Parenteral nutrition	1	1.82
None	16	29.Eyl
Treatment applied on the day of data collection		
Ongoing infusion	26	47.27
Intermittent infusion	1	1.82
Bolus infection	5	9.Eyl
Combination of intermittent and bolus	4	7.27
None	19	34.55
Flushing the catheter		
0.9% Sodium Chloride	55	100
Is catheter flushing documented?		
None	54	98.18
IV medications during the day		
Electrolytes	3	5.45
Antibiotic	14	25.45
Analgesia	15	27.47
Anti-emetic	7	1.73
Insulin	1	1.82
Stomach protection	3	5.45
Other	1	1.82
None	11	20.00

DISCUSSION

This study shows the PIC usage rate, characteristics, management, practices and differences between them in a university hospital in Turkish Republic of Northern Cyprus. According to the results of our study, PIC was inserted mostly in patients older than 18 years (n=51). In the study of Alexandrou et al. (2018), 40,620 PICs included in the study were implanted and the majority of the patients were between the ages of 37-74 (mean 59) (12). It is important that PIC applications are recorded in writing and that this registration is done correctly. For this, the nurses are required to record the PIC applications with the necessary information on the observation forms (2, 14). In our study, it was determined that the PIC application was recorded in the observation form as date (98.18%) and time (45.45%). It is stated that the application should be recorded in order to provide easy access to all information of the patients, especially for the control of infection that may occur in PICs, and to keep patient safety in the foreground. In these records, the type of IV application, the number of the catheter used, the area of application, the person performing the application, the reason why it was removed and inserted should be included (15).

In the study of Alexandrou et al. (12), it is stated that PICs are mostly used for IV drug treatment (70%). In this study, it was concluded that 72.73% of PICs were used for IV drug treatment. In the study of Wallis et al. (2014), IV administration of antibiotics was found to be a risk factor for the development of phlebitis and occlusion (8). In the study of Enes et al. (16), it was determined that phlebitis developed in patients who received fluid infusion and drug therapy together.

PIC procedure is routinely applied in hospitals. However, studies have shown that 4-28% of the applied catheters are not used for therapeutic purposes. At the same time, it has been reported that 20% of the patients who have a catheter inserted are unnecessary (17, 18). According to a study conducted in the USA (2016), it was stated that the number of applications that were made unnecessarily and/or resulted in wrong and unsuccessful attempts was 150 million, causing an extra $1.5 billion in financial expenditure (19). In this study, 11 patients (20%) were not given any medication/medication etc. Although not administered, patients appear to have the presence of PIC.

PIC applications are generally the responsibility of nurses. According to the regulation published in Turkey in 2011, nurses are defined as the occupational group that performs PIC and should monitor complications (20). In this case, the importance of nurses' theoretical knowledge and practical applications regarding PIC application increases even more. In our study, it was determined that 96.36% of PIC applications were performed by nurses. Nurses' education on PIC is also of great importance. In the study of Keleekai et al. (19), the knowledge, confidence and skills of the experimental group on PIC were determined at a considerably higher level than the control group after the training program was completed. The level of education and knowledge that affects the quality of PIC applications; It is affected by criteria such as working shifts, conditions, hours, the position of the nurse in the institution, and the patient service/clinic (21).

There are also many difficulties that nurses face during PIC application. Among them, The patient is obese or a baby, the veins are small, edema, burns, hypovolemia, the presence of chronic diseases, and dehydration (21). It is of great importance to ensure that nurses have sufficient equipment and skills with PIC application, starting from the undergraduate student period. The mistakes made by nurses who do not have sufficient equipment and skills during PIC application are stated in the study of Uzen Cura et al. (20). According to the study, During the observation, it was determined that 82.8% of the nurses did not wash their hands, the area where asepsis was provided with alcohol was palpated again at a rate of 65.6%, and 71.7% did not wait for the aseptic solution to dry.

PIC is applied to more than 70% of the procedures performed for IV applications. Some criteria should be considered for PIC applied so many times. These; the patient's age, vascular characteristics, medical condition, the region where the treatment will be applied, the purpose of the treatment (1). In addition to the frequent use of PICs, the occurrence of catheter-related complications is inevitable (6).

Appropriate material selection is also important in PIC application. Because the material or materials used in PIC application may be associated with the development of complications (such as extravasation, phlebitis, infection). In addition to the appropriate material, vein selection is also important. What makes the application more successful is that the vein is more visible and fuller (2, 22). There is no explanation in the current guidelines as to which catheter size should be. However, it is recommended to apply 20G (pink color) and higher catheters in PIC applications (23). In the study of Wallis et al. (8), 18G and larger catheters are among the risk factors for the development of phlebitis, and 22G and smaller catheters are shown in case of accidental catheter removal.

According to the recommendation of the Infusion Nurses Association Practice Guide (2016); It is stated that 14-18G catheters should be preferred for adults with visible and palpable veins, 20-24G for patients with short veins and feeding problems, and 24-26G for sensitive age groups such as children and the elderly (13). In this study, it was found that the 20G (pink) catheter was mostly applied to the patients (74.55%), and the forearm region was the most preferred (36.36%) region. According to the Infusion Nurses Association Practice Guide, If veins on the back of the hand are to be used in children, short-length catheters are recommended if the vein is convoluted and non-palpable, and long catheters are recommended for use of straight/palpable veins in adult patients (13).

Materials such as dosiflow used to adjust the hour in PIC applications or three-way taps for medication/solutions that will be applied more than once can be preferred. In order to prevent an infection that may occur, materials that can be operated without a needle are recommended. Needle-free intervention is also important and recommended in terms of employee safety (1, 7, 13). In this study, it was determined that stepcock/three-way tap (38.18%) was used the most.

According to the results of this study, IV crystalloid fluids are used with a rate of 69.09% in patients with PIC, in addition to this, IV antibiotic treatment is applied to 25.45% and analgesia/PCA treatment is applied to 27.47%. As stated in the Practice Guide of the Infusion Nurses Association, drug administration is not recommended in liquid infusions. If the drug is to be administered, it should be diluted with an appropriate amount of liquid and the infusion should be administered intermittently. It is recommended to wash the IV line with physiological saline after each fluid therapy and/or between treatments (1, 13). In this study, it was found that the IV route was 100% washed.

CONCLUSION

In this study, information about the health status and management of patients hospitalized

in a university hospital and undergoing PIC application was concluded. Although recommended in the guidelines, many PIC applications are still not recorded in writing and IV medication/solution is not administered to some patients, but we are faced with the result that PIC is applied.

The latest state of PIC applications and compliance and focus on internationally published guidelines in PIC applications and management bring about a serious improvement in surveillance, evaluation, decision making, minimizing application errors, reducing the risk of complication development and documentation. The serious complications stated in the studies conducted on the subject still show that the healthcare team involved in PIC applications needs the necessary training. With the necessary training, standard procedures, up-to-date guidelines, and regular application of aseptic techniques, complications will be drastically reduced.

Ethics Committee Approval: Appropriate permission for the study was obtained from the Committee of Ethics of Derince Training and Research Hospital (YDU/2020/83-1158),

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Expression pattern and subcellular localization of p62/SQSTM 1 during mouse oocyte maturation

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Abstract

Objective: Autophagy is a survival mechanism, and it is initiated by several factors such as oxidative stress. Cells give autophagy response against oxidative stress through Nrf2-Keap1-p62 pathway. p62 or Sequestosome 1 (SQSTM 1) which takes place during autophagy, and it is showed that p62 plays critical role during primordial follicle formation. During oocyte maturation, Germinal Vesicle (GV) oocytes resume meiosis with the beginning of puberty and progress through Metaphase I and Metaphase II stages. Metaphase II (MII) oocytes are ready to be fertilized and until fertilization they are arrested at metaphase II stages. It is known that oxidative stress is increasing during oocyte maturation. However, it is not shown that spatial and temporal expression of p62 throughout oocyte maturation. Thus, the aim of the study was to reveal expression pattern and the subcellular localization of the p62 protein in mouse GV, MI and MII oocytes.

Methods: GV oocytes were received from female Balb/C mice at 4 weeks aged and GV oocytes were cultured for 8h to develop into MI oocytes and for 14h to mature into MII oocytes. Then, expression of p62 protein was observed in oocytes at GV, MI and MII maturation stages by using immunofluorescence method.

Results: Our results showed that there is a significant increasing p62 expression when GV oocytes are compared to MI and MII oocytes, but there is no difference between MI and MII oocytes. Moreover, we revealed that p62 is localized in cytoplasm of GV oocytes, while it is localized around chromosomes in MI and MII oocytes.

Conclusion: In conclusion, our results indicate that further study is mandatory to understand the participation of p62 during meiosis, and the impact of p62 during oocyte maturation.

Key words: Autophagy, oocyte maturation, p62, SQSTM 1

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INTRODUCTION

Autophagy is a survival mechanism of the cells as it is able to degrade and recycle the components of the cell in case of damaged organelles or unfunctional proteins, starving and oxidative stress (1). Autophagy helps cells to keep homeostasis in balance (2). In oocytes, autophagy is initiated with the increased ROS (Reactive oxygen species) level and disrupted antioxidant mechanisms of the cell (3). p62 or Sequestosome 1 (SQSTM 1) which has a critical role as an adaptor protein during autophagy mechanism (4). When there are ubiquitinated proteins or organelles, p62 is phosphorylated and p62 becomes more mobile and binds to ubiquitinated proteins (5). Also, mTORC 1 phosphorylates p62 and phosphorylated p62 shows more affinity against KEAP 1 protein (6). Phosphorylated p62 proteins which are bound to ubiquitinated protein aggregation interact with Keap1(7) , phosphorylated p62 and ubiquitinated proteins are recognized by LC3 on autophagosome, then all of them are degraded by autolysosome (8). p62 also takes place in oxidative stress response through Nrf2-Keap1 pathway (9). Unless there is an oxidative stress, p62 is self-oligomerized and found in the cytoplasm, and Keap1 which binds to Nrf2 prevents Nrf2 from entering the nucleus. When oxidative stress is present in the cell, p62 is phosphorylated and binds to Keap1, so it prevents Keap1 from binding to Nrf2 transcription factor and free Nrf2 can now move into the nucleus and activate transcription of genes which enable cells to deal with oxidative stress, surprisingly Nrf2 is also activate expression of p62, so a positive feedback is observed between Nrf2 and p62 (7, 10, 11).

Germ cells are differentiated from Primordial Germ Cells (PGCs). During embryonic development, PGCs migrate from the endoderm of yolk sac to the gonadal ridges, and they start differentiation in there and form oogonia (12). Oogonia cells enter meiosis, but they arrest at prophase I and they are called as Germinal Vesicle (GV) oocytes at this stage (13). With puberty, they resume their meiosis and proceed through Metaphase I and Metaphase II stages. Until fertilization, they are arrested at metaphase II stages (14).

During development of GV oocyte into MII oocyte, ROS level increases and it exceeds the level which could be compensated by natural cellular antioxidant, and homeostasis become imbalance and oxidative stress is formed (15). Therefore, since p62 is an adaptor protein which takes place in autophagy and the oxidative stress is one of the inducer for the autophagy (16), and according to our literature search there are no studies in the literature that show expression level of p62 during oocyte maturation, the study was designed to reveal the expression pattern and localization of the p62 protein in mouse GV, MI and MII oocytes.

METHODS

Animals

The approval of experiment protocol was received from the Animal Care and Usage Committee of Ankara University (Protocol no: 2020-2-19). The female Balb/C mice at 4 weeks-aged (n=12) were purchased from the Research Animal Laboratory Unit of Ankara University. Hosting of the mice was under freely accessing to food and water conditions and hold in a 12 hr light-dark cycle.

Oocyte Collection and in vitro Culture

Cumulus-enclosed GV oocytes were obtained by using the ovaries of 4-week-old Balb/C female mice following superovulation with 5 IU pregnant mare's serum gonadotropin (PMSG, Intervet). Adipose tissue of the ovaries was removed, cumulus- oocytes complexes (COC) comprising GV oocytes were released through puncturing the ovaries by using the 23-gauge needle in morpholinepropanesulfonic acid (MOPS) buffered medium (G-MOPS^{TM}) (Vitrolife, Sweden). The cumulus cells were got rid of by pipetting. Following denuding, GV oocytes were placed into the culture medium (G-TL^{TM}; Vitrolife, Sweden) as 50 µL of culture drops inside 35 mm culture dishes (Corning, USA) that were filled with 3 mL Ovoil (10029, Vitrolife). GV oocytes (0 hr) were cultured up to Metaphase I (MI) oocytes (8 hr) and Metaphase II (MII) oocytes (14 hr) at 37 °C in 5% CO_2. Around 100 oocytes were considered for each group, and all experiments were carried out at least in three replicates.

Immunofluorescence (IF) Staining

Following fixation of GV, MI and MII oocytes in 4% paraformaldehyde (Sigma-Aldrich, USA), permeabilization was performed with 1% Triton X-100 (Thermo Fischer Scientific, USA) at room temperature (RT), afterwards blocking with 20% normal goat serum (Vector Laboratory, USA) blocking solution, IF was performed to determine the spatial temporal expression pattern of the p62 in the GV, MI and MII stage oocytes. In summary, oocytes were placed overnight at +4°C with rabbit monoclonal antibody for p62 (Cell Signaling, 23214) After a triple wash with 1x PBS consisting of 2% bovine serum albumin (BSA) for 10 min (PBS-BSA; Sigma-Aldrich, USA), incubation of oocytes was performed with anti-rabbit Alexa 488 secondary antibody (Invitrogen, USA) throughout 1 hr at RT, then washing of the oocytes was performed three times with 1x PBS-BSA solution for 10 min each. Control group was prepared by omission of primary antibody application. Mini well trays (Thermo Fisher Scientific, USA) were used for the staining part of the procedure in a humidified chamber. Then, oocytes were placed into a 4 µLdrop of PBS-based mounting medium consisting of 1 µg/mL Hoechst (Thermo Fisher Scientific, USA) indicating DNA on glass-bottomed 35-mm Petri dishes. The drops were overlaid with paraffin oil.

Oocytes were intact in terms of their 3D spherical shape and then micrographs were taken by using a Zeiss LSM-880 Airyscan® system (Zeiss, Germany). Staining of oocytes for all groups was performed at least five times and cumulated images were analyzed. Measurement of the signal intensity was performed with using Image J software (National Institutes of Health, Bethesda, Maryland, USA), and the signal intensities of the p62 has been quantified relatively.

Statistical Analysis

Analysis of the experimental results were performed by using one-way analysis of variance (one-way ANOVA) followed by Dunn's post hoc test. Statistical analyses were conducted with SigmaStat for Windows, version 3.5 (Jandel Scientific Corp). For all tests, $P < 0.05$ was evaluated as statistically significant.

RESULTS

In the study, we aimed to show p62 expression pattern during the oocyte maturation. When the p62 expression level is considered temporally, signal intensity of the MI and MII oocytes is significantly increased compared with the signal intensity of GV oocytes. However, there is not statistically meaningful difference in p62 expression level between MI and MII oocytes (Fig. 1B). In addition to that, we aimed to reveal p62 expression spatially. In GV oocytes, p62 signal was observed in cytoplasm and particularly in granulosa cells, while in MI and MII oocytes it was observed around the chromosomes (Fig. 1A and Fig. 2). Moreover, in polar body of MII oocytes, p62 signal was observed around the chromosomes (Fig. 2).

DISCUSSION

In this study, this is the first-time; we have shown that expression pattern of the p62 protein during mouse oocyte maturation. We found that expression level of p62 was significantly increased when GV oocytes were compared with both MI and MII oocytes, but there was no difference between MI and MII oocytes. In literature, it is showed that mitophagy, selective autophagy, inhibition results in decreased quality and viability of vitrified porcine oocytes and unsuccessful embryonic development (17). Moreover, autophagy is responsible for the primordial follicle formation and oocyte reserve (8). Also, it is showed that p62 expression level is changed during the primordial follicle formation and activated autophagy is related with more oocyte reserve as there is a crosstalk between apoptosis and autophagy (18). Furthermore, in post maturation oocyte aging (PMOA), autophagy is activated during in vitro mouse oocyte maturation until 12h incubation, but its activity turns to basal level between 12h and 18h. p62 level is decreased during first 12h but increased between 12h and 18h. It is suggested that autophagy prevents apoptosis during early PMOA, but in late PMOA, it cannot prevent

oocytes from the entering apoptosis since higher activity of the caspases inhibit autophagy (19). However, during oocyte maturation, expression level of p62, such an important protein for the autophagy, is not investigated.

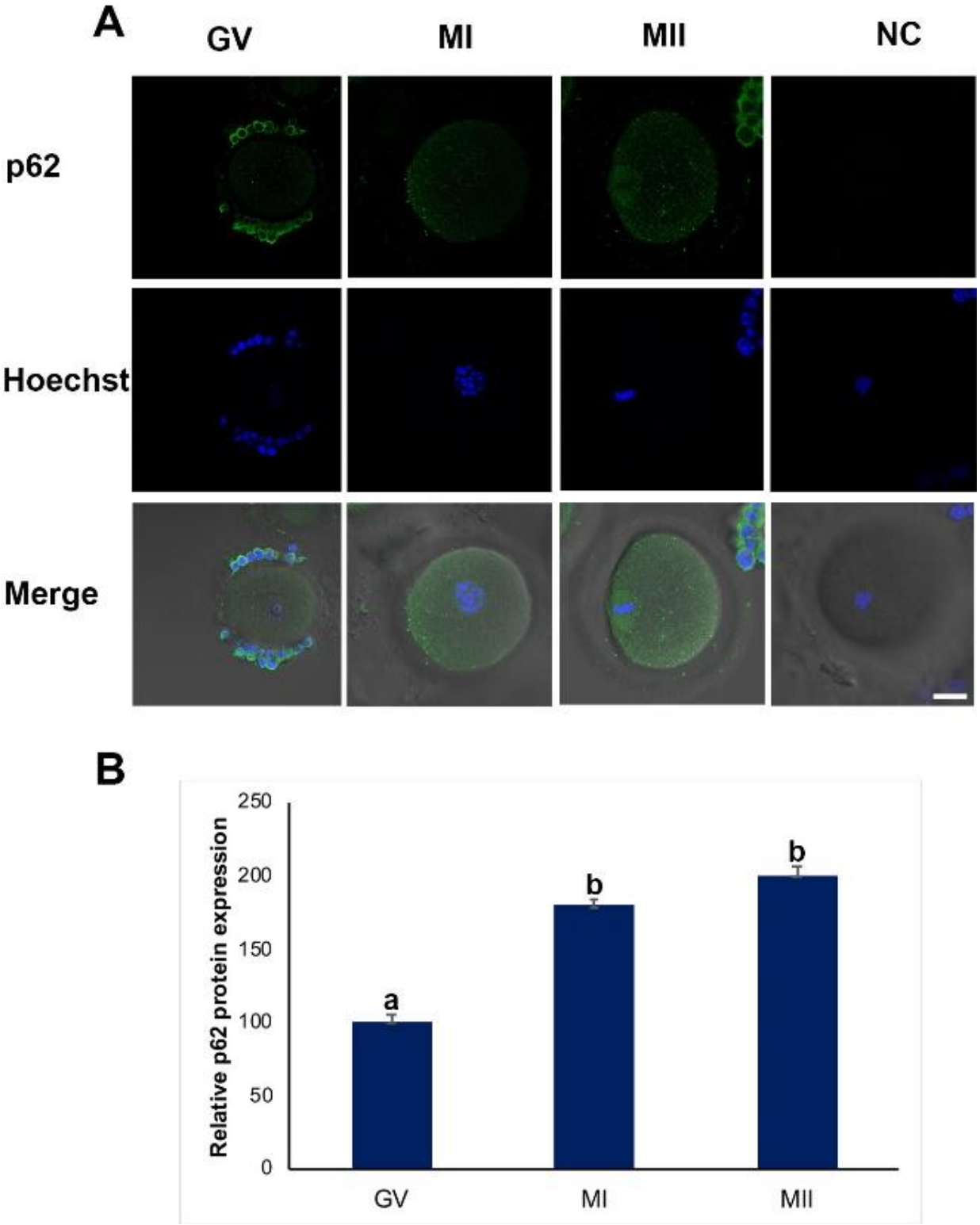

Figure 1. Immunofluorescent (IF) staining. **A.** p62 expression (green) and DNA (blue) in Germinal Vesicle (GV), Metaphase I (MI) and Metaphase II (MII) oocytes (n=100). **B.** Relative p62 protein expression from IF during oocyte maturation. Different letters are indicator for $p<0.05$. NC, negative control. Scale bar: 20 µm.

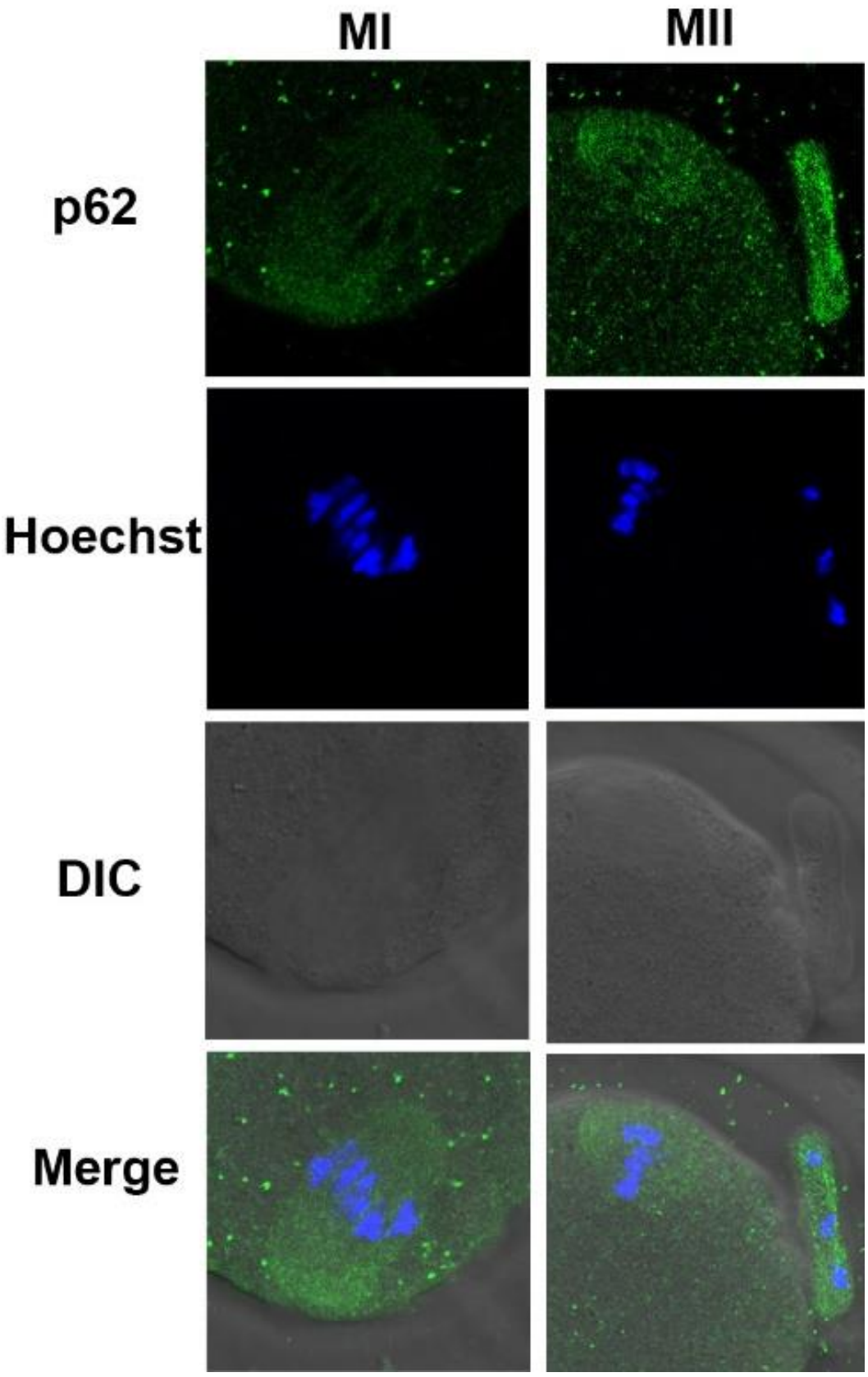

Figure 2. Immunofluorescent (IF) staining. p62 expression (green) is shown around chromosomes and meiotic spindles in Metaphase I (MI) and Metaphase II (MII) oocytes. Hoechst staining indicates chromosomes. DIC, differential interference contrast image

On the other hand, we showed cellular localization of the p62 during oocyte maturation. In GV oocytes, p62 was localized in cytoplasm and in surrounding granulosa cells (GCs). GCs support the maturation of oocytes (20). While p62 is accumulated and become unfunctional in cytoplasm, autophagy is decreasing in granulosa cells with aging, so promotion of oocyte maturation may be disrupted (21). We also observed that, p62 is located around the chromosomes and spindles in MI and MII oocytes and this result may bring the question about the role of p62 during meiosis. Previously, in sea urchin oocytes, it is demonstrated that p62 is bound to the chromatin at interphase stage while in clam germinal vesicle oocytes, p62 binding to chromatin is observed at prophase I (22).

Autophagy is crucial for homeostasis to maintain quality and function of cells and p62 takes place in autophagy as an adaptor protein (23). Moreover, it is known autophagy is activated in the case of oxidative stress and during oocyte maturation oxidative stress is increasing (24). Thus, investigating the expression level and subcellular localization of p62 protein may be important for understanding the compensation mechanisms of the oocytes against increased ROS level during oocyte maturation.

CONCLUSİON

In conclusion, the current work demonstrated the spatio-temporal expression of p62 protein in GV, MI and MII mouse oocytes. Our results give point of view about the relationship between autophagy, oocyte maturation and the potential role of p62 during meiosis. Additional studies, such as effect of silencing p62 during oocyte maturation, may reveal the function of p62 and its importance during oocyte maturation and meiosis.

Ethics Committee Approval: Approval of the experimental protocol was obtained from the Animal Care and Usage Committee of Ankara University (Protocol no: 2020-2-19).

Author Contributions: Concept: F.U, N.B, Ö.Ç, Design: F.U, N.B, Ö.Ç, Literature Search: F.U, N.B, Ö.Ç, Data Collection and Processing: F.U, N.B, Ö.Ç, Analysis or Interpretation: F.U, N.B, Ö.Ç, Writing: F.U, N.B, Ö.Ç.

Conflict of Interest: The authors declare no conflicts interests.

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Synthesis, Characterization and Investigation of Antimicrobial Activity of Orthophtaldehyde Nanoflowers

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Abstract

Objective: Endoscopy procedures are frequently performed in gastroenterology clinics, and disinfection cost, corrosion and toxicity are important problems in this area. For this purpose, hybrid nanoflowers at various pHs were synthesized with orthophtaldehyde (OPA), which has an important in disinfection in these clinics, and the hybrid nanostructures obtained by reducing the amount of orthophtaldehyde use were aimed to also increase the effectiveness of the disinfectant.

Methods: OPA nanoflowers (OPA NFs) were synthesized and their effective diameters (hydrodynamic diameters) and surface charges were determined by dynamic light scattering (DLS) and Zeta potential (ZP) measurements, respectively. Antimicrobial activities of orthophtaldehyde and OPA NFs against *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 35218 and *Candida albicans* (*C. albicans*) ATCC 90028 standard strains were evaluated by the liquid microdilution method using percent inhibition method.

Results: OPA-based OPA-Cu^{2+}hybridnano flowers (OPANF) were synthesized successfully at different pH values (pH 7.4, 9 and 11). The most effective antimicrobial activity was observed in the nanoflowers synthesized at pH=7.4 for all tested microorganisms. Although the antimicrobial activity decreased as the pH value increased, NFs activity was higher than OPA alone at all pH values ($p<0.001$). In this study, NFs synthesized with 0.02 mg/ml OPA were found to be 5.2 times more effective for *C.albicans*, 5.75 times for *E.coli* and 4.4 times for *S.aureus* than OPA(0.02 mg/ml).

Conclusion: NFs showed very high antimicrobial activity compared to OPA and also promise to be a preferred agent in medical device disinfection providing a high level of disinfection with the use of a few amounts of OPA that use in clinics.

Key words: disinfection, endoscopy, nanoflower, orthophthaldehyde.

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INTRODUCTION

Endoscopic procedures are widely used medical procedures all over the world. During these procedures, patients are exposed to some risks. A number of infectious pathogens can be transmitted to patients during the procedure. Devices used in endoscopy are classified as semi-critical devices. These devices come into contact with damaged or intact mucous membranes and skin (1). Endoscopy devices are cleaned with high level disinfection (HLD) procedures to reduce the risk of microorganisms transfer from endoscopes. The HLD process removes all microorganisms, only a small number of bacterial spores may remain (2,3). Cleaning of endoscopes requires cleaning of interior, exterior and all surfaces and the use of effective microbicidal agents (4). Flexible gastrointestinal endoscopic devices are heat labile, so chemicals are often used in HLD. Low temperature sterilization methods can also be used, but there is no low temperature sterilization method approved by the United States Food and Drug Administration (FDA). Orthophtaldehyde (OPA) is a highly effective and important chemical substance used for this purpose. The use of OPA is a chemical agent used for HLD, approved by the FDA in October 1999. In vitro studies show that it has excellent microbicidal activity (5). In United States, more than 10 million gastrointestinal endoscopy procedures are performed annually (6). Therefore, sterilization of these devices is of great importance.

Recently, hybrid nanostructures in the form of organic inorganic flowers called "nanoflowers (NFs)" were first discovered by Zare et al. using Cu^{2+} as an inorganic molecules and protein/enzyme as organic compounds and were described to support catalytic activity and stability (7). NFs are also important for industrial areas such as enzyme purification processes, drug delivery, vaccine studies, and possible applications for environmental protection (8-11). Altınkaynak et al. (2016) observed that lactoperoxidase derived NFs show more efficient catalytic activity than free lactoperoxidase (12). Since the discovery of NF, plant extracts are among the most commonly used organic compounds (13-18).

In this context, orthophtaldehyde, which is used as a HLD were firstly synthesized as NFs. It is aimed to evaluate the antimicrobial activity of this newly created substance on bacteria and fungi and to compare it with the use of OPA alone.

METHODS

Microorganisms:

In the study, Candida albicans (C. albicans) ATCC 90028, Staphylococcus aureus (S. aureus) ATCC 25923, Escherichia coli (E. coli) ATCC 35218 were used as standard strains. Microorganisms are stored from -80 °C and expected to dissolve, and bacteria were grown on tryptic soy agar and fungi were cultivated on

yeast extract agar after an overnight incubation, and the incubated microorganisms were used in antimicrobial activity experiments. The strains used for this study were obtained from the collection of Erciyes University Faculty of Pharmacy, Department of Pharmaceutical Microbiology.

Synthesis of OPA Nanoflower:

OPA Copper nanoflower syntheses were made with phosphate buffer saline (PBS) solution prepared at three different pH.

For 1000 ml of synthesis

20 mg of OPA is weighed on a precision balance so that the final concentration in the solution is 0.02 mg/ml. It is mixed thoroughly in the magnetic stirrer for about 5 minutes until it is well dissolved in 20 ml of distilled water. 6.66 ml of the pre-prepared 120 mM Copper sulfate solution is added and mixed approximately for one minute so that the final concentration in the solution is 0.8 Mm/L. The solution is transferred to a one liter balloon jug. The volume is made up to 1 liter with freshly prepared PBS with pH adjusted to 7.4.

For pH 9:

The pH of PBS is adjusted to pH 9 with 0.1 M NaOH and used for nanoflower synthesis.

For pH 11:

The pH of PBS is adjusted to pH 11 with 0.1 M NaOH and used for nanoflower synthesis (14).

Antimicrobial activity method:

The minimum inhibitory concentrations were determined by using broth microdilution method. Mueller Hinton broth and RPMI 1640 were used respectively for bacteria and fungus. Microplates were incubated for 24 hours (590 nm) for bacteria and 48 hours (600 nm) for fungi. Results were evaluated spectrophotometrically (Azure Ao, Biosystem, France) and inhibition percentages were calculated. The study was performed in three replications for each microorganism (19,20).

Statistical analysis

In the data analysis, Graphpad Prism software version 8.0.1. was used. The conformity of the data to the normal distribution was examined using KolmogorovSmirnov tests. At least three experiments were repeated in the measurements. The data is presented as mean value ± SD value.and its statistically evaluated with Mann-Whitney U and One-Way ANOVA tests (Tukey's multiple comparison test). One-way ANOVA procedure was used to perform the analysis of variance. The p value less than 0.01 were considered significant

RESULTS

Ortophitaldehyde hybrid nanoflowers and their characterization:

OPA-based OPA-Cu^{2+} hybrid nanoflowers (OPA NFs) were synthesized by incubating OPA and copper (II) (Cu^{2+}) ions in PBS at different pH values (pH 7.4, 9 and 11). First, the

synthesis of OPA NFs were completed and characterized by adjusting the buffer solution to pH 7.4 (Figure 1). As seen in Figure 1A, the OPA NF has a spherical, porous and compact structure with a scanning electron microscope. The mean diameter of the OPA NF was determined as 15 µm. Hydrodynamic diameter and surface charge of OPA NFs are seen as dynamic light scattering and zeta potential of 1.5 µm and -30 mV, respectively. The main reason why the effective diameter is much smaller than the electron microscope diameter is that OPA NFs precipitate after a certain period of time and smaller leaf-like structures suspended in solution are measured. When the zeta potential value is considered, it is an indicator that the surface charge is negative and that the OPA molecules have lost protons.

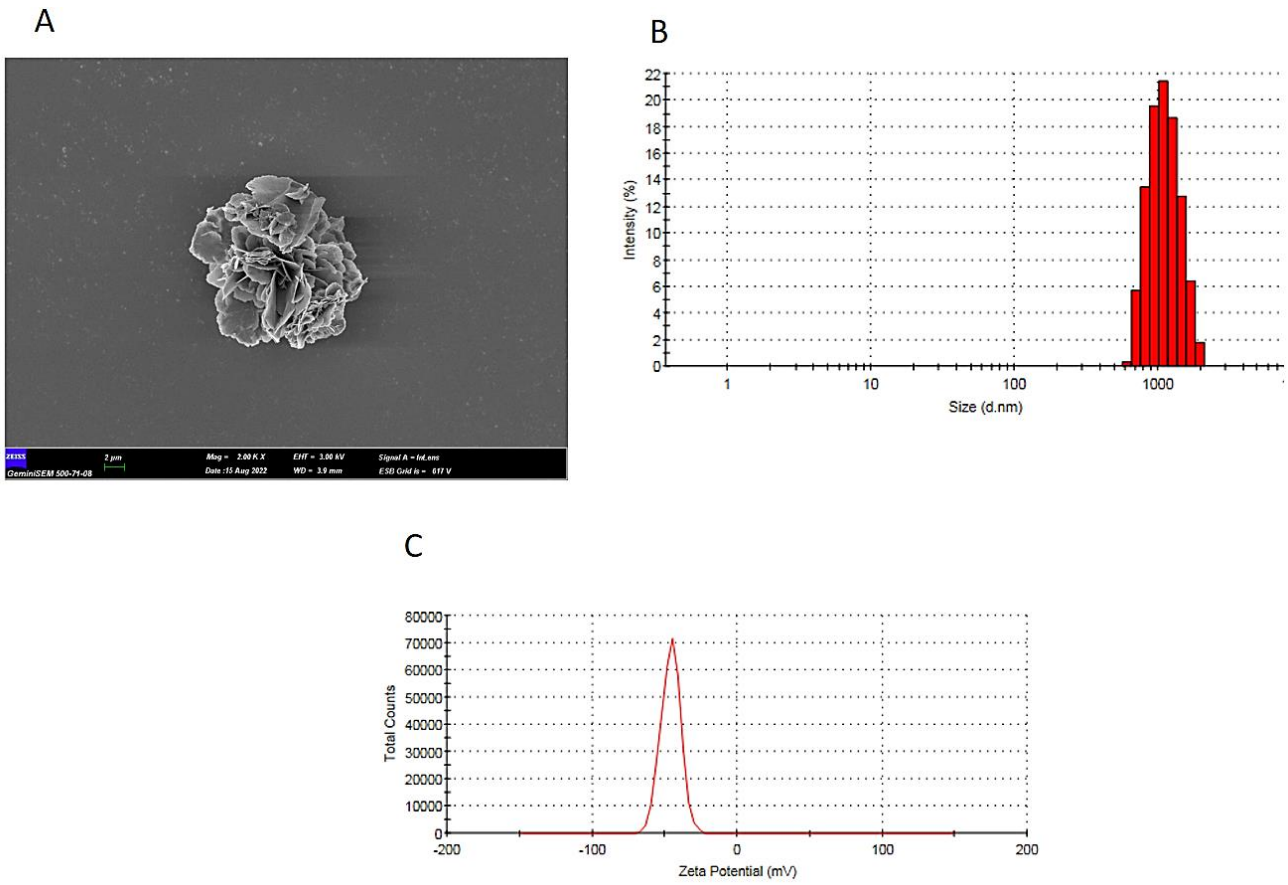


Figure 1. Characterization of OPA NF (pH 7.4): A) Electron microscope image (2000 KX), B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum

The same synthesis protocol was carried out at pH 9 and 11, respectively, and the resulting OPA NF like structures were characterized as seen in Figure 2 and Figure 3. For the formation of OPA NF at pH 9, according to the scanning electron microscope image in Figure 2A, leaves with an average size of 5-10 µm were formed to form OPA NF and entered the growth process, but they did not combine to form a flower-like morphology. Structures in square-like morphology are seen in the additional enlarged image in Figure 2A. Hydrodynamic diameter

and surface charge, dynamic light scattering and zeta potential of OPA NF like structures are seen as 3.5 µm and -35 mV in Figures 2B and 2C, respectively. In the measurement of the effective diameter, the diameter of the leaves were measured in general, and when the zeta potential value is considered, the surface charge being more negative compared to Figure 1C is an indicator of higher proton loss of OPA molecules.

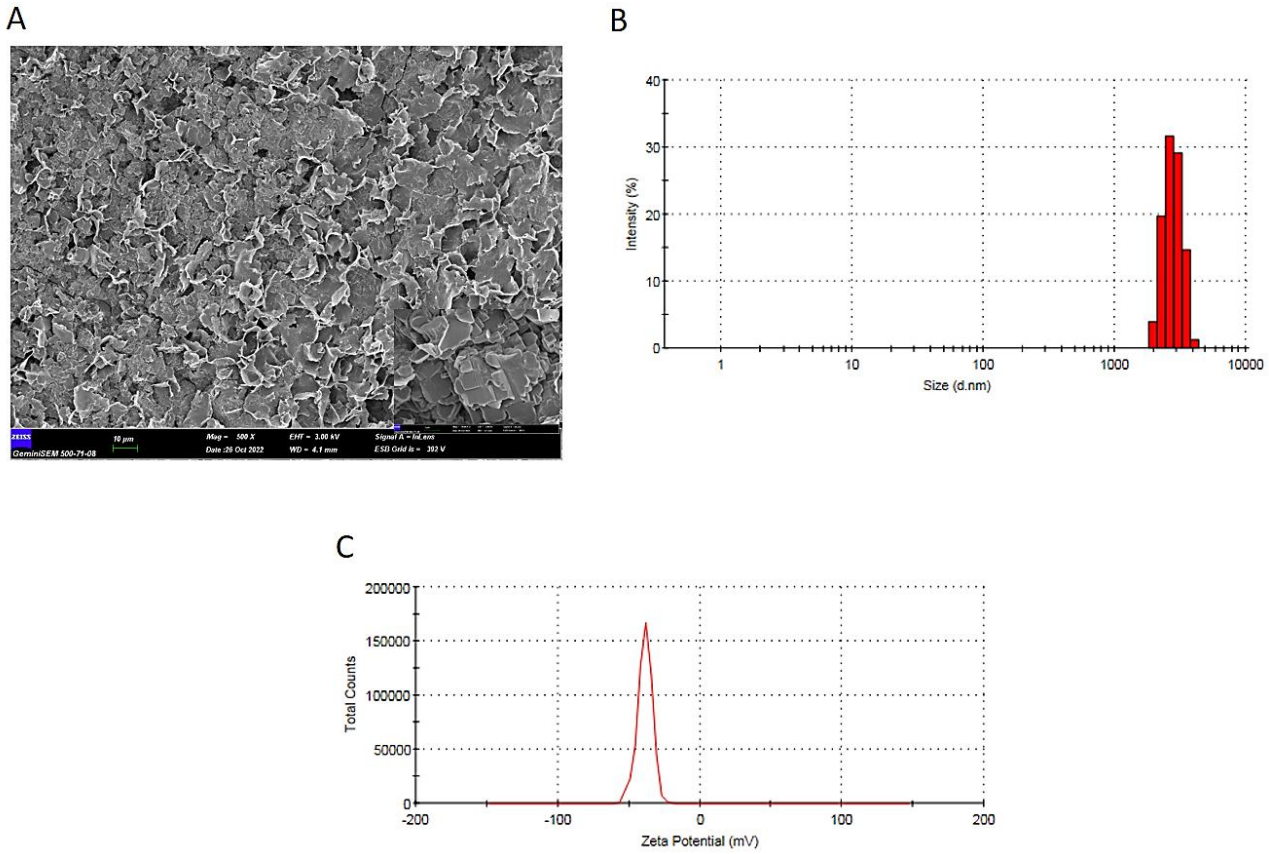


Figure 2. Characterization of OPA NF (pH9): A) Electron microscope image (500 KX), enlarged image that is in the lower right corner of figure 2A (10000 KX). B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum

It was seen that spherical Cu-based structures were formed instead of OPA NF according to the scanning electron microscope image in Figure 3A for the formation of OPA NF at pH 11. Hybrid structures have been obtained in different size, from nano-size to micro-size, where the reaction medium is highly alkaline. The hydrodynamic diameters of the OPA-Cu hybrid structures showed polydispersity as dynamic light scattering at 200 nm, 1 µm and 3.5 µm (Figure 3B). The surface charges of these structures, on the other hand, give a high negative zeta potential of approximately -50 mV (Figure 3C).

OPA NFs were also synthesized by incubating OPA and copper (II) (Cu^{2+}) ions in PBS at acidic pH values (pH 5 and 3). However, both OPA NF formation and reaction no precipitate was obtained as a result. Since OPA carries a positive or nearly positive charge in an

acidic environment, a repulsive force is formed between the Cu^{2+} ions in the reaction and the OPA molecules, thus preventing their interactions.

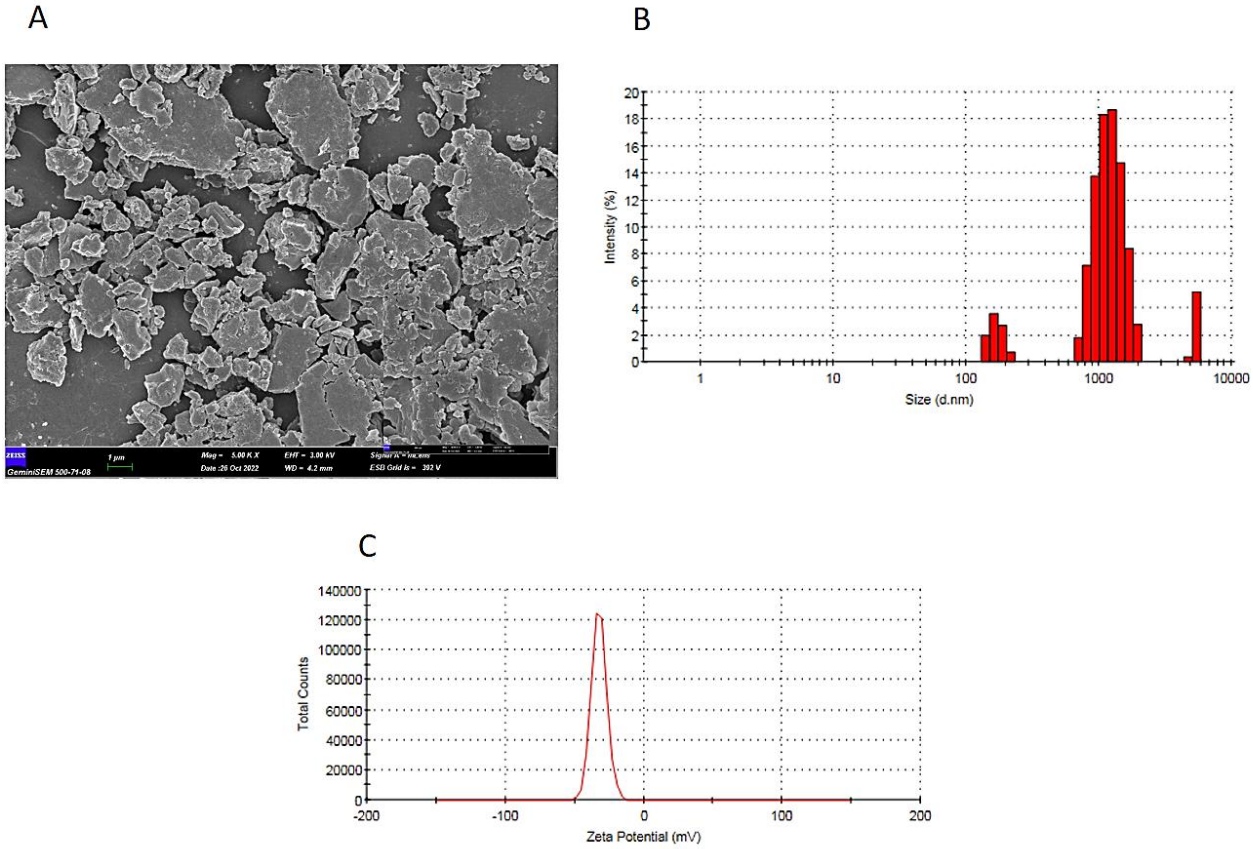
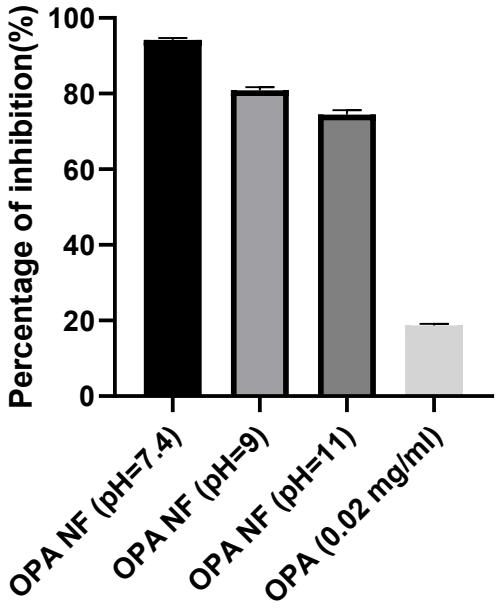
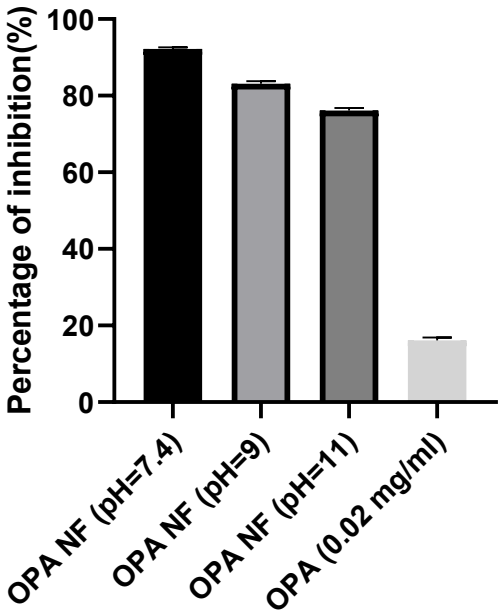


Figure 3. A) Characterization of OPA NF (pH11): A) Electron microscope image (5000 KX). enlarged image that is in the lower right corner of figure 3A (30000 KX). B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum.



Graphic 1. Percentage inhibition of OPA NFs (pH=7.4, 9 and 11) and OPA (0.02 mg/ml) against *C.albicans*



Graphic 2. Percentage inhibition of OPA NFs (pH=7.4, 9 and 11) and OPA (0.02 mg/ml) against *E.coli*

According to the results of the study, the most effective antimicrobial activity was observed in the NF synthesized at pH=7.4 for all tested microorganisms. Although the antimicrobial activity decreased as the pH value increased, OPA NF activity was higher than OPA alone at all pH values. However, the percent inhibition achieved at pH 7.4 was over 80% and these synthesized OPA NFs have minimum inhibitory effect for all tested microorganisms. These values were found to be statistically significant ($p<0.001$).

The highest antimicrobial activity was determined in *C.albicans*, a fungus (~94 % graphic1), followed by *E.coli* (~92 % graphic, 2) which is Gram-negative bacteria *and S.aureus* (~88 % graphic, 3), which is Gram-positive.

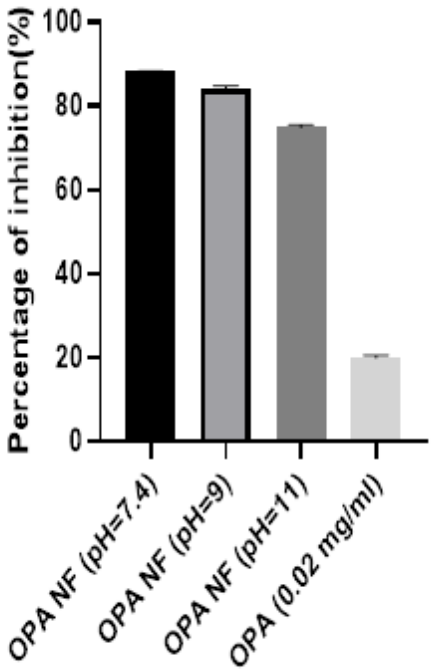


Table.3. Percentage inhibition of OPA NFs (pH=7.4,9 and 11) and OPA (0.02 mg/ml) against *S. aureus*

DISCUSSION

Today, although endoscopy procedures are widely used all over the world, the field of application and the variety of devices are increasing day by day. A high level disinfection process is applied for the disinfection of these devices. Problems such as cost, toxicity and effectiveness of chemicals used for HLD have become more important with increasing microorganism resistance. This study has unique value as it is the first study to investigate disinfectant-based nanoflower synthesis and efficacy. According to the findings, NFs-based OPA synthesized with a small amount of OPA showed highly effective antimicrobial activity. The amount of OPA used by the FDA in the routine has been specified as 0.55% (21). In this study, NFs synthesized with 0.02 mg/ml OPA were found to be 5.2 times more effective for *C.albicans*, 5.75 times for *E.coli* and 4.4 times for *S.aureus* than OPA(0.02 mg/ml).

Several toxicity studies performed indicate that OPA can be a chemical irritant and sensitizer as an adjuvant for other allergens (22,23). OPA concentrations ranging from 1.0 to 13.5 ppb have been detected in air samples taken from the endoscope cleaning unit of a hospital using OPA as the primary disinfectant (24-26). These findings demonstrate that OPA has the potential to induce respiratory sensitization following inhalation exposure. By reducing the amount of OPA that used with OPA NF, high antimicrobial activity will be achieved and

toxicity will be reduced, thus reducing the amount of inhalation on healthcare workers and the exposure of patients.

There are several types of disinfectants, each with their own advantages and disadvantages. Short contact time is an important consideration in countries with a high frequency of endoscopy procedures and device limitations. For this reason, disinfectants that require shorter contact times are used, such as OPA and formulas of peracetic acid and hydrogen peroxide. However, these disinfectants are more expensive than the glutaraldehyde. Given the growing need for endoscopy worldwide, the cost of endoscope reprocessing is a major concern (27). Most endoscopy units in China have used glutaral aldehyde (GA) as the disinfectant of choice because of its costeffectiveness. OPA is seen as the best alternative according to GA, the cost of OPA is almost prohibitive than GA (USD, 6.20$ per endoscope). Therefore, very few endoscopy units in China have been able to chosen OPA (28). In this study, both high-efficiency and low-cost disinfectants were obtained by reducing the standard usage dose of OPA with synthesizing OPA NFs.

As a result of OPA NF (pH 7.4) acting as Fenton agents in the presence of hydrogen peroxide (H_2O_2), OPA NF oxidized the model substrate by showing enzyme-like activity through the Fenton reaction mechanism. In general, the Fenton reaction Cu^{2+} ions in the structure of OPA NFs are reduced to Cu^{1+} ions by reacting with H_2O_2, and these Cu^{1+} ions react with H_2O_2 again to form highly reactive hydroxyl radicals, and the free hydroxyl radicals produced by the Fenton reaction, oxidizing the model substrate, cause enzyme-like activity (13,14).

We hypothesized that abundant negatively charged groups in the cell promote the interaction between NFs and the bacterial cell membrane, leading to inhibition of cell replication and eventual cell death due to deformation of the cell membrane. Also, NFs are thought to act as Fenton-like agent based on Fenton chemistry to generate Cu^{1+} ions and various radicals that can cause cell death through oxidative stress and membrane damage.

Limitations:

This study has some limitations. Although the diversity of microorganisms is in terms of fungi and bacteria, however virus and parasitic activity can be investigated with further studies.

CONCLUSIONS

In this study, It is thought that the peroxidase like activity of NFs synthesized increases the antimicrobial activity. NFs synthesized for this purpose promises to be a preferable agent in medical device disinfection with further studies, by providing high level disinfection with the use of a few amount of OPA, being more environmentally friendly and less toxic.

Ethics Committee Approval: Ethics committee approval was not obtained for this study as it was carried out only with standard strains

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: G.C.S, N.I; Design: G.C.S, N.I; Literature search: G.C.S, N.I; Data Collection and Processing: G.C.S, N.I; Analysis or Interpretation: GCS, NI; Writing: G.C.S, N.I

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

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Quality of Life in Women under 65 Years of Age with Diabetes and Affecting Factors: A cross-Sectional Study

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Abstract

Objective: The study was conducted in the cross-sectional manner to determine the factors affecting the quality of life in women 65 years under with diabetes, to ensure the guide women in the management of the disease.

Methods: This study was conducted in the cross-sectional manner with 134 female patients with diabetes. The data were collected using the "Personal Information Form" and the “The World Health Organization Quality of Life Instrument Short Form”. Descriptive statistical analyzes were used to evaluate the data, and correlation and regression analyzes were used to evaluate the effects of independent variables.

Results: In the correlation analysis made for the relationship of some variables with the quality of life, it was determined that there was a positive significant relationship between age and duration of marriage variables, physical area, mental area, environmental area, general health, and total quality of life. It has been determined that the variables of residence, income status, duration of marriage, presence of gynecological disease, number of births, number of living children, family planning use status, fasting and postprandial blood sugar variables affect the quality of life. It was determined that the variables included in the model explained 61% of the quality of life.

Conclusion: Age, duration of marriage, geographical conditions of the place of residence and income status, quality of life also varies according to gender, as gynecological diseases experienced, pregnancy, and family planning use status, fasting and postprandial blood sugar variables affect the quality of life.

Key words: Quality of life, woman, diabetes, gender, factors affecting quality of life

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INTRODUCTION

Diabetes mellitus is a chronic and broad-spectrum metabolism disorder that negatively affects daily life and life expectancy activities by causing hemostasis and disruption of physical functions in the whole body with its effect on multiple organ systems (1-3). According to the International Diabetes Federation (IDF) 2019 diabetes atlas data, the diabetes prevalence of 351.7 million was reported to be slightly lower in women between the ages of 20 and 79 compared to men (9.0% versus 9.6%). However, it is reported that it will reach 417.3 million in 2030 and 486.1 million in 2045, and it will increase even more in both men and women (4). Diabetes, which causes changes in the lifestyle of individuals due to the need for continuous follow-up and treatment, negatively affects the quality of life of the individual, which is a comprehensive structure that includes physical, emotional, and social aspects (5,6).

In the studies in the literature, it has been reported that gender affects the quality of life of individuals as well as factors such as diabetes-related complications, age, drug intake routes, profession, and physical activity level (5-7). It is emphasized that the differences observed between women and men are related to various underlying causes, and the effects such as bone loss and gastrointestinal side effects that develop in the treatment selection or treatment process related to gender are more a matter of concern for women (8-10). Verma and Dadarwal reported that women experienced more depression / anxiety than men, had low physical activity and poor social conditions, and therefore their quality of life scores were low. It has been reported that there is some evidence indicating that there are differences in coping styles and psychosocial adjustment to the disease in men and women with diabetes (11). In many studies in the literature examining the factors affecting the quality of life of individuals with diabetes, it is emphasized that demographic variables are important factors, but there are only a few studies examining the effect of diabetes on the quality of life in women (12,13). In addition, the combination of physiological and emotional changes caused by diabetes and hormonal changes that occur due to some special conditions such as pregnancy, menopause, and menstruation are thought to be effective on quality of life of women. This study was planned to guide women in the management of the disease by determining the factors affecting the quality of life in women with diabetes and in determining coping strategies with the symptoms that develop due to the disease.

1. What is the mean score of the quality of life scale of women with diabetes?
2. What are the determining factors affecting the quality of life of women 65 years under with diabetes.

METHODS

Adherence to ethical standards:

Before starting the research, ethical approval from a state University Non-Interventional Ethics Committee (dated and numbered 2020 / E.9688) and application permission from the institution where the research was conducted was obtained. The patients constituting the sample of the study were explained that the purpose, duration, and personal information of the study would not be shared, and their written consent was obtained in line with the voluntary principle.

Objective and type of the study:

The study was conducted in the cross-sectional manner to determine the factors affecting the quality of life in women 65 years under with diabetes, to ensure the guide women in the management of the disease.

Population and sample of the study:

The population of the study consisted of 152 woman patients who were diagnosed with diabetes who applied to the internal medicine outpatient clinic of the training and research hospital between 01.12.2020 and 15.01.2021. The sample of the study, on the other hand, consists of 134 patients who applied to the internal medicine outpatient clinic on the dates of data collection, accepted to participate in the study and met the inclusion criteria (married and female patients under 65 years of age diagnosed with diabetes).

Data Collection:

The data were collected using the "Personal Information Form" and the “The World Health Organization Quality of Life Instrument Short Form (WHOQOL-BREF-TR)”.

Personal Information Form:

In the Personal Information Form developed by researchers in line with the literature (14-16), there were 22 questions which focused on the sociodemographic characteristics (age, gender, marital status, work experience) and obstetric characteristics (whether they had any children of womans, presence of gynecological disease).

The World Health Organization Quality of Life Instrument Short Form Turkish Version (WHOQOL-BREF-TR):

Health-related quality of life scale was developed by WHO, and its validity and reliability study in our country was conducted by Eser et al. In 1999. The scale has two versions: long (WHOQOL-100) and short (WHOQOL-27) forms. The scale measures physical, mental, social and environmental well-being and consists of 26 questions. When using the Turkish version (the 27th question is the national question), the environmental area score is named environment-TR. In this case, Environment-TR field score is used instead of environmental score. Total quality of life score is minimum 27, can vary between a maximum of 135. The higher the score, the higher the quality of life (14). The Cronbach's alpha internal consistency coefficient was found to be

α=0.89, and the Cronbach's alpha internal consistency coefficient of this study was found to be α=0.89.

Statistical Analysis:

After the data were coded by the researchers, data analysis was performed by using IBM SPSS (Statistical Package for the Social Sciences) Statistics 25. Descriptive statistical analyzes (mean, median, standard deviation, frequency, 25th and 75th percentiles) were used to evaluate the data. The conformity to the normal distribution of the data was examined with the Shaphiro Wilk test. Analysis of variables conforming to normal distribution was performed with Student's t test or ANOVA. Mann-Whitney U or Kruskal Wallis tests were used for variables that did not show normal distribution. Bonferroni correction was made according to the number of groups. Spearman correlation and multiple linear regression analysis (enter method) were used to evaluate the effects of independent variables. The scale reliability coefficient was determined with Cronbach's Alpha. 95% confidence interval and p-value less than 0.05 were taken into account in the evaluation of the obtained results.

RESULTS

It was determined that the median age of the patients participating in the study was 52 (49-55), the median BMI was 30.45 (27.63-32.88), and the median duration of marriage was 32 (26-36) years**.** In addition, 97% of them were housewives, 46.3% of them were primary school graduates, 61.9% of them reside in the city center and 56.7% of them have moderate income. It was determined that 85.1% of the participants did not smoke, 86.6% did not have a gynecological disease, and 90.3% did not use a family planning (FP) method. It was found that the median number of births of the patients was 5 (4-7), and the number of surviving children was 5 (4-6) (Table 1, Table 3).

Considering the characteristics of the disease, it was determined that 43.3% of the participants were followed up with a diagnosis of diabetes for 6-10 years, 48.5% received oral antidiabetic (OAD) treatment, 66.4% had a disease other than diabetes, 64.9% had another diabetic family member. It was determined that 85.1% did not receive training for diabetes management, 45.5% did an average of 30 minutes of walking exercise per day. The median fasting blood glucose (FBG) of the patients was 180 (140-200), and the median postprandial blood glucose (PBG) was 340 (287.5-400) (Table 2, Table 3).

Comparing the results including the mean scores of the quality of life related to the sociodemographic characteristics of the patients, it was determined that the mean scores of the total quality of life, physical health and general health sub-dimensions of the retired were statistically significantly higher than the mean scores of housewives. In addition, it was determined that the mean scores of social field, environmental field, general health sub-

dimensions, and total quality of life were higher with a statistically significant difference in literate patients. According to the place of residence of the patients, it was observed that the mean scores of the physical health (center-village, center-county), social field (center-county), environmental field (center-village, center-county) sub-dimensions and total quality of life of those living in the village were statistically significantly higher (center-village, center-county) ($p<0.05$). It was found that the social field and environmental field sub-dimensions and total quality of life mean scores of the patients who evaluated their own income as low were lower, and the difference between them was significant. It was determined that the mean scores of the physical

health sub-dimension of non-smokers were higher than the mean scores of the patients using it with a statistically significant difference. It was found that the mean scores of the psychological and social field sub-dimension of the patients without gynecological disease, and the mean scores of the social field sub-dimension of the patients who used FP were high and the difference was significant (Table 1).

The comparison of the average quality of life scores according to the characteristics related to the disease is shown in Table 2. According to the duration of the disease, it was determined that the physical health score of women who were diabetic for 11 or more years, and the general health and quality of life score averages of those who had 1-5 years were higher, and the difference within the group was significant. In the comparison of the average quality of life scores according to the treatment method; It was observed that OAD users' mental, social, environmental, general, and total health score averages were found to be higher with a statistically significant difference. It was found that the average physical, social and total quality of life score of those who did not have any other chronic diseases other than diabetes was higher with a statistically significant difference. Social, environmental, general, and total quality of life mean scores were found to be significantly higher in those with a family history of diabetes and those who received training on diabetes management (Table 2).

In the correlation analysis made for the relationship between some variables and the quality of life in patients, it was determined that there was a positive significant relationship between age and duration of marriage variables and physical field, mental field, environmental field, general health and total quality of life. While a negative relationship was found between exercise duration and social field, a positive relationship was found between FBG and mental, social, and general health. A positive relationship was found between PBG and social field (Table 3).

The multiple regression model established to find the predictors of the quality of life variable

is statistically significant (p <0.05). It was determined that the variables of residence, income status, duration of marriage, presence of gynecological disease, number of births, number of living children, FP status, FBG and PBG variables affect the quality of life of the patients and the variables included in the model explain 61% of the quality of life (Table 4).

Table 1. Comparison of mean quality of life scores according to sociodemographic variables

	n (%)	Physical Area	Mental Area	Social Area	Environmental Area	General Health	Total Quality of Life
Profession							
Housewives	130 (97)	11.61±3.35	12.21±3.26	6.07±2.66	16.16±4.89	3.60±1.27	49.67±12.37
Retired	4 (3)	18±00	14±00	5±00	18±00	5±00	60±00
p^a		**0.002**	0.131	0.429	0.370	**0.019**	**0.017**
Education Status							
Illiterate	39 (29.1)	11.92±3.46	11.69±4.32	5.35±2.81	15.94±5.84	3.20+1.55	48.12±16.02
Literate	23 (17.2)	12.69±3.58	13.47±2.92	7.30±3.23$^{\Omega}$	17.39±5.42	3.95±1.18$^{\Omega}$	54.82±12.94$^{\Omega}$
Primary School Graduates	62 (46.3)	11.37±3.13	12.19±2.61	5.88±2.24^{w}	16.43±3.77	3.88±0.97$^{\Omega}$	49.77±8.98
High School	10 (7.5)	12.00±5.16	12.20±1.54	6.80±1.54	13.20±4.13^{w}	3.20±1.54	47.40±10.84
p^b		0435	0.105	**0.011**	**0.036**	**0.045**	**0.022**
Living Place							
Province veya City center olmalı	83 (61.9)	11.08±3.27	11.97±3.31	5.55±2.56	15.22+4.40	3.50±1.20	47.34±11.15
County	38 (28.4)	12.52±3.21*	12.42±2.34	6.89±2.65*	17.28+4.24*	3.84±1.1	52.97±10.54*
Village	13 (9.7)	14.30±4.15*	14.69±4.55	6.69±2.39	19.38+6.98*	4.00±2.04	58.07±18.46*
p^b		**0.004**	0.130	**0.033**	**0.026**	0.229	**0.011**
Income Status							
Low	29 (21.6)	12.20±3.85	13.17±4.00	7.34±3.06	18.31±6.63	4.03±1.52	55.06±16.77
Medium	76 (56.7)	11.51±3.09	12.15±1.90	6.15±2.21^{E}	16.02±2.99^{E}	3.55±1.01	49.40±7.06^{E}
High	29 (21.6)	12.17±4.05	11.65±4.74	4.44±2.44^{E,R}	14.62±5.94^{E}	3.51±1.59	46.41±16.26^{E}
p^b		0.785	0.132	**0.000**	**0.013**	0.137	**0.030**
Smoking							
Yes	20 (14.9)	9.85±2.20	13.05±2.28	6.10±1.82	16.80±3.22	4.15±0.67	49.95±6.86
No	114 (85.1)	12.14±3.55	12.13±3.35	6.03±2.75	16.11±5.06	3.56±1.34	49.99±3.06
p^a		**0.007**	0.206	0.777	0.181	0.065	0.851
Presence of Gynecological Disease							
Yes	18 (13.4)	12.00±4.82	10.55±3.03	4.38±2.32	14.94±3.93	3.27±1.36	45.16±12.03
No	116 (86.6)	11.77±3.24	12.53±3.18	6.30±2.59	16.41±4.93	3.70±1.26	50.73±12.24
p^a		0.714	**0.022**	**0.003**	0.202	0.230	0.091
FP Use Status							
Yes	13 (9.7)	11.23±4.12	12.38±2.59	8.07±1.75	16.00±6.73	3.46±1.66	51.15±15.06
No	121 (90.3)	11.86±3.41	12.25±3.29	5.82±2.62	16.23±4.61	3.66±1.24	49.85±12.05
p^a		0.436	0.460	**0.002**	0.794	0.599	0.665

*FBG: Fasting Blood Glucose, Ω: difference with the illiterate, w: difference with the literate, *: difference with the center, E: difference with the bad, R: difference with the middle, P^a: Mann-Whitney U, P^b: Kruskal Wallis tests, P^c: Independet t test*

Table 3. Relationship of some variables with quality of life

	Median(Q1,Q3)	Physical Area	Mental Area	Social Area	Environmental Area	General Health	Total Quality of Life Scale
		12 (8-14)	12 (11-14)	6 (4-8)	16 (14-19)	4 (3-5)	51 (44-57)
Age (year)	52 (49-55)	**0.263****	**0.306****	0.013	**0.385****	**0.328****	**0.343****
Body Mass Index	30.45 (27.63-32.88)	0.137	-0.070	0.017	-0.025	0.086	0.023
Duration of the marriage	32(26-36)	**0.358****	**0.408****	**0.106**	**0.463****	**0.483****	**0.463****
Number of births	5 (4-7)	0.012	0.005	-0.010	0.057	-0.103	0.014
	5(4-6)	0.028	0.139	0.111	**0.199***	0.001	0.146
Exercise min / day	30 (30-45)	-0.063	-0.009	**-0.184***	-0.023	0.050	0.005
FBG	180 (140-200)	0.023	**0.230****	**0.251****	0.105	**0.178***	0.167
PBG	340 (287.5-400)	-0.160	0.082	**0.197***	0.030	0.017	0.008

*FBG: Fasting Blood Glucose (n=69), PBG: Postprandial Blood Glucose (54), Q1: first quartile, Q3: third quartile, *p<0.05, **p<0.001*

Table 2. Comparison of average quality of life scores according to the characteristics of the disease

	n (%)	Physical Area	Mental Area	Social Area	Environmental Area	General Health	Total Quality of Life
Duration of the Disease							
Less than 1 year	17 (12.7)	10.11±2.39	10.94±5.11	6.00±3.64	14.70±5.12	2.76±1.56	44.52±14.65
1-5 years	49 (36.6)	11.14±3.14	11.95±2.03	6.00±2.39	16.10±4.06	3.95±1.09[d]	49.16±9.43
6-10 years	58 (43.3)	12.65±3.55[d,q]	13.00±3.01	6.41±2.50	16.84±5.40	3.63±1.18[d]	52.55±12.92
11 years and above	10 (7.5)	13.33±4.7[d]	11.80±4.51	4.20±1.93	15.70±4.16	3.70±1.63	48.40±14.89
p[b]		**0.033***	0.069	0.082	0.882	**0.028***	0.264
Treatment Method							
OAD	65 (48.5)	11.73±3.67	13.53±3.34	6.27±2.55	17.30±4.72	3.75±1.25	52.23±12.32
Insulin	63 (47)	12.04±3.41	12.04±2.20[h]	6.19±2.54	15.87±4.36	3.79±1.09	49.95±10.38
Diet only	6 (4.5)	10±0.00	5±0.00[h,m]	2±0.00[h,m]	8±0.00[h,m]	1±0.00[h,m]	26±0.00[h,m]
p[b]		0.320	**0.000****	**0.001***	**0.000****	**0.000****	**0.000****
Any Other Chronic Disease Other Than Diabetes							
Yes	89 (66.4)	11.38±3.69	11.88±2.95	5.71±2.62	15.74±4.18	3.56±1.16	48.29±10.70
No	45 (33.6)	12.64±3.58	13.02±3.63	6.68±2.54	17.15±5.83	3.82±1.48	53.33±14.56
p[c]		**0.047***	0.054	**0.044***	0.110	0.268	**0.025**
Family History of Diabetes							
Yes	87 (64.9)	12.09±3.68	12.63±2.69	6.81±2.60	17.04±4.87	3.82±1.27	52.41±11.92
No	47 (35.1)	11.27±3.02	11.59±3.98	4.61±2.03	14.68±4.37	3.31±1.23	45.48±11.86
p[c]		0.197	0.076	**0.000****	**0.006***	**0.028***	**0.002***
Educational Status for Diabetes							
Yes	20 (14.9)	11.75±2.04	10.55±5.20	4.05±2.16	13.55±4.92	2.55±1.53	42.45±14.55
No	114 (85.1)	11.81±3.68	12.57±2.66	6.39±2.55	16.68±4.67	3.84±1.13	51.30±11.44
p[a]		0.609	0.085	**0.000****	**0.025***	**0.000****	**0.040***
Exercise practice							
Yes	61 (45.5)	11.18±2.58	12.13±3.59	6.49±2.78	15.80±4.85	3.54+1.45	49.14±12.65
No	73 (54.5)	12.32±4.02	12.38±2.90	5.67±2.45	16.56±4.81	3.73+1.11	50.68±12.07
p[c]		0.057	0.654	0.072	0.367	0.373	0.474

*OAD: Oral Antidiabetic Drugs, d: difference with the Less than 1 year, q: difference with the 1-5 years, h: difference with the OAD, m: difference with the insulin, P[a]: Mann-Whitney U, P[b]: Kruskal Wallis tests, P[c]: Independent t test *p<0.05, **p<0.001*

Table 4. Multiple Regression Analysis Results for Quality of **Life**

Quality of Life	B	SE	B	t	p
(Constant)	23.874	7.429		3.214	.002
Living Place[a]	4.377	1.334	.238	3.280	.001
Income Status[b]	-5.219	1.274	-.280	-4.097	.000
Duration of the marriage	.485	.096	.355	5.058	.000
Presence of Gynecological Disease[c]	13.412	2.229	.373	6.017	.000
Number of births	-5.929	1.130	-.887	-5.248	.000
Number of children	7.180	1.209	.983	5.938	.000
FP Use Status[d]	-8.412	2.530	-.203	-3.325	.001
FBG	.093	.015	.726	6.332	.000
PBG	-.053	.009	-.745	-6.078	.000
	R:0.781	R^2:0.610	AdjR^2:0.581	p:0.000	

*FP Use Status: using family planning method, FBG: Fasting Blood Glucose, PBG: Postprandial Blood Glucose, a:, b: bad, c: yes, d: no, It was significant at the 95% confidence interval *p<0.05, **p<0.001*

DISCUSSION

Quality of life, which is a concept related to the determination of individual clinical evaluation of the patient's own health status, is adversely affected by the symptoms and serious complications caused by diabetes, which is a chronic and progressive disease, and causes significant changes in patients' lives (12). In the

literature, in parallel with the data of this research presented, besides complications, different descriptive characteristics such as profession, education, economic status, smoking, environment, education for diabetes and being a diabetic family member, treatment type, duration of diabetes, and having a secondary chronic disease were observed to be effective on chronic disease management. Thus, it was observed that the quality of life in different areas was negatively affected. In a pilot study conducted in Turkey, where Koç (2015) examined the quality of life and related factors in patients with diabetes, while it was determined that diabetes affects the quality of life of the patients negatively by 96.1%, it has been reported that gender, age, marital status, income, diabetes treatment type, duration of diabetes, diabetes complications and the presence of comorbid diseases have a significant effect on quality of life (15). In Yıldız Aslan's study, 70% of the patients with diabetes had a chronic disease other than diabetes. In these studies, it was stated that the duration of the disease and a secondary chronic disease negatively affect the quality of life (17). It is essential not to ignore the conditions that affect the quality of life in disease management. It is important to give holistic care to patients and improve their quality of life.

In the study, it was determined that individuals who are retired and continue their lives in the village, who do not smoke, who have been diagnosed with diabetes for more than 11 years and who have a second chronic disease have a moderate quality of life in the physical field. This result suggests that living in settlements far from mega-urban environments where physical activity areas are limited due to the effect of developed industry and technology, along with fresh air, abundant oxygen and reliable food consumption opportunities, healthy lifestyle behaviors and therefore the quality of life in the physical space are positively affected. In parallel with this study, in Gökpınar's study, the total quality of life score of those living in the village is higher than those living in the city (16). In contrast to this study presented in Yıldız Aslan's study, the Total Quality of Life score of those living in villages and townships is significantly lower than those living in the city (17).

It was determined that the mental domain quality of life in women who do not have any gynecological disease and who use OAD was found to be at a significantly moderate level. Parsons et al. (2006) reported that increased FBG levels and diabetes caused the development of lower urinary tract symptoms. It has been determined that the emotional effects and mood changes of some gynecological conditions that are due to the normal physiology of the woman, such as menopause, menstruation and pregnancy, which are experienced with diabetes, affect the quality of life. Therefore, in the study in

question, it can be said that women without any gynecological problems felt more secure and thought that they were successful in diabetes management using only OAD, and the psychiatric life quality increased to a moderate level (18).

In women whose living area is village, who are literate, whose income is low, who do not have a gynecological disease, who use one of the family planning methods, who are treated with OAD, who have no other disease other than diabetes, who have diabetes in their family and who have not received any education on diabetes, the social field quality of life was found to be low with a significant difference. Considering that this finding of the study is associated with the lowest quality of life in social life in women with a diabetes duration of 11 years or more, it can be said that as the duration of the illness increases, mental well-being, energy and physical function skills decrease, the risk of chronic complications increases and the quality of life of individuals decreases by restricting their participation in daily life. In the study by Bilgin et al. examining the relationship between diabetes and quality of life, it was reported that the mean scores of social function perception were statistically significantly lower in women (19).

The "environmental field" quality of life is significantly higher for women who live in the village, who are literate, who use OAD, who state that their income is low, who have diabetes in their family and who do not receive education for diabetes. It can be said that the reason for this result is that women have the opportunity to consume organic healthy foods due to life in the village, engage in physical activity thanks to agricultural and livestock work, and exhibit healthy lifestyle behaviors in line with the conditions of the region where they live.

In the study, according to the lowest and highest score that can be obtained from the scale, it can be said that the quality of life of the patients in the physical and mental health field is moderate, the quality of life in the social and general health is low, and the quality of life in the environmental field is higher than the average. Similar to the findings of this research, in the study of Tavakkoli and Denhghan, it was determined that the psychiatric life quality of the patients was at a moderate level. It is thought that the skills of coping with the complications caused by chronic diseases have important effects on the psychological health of individuals (20). In the thesis of Güneş examining the relationship between quality of life and hopelessness in patients with type 2 DM, it was reported that the quality of life in the mental, social, and environmental fields was above the middle level (21). In the study where Gen examined the relationship between the quality of life and depression and anxiety levels in individuals with diabetes, it was found that the general health score of the quality of

life in women was statistically significantly lower than that of men. It can be said that the different levels of the results obtained from the fields of quality of life in this study and other studies in the literature are due to the differences in the geographical region, sociodemographic characteristics, and lifestyle behaviors of the participants (22).

CONCLUSION

In addition to age, duration of marriage, geographical conditions of the place of residence, income status, gynecological diseases experienced in women due to their normal physiology, pregnancy and medical treatments used for these affect the perceptions, preferences, and decision-making processes of the patient in the management of a disease.

Limitations of the study

The most important limitation of the study is that it is cross-sectional and conducted in a single province and in a single hospital so data cannot be generalized.

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The Difficulties Experienced by Nurses Working in A Tertiary Hospital in Turkey while Providing Health Care to Immigrant and Refugee Patients and Intercultural Sensitivity

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Abstract

Objective: This research was carried out to evaluate the difficulties experienced by nurses during the delivery of health care to immigrant and refugee patients and their intercultural sensitivity.

Methods: The descriptive-cross-sectional study was conducted with 132 nurses. In the study, "socio-demographic questions, questions about the difficulties experienced while caring for immigrant/refugee patients" and "Intercultural Sensitivity Scale (ISS)" were applied. Mean ± standard deviation, frequency, and percentage values, Kruskal Wallis, Mann Whitney-U test, and Spearman correlation analysis were used in the data analysis. The statistical significance level was accepted as $p<0.05$.

Results: It was found that 65.2% of the nurses had no desire to care for migrant or refugee patients, 87.9% had difficulties while giving care, and 96.2% were found to be hindered by language differences while caring for patients. The mean total score of the ISS scale was determined as 61.58±9.96. When the ISS scale was evaluated in terms of the total score, the mean scores of those aged >30 years ($p=0.021$), those with undergraduate and graduate degrees ($p=0.036$), and those living in the city center were high ($p=0.044$). While the scale's total score was higher in those who benefited from the media and previous experiences as a source of cultural information ($p=0.015$, $p=0.002$, respectively), it was lower in those who had difficulties caring for immigrants and refugee patients ($p=0.035$).

Conclusion: In our result, it is seen that the level of intercultural sensitivity in nurses is moderate, and age, education level, and place of residence for immigrant or refugee patients affect intercultural sensitivity. It is seen that nurses with low intercultural sensitivity have difficulties in giving care to immigrant and refugee patients.

Keywords: Health care, health services, immigrant, nurse, refugee, Turkey.

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INTRODUCTION

Turkey has been an important migration route throughout history. It has faced massive migrations in different periods due to the great problems experienced in the surrounding countries due to its geopolitical location (1). In recent years, the number of people who immigrated or had to migrate to our country due to civil war, famine, and living conditions has gradually increased. According to the United Nations report, there has been a significant increase in asylum seekers and refugees, especially after 2010. It has been reported that 90% of these citizens are Syrian nationals, followed by Afghanistan and Iraqi nationals. As of 2020, according to official figures, the number of immigrants, refugees, and asylum seekers living in Turkey is around 6.1 million, constituting approximately 7% of Turkey's population (2, 3). This high rate of immigrants and refugees brings economic, social, and cultural problems, as well as housing and health problems. Among these problems, it is important to consider ethnic differences and language problem, which is one of the most common situations in preventing health problems and providing health care. Because culture affects healthcare behaviors, intercultural sensitivity is important for the effectiveness of healthcare services (4).

Intercultural sensitivity is sensitivity to cultural differences and the perspectives of people from different cultures (4). Intercultural sensitivity is also a part of the concept of cultural competence and has been expressed as a process that begins with the formation of cultural awareness (5). Intercultural sensitivity should be high to effectively solve the problems encountered while caring for individuals from different cultures (6). In order to provide effective medical services to foreign patients in health care delivery, foreign patient clinics are opened in hospital institutions around the world, and medical tour coordinators and professional translators are employed to eliminate language differences (7). However, despite the importance of intercultural sensitivity in understanding and accepting intercultural differences, as far as is known, this issue still needs to be sufficiently addressed in the health system. It is known that the level of intercultural sensitivity plays an important role in understanding and accepting intercultural differences in the care practices of nurses, who have an important place in the health system (3).

Therefore, our study aimed to evaluate the difficulties and intercultural sensitivity of nurses while providing healthcare to immigrant and refugee patients.

METHODS

The research type is descriptive-cross-sectional. The research was carried out by applying a questionnaire to nurses working in Samsun University Training and Research Hospital between 1-30 August 2022. Samsun

University Training and Research Hospital is a large tertiary referral hospital with 1140 beds and is located approximately 5 km from the city centre. Samsun is the largest city of northern Turkey located on the coast of the Black Sea with a population of 1,312,990. There are a total of 11 hospitals with emergency services accessible 24/7 in Samsun city center, five of which are state hospitals and six of which are private hospitals (8). Since Samsun Training and Research Hospital is in the center of the city and is a public hospital, it serves the majority of its patients.

For the study, first of all, it was questioned whether all nurses working in the hospital care for immigrant and refugee patients. Its population consisted of 176 nurses caring for immigrant and refugee patients. The sample was not selected, and it was tried to reach all of these nurses. The questionnaire was distributed to 153 nurses who voluntarily agreed to participate in the study and collected the next day after the necessary explanations were made. Twenty-one questionnaires were excluded due to missing data. The study was completed with 132 (75.0%) nurses.

Measures

As a data collection tool, in the first part of the questionnaire, there were socio-demographic (e.g., age, gender, marital status, working year, place of residence) and the difficulties experienced while caring for immigrant/refugee patients, a total of 20 questions created in line with the literature. The second part, the "Intercultural Sensitivity Scale (ISS)," was applied. The Turkish validity and reliability study of this scale, developed by Chen and Starosta (9), was conducted by Bulduk et al. (10), and Cronbach's alpha coefficient was found to be 0.72.

"Intercultural Sensitivity Scale (ISS)" (9); It consists of 24 items and includes five sub-dimensions. The individual's agreement with the statements in the scale is evaluated with a 5-point Likert scale. In the sub-dimensions of the scale, interaction engagement (7 items), respect for cultural differences (6 items), interaction confidence (5 items), interaction enjoyment (3 items), and interaction attentiveness (3 items). Nine items (2, 4, 7, 9, 12, 15, 18, 20, and 22) are scored inversely on the scale. The lowest score obtained from the scale is 24, and the highest score is 120. The total score obtained from the scale indicates the level of intercultural sensitivity; as the score obtained from the scale increases, cultural sensitivity increases, and the scale does not have a certain cut-off point.

Statistical Analyses

SPSS 22.0 package program was used for statistical analysis. Results were expressed using mean ± standard deviation (min-max) and number (%) according to data. Mann Whitney U test and Spearman correlation analysis were used in the data analysis because the data did not fit the normal distribution. The Kruskal–

Wallis variance analysis assessed comparison between 3 nonnormally distributed groups; if necessary, Bonferroni corrected Mann–Whitney U test was used for post-hoc comparisons. The statistical significance level of $p<0.05$ was accepted ($p<0.016$ for Bonferroni correction).

Ethical approval

Samsun University Clinical Research Ethical Committee ethical approval (SÜKAEK-2022/5/13) and Samsun Education and Research Chief Physician permission were obtained for the research. The nurses participating in the study signed the "Informed consent form."

RESULTS

The mean age of the 132 people participating in the study was 32.22±7.78 years. Of the participants, 87% (n=115) were female, and 82.6% were married. It was determined that 90.9% of the nurses had not been abroad before (Table 1).

Table 1. The sociodemographic characteristics of the study group

Variables	Category	Mean±SD (min-maks)	
Age(years)		32.22±7.78 (23.0-63.0)	
Number of children		1.68±0.67 (1.0-5.0)	
Number of Working Years		9.05±7.58 (1.0-40.0)	
		n=132	**%**
Gender			
	Male	17	12.9
	Female	115	87.1
Education status			
	High school	14	10.6
	Associate Degree	30	22.7
	Undergraduate and postgraduate	88	66.7
Marital status			
	Married	109	82.6
	Single/Widowed/Divorced	23	17.4
Child presence status			
	Yes	106	80.3
	No	26	19.7
Living place			
	Provincial center	111	84.1
	Town/village	21	15.9
Status of being abroad			
	Yes	12	9.1
	No	120	90.9

Mean±SD (min-max); Mean±Standard Deviation (minimum-maximum), n; Number, %; Percent

Of the nurses, 65.2% (n=86) had no desire to care for immigrant or refugee patients, 47.7% (n=63) did not feel competent while giving care, and 96.2% had language differences while caring for patients. It was found that very few (10.6%) benefited from in-house training as a source of cultural information about the patients, and 67.4% benefited from their previous experiences (Table 2). In addition, when the other difficulties they experienced were examined, 62.1% faced negative attitudes from the patients or their relatives, and 25.8% experienced the rejection of physical contact while giving care to patients (Table 2)

Table 2. Distribution of variables regarding the difficulties experienced by nurses while giving care to immigrant/refugee patients

Variables	n=132	%
Intercultural nursing education status		
Yes	24	18.2
No	108	81.8
Information status about the presence of an interpreter for communication with immigrant or refugee patients in the institution		
Yes	97	73.5
No	35	26.5
Willingness to care for immigrant or refugee patients		
Yes	46	34.8
No	86	65.2
The state of feeling competent in caring for immigrant or refugee patients		
Yes	69	52.3
No	63	47.7
Difficulty in caring for immigrant or refugee patients		
Yes	116	87.9
No	16	12.1
The barrier of language difference when caring for immigrant or refugee patients		
Yes	127	96.2
No	5	3.8
Communication method selection when caring for immigrant or refugee patients		
I get help from a translator	74	56.1
I use body language	31	23.5
I expect him to explain as much as he knows Turkish.	27	20.5
Cultural information resource on immigrant or refugee patients		
Consulting with friends		
Yes	51	38.6
No	81	61.4
Obtaining information from the media		
Yes	77	58.3
No	55	41.7
Benefiting from in-house training		
Yes	14	10.6
No	118	89.4
Leveraging previous experience		
Yes	89	67.4
No	43	32.6
A helpful situation regarding the difficulties experienced while caring for immigrant or refugee patients		
Providing in-service training	41	31.1
Sufficient number of translators	40	30.3
Establishment of separate units for immigrant or refugee patients	51	38.6
Refusal of physical contact when caring for immigrant or refugee patients		
Yes	34	25.8
No	98	74.2
The situation of encountering negative attitudes of patients or their relatives while caring for immigrant or refugee patients.		
Yes	82	62.1
No	50	37.9

n; Number, %; Percent

The total score of the ISS scale was 61.58±9.96 (32.0-80.0). In the sub-dimensions of ISS, interaction engagement was 17.93±3.77 (9.0-28.0), respect for cultural differences 14.83±3.25 (6.0-25.0), interaction confidence was 13.62±2.84 (5.0-20.0), interaction enjoyment 7.23±2.56 (3.0-14.0), interaction attentiveness 7.95±2.13 (3.0-15.0).

When the variables according to the intercultural sensitivity scale and scale sub-

dimensions are examined (Table 3), the total ISS score of nurses with age >30 years (p=0.021) and nurses with undergraduate and graduate degrees in total ISS score and self-confidence in communication sub-dimension scores were found to be significantly higher (respectively; p=0.036, p=0.008). While nurses living in the city center had higher scores on the total score of ISS and its sub-dimensions, there was a significant difference only concerning cultural differences sub-dimension (p=0.044). It was found that nurses with a working year of more than ten years had higher scores in the total score of ISS and its sub-dimensions, but there was no significant difference in pairwise comparisons (p>0.05) (Table 3).

Table 3. Socio-demographic variables and intercultural sensitivity scale total score and scale sub-dimensions

Variables	Interaction engagement	Respect for cultural differences	Interaction confidence	Interaction enjoyment	Interaction attentiveness	ISS Total score
Age						
<30 years	17.05±4.82	16.18±3.44	13.47±4.82	6.88±3.56	7.81±2.50	61.39±11.59
>30 years	17.65±3.78	14.22±2.43	16.52±3.23	7.95±2.75	8.58±2.44	64.92±8.01
p+	0.522	0.028	0.048	0.238	0.134	0.021
Gender						
Male	17.65±5.02	14.88±4.37	13.23±3.23	6.82±2.30	7.29±2.39	59.88±14.76
Female	17.98±3.59	14.82±3.32	13.68±2.80	7.31±2.60	8.05±2.09	61.83±9.11
p+	0.734	0.947	0.552	0.480	0.172	0.453
Education status						
High school	17.14±3.20	14.36±3.39	12.50±1.95	6.71±2.67	8.57±2.9	59.28±7.62
Associate Degree	18.87±3.59	15.17±3.87	12.56±2.58	7.40±2.99	7.30±1.95	59.30±10.16
Undergraduate and postgraduate	18.43±3.86	14.36±3.39	14.15±2.92	7.26±2.4	8.08±2.02	62.73±10.11
p	0.103*	0.734*	0.008* 1-2**=0.997 1-3**=0.057 2-3**=0.006	0.704*	0.116*	0.036* 1-2**=0.644 1-3**=0.016 2-3**=0.022
Marital status						
Married	18.12±3.85	14.45±3.06	13.92±2.66	7.33±2.57	8.08±2.17	61.93±9.66
Single/Widowed/Divorced	17.04±3.30	16.60±3.60	12.17±3.28	6.73±2.50	7.34±1.82	59.91±11.34
p+	0.212	0.004	0.007	0.309	0.134	0.378
Child presence status						
Yes	17.99±3.79	14.52±3.18	13.82±2.66	7.32±2.60	8.07±2.17	61.73±9.54
No	17.73±3.81	16.02±3.29	12.81±3.46	6.88±2.44	7.50±1.96	61.00±11.72
p+	0.755	0.029	0.104	0.439	0.227	0.740
Living place						
Provincial center	17.95±3.77	15.08±3.23	13.64±2.80	7.28±2.60	8.01±2.14	61.96±9.81
Town/village	17.85±3.88	12.52±3.09	13.47±3.12	6.95±2.35	7.61±2.10	58.42±10.69
p+	0.914	0.044	0.800	0.584	0.434	0.021
Number of Working Years						
<2 years	16.70±3.88	14.15±3.74	12.35±2.73	8.35±3.85	7.15±2.7	60.20±9.40
2-10 years	17.61±3.55	14.76±3.26	13.58±2.78	6.66±2.05	7.63±2.01	60.26±10.13
≥10 years	19.00±3.89	15.25±3.02	14.25±2.85	7.61±2.36	8.13±1.97	64.25±9.59
p	0.046* 1-2**=0.597 1-3**=0.060 2-3**=0.137	0.445*	0.045* 1-2**=0.195 1-3**=0.035 2-3**=0.443	0.016* 1-2**=0.024 1-3**=0.521 2-3**=0.124	0.135*	0.093*
Status of being abroad						
Yes	17.91±2.77	13.83±3.53	12.50±3.06	6.00±2.13	6.83±1.52	57.08±9.68
No	17.94±3.87	14.93±3.22	13.73±2.81	7.35±2.57	8.06±2.15	62.03±9.91
p+	0.983	0.266	0.153	0.08	0.056	0.101

+; Mann Whitney U test, *; Kruskal Wallis test, **; Mann Whitney U test with Bonferroni correction, ISS; Intercultural Sensitivity Scale

Those who received cross-cultural nursing education had a higher total score on ISS (p=0.081), and the interaction confidence score was significantly higher (p=0.048). Nurses who wanted to care for immigrant or refugee patients had higher ISS total scores (p=0.042), interaction engagement scores (p=0.032), and interaction confidence scores (p=0.042). The total score of ISS was significantly lower in patients who had difficulty (56.68±11.11) while giving care to immigrant or refugee patients compared to those who did not (62.25±9.64) (p=0.035). When questioning the use of cultural information resources regarding immigrant or refugee patients, Those who benefited from the media and previous experiences had higher ISS total scores (p=0.015, p=0.002, respectively). It was determined that those who did not feel that they were adequate while giving care, who thought that language difference constituted an obstacle to care, who did not experience physical contact rejection while giving care, and who did not encounter negative attitudes from the patient or their relatives while giving care, were found to have higher ISS total scores and sub-dimensions (Table 4).

It was found that there was a positive and low-level significant relationship between the nurses' working years and the total score of ISS, the sub-dimensions of interaction engagement and interaction attentiveness (r=0.195; p=0.045, r=0.288; p=0.003, r=0.239; p=0.014, respectively).

DISCUSSION

In the last decade, increasing migration and human movement due to wars and economic and political reasons in the world have created more diverse and multicultural patient populations. In the context of health services, interactions between people from different cultures have become more common, and intercultural communication has become important. Holistic care is important in health services, and individuals' cultural values, beliefs, and practices should be evaluated together. While it is stated that culture is an important factor during the professional practices of nurses in health service delivery, it is necessary to understand the problems that nurses experience with immigrant and refugee patients to provide better quality health care in nursing care (11). Therefore, our study evaluated nurses' difficulties and intercultural sensitivity with immigrant and refugee patients.

As a result of our study, we determined that approximately 50% of the nurses have difficulties giving care to immigrant and refugee patients. When we examined these difficulties, we found that the language difference was an obstacle during health care, the patients or their relatives faced negative attitudes, and a substantial level of nurses (24%) encountered physical contact rejection while providing care to the patients.

Table 4. Difficulties experienced by nurses and intercultural sensitivity scale total score and scale sub-dimensions

Variables	Interaction engagement	Respect for cultural differences	Interaction confidence	Interaction enjoyment	Interaction attentiveness	ISS Total score
Intercultural nursing education status						
Yes	18.15±3.77	14.99±3.24	13.85±2.81	7.17±2.48	8.12±2.18	62.29±9.82
No	16.95±3.73	14.12±3.27	12.58±2.84	7.50±2.93	7.20±1.74	58.37±10.13
p+	0.161	0.240	0.048	0.577	0.058	0.081
Information status about the presence of an interpreter for communication with immigrant or refugee patients in the institution						
Yes	18.52±3.47	15.29±3.14	14.09±2.53	7.25±2.19	8.29±2.14	63.47±8.55
No	16.31±4.15	13.54±3.23	12.30±3.27	7.17±3.41	7.00±1.81	56.34±11.71
p+	0.003	0.006	0.001	0.865	0.002	<0.001
Willingness to care for immigrant or refugee patients						
Yes	18.45±3.96	15.10±3.12	13.98±2.78	7.09±2.39	8.12±2.31	62.76±10.10
No	16.97±3.24	14.32±3.45	12.93±2.86	7.5±2.84	7.63±1.71	59.36±9.38
p+	0.032	0.192	0.042	0.387	0.203	0.042
The state of feeling competent in caring for immigrant or refugee patients						
Yes	17.81±4.35	14.59±3.47	13.10±3.27	7.23±2.73	8.00±1.98	60.73±11.25
No	18.07±3.06	15.09±3.00	14.19±2.18	7.24±2.37	7.90±2.29	62.50±8.30
p+	0.686	0.379	0.028	0.989	0.799	0.310
Difficulty in caring for immigrant or refugee patients						
Yes	16.68±3.84	13.43±3.44	13.50±2.22	6.18±1.83	6.87±1.14	56.68±11.11
No	18.11±3.75	15.02±3.19	13.63±2.93	7.37±2.61	8.10±2.19	62.25±9.64
p+	0.158	0.067	0.857	0.081	0.030	0.035
The barrier of language difference when caring for immigrant or refugee patients						
Yes	17.96±3.78	14.85±3.24	13.57±2.87	7.30±2.58	7.98±2.16	61.68±9.95
No	17.40±4.03	14.20±3.89	14.80±1.78	5.40±0.54	7.20±1.09	59.00±10.97
p+	0.746	0.659	0.347	0.103	0.422	0.556
Communication method selection when caring for immigrant or refugee patients						
I get help from a translator.	18.75±3.37	15.29±3.05	14.00±2.78	7.20±1.90	8.27±1.92	63.52±8.39
I use body language.	17.35±3.51	15.12±2.74	12.83±2.74	7.48±3.41	8.22±2.21	61.03±8.63
I expect him to explain as much as he knows Turkish.	16.37±4.57	13.22±3.89	13.48±3.03	7.03±3.04	6.77±2.24	56.88±13.48
p	0.011*	0.014*	0.157*	0.795*	0.005*	0.011*
	1-2**=0.180	1-2**=0.967			1-2**=0.999	1-2**=0.453
	1-3**=0.013	1-3**=0.012			1-3**=0.005	1-3**=0.008
	2-3**=0.658	2-3**=0.062			2-3**=0.021	2-3**=0.238
Cultural information resource on immigrant or refugee patients						
Consulting with friends						
Yes	18.00±3.76	14.29±3.7	13.92±2.90	7.25±2.77	8.23±2.10	61.70±10.82
No	17.90±3.8	15.17±2.91	13.43±2.81	7.22±2.43	7.77±2.14	61.50±9.44
p+	0.884	0.132	0.338	0.943	0.231	0.911
Obtaining information from the media						
Yes	18.42±3.17	15.35±3.10	14.12±2.46	7.19±2.18	8.25±2.10	63.36±9.02
No	17.25±4.43	14.10±3.34	12.90±3.19	7.29±3.02	7.52±2.11	59.09±10.72
p+	0.078	0.030	0.015	0.833	0.051	0.015
Benefiting from in-house training						
Yes	18.92±2.23	15.50±3.08	14.64±2.46	7.35±1.94	8.57±2.70	65.00±7.66
No	17.82±3.91	14.75±3.27	13.50±2.87	7.22±2.63	7.88±2.05	61.17±10.14
p+	0.302	0.420	0.157	0.851	0.254	0.176
Leveraging previous experience						
Yes	18.32±3.42	15.86±2.81	14.72±2.62	8.02±2.43	8.41±2.19	65.34±7.15
No	17.75±3.94	14.33±3.35	13.08±2.81	6.85±2.54	7.73±2.07	59.76±10.62
p+	0.417	0.011	0.002	0.013	0.082	0.002
Refusal of physical contact when caring for immigrant or refugee patients						
Yes	16.85±4.23	14.26±4.35	13.20±2.56	7.50±3.33	7.82±2.74	59.64±12.62
No	18.31±3.55	15.03±2.77	13.76±2.93	7.64±2.24	8.00±1.88	62.25±8.82
p+	0.051	0.239	0.326	0.486	0.679	0.189
The situation of encountering negative attitudes of patients or their relatives while caring for immigrant or refugee patients						
Yes	18.15±3.97	14.65±3.46	13.25±2.95	6.86±2.66	7.86±2.11	60.80±10.61
No	17.58±3.45	15.12±2.97	14.22±2.58	7.84±2.27	8.10±2.16	62.86±8.72
p+	0.396	0.432	0.059	0.034	0.543	0.252

+; Mann Whitney U test, *; Kruskal Wallis test, **; Mann Whitney U test with Bonferroni correction, ISS; Intercultural Sensitivity Scale

For the communication between patients and healthcare professionals to be healthy, it is necessary for the patient to express his/her problems clearly and for the healthcare worker to understand the patient correctly. The literature shows that the language barrier is one of the main problems in meeting the health needs of health service providers, immigrants, or refugees (12-16). A study conducted with midwifery students determined that 90% of the students encountered communication problems while caring for refugee or asylum-seeking patients; they mostly used body language for communication and communicated through

someone who could translate or a translator in charge of the institution (13). Akkoc et al. (14), on the other hand, found in a study conducted with healthcare professionals that 61% of the people had problems in communication, that they received help from an interpreter most frequently, and that they used dictionaries and body language in the communication methods they used less frequently. These results are similar to the results we found in our study, and it is seen that the majority of the nurses in our study group still mostly benefit from interpreters to provide understandable and accurate service when they encounter language barriers. However, in a qualitative study on the provision of health services, it is mentioned that translators must do more complete translations. The problems are related to the decrease in the quality of service and the inability to establish proper communication (17). Therefore, increasing the number of interpreters in our health centers with the increasing immigrant and refugee population and the fact that interpreters are composed of people with basic health knowledge may be beneficial in increasing the quality of health services.

While evaluating the scores from the ISS, there is no cut-off point; as the score increases, the level of intercultural sensitivity increases. When the studies in the literature are examined, the mean score of ISS varies among nurses. Ozdemir et al. (18) 's total mean score of ISS was 91.53 points; in the study of Yılmaz et al. (19), 84.01 points. In the study by Uzun and Sevinc (20) with 120 nurses, the average score was 84.32. The study of Cakmak et al. (12) is stated as 77.24 points. In our study, it is seen that the total mean score of nurses' ISS was at a moderate level [61.58], and the level of intercultural sensitivity was slightly lower than the studies in the literature. This suggests that the nurses included in our study may have less knowledge, skills, and interaction regarding intercultural nursing care. Therefore, as an outcome of our study, it may be necessary to organize in-service training to increase intercultural sensitivity in nurses working with different cultures in our hospital.

The level of intercultural sensitivity varies according to some factors (3, 12, 18, 21, 22). In our study, we found that nurses aged >30 who graduated with undergraduate and postgraduate degrees and lived in the city center had higher total mean scores of ISS. There was a statistically significant difference between mean scores. In other studies with nurses (3, 12, 20), although the total score increased with age, it was found that, unlike our study, there was no significant difference. Professional experience increases with the increase in the age of nurses, and the higher total scores of ISS may be related to this. Similar to our result, it is known in the literature that as the education level of nursing students increases, cultural sensitivity also increases (3, 23). In our country, culture is given a limited place in the curricula of nursing

and medical faculties. The literature emphasizes that the number of courses should be more for the education on culture to be effective on cultural sensitivity (24). In addition, our study determined that the interaction confidence scores in communication were significantly higher in undergraduate and graduate graduates and in people who received intercultural education. Intercultural education courses that nurses encountered during their undergraduate and higher education contributed positively to their self-confidence while communicating with different cultures.

According to our study's results, as the nurses' working years increase, the total score of ISS and the scores obtained from the subscales increase. However, there was no statistically significant difference between the intercultural sensitivity scale scores. Unlike our study, Lin et al. (25) and Chang et al. (26) stated that the working year of nurses and the unit they work in affect their cultural sensitivities. We think that with the increase in nurses' clinical experience, the people and culture they encounter also increase. Accordingly, it will positively contribute to the increase of intercultural sensitivity.

In the literature, he stated that gaining information about the cultural structure of the patients is the most important way to experience this process one-to-one while giving care to patients from different cultures throughout the health and disease process (27). Consistent with this result in our study, we found that most nurses benefited from previous experiences as a source of cultural information about these patients. In addition, in using cultural information resources related to immigrant or refugee patients, The nurses who benefited from the media and previous experiences had significantly higher ISS total scores, respect for cultural differences, and interaction confidence dimensions. Individuals with high interaction confidence develop a more positive perspective on people outside their cultures, and these qualities increase intercultural communication and sensitivity. It is known that people with high intercultural sensitivity recognize cultural differences and respect these differences with tolerance (27). While providing health care services to people with cultural differences, it may be beneficial to increase intercultural sensitivity and health service quality, especially if it consists of experienced nurses who have given care to these people before or nurses who know the cultures of the people they will care for (through intercultural nursing education, media or friends).

CONCLUSION

This study shows that most nurses have language difficulties, especially when caring for immigrant or refugee patients, they encounter negative attitudes from patients or their relatives, and they most benefit from

interpreters for communication. It was determined that the level of intercultural sensitivity in the nurses in our study group was moderate, and age, education level, place of residence, and working year affected intercultural sensitivity. In addition, in our study, it is seen that nurses with low intercultural sensitivity have difficulties in giving care to immigrant and refugee patients. In order to provide better quality care in nursing care, it is necessary to understand nurses' attitudes towards immigrant and refugee patients. It may be beneficial to provide interventions to these patients in separate units, support nurses with more frequent in-house training and new policies, and have nurses with previous experience caring for these patients work in these areas. In addition, it will be beneficial to increase cultural respect and sensitivity by providing nurses with the opportunity to work abroad, learn about different cultures, and learn new languages.

Ethics Committee Approval: Samsun University Clinical Research Ethical Committee ethical approval (SUKAEK-2022/5/13, date:10.08.2022) and Samsun Education and Research Chief Physician permission were obtained. Participants signed an informed consent form and were told that participation in the study was entirely voluntary and that they could leave from the study at any time. At every stage of the study, the principles of the Declaration of Helsinki were followed.

Author Contributions:

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

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‘‘Is the fear of coronavirus bigger than the reality of coronavirus?” The Correlation between the fear of Covid-19 Experienced by Women in The Postpartum Period and the Level of Depression and Social Support

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Abstract

Objective: This study has been carried out aiming to determine the correlation between the fear of COVID-19 experienced by women in the postpartum period and the postpartum depression and the level of perceived social support, and the factors affecting the fear of COVID-19.

Methods: The data of this cross-sectional and correlation-seeking study was collected through an online questionnaire conducted between February and April 2021. The introductory information form, the Fear of COVID-19 Scale (FCV-19S), Edinburgh Postpartum Depression Scale (EPDS) and Multidimensional Scale of Perceived Social Support (MSPSS) were used for data collection in the study. Continuous variables were reported as mean±standard deviation and categorical variables were reported as numbers and percentages (%).

Results: It has been determined in the study that 65.1% of women experienced the fear of COVID-19 in high level. A statistically significant difference has been found between the women's age, education level, and their fear of their infants being infected with the coronavirus and the fear of COVID-19 ($p<0.05$). It has been discovered that as women's fear of COVID-19 increases, so does the risk of postpartum depression.

Conclusion: It becomes more of an issue to monitor the level of the fear of coronavirus and postpartum depression and to plan appropriate interventions for the purpose of protecting and maintaining maternal health during the pandemic process**.**

Keywords: COVID-19; depression; fear; postpartum period

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INTRODUCTION

The 'COVID-19 disease' was recognized as an international public health issue by WHO and declared as a pandemic on 30 January 2020 (1, 2). The thought of people getting sick, dying, or losing their beloved ones has affected the whole world population due to the rapid spread of the pandemic, heavy effects of the virus on the body, high mortality rates (3), healthy people and even infants and children getting infected (4).

Women are also afraid of being exposed to the coronavirus due to the risks they are exposed to during the pandemic, reproductive health such as pregnancy, childbirth and postpartum (5). Fear and uncertainty caused by COVID-19 may increase the risk of progressing depression among women in the postpartum period (6, 7). The psychological effect of COVID-19 as well as the associated lockdown measures have also become a source of concern for mothers' mental health (8-10).

Social support lowers the risk of depression, assisting in the care of the infant and the mother and increasing mothers' feeling of competence in the mothering role (8). However, mothers may not receive adequate care, support, and assistance due to the measures taken during the pandemic process and keeping the social distance (8, 11). Studies conducted on the relevant subject are important in terms of assessing the psychosocial health of mothers, guiding postpartum care services, improving services and raising awareness among the health professionals during the pandemic. This study has been carried out to determine the correlation between the fear of COVID-19 experienced by women in the postpartum period and the level of postpartum depression and perceived social support, and the factors affecting the fear of COVID-19.

Hypotheses of the Study

H1: As women's fear of COVID-19 increases, so does the risk of postpartum depression.

H2: As the perceived social support level of women increases, the fear of COVID-19 decreases.

H3: There is a correlation between the fear of COVID-19 experienced by women and postpartum depression, perceived social support level

H4: Sociodemographic and obstetric attributes of women affect their fear of COVID-19.

H5: Some attributes of women associated with COVID-19 infection affect their fear of COVID-19.

METHODS

Study Design and Sample

The study is descriptive and cross-sectional. Participants were recruited through the Google Forms between February and April 2021. No sample selection was made in the study and the study was conducted with 284 women who agreed to participate in the study. Sample

selection was not made in the study and 284 women who met the inclusion criteria were included in the sample. In the post-study power analysis (PostHoc) conducted to examine the power of the sample, it was discovered that the sample had a 99% power at a 95% confidence interval with a 0.40 effect size (G * Power 3.0.10). The inclusion criteria are as follows: i- being between the ages of 18-45, ii- being in the first 6 months postpartum, iii- having no defined psychiatric illness.

Data Collection Tools

The introductory information form, the Fear of COVID-19 Scale (FCV-19S), Edinburgh Postpartum Depression Scale (EPDS) and Multidimensional Scale of Perceived Social Support (MSPSS) were used for data collection in the study.

Introductory Information Form: This form includes sociodemographic questions, obstetric attributes and pandemic process.

The Fear of COVID-19 Scale (FCV-19S): The validity and reliability of the Fear of COVID-19 Scale developed by Ahorsu et al. (12) and its adaptation into Turkish were performed by Bakiroglu et al. (13). The scale is a self-assessment, five-point Likert scale having seven items. The scale yields a score ranging from 7 to 35. The cut-off point for fear related to COVID-19 was determined as 16.5 (14), and responses above the cut-off value were defined as extreme fear. Cronbach's alpha value was determined to be 0.88 in the study conducted in the Turkish sample (12). Cronbach's alpha value of the scale was found to be 0.89 in our study.

***Edinburgh Postpartum Depression Scale* (EPDS):** The scale developed by Cox et al (15), whose validity and reliability were adapted into Turkish by Engindeniz et al (16) determines the risk of postpartum depression. The scale is a self-assessment, four-point Likert type scale having 10 items. The higher the score, the more severe the postpartum depressive symptoms. Cronbach's alpha value was determined to be 0.79 in the study conducted in the Turkish sample (16). Cronbach's alpha value of the scale was found to be 0.90 in our study.

Multidimensional Scale of Perceived Social Support (MSPSS): Multidimensional Scale of Perceived Social Support was developed by Zimet et al. in 1988 (17). The validity and reliability studies of the revised form of the scale whose adaptation into Turkish was performed by Eker and Arkar (18), were carried out by Eker et al. in 2001 (19). The MSPSS consists of 12 items and is a seven-point Likert type scale. Each item is given a score ranging from 1 to 7. The obtained score being high indicates a high level of perceived social support while low scores indicate that there is no perceived support or there is a lack of support (18, 19). Cronbach alpha values were found to be 0.91 by Zimet et al., (17) and 0.92 by Eker et al. (19). Cronbach's alpha value of the scale was found to be 0.95 in our study.

Ethical Principles

Written approval (Date: 04.12.2020 Decision No: 21/10775) was obtained from the Ethics Committee of the relevant University before starting the study. Written permission (Date: 31/12/2020; Decision No: T1255-44) was obtained from the Ministry of Health in order to conduct the study. In addition, all participants were informed about the study at the beginning of the online questionnaire and their consent was obtained. The study was based on the principles of the Declaration of Helsinki (2013).

Data Collection

Before the links for the forms were sent out to the women, data collection forms were administered to 10 women who were not included in the sample group and the forms were finalized. The data of the research was collected through electronic surveys created through Google Forms between February and April 2021. During the pandemic, where direct contact was reduced as much as possible due to the physical distance rules, the participants were invited to the research via social media groups (WhatsApp groups, public forums, Twitter and Facebook accounts). All participants were informed about the study at the beginning of the online survey and their consent was obtained. No names, Internet Protocol (IP) addresses, or other identifying information were collected; thus, participants' responses were anonymous, and no personal information was attached to the data. All questions had to be completed before submission.

Statistical Analysis

Analysis of the data collected was performed using the Statistical Package for Social Science (SPSS) version 25.0. Continuous variables were reported as mean±standard deviation and categorical variables were reported as numbers and percentages (%). The Pearson's chi-square test or Fisher's exact test were used to compare the categorical variables between the groups. Multivariate logistic regression was used to analyze the influencing factors of fear of COVID-19. Pearson's correlation coefficients were calculated to assess the correlations. Simple linear regressions were used to determine the effect of fear of COVID-19 on postpartum depression. In this study, the FCV-19S categories (and scores of <16.5, scores of ≥ 16.5) were used as categorical variables. The level of acceptable significance was set at $p<0.05$.

RESULTS

73.9% of women are between the ages of 20-34. 59.5% of the women have stated that they have a bachelor's degree or higher education, 87.3% of them have a nuclear family, 87.7% of them had an intended pregnancy, 63.0% of them gave birth by cesarean section, 51.6% of them had issues from time to time during the pregnancy process. When the FCV-19S cut-off score in the study was set at 16.5, it was

determined that 65.1% of women (185 women) experienced a high level of fear of COVID-19. In the study, a statistically significant difference was found between the age and education level of women and their fear of COVID-19 ($p<0.05$; Table 1).

No significant difference was determined between the women's status of getting infected with COVID-19, taking COVID-19 test and loss of a beloved one due to COVID-19, and their COVID-19 fear levels ($p>0.05$). A statistically significant difference was found between the fear of their infant getting infected with COVID-19 and their COVID-19 fear levels ($p<0.05$; Table 2).

According to multivariate logistic regression analysis, women aged 19 and younger are 0.092 times less likely to have the fear of COVID-19 than those aged 35 and older (OR=0.092; 95% CI: 0.010~0.844). Likewise, the fear of COVID-19 among women who are primary school graduates is 0.433 times less than women having a bachelor's degree or higher education. (OR=0.433; 95% CI: 0.210~0.894). The fear of COVID-19 has been found to be 10 times higher in women who extremely experience the fear of their infants getting infected with COVID-19 compared to those who do not experience it (OR=10.108; 95% CI: 1.921~53.194; Table 3).

The mean score of the participant women obtained from the FCV-19S scale was determined as 19.51±7.20, from the EPDS scale as 10.82±6.84. While the mean score of women obtained from the MSPSS scale was found to be 60.00±17.46, the mean score of family support obtained from MSPSS sub dimensions was 22.20±5.78, the mean score of friend support was 19.92±6.65, and the mean score of receiving special person support was 18.79±7.21. A weak, positive and statistically significant correlation has been determined between the mean score of women obtained from the FCV-19S scale and the mean EPDS score ($r=0.310$; $p<0.05$). It has been determined that there is no statistically significant correlation between the mean score of FCV-19S scale and the total score of MSPSS scale, family support, friend support and special person support sub dimensions ($p>0.05$; Table 4).

A statistically significant difference has been found between the mean score of women obtained from the EPDS scale and their levels of fear of COVID-19 ($p< 0.05$). The mean score of the EPDS scale obtained by women with high COVID-19 fear levels has been found to be higher. It has been determined that there is no statistically significant difference between the total score of the MSPSS scale, family support, friend support and special person support sub dimensions and the levels of the fear of COVID-19 ($p>0.05$; Table 5).

Table 1. Comparison of the fear of COVID-19 according to sociodemographic and obstetrics variables

Descriptive characteristics	Normal fear (FCV-19S<16.5) n=99 (%34.9) n(%)	High fear (FCV-19S≥16.5) n=185 (%65.1) n(%)	Total n=284 n(%)	Test statistics χ2	p value p
Age (years)					
≤19	10(10.1)	1(0.5)	11(3.9)	16.921	**<0.001**
20-34	65(65.7)	145(78.4)	210(73.9)		
≥35	24(24.2)	39(21.1)	63(22.2)		
Educational status					
Elementary	26(26.3)	23(12.4)	49(17.3)	9.219	**0.010**
High school	23(23.2)	43(23.2)	66(23.2)		
University and higher	50(50.5)	119(64.3)	169(59.5)		
Employment					
Employed	44(44.4)	90(48.6)	134(47.2)	0.457	0.499
Not employed (Housewife)	55 (55.6)	95(51.4)	150(52.8)		
Family type					
Nuclear	82(82.8)	166(89.7)	248(87.3)	2.775	0.96
Extended	17(17.2)	19(10.3)	36(12.7)		
Income level					
Income is less than expenses	22(22.2)	30(16.2)	52(18.3)	1.557	0.459
Income is equal	48(48.5)	97(52.4)	145(51.1)		
Income is more than expenses	29(29.3)	58(31.4)	87(30.6)		
Presence of social support					
Yes	57(57.6)	125(67.6)	182(64.1)	2.797	0.94
No	42(42.4)	60(32.4)	102(35.9)		
Relationship with the spouse*					
We get along well	79(79.8)	148(80)	227(79.9)	4.029	0.134
We do not get along sometimes	19(19.2)	27(14.6)	46(16.2)		
We do not get along at all	1(1)	10(5.4)	11(3.9)		
Prepregnancy depression history					
Yes	38(38.4)	79(42.7)	117(41.2)	0.497	0.481
No	61(61.6)	106(57.3)	167(58.8)		
Intended pregnancy					
Yes	86(86.9)	163(88.1)	249(87.7)	0.092	0.762
No	13(13.1)	22(11.9)	35(12.3)		
Number of abortion					
No	78(78.8)	143(77.3)	221(77.8)	0.083	0.773
Yes (≥ 1)	21(21.2)	42(22.7)	63(22.2)		
Number of pregnancies					
1	33(33.3)	86(46.5)	119(41.9)	4.796	0.091
2	39(39.4)	55(29.7)	94(33.1)		
≥3	27(27.3)	44(23.8)	71(25.0)		
Number of deliveries					
1	66(66.7)	138(74.6)	204(71.8)	3.489	0.175
2	27(27.3)	33(17.8)	60(21.1)		
≥3	6(6.1)	14(7.6)	20(7.0)		

Table 1. Comparison of the fear of COVID-19 according to sociodemographic and obstetrics variables (Continued)

Descriptive characteristics	Normal fear (FCV-19S<16.5) n=99 (%34.9)	High fear (FCV-19S≥16.5) n=185 (%65.1)	Total n=284	Test statistics	p value
Number of living children					
1	41(41.4)	102(55.1)	143(50.4)		
2	42(42.4)	58(31.4)	100(35.2)	4.970	0.083
≥3	16(16.2)	25(13.5)	41(14.4)		
Mode of delivery					
Vaginal	40(40.4)	65(35.1)	105(37.0)	0.768	0.381
Cesarean section	59(59.6)	120(64.9)	179(63.0)		
Postpartum month					
0-2 months	27(27.3)	51(27.6)	78(27.5)		
3-4 months	29(29.3)	54(29.2)	83(29.2)	0.003	0.999
5-6 months	43(43.4)	80(43.2)	123(43.3)		
Evaluation of the pregnancy process					
I had a very difficult process	21(21.2)	43(23.4)	64(22.6)		
I had problems from time to time	50(50.5)	96(52.2)	146(51.6)	0.533	0.766
I experienced a very comfortable pregnancy process	28(28.3)	45(24.5)	73(25.8)		
Evaluation of the delivery process					
It was a very comfortable delivery	32(32.3)	58(31.4)	90(31.7)		
At medium level	44(44.4)	80(43.2)	124(43.7)	0.164	0.921
It was a very bad experience	23(23.2)	47(25.4)	70(24.6)		

Note: The bold values indicate statistically significant at $p < 0.05$.
Abbreviations: FCV-19S: Fear of COVID-19 Scale,
*Fisher's exact

Table 2. Comparison of some attributes of women associated with COVID-19 infection with their fear levels of COVID-19

Characteristics	Normal fear (FCV-19S<16.5) n=99 (%34.9) n(%)	High fear (FCV-19S≥16.5) n=185 (%65.1) n(%)	Total n=284 n(%)	Test statistics χ2	p value p
Getting infected with COVID-19					
Yes	14(14.1)	24(13.0)	38(13.4)	0.076	0.783
No	85(85.9)	161(87.0)	246(86.6)		
Taking COVID-19 test					
Yes	37(37.4)	73(39.5)	110(38.7)	0.118	0.731
No	62(62.6)	112(60.5)	174(61.3)		
Loss of a beloved one due to COVID-19					
Yes	16(16.2)	42(22.7)	58(20.4)	1.698	0.193
No	83(36.7)	143(77.3)	226(79.6)		
Fear of her infant getting infected with COVID-19*					
Extremely	41(41.4)	125(67.6)	166(58.5)		
Occasionally	51(51.5)	58(31.4)	109(38.4)	21.679	**<0.001**
Never	7(7.1)	2(1.1)	9(3.2)		

Note: The bold values indicate statistically significant at $p < 0.05$.
Abbreviations: FCV-19S: Fear of COVID-19 Scale,
*Fisher's exact

Table 3. Significant risk factors associated with FCV-19S among women. (Logistic regression)

Variables	β	SE	Wald	p values	OR	% 95 CI Lower	Upper
Constant	0,650	1,447	0,202	0,653	1,916		
Age (years)							
≤19	-2,382	1,129	4,454	**0,035***	0,092	0,010	0,844
20-34	0,053	0,319	0,027	0,869	1,054	0,564	1,969
≥35	Reference						
Educational status							
Elementary	-0,837	0,369	5,128	**0,024***	0,433	0,210	0,894
High school	-0,151	0,3350	0,203	0,652	0,860	0,446	1,658
University and higher	Reference						
Fear of her infant getting infected with COVID-19							
Never	Reference						
Extremely	2,313	0,847	7,455	**0,006***	10,108	1,921	53,194
Occasionally	1,335	0,849	2,474	0,116	3,801	0,720	20,067

Abbreviations: CI, confidence interval; SE, standard error; β, regression coefficient.
Hosmer Lemeshow Test= 3.738 p=0.712, -2 Log likelihood =328.172, Cox & Snell R Square =0.129, Nagelkerke R Square =0.177

Table 4. The mean scores obtained by women from the fear of COVID-19 Scale, EPDS, MSPSS, and the correlation between the scales

	Mean ± SD	1	2	3	4	5	6
1-FCV-19S	19.51±7.20	1					
2-EPDS	10.82±6.84	0.310**					
MSPSS and its sub dimensions							
3-MSPSS total score	60.00±17.46	0.008	-0.379**				
4-Family Support	22.20±5.78	0.048	-0.305**	0.823**			
5-Friend Support	19.92±6.65	0.028	-0.385**	0.937**	0.691**		
6-Special Person Support	18.79±7.21	-0.046	-0.319**	0.898**	0.554**	0.792**	1

Note: Correlation is significant at the 0.05 level (2-tailed), r<0.2 very weak correlation, r=0.2-0.4 weak correlation, r=0.4-0.6 moderate correlation, r=0.6-0.8 strong correlation, r=0.8> very strong correlation Abbreviations: FCV-19S: Fear of COVID-19 Scale, PDS, Edinburgh Postnatal Depression Scale, Multidimensional Scale of Perceived Social Support (MSPSS)
** p<0.001

Table 5. Comparison of the mean scores obtained by women from the EPDS, MSPSS according to their levels of fear of COVID-19

Characteristics	Normal fear (FCV-19S<16.5) n=99 (%34.9)		High fear (FCV-19S≥16.5) n=185 (%65.1)		Test statistics	p value[a]
	Mean	± SD	Mean	± SD	t	
EPDS	9.07	6.40	11.76	6.90	0.241	**0.001**
MSPSS and its sub dimensions						
MSPSS total score	59.89	19.03	61.47	16.58	3.396	0.487
Family Support	21.89	6.29	22.36	5.49	3.705	0.521
Friend Support	19.43	7.39	20.13	6.22	6.267	0.466
Special Person Support	18.46	7.53	18.98	7.05	1.891	0.568

Note: Correlation is significant at the 0.05 level (2-tailed). Abbreviations: FCV-19S: Fear of COVID-19 Scale, PDS, Edinburgh Postnatal Depression Scale, Multidimensional Scale of Perceived Social Support (MSPSS)
[a] t test for independent group

DISCUSSION

The current pandemic emergency, as well as local governments' restrictive measures to prevent the spread of coronavirus infection, have a negative effect on mothers and may increase the likelihood of developing symptoms of fear, anxiety, and depression (9, 10). This study has been carried out aiming to determine the correlation between the fear of COVID-19 experienced by women in the postpartum period and the postpartum depression and the level of perceived social support, and the factors affecting the fear of COVID-19.

Being a disadvantaged group, women experience high levels of fear of coronavirus (13). It has been determined in our study that more than half of the women experience fear of COVID-19. Guvenc et al. (20) have reported in their study that 66% of puerperant women have the fear of getting infected with COVID-19. The mean score of the mothers' fear of COVID-19 scale was found to be 19.51±7.20 in our study. Matsushima et al. (21) have shown that the mean score of fear of COVID-19 in pregnant and postpartum women was 17.53±5.12. In their study where they researched the postpartum fear of COVID-19 and its correlation with breastfeeding, Uzun et al. (22) reported that the mean score of the fear of COVID-19 in the puerperant women was 18.00±4.30. The mean score of the fear of COVID-19 in our study was found to be higher than in other studies. The reason for this situation can be explained by the fact that Turkey was experiencing the second wave of the COVID-19 pandemic, heavy restrictions were imposed, and the number of cases reached the highest level at the time our study's data was collected. It can be said that women who witnessed the negative effects experienced in the first wave of the pandemic, spent their puerperium periods in the second wave, causing them to experience more fear of COVID-19. Additionally, Broche-Pérez et. al (23) reported that the reason why women experience more fear of the pandemic is that anticipating that the disease will have a negative impact on the health of their family members and friends. It was determined in our study that the fear levels of COVID-19 were higher in mothers who extremely had the fear of their infant getting infected with COVID-19. In this direction, it can be said that the high level of the fear of coronavirus of mothers is also affected by the fear of their infant getting infected in our study.

As a person becomes older, worsening of COVID-19 disease, hospitalization, and mortality rates increase (24). It was found in our study that age affected the fear of COVID-19 and that women aged 19 and younger had less fear of COVID-19 than women aged 35 and over. Kalafatoglu and Yam (25) reported that the fear of COVID-19 in individuals aged 31-45 was higher than the fear of COVID-19 in those aged 18-30. Individuals aged 24-28 reported much higher anxiety over COVID-19

than those aged 18 and under, according to Turktemiz et al (26). This circumstance can be attributed to the developmental phases and needs of those individuals. According to Erikson's Theory of Stages of Psychosocial Development, these age ranges coincide with Identity Achievement (12-19 years), Intimacy vs. Isolation (20-30 years), and Generativity vs. Stagnation (30-60 years). The period of identity achievement carries the characteristics of individuals to take more risks, to have contradictory behaviors and to appear strong-fearless (27). Individuals in the other stages, on the other hand, have developmental needs such as forming social bonds, working and being productive. Individuals in this age range may have a higher fear of COVID-19 due to the necessity of addressing these needs.

It is emphasized in the literature that higher levels of anxiety and depression are encountered in people with higher education levels (28, 29). It was determined in our study that mothers having a bachelor's degree or higher education experienced more fear of COVID-19 than mothers who graduated from primary school. Similar to our study result, Uzun et al. (22) demonstrated that mothers having higher education levels had a higher fear of COVID-19 score. It has been stated in other studies conducted that there is a positive correlation between educational level and the fear of COVID-19 (28, 29). Salari et al. (30) reported in the study they conducted that issues such as fear, anxiety and depression were common in individuals having high educational levels, which may be due to the high awareness of this group about their own health.

The risk of transmission of COVID-19 infection at birth and in the postpartum period, the fact that relatives are not admitted to the hospital after birth, and being deprived of social support resources due to restrictions make the puerperium period more difficult. This leads to increased anxiety and depression (8, 11). Sun et al. (31) shown that there is the correlation between COVID-19 and depression and 34% of women were affected by postpartum depression. Likewise, it was reported in a similar study that the COVID-19 pandemic had an effect on postpartum depression and increased the rates of depression in puerperant women (32). The mean score of the Edinburgh Postnatal Depression Scale of the women was found to be 10.82±6.84 in our study. Guvenc et al (20) reported that women's Edinburgh Postnatal Depression Scale mean score was 10.42±5.81, which was similar to our findings. Fear and uncertainty caused by COVID-19 enhance the risk of progressing depression among women in the postpartum period (20, 22). It was determined in our study that as women's COVID-19 fear levels increased, so did their depression risks. It was reported in a study that women who fear the transmission of COVID-19 infection to themselves or their infants had a higher risk of postpartum

depression (20). In this regard, the fear of the coronavirus can be considered a significant risk factor for the development of postpartum depression.

In our study, no significant correlation was found between the COVID-19 fear levels of women and their levels of perceived social support. It has been reported in other studies conducted that there is no difference between the fear of COVID-19 and the level of perceived support (21, 33). According to this result obtained from our study, the fact that spouses stayed at home within the scope of pandemic measures as a result of the implementation of strict isolation and stay-at-home measures at the time the study was conducted, and that some health institutions continued to provide training with virtual support classes on birth preparation and postpartum care may have helped to meet the formal and informal support needs of women.

Limitations and strengths

An internet-based questionnaire was used in the study. Therefore, volunteer mothers participated in the study. Furthermore, the data collected from the study are cross-sectional data and another limitation is that it does not provide long-term results regarding the fear of COVID-19, postpartum depression, and perceived social support. Individuals may also respond differently to the questionnaire depending on the stage of the COVID-19 pandemic; on the other hand, data in our study were collected in a short time and before vaccination, which may affect the research variables, in order to minimize differences and changes in restrictions due to COVID-19. Despite its limitations, our study provides important insights into mothers' levels of the fear of COVID-19, depression, and social support during the COVID-19 pandemic. The current study presents findings that can guide the determination of policy recommendations and intervention strategies for the protection and improvement of maternal health in the postpartum period.

CONCLUSION

Mothers in the postpartum period are a vulnerable group, having a high risk of depression and the fear of coronavirus, and they require social support to get through this period in a healthy way. The fear of coronavirus of mothers and its correlation with the risk of postpartum depression has been strongly correlated in this study. For this reason, health professionals should assess the fear of coronavirus and depression levels of mothers in the postpartum period; should plan initiatives to reduce their fear levels and depression risks and continue to strengthen social support.

Ethics Committee Approval: This study was approved by Nevşehir Hacı Bektaş Veli University Ethics Committee (Date: 04.12.2020 Decision No: 21/10775).

Author Contributions:

Concept: P.C, P.U.Ö, Design: P.C, P.U.Ö, Data Collection and Processing: P.C, P.U.Ö, A.S.A.K, Analysis or Interpretation: P.C, Writing: P.C, P.U.Ö, A.S.A.K.

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The Evaluation of the Relation Between Smartphone Addiction and Eating Attitudes among Medical Students

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Abstract

Objective: This study aims to evaluate the relationship between smartphone addiction and the demographical characteristics, eating attitudes, and body mass index of medical students.

Methods: The data was obtained through personal questionnaires, the scales regarding eating attitudes and smartphone addiction. The questionnaire and the scales were carried out online among current medical students of Zonguldak Bülent Ecevit University.

Results: Of the participants (n=347), 59.9% were female,40.1% were male. Participants were found to use the internet mostly through their smartphones and they were also found to use their smartphones mostly for messaging and monitoring social media. The scores for the Smartphone Addiction Scale were found to be significantly higher in students who were using their smartphones for social media than those who were not. A weak, positive, and significant relationship was found between the scores of the Smartphone Addiction Scale and Eating Attitudes Test of the students (r=0.135,p=0.12). As a result of the correlation analysis, a very weak, positive and significant relationship was found between Body Mass Index and the age of owning the first smartphone (r=0.113, p=0.036).

Conclusion: We reported a relation between the intentions of using a smartphone and smartphone addiction and another relation between one's age of owning the first smartphone and these individuals' body mass indexes. A difference was observed between those who have abnormal eating attitudes and who have not regarding smartphone addiction scale scores.

Key Words: Smartphone addiction, eating disorder, eating attitude, body mass index, social media, medical students

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INTRODUCTION

Smartphones provide many benefits that make life easier such as easy access to information, social connection, work applications, mobility, ease of carrying, etc. and they have become indispensable in everyday life (1). According to 2021 data from the "Use of Information Technologies in Households" research conducted by the Turkish Statistical Institute, the rate of smartphone use in Turkey has increased gradually over the years, and this rate is 96.7% among the 16-24 age group (2). In addition to the known advantages of smartphones, they can cause some potential harm if used unconsciously or excessively (1). “Smartphone addiction” is when a person does not want to leave their phone and deals with the phone in a way to neglect their responsibilities during daily life and experiences deprivation when they are away from their phone (3).

Having a smartphone is considered an advantage among university students, however, smartphone use was found to be associated with increased insomnia, emotional instability, anxiety, and depression (4). Besides, increasing usage rates among young people aged 16-24 make this age group risky in terms of addiction (5). The studies showed that smartphone addiction has negative effects on energy levels, body weight, eating and exercise habits, and academic performance (6).

Although there are studies in the literature showing the relationship between internet addiction and eating disorders (7-9), the number of studies discussing the relationship between smartphone addiction and eating disorders is limited. Therefore, this study was planned and conducted to reveal the relationship between smartphone addiction and medical students' eating habits and their body mass index.

METHODS

Data collection tools

The study sample consists of 370 participants who filled out the questionnaire including a personal information questionnaire, smartphone addiction scale, and eating attitude test prepared by the researchers, and the participants returned the questionnaires within 2 weeks. Consent was obtained from the participants electronically before the survey. The data of the students, who had missing information in their questionnaires, were not taken into consideration.

Personal Information Questionnaire:

Students were asked to fill out a questionnaire including questions about their age, gender, grade, place of residence during their education period, education level of their parents, height, weight, income level, alcohol and cigarette use, chronic disease status, sleeping hours, features regarding their eating and exercise habits, and their smartphone use habits.

Eating Attitude Test-40 (EAT): EAT is a 40-item self-report scale in which anorexic/bulimic

attitudes and beliefs are graded. A score of 30 and above is the cutting score commonly used to define individuals with anorexia or bulimia (predisposition to an eating disorder) (10). The reliability and validity analysis of EAT for Turkish society were made by Savasir and Erol (1989). The internal consistency coefficient (Cronbach's alpha) and test-retest reliability of the EAT for the Turkish sample were found to be 0.70 and 0.65, respectively (11). EAT showed good internal consistency reliability in our study sample (Cronbach's coefficient α = 0.85).

Smart Phone Addiction Scale (SPAS): It was developed by Known based on Young's articles on internet addiction (12). The scale, whose validity and reliability for university students in Turkey were done by Demirci et al. (2014), is valid and reliable in determining the risk of smartphone addiction. The internal consistency coefficient value (Cronbach's alpha) for the Turkish version of the scale is 0.947. The scale is a self-report scale consisting of 33 items in a 6-point Likert Type Scale. The options in this scale are listed from 1 (absolutely not) to 6 (absolutely yes). No cutting score was specified on the original scale. The higher the score obtained from the scale gets, the higher the risk for addiction becomes. In our study, the Cronbach Alpha coefficient of the scale was calculated as 0.924.

Statistical Analyses: The sample was calculated using the Open Epi program and it was aimed to reach 285 people with an unknown prevalence of 50% and a deviation margin of 5%. The data were evaluated with SPSS 23.0 package program. The compatibility of the numeric variables for the normal distribution was examined by the Kolmogorov-Smirnov test and visual parameters. Descriptive statistics were shown as mean ± standard deviation for those who suit the normal distribution, as median (minimum-maximum) for non-normally distributed data, and as number and percentage for categorical variables. The differences between the categorical variables were examined by the Chi-square test. For the comparison of numerical variables between the groups Mann-Whitney U and Kruskal-Wallis tests were used for non-normally distributed data. Spearman's correlation analysis was used to assessment of the relationship between numerical variables that did not fit the normal distribution. In the study, the cases where the p-value is below 0.05 were evaluated as statistically significant results.

The results were reported by considering the STROBE criteria compliance (14).

RESULTS

Three hundred seventy students (N=1100), who formed the target population of the study, were from Zonguldak Bülent Ecevit University Faculty of Medicine and answered the questionnaire, and 23 participants did not fill the questionnaire completely (n=347). Of the

347 students, 59.9% (n=208) were female and 40.1% (n=139) were male. The mean age of the research group was 21.10±2.01 and the mean body mass index was 22.44±4.20. According to the BMI classification, 64.8% (n=225) of the participants were in the class of normal weight, 13% (n=45) were underweight, and 4% (n=14) were obese. Of the participants, 68.3% (n=237) were in the preclinical term while 31.7% (n=110) were in the clinical term. The data on the sociodemographic characteristics of the students and their families participating in the study are shown in Table 1.

In our study, 90.5% (n=314) of the students used smartphones as a way of accessing the internet, and their mean age of owning a smartphone for the first time was 13.87±1.93. According to the evaluation of the smartphone usage purposes, the purposes were found to be “messaging”, "social media monitoring” and "listening to music” at the rates of 95.1% (n=330), 91.1% (n=316) and 90.2% (n=313), respectively. According to the characteristics of the eating and exercise behaviors of the students, nearly half of them could "sometimes" follow the healthy eating recommendations, and 73.8% (n=156) did not exercise at all or exercised less than 60 minutes a week. The eating attitude test mean score of the participants was determined as 15.21±9.13 and according to the scale score (cutting score is 30 points), 8.9% (n=31) had "abnormal eating attitudes". The data regarding the participants' smartphone use, exercise, and eating habit characteristics and their related scale scores are given in Table 2.

There was a statistically significant difference regarding the relationship between BMI mean values and genders, and the mean BMI of men was higher than that of women ($p<0.001$). In addition, the mean BMI of the participants, who reported that they skipped meals, was found to be statistically significantly higher than that of others ($p=0.045$). Also, the mean BMI of the participants who reported that they did not “follow healthy eating recommendations at all” was found to be statistically significantly higher than that of others ($p=0.002$). In our study, no statistically significant relationship was found between the time that the students spend on weekly exercise and their mean BMI ($p=0.761$). The data regarding the relationship between the participants' mean BMI and gender, exercise, and eating behaviors are shown in Table 3.

Table 1. Sociodemographic characteristics of the students and their families participating in the study

Age	Mean±SD	21.10±2.01	
	Min-Max	18-32	
Body Mass Index	Mean±SD	22.44±4.20	
	Min-Max	16.21-60.84	
		n (number)	**% (Percent)**
Gender			
	female	208	59.9
	male	139	40.1
	total	347	100
Grade			
	Term 1	50	14.4
Preklinic term	Term 2	91	26.2
	Term 3	96	27.7
	Term 4	53	15.3
Clinical term	Term 5	29	8.4
	Term 6	28	8.1
Accommodation			
	family home	44	12.7
	student house/apartment	149	42.9
	youth hostel	154	44.4
Education level of the mother			
	illiterate	2	0.6
	literate	5	1.4
	primary school	97	28.0
	secondary school	34	9.8
	high school	104	30.0
	university/collage	92	26.5
	postgraduate/doctoral	13	3.7
Education level of the father			
	illiterate	1	0.3
	literate	1	0.3
	primary school	41	11.8
	secondary school	46	13.3
	high school	89	25.6
	university/collage	148	42.7
	postgraduate/doctoral	21	6.1
Income level			
	low income	41	11.8
	middle income	287	82.7
	high income	19	5.5
Body Mass Index classification			
	underweight	45	13.0
	normal weight	225	64.8
	over-weight	63	18.2
	obese	14	4.0
Chronic disease status			
	yes	65	18.7
	no	282	81.3
Cigarette use			
	none	265	76.4
	≤1/2 package/day	32	9.2
	1/2-1 package/day	38	11.0
	≥1 package/day	12	3.4
Alcohol use			
	none	270	77.8
	1-7 standard drinks/per week	70	20.2
	≥8 standard drinks/per week	7	2.0

Table 2. Participants' smartphone use, exercise, and eating habit characteristics and their related scale scores

Age of owning a smartphone for the first time	Mean±SD	13.87±1.93	
	Min-Max	5-24	
Smartphone addiction scale scores	Mean±SD	88.20±24.83	
	Min-Max	33-186	
Eating attitude test scores	Mean±SD	15.21±9.13	
	Min-Max	1-68	
		n (number)	**% (Percent)**
Way of accessing the internet			
	computer	28	8.1
	smartphone	314	90.5
	tablet	5	1.4
Smartphone usage time			
	less than 1 hour	6	1.7
	1-2 hours	41	11.8
	2-4 hours	131	37.8
	4-6 hours	112	32.3
	6-8 hours	43	12.4
	above 8 hours	14	4.0
Smartphone usage purposes			
	messaging	330	95.1
	talking	304	87.6
	playing games	93	26.8
	social media monitoring	316	91.1
	taking photos	237	68.3
	listening to music	313	90.2
	watching videos/movies	253	72.9
	checking email	154	44.4
	stock market tracking	104	30.0
	following news	194	55.9
	shopping	228	65.7
	research/education	252	72.6
Abnormal eating attitudes			
	yes	31	8.9
	no	316	91.1
Healthy eating recommendations			
	not follow at all	29	8.4
	rarely	80	23.1
	sometimes	165	47.6
	often	67	19.3
	always	6	1.7
Weekly exercise (min.)			
	None	120	34.6
	less than 60 min.	136	39.2
	between 61-120 min.	58	16.7
	between 121-240 min.	19	5.5
	Above 240 min.	14	4.0

Table 3. The data regarding the relationship between the participants' mean BMI and gender, exercise, and eating behaviors

		Body Mass Index		p
		Meant±SD	Median (Min-Max)	
Gender				
	Female	20.99±3.00	20.31 (16.21-32.27)	**<0.001***
	Male	24.62±4.77	24.15 (17.31-60.84)	
Skipped meals				
	Yes	23.02±4.77	22.26 (16.41-60.84)	
	No	21.36±3.14	21.21 (16.21-27.77)	**0.045****
	Sometimes	21.92±3.37	21.33 (16.53-29.75)	
Healthy eating recommendations				
	not follow at all	25.34±7.71	23.66 (18.19-60.84)	
	rarely	22.73±3.55	21.96 (16.65-34.32)	
	sometimes	22.30±3.77	21.60 (16.21-38.16)	**0.002****
	often	21.18±3.29	20.41 (16.41-34.26)	
	always	22.53±2.75	23.63 (18.73-25.51)	
Weekly exercise (min.)				
	none	22.30±4.97	21.15 (16.65-60.84)	
	less than 60 min.	22.51±3.88	21.61 (16.94-34.26)	
	between 61-120 min.	22.61±3.74	22.56 (16.41-31.22)	0.761**
	between 121-240 min.	22.15±3.60	23.45 (16.21-27.45)	
	above 240 min.	22.69±2.55	22.95 (18.44-28.41)	

**Mann-Whitney U test, **Kruskal Wallis test*

The participants who use smartphones for "social media monitoring" have statistically significantly higher mean scores on SPAS than those who do not use it for this purpose (p=0.011) according to the relationship between smartphone using purposes and the smartphone addiction scale. For the other purposes (messaging, talking, playing games, taking photos, listening to music, watching videos/movies, checking email, stock market tracking, following the news, shopping, research/education), no statistically significant correlation was found between the use of smartphones and the mean scores of SPAS (p values are 0.426, 0.735, 0.506, 0.367, 0.367, 0.484, 0.713, 0.599, 0.688, 0.177, 0.122, respectively). In addition, no statistically significant relationship was found between the mean scores of the smartphone addiction scale and the education level of the parents, and family income level (p values are 0.188, 0.123, and 0.405 respectively). The data on the relationship of the participants' smartphone addiction scale scores with sociodemographics, descriptive characteristics, and smartphone use for the purpose of social media monitoring are shown in Table 4.

In our study, no statistically significant relationship was found between the eating attitude test scores and gender, accommodation, alcohol use, smoking, and using smartphones for social media purposes (p values are 0.484, 0.354, 0.392, 0.119, 0.123, respectively). There was a significant relationship between eating attitude scale scores and chronic disease status (p=0.011). Accordingly, the mean EAT score was higher in those who have chronic diseases than the ones who do not. The data on the relationship between the participants' eating

attitude test scores with sociodemographics, descriptive characteristics, and using the smartphone for social media monitoring are shown in Table 5.

Table 4: The relationship of the participants' smartphone addiction scale scores with sociodemographic, descriptive characteristics, and using the smartphone for social media monitoring

		Smartphone addiction scale scores		p
		Mean±SD	Median (Min-Max)	
Gender				
	Female	88.69±21.97	86.00 (33-170)	0.312*
	Male	87.48±28.66	83.00 (36-186)	
Accomodation				
	family home	88.64±21.01	89.50 (39-134)	0.765**
	student house/apartment	87.79±26.46	84.00 (33-186)	
	youth hostel	88.48±24.35	85.00 (40-170)	
Using smartphones strolling in social media				
	yes	89.06±23.56	86.00 (36-170)	**0.011***
	no	79.45±34.58	70.00 (33-186)	
Alcohol use				
	yes	91.70±25.05	88.00 (36-148)	0.164*
	no	87.21±24.73	84.50 (33-186)	
Cigarette use				
	yes	88.70±26.31	84.00 (36-170)	0.937*
	no	88.05±14.41	85.00 (33-186)	
Chronic disease status				
	yes	91.12±26.34	92.00 (40-155)	0.264*
	no	87.53±24.47	84.00 (33-186)	

**Mann-Whitney U test, **Kruskal Wallis test*

According to the comparison between the age of owning a smartphone for the first time and family income level, students with a low family income level had their smartphones at a statistically significantly later age (p= 0.040). The data on the relationship between the age of the students owning a smartphone for the first time and the body mass index classification and family income level are shown in Table 6.

The mean SPAS score of participants who did not have abnormal eating attitudes was 87.15 (33-186) and the median score of those who had abnormal eating attitudes was 99.00 (58-153). There was a statistically significant difference between the two groups (p=0.038). In addition, a very weak, positive, and significant relationship was found between the SPAS scores of the students and their EAT scores

(r=0.135, p=0.12). As a result of the correlation analysis, there was a very weak, positive, and significant relationship between BMI and the age of the students having a smartphone for the first time (r=0.113, p=0.036). The results of the correlation analysis between the scale scores of the students and the numerical variables are shown in Table 7.

Table 5: The relationship between the participants' eating attitude test scores with sociodemographic, descriptive characteristics, and using the smartphone for the purpose of social media monitoring

		Eating Attitude Test Scores		p
		Mean±SD	Median (Min-Max)	
Gender				
	Female	15.24±9.90	13.00 (1-68)	0.484*
	Male	15.18±7.87	13.00 (2-42)	
Accommodation				
	family home	15.11±11.67	11.50 (2-61)	
	student house/apartment	15.42±8.19	14.00 (2-48)	0.354**
	youth hostel	15.05±9.24	13.00 (1-68)	
Using smartphones while strolling in social media				
	yes	15.13±9.30	13.00 (1-68)	0.123*
	no	16.03±7.22	15.00 (3-36)	
Alcohol use				
	yes	15.39±7.94	14.00 (3-40)	0.392*
	no	15.16±9.46	13.00 (1-68)	
Cigarette use				
	yes	16.29±10.06	15.00 (3-68)	0.119*
	no	14.88±8.82	13.00 (1-61)	
Chronic disease status				
	yes	17.86±10.60	15.00 (2-52)	**0.011***
	no	14.60±8.66	12.00 (1-68)	

*Mann-Whitney U test, **Kruskal Wallis test

Table 6: The relationship between the age of the students owning a smartphone for the first time and the body mass index classification and family income level

	Age of the Students Owning a Smartphone for the first time		p
	Mean±SD	Median (Min-Max)	
Family income level			
low income	14.59±1.77	14.00 (12-19)	**0.040***
middle income	13.79±1.93	14.00 (5-24)	
high income	13.53±2.14	13.00 (10-19)	
Body mass index classification			
underweight	13.40±1.81	14.00 (5-17)	
normal weight	13.80±1.84	14.00 (9-22)	0.154*
over-weight	14.13±2.30	14.00 (10-24)	
obese	13.93±1.54	14.00 (12-18)	

**Kruskal Wallis test*

Table 7: Correlation analysis between the scale scores of the students and the numerical variables

	BMI	SPAS total score	EAT-40	Sleeping hours
	*r**	*r**	*r**	*r**
BMI	-			
SPAS total score	0.014	-		
EAT-40	0.081	**0.135****	-	
Sleeping hours	-0.014	0.029	-0.091	-
Age of owning a smartphone for the first time	**0.113****	-0.079	0.054	0.062

BMI: Body mass index EAT-40: Eating Attitude Test-40 SPAS: Smart Phone Addiction Scale
*Spearman's correlation coefficient
** $p<0.05$ The significant results of the analysis are shown in bold.

DISCUSSION

Smartphones are tools that have many purposes such as gathering information, communication, education, and entertainment, and have become indispensable in daily life (15). In the studies, it was emphasized that the use of smartphones has become a necessity rather than a preference (16).

In a study conducted by Uzgoren et al. in 2013, 75% of 389 students bought their first smartphone between the ages of 13-16 (17). In our study, the mean age of owning the first smartphone in accordance by this data was found as 13.87±1.93.

In the literature, a positive relationship was shown between smartphone use and some health-endangering behaviors such as smoking, substance, and alcohol use (18). Personality-related characteristics such as low self-esteem and loneliness are predictors of all addictive behaviors (19). However, unlike the literature, no statistically significant difference was found in our study between the students who smoked or used alcohol and those who did not in terms of smartphone addiction scale scores. This may be due to the fact that there are few students smoke and use alcohol compared to the total number of participants. Studies using a larger sample group would give more accurate data on this issue.

According to the research by the Turkish Statistical Institute conducted in 2021, messaging and social media use are at the top of smartphone usage purposes, and this finding is similar to our study finding (2). In the thesis study carried out by Unal in 2015, 56.8% of the students use their smartphones to surf on the internet, and 55.5% follow social media (20). Compared to previous studies, the usage purposes of smartphones have changed to internet-based applications such as social media monitoring and messaging according to more recent studies.

In the comparison of the total SPAS scores regarding the purpose of smartphone use, the students who use their phones to access social media platforms scored significantly higher than those who are not. In a study published in 2015, smartphone communication methods such as messaging and social media monitoring

were associated with higher addiction rates (21). Nowadays, most the students use their smartphones to access the internet and social media platforms, and it is important that the use of smartphones for this purpose might be risky in terms of problematic use

This study showed that the rate of medical school students who had abnormal eating attitudes was 8.9%. Although this is lower than some studies with different demographics, it is still an important ratio. In the study carried out by Buyukgoze-Kavas, which included 269 participants consisting of Turkish university students, 12.3% of the participants were at high risk in terms of eating disorders (22). In their study on the eating attitudes and body image of university students attending the Faculty of Vocational Education of a public university, Akdevelioglu and Gumus found that 5.9% of 577 students had abnormal eating habits (23). This lower rate was explained by the fact that the students come from low-income families and live in non-industrialized cities in Turkey. In a study that evaluated 301 students studying at different universities located in Istanbul and Ankara, which are the most industrialized cities in Turkey, 18.3% showed abnormal eating attitudes which indicates that they are at high risk for eating disorders (24).

In our study, the SPAS score median of participants who had abnormal eating attitudes was higher than that of those who did not have abnormal eating attitudes. Tayhan Kartal and Yabanci Ayhan (2021) found a positive relationship between smartphone addiction and the Eating Attitude Test-40 scores of university students (3). Significant neurocognitive similarities between addiction behavior (e.g., smartphone addiction) and eating disorders (e.g., restrained eating and external eating) have been reported in the literature (25).

Pro-anorexia ("pro-ana") and pro-bulimia ("pro-mia") websites are online communities whose members in general do not consider eating disorders to be serious mental disorders that require treatment. These communities define eating disorders as a positive condition, such as lifestyle choice, or as a condition in which a person should freely choose whether to recover or not despite possible life-threatening consequences (26). Considering these situations, ease of access to the internet and smartphone applications may cause adolescents to be exposed to harmful websites and contents although they may provide access to accurate information about eating disorders. In a study, girls between the ages of 13 and 17 demonstrate a higher urge to be slim and perceive their appearance as worse after visiting anorexia-related websites (27). For these reasons, the frequency and purpose of internet and smartphone use, especially among the young population, are critical because they make them prone to eating disorders although not at an addiction level.

In an earlier study, internet addiction was associated with BMI regardless of eating attitude (28). In another study published very recently in our country, SPAS scores had a positive relationship with BMI and EAT scores (3). In our study, no relationship was found between BMI and SPAS scores. Thus, intensive smartphone use may not always be associated with overweight or obesity due to being mobile during smartphone use and that it made it easy to use applications related to health, nutrition, and exercise.

In a study conducted in China with 1199 high school and university students, women with internet addiction were reported to have significantly higher eating attitude test scores than those who did not have an addiction (7). In a study that examines the relationship between smartphone addiction and eating attitudes (1), the researchers did not find a significant relationship between the two variables, and another study (3) demonstrated a significant positive relationship between smartphone addiction scores and the EAT scores. In our research, a very weak relationship was found between smartphone addiction and eating attitude. Being able to access the internet from anywhere with smartphones causes the young population, who cares about their physical appearance, to communicate more easily with their peers, therefore, in the social media environment, it can lead to results such as disordered eating or eating disorders due to the effort to be slim to look “more beautiful”.

The use of digital technology was associated with physical inactivity, obesity, and sleep problems in preschool and school-age children (29). We have not found any other studies in the literature that investigate the relationship between the age of the students owning a smartphone for the first time and BMI. However, in our study, a weak, positive relationship was found between the age of owning a smartphone for the first time and BMI. Accordingly, it was found that those who owned smartphones at a younger age had lower BMI values than those who owned smartphones at a later age. In addition, in our study, it was found that students with a low family income level have smartphones for the first time at a later age. In light of this data, it made us think that the BMI values of the participants with higher age of owning their first smartphone were higher in participants with low family income, due to the reasons such as uniform nutrition, carbohydrate-based diet, etc. Besides, although there is no statistically significant relationship between body mass index classification and the age of owning their first smartphone, the participants in the “underweight” class were found to have a smartphone at an earlier age. It suggested that those who have a smartphone at an earlier age may have lower BMI values due to the reasons such as their way of perceiving the body and

they associate beauty with being slim as they are exposed to the influence of social media before their self-perception is developed.

For the future, researchers are advised to investigate how personal characteristics affect smartphone addiction symptoms, and how these symptoms may affect social and occupational functioning and mental and physical health.

Limitations

It should be taken into consideration that there are some limitations to this study when interpreting the findings. Firstly, our study is a cross-sectional study and does not show cause-effect relationships. Our biggest limitation was the delivery of the survey questions to the participants online and there was no face-to-face evaluation opportunity. Since the study data were obtained from medical school students, the results cannot be generalized to all individuals. In addition, our other limitations were that the data of the participants' anthropometric measurements were based on their own reports and there was no psychiatric evaluation regarding their characteristics of smartphone addiction and eating attitudes.

CONCLUSION

We reported a relation between the intentions of using a smartphone and smartphone addiction and another relation between one's age of owning the first smartphone and these individuals' body mass indexes. A difference was observed between those who have abnormal eating attitudes and who have not regarding smartphone addiction scale scores. Previous studies have shown that smartphone addiction can cause potential health-related risks. Identifying the factors that cause and/or maintain eating disorder symptoms can enable the development of targeted intervention programs in today's world where smartphone applications are widely used and internet access is easy. In addition, awareness-raising campaigns may be launched by the authorities and government agencies to ensure that excessive and inappropriate use of smartphones is avoided. Mass media can be used to raise general awareness about the negative effects of smartphone use on human health and behavior, in particular, its dangerous consequences (body perception, desire to be slim, the concept of beauty, etc.) for the adolescent and young adult population.

Ethics Committee Approval: The study complies with the Declaration of Helsinki and was approved by Zonguldak Bülent Ecevit University Clinical Studies Ethical Committee on Dec 15, 2021 with an approval number of 2021/24.

Author Contributions:

Concept: V.Ç.K, Design: V.Ç.K, S.Ö, B.Ş.Ç, S.T, M.Z.K, BK, S.Ö, D.A.T, Y.A.A, A.R.A, A.A.H, İ.A, Analysis or Interpretation: A.D.Ç, H.Ç, S.Ö, B.Ş.Ç, S.T, M.Z.K, B.K, S.Ö, D.A.T, Y.A.A, A.R.A, A.A.H, İ.A, Writing: A.D.Ç,

H.Ç, S.Ö, B.Ş.Ç, S.T, M.Z.K, B.K, S.Ö, D.A.T, Y.A.A, A.R.A, A.A.H, İ.A, V.Ç.K.

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Amoebicidal and Cytotoxic Activity of Propolis collected from Different Regions in Turkey on *Acanthamoeba castellanii* Trophozoites

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Abstract

Objective: The present study aimed to investigate the amoebicidal effects of propolis collected from different regions in Turkey on *Acanthamoeba* trophozoites.

Method: The propolis was collected from different geographic sites (Van, Erzurum, Gümüşhane, Ordu, Rize, and Muğla) in Turkey. Different concentrations of propolis ethanolic extract (in quantities from 1, 2, 3, 4, 5, 6, 7, and 8 mg/mL) and the same volume of trophozoites in 100 µl culture were blended for the identification of the amoebicidal efficiency of propolis.

Results: The growth of trophozoites stopped in Turkish propolis extracts with 50% inhibitory concentrations (IC50)/48h for 5 mg/mL extract solution. Propolis showed more potent inhibitory effects on *Acanthamoeba* trophozoites at concentrations of 7, 6, 5, 4, and 3 mg/mL for 72 h. Propolis extract substantially inhibited human bronchial epithelial cells, especially at higher concentrations (7, 8, and 16 mg/mL).

Propolis can kill *Acanthamoeba* trophozoites at a concentration (of 3-6 mg/mL) but is safe for human bronchial epithelial cells at the same concentrations after 72 h treatment, this paves the way for propolis to be an alternative source of therapeutic drugs in the treatment of *Acanthamoeba* spp.

Conclusion: *Acanthamoeba* infection still cannot be treated with drugs. In this study, propolis collected from different regions of Turkey showed amoebic and cytotoxic activities. Propolis extract, which is a natural product that can be used against *Acanthamoeba* trophozoites can be an alternative source of therapeutic drugs in the treatment of Acanthamoeba, supported by in vivo studies.

Keywords: *Acanthamoeba* sp., amoebicidal activity, Turkish propolis, MTT assay, Cytotoxicity, RP-HPLC

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INTRODUCTION

Propolis is a mix of content used by bees to protect the hive. This preservation refers to filling gaps in the hive's walls, decreasing entry on cold days, and mummifying the claimed uninvited guests, thus avoiding their decomposition. Bees take resins from sprouts, exudates, and other parts of plants. They mix those resins with their salivary enzymes and wax and then form propolis (1).

Since propolis is rich in flavonoids, resins, vitamins, and minerals, it has been preferred in natural treatments in public health and applied to medicine for many years. It is known that propolis contains different compounds depending on the plant species and geographical conditions where it is collected (2). The chemical compound of propolis is based on the local flora of the districts and collection time, which makes it difficult to standardize (3).

Recently, many studies have been conducted on propolis because it is anti-bacterial (4); antioxidant, anticancer (5); wound healing (6), and anti-protozoal activities (7).

Propolis prevents the multiplication of promastigotes in *Leishmania*, inhibits the parasitemia in *Plasmodium* and *Trypanosoma*, prevents the growth of *T. cruzi*, inhibits the proliferation of *Blastocystis*, and reduces oocysts shedding in *Cryptosporidium* and *Giardia*. Moreover, propolis increases the specific IgM and IgG titers in toxoplasmosis. Besides these, there is limited knowledge of the mechanism with the thought of these findings, the mechanism of action of propolis is not clear in *Acanthamoeba* spp., and *Trichomonas* spp. (8).

Acanthamoeba spp. is a phase of free-living amoeba that distributes in natural water sources, seawater, and soil, it has also been isolated from various niches including, swimming pools, tap water, bottled mineral water, and even lens storage containers and lens cleaning solutions, dialysis units, dental treatment units (9,10).

Acanthamoeba culbertsoni, *Acanthamoeba castellanii*, and cause granulomatous amoebic encephalitis (GAE) and *A. rhysodes*. *Acanthamoeba* keratitis (AK) is a parasitosis caused by various *Acanthamoeba* species. Asymptomatic individuals present with severe ocular pain, inflammation, visual impairment, and annular stromal infiltration. In such cases, vision may deteriorate over time and vision loss may occur (11).

There is no eradication of *Acanthamoeba* from infection yet because treatment of acanthamoebiasis still has very significant problems related to the induction of parasite resistance and toxic side effects (10). *Acanthamoeba* infection is similar to bacteria, and both of them show high resistance to many antimicrobial agents at tolerable concentrations. Therefore, there is a need for new approaches and more effective treatment protocols for *Acanthamoeba* infections.

Today, several studies conducted in many fields to eradicate severe parasitic and bacterial infections based on bioactive compounds extracted from various natural sources (11, 12, 13, 14).

The present study aimed to investigate the amoebicidal effects of propolis collected from different regions in Turkey on *Acanthamoeba* trophozoites. In many studies, it has also been shown that propolis has anti-bacterial and anti-cancer effects. However, there is limited study

on the cytotoxicity of Turkish propolis on human bronchial cells. Therefore, this study also discusses the determination of the cytotoxic activity of propolis collected from different regions of Turkey.

METHODS

Propolis Extraction

Propolis samples collected from Ordu, Erzurum, Gümüşhane, Van, Rize, and Muğla provinces of Turkey were tested. The samples (60 g) were dissolved in 300 ml of ethanol for 72 h at room temperature in a shaking incubator. After the reaction mixture was filtered to remove insoluble components, the extract was evaporated to take out the resolvent, and the dry residue was thawed in distilled water. The supernatant was used for the propolis solution's final concentration (32 mg/mL). Seven different concentrations of propolis (1, 2,,4, 5, 6, 7, 8 mg/mL) were used for treatment with *Acanthamoeba* trophozoites.

Culture of Acanthamoeba

A. castellanii (ATCC 30010 purchased from the American Type Culture Collection) was placed on Ringer agar plates seeded with *E. coli* so that it could use gram-negative bacteria as a food source. After 3 days of incubation at 26°C, the plates were examined under the microscope to detect the presence of *Acanthamoeba* trophozoites (13). The plates were incubated at 26°C in the incubator, and 3 days later, they were microscopically examined for the presence of *Acanthamoeba* trophozoites (13). Trophozoites in the exponential growth stage (72 h) were gently collected from Ringer agar plates with the aid of a sterile cell scraper. The trophozoites were washed twice with Ringer's dilution and concentrated by centrifugation. Viable and dead trophozoites were separated from each other by Trypan blue staining and counted in a hemocytometer (13). The final concentration was prepared to 10^6 trophozoites/mL. Numbers of viable *Acanthamoeba* trophozoites were determined during the 1, 3, 6, 8, 24, 48, and 72-hour periods at 26°C.

Evaluation of the effect of propolis on Acanthamoeba

The propolis extract of 100 µl was mixed with an equal volume of amoebae culture in concentrations of 1, 2, 3, 4, 5, 6, 7, and 8 mg/mL. The viability of the parasite was checked and recorded at 1-, 3-, 6-, 8-, 24-, 48-, and 72-h intervals by using a Thoma hemocytometric chamber to represent the amoebicidal activity of the propolis extract. One hundred *A. castellanii* trophozoites were counted for each experiment. A control group was formed by adding amoeba culture to the reaction mixture without propolis extract. The results are stated as percent inhibition per control cells (considered 100%). The 50% inhibitive concentrations (IC50) were shown with logarithmic regressions. All experiments were performed in three repetitions and statistical analyses were made to determine the cell viability percentage.

Cytotoxicity of propolis extract

The cytotoxicity of propolis ethanolic extracts was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (15). BEAS-2B, human bronchial epithelial cells (ATCC, CRL-9609), were used for determining the cytotoxicity of propolis. The cells were maintained in serum-free LHC-8 medium and cultured at 37°C in a 90% humidified incubator with 5% CO_2. After BEAS-2B cells were seeded in a 96-well plate ($1x10^4$ cells each well), the cells were treated with

different concentrations (1, 2, 4, 5, 6, 7, 8, and 16 mg/mL) of ethanolic extracts of propolis for 72 h. After treatment, MTT solution (20 µl) was added to each well and incubated for 4 h and then 100 mL of DMSO was added to each well. After 15 min, the color intensity of the wells was measured at 570 nm using a Biotek microplate reader. The results were expressed as percentage inhibition relative to control cells (considered as 100%). Logarithmic regressions were performed to define 50% inhibitory concentrations (IC50) (concentrations that inhibit the response by 50%). The experiment was repeated three times using different dilutions of the extracts.

Analysis of phenolic compounds by RP-HPLC

Fourteen standards of phenolic compounds were analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC, (Elite LaChrom Hitachi, Japan) to determine propolis' phenolic profile. The sample was inserted into the HPLC system and installed with a reversed-phase C18 column (150 mm x4.6 mm, 5µm; Fortis). Water, acetonitrile, and acetic acid were applied for the mobile phase by using a programmed gradient. The mobile phase was formed of (A) 2% acetic acid in water and (B) acetonitrile in water (70:30). The injection volume of the samples was 20 µL, the column temperature was 30°C, and the flow rate was 0.75 mL/min. The programmed solvent used was maintained with a linear gradient held at 95% A for three minutes, decreasing to 80% A at 10 min, 60% A at 20 min, 20% A at 30 min, and finally started with a linear gradient decreasing to 95% A at 50 minutes.

Statistical analysis

A one-way test of variance (ANOVA) with the SPSS software package for Windows was applied to complete all statistical analogies. Mean ± standard error (SE) was used for all results. MTT data were analyzed using one-way ANOVA followed by the Student t-test. Differences between $p < 0.05$ were found to be statistically significant.

RESULTS

Amoebicidal activity

The trophozoite growth stopped in Turkish propolis ethanolic extracts with IC_{50}/48h at 5 mg/mL. Propolis showed stronger inhibitory effects at the concentrations of 7, 6, 5, 4, and 3 mg/mL with 72 h against *Acanthamoeba* trophozoites. The ethanol extracts of propolis reduced cell viability by approximately 2, 2.67, 22.67, 33.33, and 58.33% at concentrations 7, 6, 5, 4, and 3 mg/mL within 72 h, respectively. Among the different concentrations of propolis ethanolic extracts used in this study, 7 mg/mL propolis extract solution represented the strongest amoebicidal activity on trophozoites with IC50/72 h. High activity at low concentrations (3-7 mg/mL) of propolis extract can kill and can be an effective treatment for *Acanthamoeba* spp., after 72h treatment.

The effect of the propolis ethanolic extract on *A. castellanii* trophozoites is presented in Table 1, Table 2, and Fig. 1

The tests with propolis ethanolic extracts were done two times in triplicate. Results are presented as mean ± mean standard error (SE) in Table 1.

Table 1. Mean ± SE values for the percentages of cell viability of *Acanthamoeba* trophozoites when exposed to different concentrations of propolis extract for varied hours

Dose	*A. castellani* forms	Treatment periods (hours)						
		1 h	3 h	6 h	8 h	24 h	48 h	72 h
		Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
7 mg/ml	Trophozoites	94.33±1.67	90.33±1.67	82.00±1.00*	65.00±1.00*	31.00±3.21*	3.33±2.33*	2.00±1.00*
6 mg/ml	Trophozoites	94.00±1.53	92.67±1.20	88.00±1.00*	75.00±0.58*	44.67±0.88*	32.50±3.50*	2.67±0.67*
5 mg/ml	Trophozoites	100.00±0.00	94.33±1.67	93.00±1.53	84.67±1.20*	66.67±2.40*	50.33±0.33*	22.67±1.20*
4 mg/ml	Trophozoites	100.00±0.00	100.00±0.00	94.33±1.67	89.00±1.00*	72.00±2.08*	56.00±2.08*	33.33±2.28*
3 mg/ml	Trophozoites	100.00±0.00	100.00±0.00	94.67±1.33	91.67±0.33	81.67±1.86*	69.00±2.00*	58.33±1.45*
1 mg/ml	Trophozoites	100.00±0.00	100.00±0.00	100.00±0.00	94.33±1.67	91.33±0.33	86.00±1.00*	75.33±2.91*

*= Statistic different from control group $p < 0.05$

Table 2. Percentages of cell viability in the *Acanthamoeba* trophozoites when exposed to 7, 6, 5, 4 mg/mL concentrations of propolis extract for 72 h.

The stage of *A. castellanii*	**The concentrations of propolis extract**	**Percentage of cell viability**
Trophozoites	7 mg/mL	2.00±1.00
	6 mg/mL	2.67±0.67
	5 mg/mL	22.67±1.20
	4 mg/mL	33.33±2.28

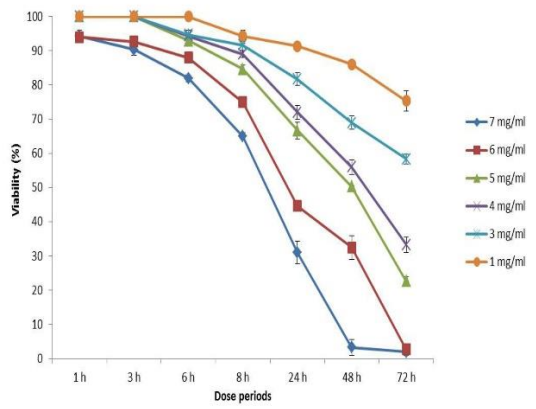

Figure 1. The effect of different concentrations of propolis extract on the cell viability of *A. castellanii* trophozoites at varying times (hours)

Propolis extract cytotoxicity

The effects of propolis extract on cell viability in BEAS-2B cells are shown in Figure 2.

The results of the MTT assay showed that propolis extract decreased cell viability in a concentration-dependent manner. These decreases in cell viability were significant at 4 mg/mL and above concentrations when compared to the control ($P<0.05$). The propolis's 50 % inhibitory concentrations

(IC50) were found as 6.60 mg/mL. Propolis showed stronger inhibitory effects at the higher concentrations tested (7, 8, and 16 mg/mL). The cell viability values of BEAS-2B cells were 43.28, 23.90, and 13.01 % at 7, 8, and 16 mg/mL concentrations, respectively. Based on our results, it can be stated that higher concentrations of propolis extract can induce cytotoxicity in BEAS-2B cells.

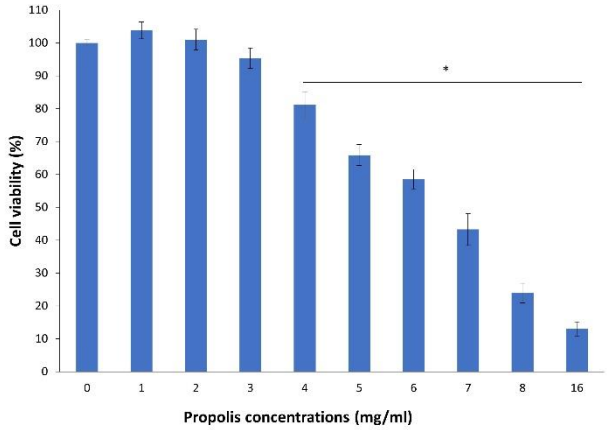

Figure 2. Effects of propolis on cell viability of BEAS-2B cells after 72 h treatment. *significant compared to control ($p < 0.05$).

Phenolic profiles

Fourteen standards of phenolic compounds were analyzed qualitatively and quantitatively using RP-HPLC-UV (Table 3). Protocatechuic acid, p-OH benzoic, Vanillic acid, Caffeic acid, Epicatechin, p-coumaric acid, Ferulic acid, Daidzein, t-cinnamic acid were present in differing amounts in samples investigated, while Caffeic acid, Catechin, Gallic acid, Syringic acid, Rutin, Luteolin were not detected in propolis. The major phenolic compound in propolis was Ferulic acid, and lower levels of Protocatechuic acid, Caffeic acid, and p-OH benzoic acid were also detected.

Table 3. Phenolic profiles of propolis extract collected from different regions in Turkey

Standard	***Results lg phenolic compound /g sample***
Gallic acid	n.d.
Protocatequic acid	0.024
p-OH benzoic acid	0.087
Catechin	n.d.
Vanillic acid	5.575
Caffeic acid	0,025
Syringic acid	n.d.
Epicatechin	2.220
p-coumaric acid	0.569
Ferulic acid	13.215
Rutin	n.d.
Daidzein	2.142
t-cinnamic acid	3.505
Luteolin	n.d.

**n.d: not detected.*

DISCUSSION

Propolis is a resinous hive product collected by honeybees from various plant sources. Propolis has an extensive variety of biological activities because of its flavonoids and caffeic acid phenethyl ester (16). It has recently been given as a dietary supplement for the therapy of various diseases (17).

Many studies have suggested that the therapeutic properties of propolis the anti-bacterial (4), anti-inflammatory (18), anti-viral (19), anti-tumoral (5), anti-fungal (20), anti-oxidant (21), anti-protozoal (22), activities.

These activities of propolis samples correlate with the total phenolic contents. The caffeic acid derivatives and flavonoids of propolis have strong antimicrobial activity. Thus, there is increasing interest in it as the origin of new therapies (23). However, we know that limited studies in Turkey have been reported on propolis's anti-amoebic and cytotoxic properties so far.

Several studies on the amoebicidal activity of propolis against *Acanthamoeba* species originated in Turkey. Topalkara et al. (7) first reported the propolis effect on *Acanthamoeba* trophozoites and cysts. In their study, there was a decrease in the number of viable trophozoites of *A. castellanii* based on a dose-dependent application of propolis. Propolis (higher than 8 mg/mL) had a lethal efficacy on *Acanthamoeba* trophozoites, while no lethal concentration of propolis for *Acanthamoeba* cysts after 1 h incubation. A second study was reported by Vural et al. (24) on the propolis effect in a rat model of *Acanthamoeba* keratitis and assigned it is in vitro cytotoxic activity in cultured corneal epithelial cells. This study showed the propolis extract had an anti-amoebic effect in this rat model of *Acanthamoeba* keratitis. Following these studies, we aimed to show propolis's cytotoxic and amoebicidal activities collected from different regions in Turkey.

For this reason, propolis extracts with different concentrations were analyzed to indicate their efficiency against the trophozoites of *A. castellanii*. The trophozoites were inhibited with 7mg/mL of propolis extract with a 2.00±1.00 percentage of cell viability at 72 h. Propolis extract (7 mg/mL) showed stronger amoebicidal activity on trophozoites since there were fewer viable trophozoites in this mixture at 72 h.

Previous studies by Duran et al. (25) indicated that the ethanolic extract of propolis samples in Adana from Turkey showed a remarkable antileishmanial effect on *Leishmania tropica*. In this study, the maximum reduction in the proliferation of *L. tropica* parasites was detected in cultures exposed to 250, 500, and 750 µg/mL of propolis. In addition, antileishmanial activities of Bursa and Hatay propolis samples against *Leishmania infantum* and *Leishmania tropica* strains were detected with the gas chromatography-mass spectrometry technique

by Duran et al. (26). The results showed that these propolis samples reduced remarkably the multiplication of *L. infantum* and *L. tropica* parasites. The chemical compounds of propolis samples were examined by high-resolution GC-MS. They reported that these propolis samples contain a high concentration of compounds such as aromatic acids, aromatic acid esters, flavanols, and cinnamic acid esters. They found some types of propolis had more antileishmanial effects than others against *Leishmania* species because of the various geographical sites, regional flora, and plant variety.

Similarly, many studies were reported on antibacterial (27) antiviral (23,28) antifungal (29) and, in antiparasitic (30) properties of propolis related to the presence of flavonoids and phenolic compounds and chemical composition in the propolis.

The result of the analysis of active components in propolis in different studies is quite different since the composition and contents of these active components depend on different factors including season and vegetation of the field, propolis collection techniques, and geographical origin (31)

Many studies have been reported to analyze the phenolic compounds of propolis from different regions in Turkey (31,32,33). The present study is similar to those of our previous studies in showing the phenolic compounds of propolis collected from different regions in Turkey. However, according to the analysis of the phenolic compounds in the present study, the propolis samples found high concentrations of Ferulic acid and Vanillic acid.

Ferulic acid is completely spread in plant cell walls and has anti-inflammation, anti-diabetic, anti-oxidation, and antiviral effects (28). In addition, Celińska-Janowicz et al. (34) reported the polyphenols of propolis such as ferulic acid, chrysin, p-coumaric acid, and caffeic acid induce anti-proliferative activity by increasing the role of proline metabolism and proline dehydrogenase/proline oxidase, active caspases-3, and -9 expressions, P53, and decrease prolidase activity, proline concentration and, collagen biosynthesis in CAL-27 cells.

Vanillic acid is one of the main pharmacologically active molecules in propolis due to its ability to have anti-inflammatory, anti-metastatic, antidiabetic, antioxidant, cardioprotective, and anti-apoptotic effects (35). Kılıç Altun and Aydemir (31) reported that the amount of Vanillic acid among all phenolic ingredients of propolis was the highest level after Hydroxycinnamic acid, Kaempferol, and Quercetin. The amount of vanillic acid level in propolis in our study is similar to those of the other previous studies. There were high concentrations of Ferulic acid.

Cytotoxic effects of some propolis samples from Turkey on different human cells have been reported in previous studies.

Anticarcinogenic effects of propolis have been shown in human lymphocytes culture (36), and peripheral blood lymphocytes (37). Ethanolic extract of Turkish propolis showed proapoptotic activity and antiproliferative effect on the human lung cancer (A549) cell line with 31.7 µg/mL IC50 value (38). The anticarcinogenic effects of the propolis samples were reported on aggressive cell lines SK-BR-3, MDA-MB-231, and nonaggressive breast cancer cell line (BCCL) MCF-7 (39). Some ethanolic extract of Turkish propolis displayed a higher cytotoxic activity against A549 lung cancer cells (IC50=25.44 µg mL^{-1}) when compared to BEAS-2B healthy lung epithelial cell cultures (IC50=55.68 µg mL^{-1}) (40). The differences in IC50 values in the studies may be due to the differences in the regions where the samples were collected, the extraction methods used, the solvents, the management of the cell cultures, the stages of the experiments, and the treatment times used. We wanted to see the response of high concentrations of propolis samples in healthy cells in the present study. We found that the extract of propolis did not show significant cytotoxic effects up to a concentration of 3 mg/mL. Based on the results of previous studies, we can say that its cytotoxicity is probably higher in lung cancer cells. It is very important to protect the healthy cells because of higher cytotoxicity in cancer cells and lower toxicity in healthy cells.

CONCLUSION

Acanthamoeba infection is still a problematic parasitic disease that is not fully treated with drugs. In this study, we found that propolis extract collected from different regions in Turkey has major amoebicidal and cytotoxic activities. These activities could widely be interesting in pharmaceutical areas. Propolis extract can be thought of as a natural product that can be used against *Acanthamoeba* trophozoites. Even though further in vivo studies are needed to show the real effect of propolis extracts on *Acanthamoeba* infections, these results may be alternative therapeutic drug sources for the treatment of acanthamoebiasis alongside existing drugs.

Ethics Committee Approval: Ethical approval is not required for this study

Author Contributions: Concept: Z.K, Ö.E, Z.K, Ü.K, Design: Z.K, Ö.E, Z.K, Ü.K, Literature search Z.K, Ö.E, Z.K, Ü.K, Data collection and Processing: Z.K, Ö.E, Z.K, Ü.K, Analyses and Interpretation: Z.K, Ö.E, Z.K, Ü.K, Writing: Z.K, Ö.E, Z.K, Ü.K,

Conflict of Interest: There are no conflicts of interests related to this study.

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Examination of the Relationship between Parents' Attitudes and Beliefs about Human Papillomavirus Vaccine and Health Literacy

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Abstract

Objective: This study was conducted to examine the relationship between the attitudes and beliefs of parents about Human papillomavirus vaccine and their health literacy.

Methods: The sample of this descriptive and cross-sectional study consisted of 599 parents who volunteered to participate in the study and had access to our survey sent from the parent whatsapp groups of a secondary school located in rural areas in the central region of Türkiye. A Descriptive Information Form, the Carolina HPV Vaccination Attitudes and Beliefs Scale, and Turkey Health Literacy Scale-32 were used to collect research data.

Results: The mean age of 56.8% (n: 340) of the parents was ≥ 45 years, 68.4% (n:410) were women, 49.6% (n:297) had primary education, 92.0% (n:551) did not have a history of cervical cancer in their mothers, 89.8% (n:538) did not have a history of cervical cancer in their family, 92.2% (n:552) had not had their children vaccinated against HPV. Also, 28.4% (n= 170) of the participants had received information about the HPV vaccine from health personnel. It was determined that the risk of not having the child vaccinated was 1.320 times higher in participants with an equal income and expenses than in those whose income was higher than their expenses and that it was 4.514 times higher in participants with no family history of cervical cancer than in those with a history of this cancer type.

Conclusion: In the study, it was concluded that those whose income was equal to their expenses and those who did not have a family history of cervical cancer had a higher risk of not having their child vaccinated and that the high level of health literacy of the parents positively affected their attitudes and beliefs about human papilloma virus vaccination. As the parents' level of health literacy increased, the rate of getting their children vaccinated against HPV increased significantly. Considering this situation, it is thought that knowledge of vaccines can be increased, attitudes and beliefs can be improved, and vaccination rates can be increased by targeting health literacy in the interventions to be implemented.

Keywords: Human papillomavirus, vaccine, parent, attitude, belief, health literacy

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INTRODUCTION

Human papillomavirus (HPV) is one of the most common and often sexually transmitted infections (1). While there are over 200 HPV types, HPV 16 and 18 are the most common oncogenic types. HPV types 31, 33, 45, 52, and 58 are less prevalent. HPV types 6 and 11 are responsible for 90% of anogenital warts (2). Genital HPV infections are important because they cause cancer-precancerous lesions. Each type has a different risk for developing cancer. For example, while the risk for developing cervical cancer in a woman infected with HPV type 6 is 4 times higher, this risk can increase up to 282 times in women infected with HPV type 16 (3). The biggest cause of cervical cancer is HPV (4). Nine out of ten cervical cancer cases develop from HPV (5, 6). Cervical cancer is the fourth most common cancer among women globally and ranks eighth place in Turkey (7, 8). While approximately 570.000 new cervical cancer cases are seen worldwide every year, more than half of these result in death (8,9).

HPV vaccines are highly effective in preventing infection with the HPV types that they target. Many studies have shown that HPV vaccines are safe and effective (10, 11). Besides, HPV vaccines protect against new HPV infections but do not treat existing ones or diseases (12). There are 3 types of HPV vaccines, namely, Gardasil, Gardasil 9, and Cervarix, which have been approved by the Food and Drug Administration (FDA) to prevent HPV infection. Gardasil, a quadrivalent vaccine, was approved in 2006. It targets HPV types 6, 11, 16, and 18. The bivalent vaccine Cervarix, which was approved in 2009, protects against HPV types 16 and 18. Gardasil 9, which was approved in 2014, targets HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 and is recommended for girls and boys aged 9-26 (13). All of these vaccines were licensed in Turkey in 2007 and 2017, Gardasil 9 vaccine can be ordered from pharmacies in Turkey. According to the currently adopted approach, HPV vaccines should be given before individuals become sexually active. Although the age to get the HPV vaccine varies according to the vaccination program of each country, the recommended age range for vaccination in Turkey is often 9-26 (13). Recent data suggest that protection against targeted HPV genotypes lasts at least 10 years with Gardasil, 9 years with Cervarix, and 6 years with Gardasil 9 (14). As of 2019, one hundred countries around the world included the HPV vaccine in their national vaccination programs (World Health Organization, 2020). In Türkiye, the HPV vaccine is not included in the national immunization program and can be administered in health institutions if individuals obtain the vaccine themselves (13, 15).

Parents' adequate and evidence-based

knowledge of HPV infection and vaccines, their awareness about the developments on the subject, and attitudes and beliefs about vaccination are important in that they are in a decision-making position for the vaccination of their children both in childhood and adolescence. The fact that the HPV vaccine is not included in the National Immunization Program in Türkiye further increases the meaning and importance of the issue. Health literacy (HL), on the other hand, comes to the fore as it is effective in the level of parents' vaccination knowledge (16). Therefore, in the current study, we aimed to examine the relationship between parents' attitudes and beliefs about the HPV vaccine and HL levels.

METHODS

Study design and Participants

A cross-sectional and descriptive design was used in the study. The survey link created via Google forms was sent to the parents via the school whatsapp groups between May 15, 2021 and May 30, 2021.

Inclusion Criteria for Research

- Volunteering to participate in the study,
- Having a daughter between the ages of 10-18,
- Ability to read and write and
- Accessing the survey sent via whatsapp.

Sample Size Calculation

The population of the study consisted of parents (832 people) of students in a secondary school located in a rural area in the central region of Türkiye. The sample size was calculated by doing a power analysis, and it was determined as 537 people, based on an error level of α=0.005 and a power value of 99.9%. We aimed to contact the maximum number of people that could be reached within the specified date range and completed the study with 599 parents (n: 599).

Data Collection Tools

The Descriptive Information Form: This form was prepared by the researchers in light of the literature. It consists of a total of 15 questions about the socio-demographic characteristics of the participants, the presence of cervical cancer in the mother and family, mothers' status of having their daughters vaccinated against HPV, and the status of obtaining information about HPV (3, 4, 11, 19, 21, 24, and 25).

The Carolina HPV Immunization Attitudes and Beliefs Scale (CHIAS): This scale was developed by McRee et al. in 2010 to evaluate the attitudes and beliefs of parents with adolescent children about HPV vaccination. The scale consists of 16 items and 4 sub-dimensions. 1. Harms sub-dimension: it consists of 6 items and covers the perceived potential harms of the vaccine, including health problems, and girls' status of being sexually active. 2. Perceived barriers sub-dimension: it consists of 5 items and covers

perceived barriers to HPV vaccination, including access to a healthcare provider and its cost. 3. Effectiveness sub-dimension: it consists of 2 items and covers the perceived effectiveness of the HPV vaccine in protecting against genital warts and cervical cancer. 4. Uncertainty sub-dimension: it consists of 3 items and is used to evaluate the availability of enough information about the HPV vaccine and the perception of vaccination norms of society. The item order of the original scale and that of the scale used in this study are the same. The harms sub-dimension includes items 1, 2, 3, 4, 5, and 6 and has a 4-point Likert-type scale (1 = strongly disagree, 2 = somewhat disagree, 3 = somewhat agree, 4 = strongly agree). 2. The barriers sub-dimension contains items 7, 8, 9, 10, and 11 and has a 3-point Likert-type scale (1 = not difficult at all, 2 = somewhat difficult, 3 = very difficult). The effectiveness sub-dimension contains items 12 and 13 and has a 4-point Likert-type scale (1=very little effective, 2=moderately effective, 3=very effective, 4=highly effective). The uncertainty sub-dimension includes items 14, 15, and 16 and has a 4-point Likert-type scale (1=strongly disagree, 2=somewhat disagree, 3= somewhat agree, 4=strongly agree). On the original scale, the possible range for all subscale scores is between 1.0 and 4.0. The acceptable Cronbach's alpha values for the sub-dimensions are as follows: harms, α=0.69; perceived barriers, α=0.69; effectiveness, α= 0.61; uncertainty, α=0.66. A high score on the scale indicates high levels of HPV attitudes and beliefs. According to the literature, the scale does not have a cutoff score (17, 18).

Turkish Health Literacy Scale-32 (THLS-32): This scale, which was developed by Okyay et al., is used to evaluate health literacy in individuals who are literate and over the age of 15. It is based on the conceptual framework developed by the European Health Literacy Research Consortium. The scale consists of two health-related sub-dimensions, namely, “healthcare” and “disease prevention and health promotion” and four processes of obtaining information about health-related decision-making and practices (access, understand, appraise, and apply). Cronbach's alpha value for the overall scale is 0.93, and 0.88 for the "healthcare" sub-dimension, and 0.86 for the "disease protection and health promotion" sub-dimension. Each item is graded as 1=very difficult, 2=difficult, 3=easy, 4=very easy, and 5=I don't know. During the calculation of the score, the codes should be re-coded as 1-4, 4-1. To facilitate the calculation process, the total score was standardized with the help of the formula "Index=(arithmetic mean-1) x [50/3]" to obtain values between 0-50. A score of 0 on the scale indicates the lowest level of health literacy, while a score of 50 indicates the highest level. The level of HL can also be interpreted

categorically as follows: inadequate: (0-25 points); problematic-limited (>25-33 points); adequate: (>33-42 points); excellent (>42-50 points) (19, 20).

Procedures

The data were collected by sending the survey link to the parents who volunteered to participate in the research and met the participation criteria, via the school parent whatsapp groups.

Ethics of the Study

At the outset, written approval of the Non-Invasive Clinical Research Ethics Committee of Cukurova University Faculty of Medicine (number: 111/114; date: 21.05.2021) was obtained. After obtaining the necessary institutional permissions, the questionnaire link was sent to the school parent WhatsApp groups. In addition, before the survey was initiated, participants' consent was obtained via an online connection. During the study, the principles of the Declaration of Helsinki were followed.

Statistical Analysis

Statistical analyses were conducted on the SPSS (IBM SPSS Statistics 24) statistical software package. Frequency tables and descriptive statistics were used to interpret the findings. Non-parametric methods were used for the measurement values that did not show a normal distribution. Accordingly, the "Mann-Whitney U" test (Z-table value) was used to compare the measurement values of two independent groups, and the "Kruskal-Wallis H" test (χ2-table value) was used to compare the measurement values of three or more independent groups. The Bonferroni correction method was employed for paired comparisons of variables with a significant difference in three or more groups. The Spearman correlation coefficient was used to examine the relationship between measurement values that did not have a normal distribution. The Binary-Logistic regression model was used to determine the factors affecting not having the child vaccinated. P <0.05 was accepted as the statistical significance value.

RESULTS

The mean age of the parents was 45.17±6.62 (years), and 340 (56.8%) of them were in the ≥45 age group. Also, 410 (68.4%) of them were female, 258 (43.1%) were born in a province, and the longest place of residence of 364 participants (60.8%) was a province. It was determined that 297 of the parents (49.6%) had primary education, 343 (57.3%) were employed, and 352 (58.8%) had equal income and expenses. In addition, the mothers of 551 (92.0%) of the participants did not have a history of cervical cancer, 538 (89.8%) of them did not have a family history of cervical cancer, 552 (92.2%) had not had their children get the HPV vaccine, 305 (50.9%) had not received information about the HPV vaccine, and 170 (28.4%) had received information about the vaccine from

health personnel.

Cronbach's alpha was found as 0.971 for the THLS-32 and 0.717 for the CHIAS. It was determined that parents' responses to the scales were generally quite reliable (Table 1).

A statistically significant difference was found between the scores of the participants on the overall CHIAS and harms and effectiveness sub-dimensions according to age groups and gender (Z=-3.527, p<0.001; Z=-3.572, p<0.001; Z=-2.239, p=0.025; Z=-2.890, p=0.004; Z=-3.542, p<0.001; Z=-2.031; p=0.042, respectively). The scores of female participants and those who were aged <45 from the total CHIAS and harms and effectiveness sub-dimensions were significantly higher than the scores of those in the ≥45 age group (Table 2)

There was a statistically significant difference between the scores of the participants on the total CHIAS and harms, perceived barriers, effectiveness, and uncertainty sub-dimensions according to the place of birth ($\chi2$=31.75, p=0.000; $\chi2$=49.318, p=0.000; $\chi2$=23.490, p=0.000; $\chi2$=16.075, p=0.000; $\chi2$=30.050, p=0.000, respectively). The difference was significant between those who were born in a province and those born in a district or village (Table 2).

A statistically significant difference was found in terms of scores obtained from the THLS-32, total CHIAS, and harms, perceived barriers, and uncertainty sub-dimensions according to the longest place of residence ($\chi2$=14.652, p=0.001; $\chi2$=30.328, p=0.000; $\chi2$=48.184, p=0.000; $\chi2$=12.765, p=0.002; $\chi2$=10.265, p=0.006, respectively). The score of the participants who lived in a province or district on the THLS-32 was significantly higher than the score of those who lived in a village. The score of those who lived in a province on the total CHIAS and the harms sub-dimension was significantly higher than the score of those living in a district or village (Table 2).

The scores of the participants on the overall CHIAS and harms, effectiveness, and uncertainty sub-dimensions yielded a statistically significant difference according to education level ($\chi2$=44.182, p=0.000; $\chi2$=59.706, p=0.000; $\chi2$=8.877, p=0.012; $\chi2$=26.579, p=0.000, respectively). The significant difference was observed between participants who had primary school education and those who had high school and university or higher education (Table 2).

There was a statistically significant difference in terms of THLS-32 scores, total CHIAS score, and harms and uncertainty sub-dimension scores according to employment status (Z=-1.976, p=0.048; Z=-5.484, p=0.000; Z=-1.976, p=0.048; Z=-4.093, p=0.000, respectively). The scores of those who had a job on the THLS-32, total CHIAS, and harms and uncertainty sub-dimensions were significantly higher than the scores of those

who did not (Table 2).

Statistically significant differences were found in terms of THLS-32 scores, total CHIAS scores, and harms sub-dimension scores according to income status ($\chi 2$=17.271, p=0.000; $\chi 2$=14.464, p=0.001; $\chi 2$=16.018, p=0.000, respectively). The scores of the participants who had equal income and expenses and those who had more income than their expenses on the THLS-32, total CHIAS, and harms sub-dimension were significantly higher than the scores of those whose income was less than their expenses (Table 2).

A statistically significant difference was observed between participants' scores on the perceived barriers sub-dimension of the CHIAS according to the status of having a cervical cancer history in their mothers and family (Z=-2.914, p=0.004; Z=-3.646, p=0.000, respectively). The scores of the participants who had a cervical cancer history in their mothers and family on the perceived barriers sub-dimension of the CHIAS were significantly higher than the scores of those whose mothers did not have a history of cervical cancer (Table 3).

There was a statistically significant difference between the scores of the participants on the THLS-32 and the perceived barriers, effectiveness, and uncertainty sub-dimensions of the CHIAS according to the status of having the child get the HPV vaccine (Z=-2.338, p=0.019; Z=-3.492, p=0.000; Z= -3.492, p=0.000; Z=-2.737, p=0.006, respectively). The scores of the participants who had their children get the HPV vaccine on the THLS-32 and the perceived barriers, effectiveness, and uncertainty sub-dimensions of the CHIAS were significantly higher than the scores of those who did not (Table 3).

A statistically significant difference was found between the scores of the participants on the total CHIAS and harms, perceived barriers, effectiveness, and uncertainty sub-dimensions according to the status of having received information about the HPV vaccine (Z=-5.323, p=0.000; Z=-6.513, p=0.000; Z= -2.069, p=0.039; Z=-4.597, p=0.000; Z=-3.598, p=0.000, respectively). The scores of those who had received information about the HPV vaccine on the total CHIAS and harms, perceived barriers, effectiveness, and uncertainty sub-dimensions were significantly higher than the scores of those who did not (Table 3).

It was determined that the answers given by the participants to the scales were generally at a very reliable level (Table 4).

A positive, very weak, and statistically significant relationship was found between the scores of the participants from the THLS-32 and the harms sub-dimension of the CHIAS (r=0.128; p=0.002). A positive, weak, and statistically significant relationship was found between the scores of the participants from the THLS-32 and the perceived barriers sub-

dimension of the CHIAS (r=0.435; p=0.000). A negative, very weak, and statistically significant relationship was found between the scores obtained from the THLS-32 and the effectiveness sub-dimension of the CHIAS (r=-0.128; p=0.002). It was determined that as the THLS-32 score increased, positive attitudes toward the harms and perceived barriers of the CHIAS increased, as well, while positive attitudes toward the effectiveness decreased. A positive, weak, and statistically significant relationship was found between the overall CHIAS score and the THLS-32 score (r=0.250; p=0.000). As the THLS-32 score increased, the overall CHIAS score increased, as well. Likewise, as the THLS-32 score decreased, the overall CHIAS score decreased, too (Table 5).

Table 1. Distribution of the findings regarding the scores of the parents on the Carolina HPV Immunization Attitudes with Beliefs Scale and the Turkish Health Literacy Scale-32(n=599)

		Mean	SD	Median	Min	Max
THLS-32		34.41	9.69	34.4	4.4	50.0
CHIAS	*Harms*	3.19	0.64	3.3	1.2	4.0
	Perceived barriers	1.97	0.55	2.0	1.0	3.0
	Effectiveness	2.46	0.70	2.5	1.0	4.0
	Uncertainty	2.12	0.69	2.0	1.0	4.0
	Total	2.59	0.38	2.6	1.3	3.5

***THLS-32:** Turkey Health Literacy Scale – 32, **CHIAS:** Carolina HPV Vaccination Attitudes and Beliefs Scale, **S.D:** Standart Deviation**, Min:** Minimum, **Max:** Maximum

Table 2. Comparison of Parents' Scores from the Turkish Health Literacy Scale-32and the Carolina HPV Immunization Attitudes with Beliefs Scale According to Their Socio-demographic Findings

Variable (n=599)	**n**	**THLS-32**		**CHIAS**									
				Harms		*Perceived barriers*		*Effectiveness*		*Uncertainty*		**Total**	
		X̄ ± S.D.	**Median [IQR]**	**X̄ ± S.D.**	**Median [IQR]**	**X̄ ± S.D.**	**Median [IQR]**	**X̄ ± S.D.**	**Median [IQR]**	**X̄ ± S.D.**	**Median [IQR]**	**X̄ ± S.D.**	**Median [IQR]**
Age groups													
<45	259	34.24±10,54	33.9 [17.2]	3.29±0,64	3.3 [1.0]	2.02±0,60	2.0 [0.8]	2.54±0,69	2,5 [1,0]	2.12±0,64	2.0 [1.0]	2.65±0,39	2.7 [0.5]
≥45	340	34.54±9.01	34.9 [11.9]	3.12±0,63	3.2 [1.0]	1.93±0,51	2.0 [0.8]	2.15±0,71	2,0 [1,0]	2.13±0,72	2.0 [1.0]	2.54±0,35	2.6 [0.4]
Statistical analysis*		Z=-0.202		Z=-3.572		Z=-1.388		Z=-2.239		Z=-0.473		Z=-3.527	
Probability		p=0.840		**p=0.000**		p=0.165		**p=0.025**		p=0.636		**p=0.000**	
Gender													
Female	410	34.47±9.72	34.4 [13.8]	3.26±0,61	3,3 [0,8]	1.96±0,57	2.0 [0.8]	2.49±0,71	2.5 [1.0]	2.13±0,68	2.0 [1.0]	2.62±0,37	2.6 [0.5]
Male	189	34.29±9.67	33.9 [12.5]	3.05±0,67	3,0 [0,8]	1.98±0,51	2.0 [0.5]	2.08±0,68	2.0 [1.0]	2.10±0,72	2.0 [1.0]	2.51±0.38	2.5 [0.5]
Statistical analysis*		Z=-0.235		Z=-3.542		Z=-0.235		Z=-2.031		Z=-0.533		Z=-2.890	
Probability		p=0.814		**p=0.000**		p=0.814		**p=0.042**		p=0.594		**p=0.004**	
Place of birth													
Province[1]	258	34.85±10,31	34.4 [17.2]	3.37±0,64	3.5 [1.0]	1.86±0,62	2.0 [1.0]	2.58±0,72	3.0 [1.0]	2.28±0,66	2.5 [0.5]	2.67±0,37	2.7 [0.4]
District [2]	213	34.74±8.52	34.9 [10.8]	3.09±0,60	3.2 [0.8]	2.40±0,48	2.5 [0.3]	2.35±0,66	2.5 [1.0]	2.07±0,69	2.0 [1.0]	2.55±0,38	2.6 [0.5]
Village [3]	128	32.98±10,16	33.4 [13.9]	3.01±0,58	3.0 [0.7]	1.97±0,47	2.0 [0.5]	2.40±0,70	2.5 [1.0]	1.89±0,69	1.5 [1.4]	2.47±0,34	2.5 [0.4]
Statistical analysis*		χ^2=3.040		χ^2=49.318		χ^2=23.490		χ^2=16.075		χ^2=30.050		χ^2=31.754	
Probability		p=0.219		**p=0.000**		**p=0.000**		**p=0.000**		**p=0.000**		**p=0.000**	
Difference				**[1-2,3]**		**[2-1,3]**		**[1-2,3]**		**[1-2,3] [2-3]**		**[1-2,3] [2-3]**	
The longest place of residence	364	35.12±9.78	35.4 [15.8]	3.31±0,63	3.5 [0.8]	1.92±0,59	2.0 [0.8]	2.51±0,71	2.5 [1.0]	2.19±0,70	2.5 [0.5]	2.64±0,37	2.7 [0.4]
Province [1]	145	35.00±8.64	34.9 [11.1]	3.08±0,61	3.2 [0.8]	2.11±0,49	2.5 [0.4]	2.33±0,70	2.5 [1.0]	2.04±0,63	2.0 [1.0]	2.54±0,37	2.6 [0.5]
District [2]	90	30.58±10,15	30.7 [13.7]	2.89±0,57	2.8 [0.8]	1.93±0,46	2.0 [0.5]	2.48±0,63	2.5 [1.0]	1.96±0,73	2.0 [1.0]	2.42±0,34	2.4 [0.4]
Village [3]													
Statistical analysis*		χ^2=14.652		χ^2=48.184		χ^2=12.765		χ^2=3.234		χ^2=10.265		χ^2=30.328	
Probability		**p=0.001**		**p=0.000**		**p=0.002**		**p=0.357**		**p=0.006**		**p=0.000**	
Difference		**[1,2-3]**		**[1-2,3] [2-3]**		**[2-1,3]**				**[1-2,3]**		**[1-2,3] [2-3]**	
Level of education													
Primary school[1]	297	33.51±9.45	33.3 [12.5]	3.04±0,59	3.2 [0.8]	2.00±0,47	2.0 [0.5]	2.43±0,67	2.5 [1.0]	1.99±0,72	2.0 [1.0]	2.51±0,35	2.5 [0.5]
High school[2]	190	35.10±9.55	35.7 [15.0]	3.25±0,65	3.3 [1.0]	1.94±0,61	2.0 [0.8]	2.40±0,70	2.5 [1.0]	2.19±0,66	2.5 [0.5]	2.60±0,37	2.7 [0.5]
University or Above[3]	112	35.62±10,41	34.7 [18.9]	3.50±0,60	3.7 [0.7]	1.94±0,67	2.0 [1.3]	2.64±0,77	3.0 [1.0]	2.35±0,60	2.5 [0.5]	2.77±0,40	2.8 [0.4]
Statistical analysis*		χ^2=4.700		χ^2=59.706		χ^2=1.914		χ^2=8.877		χ^2=26.579		χ^2=44.182	
Probability		p=0.095		**p=0.000**		p=0.384		**p=0.012**		**p=0.000**		**p=0.000**	
Difference				**[1-2,3] [2-3]**				**[1,2-3]**		**[1-2,3]**		**[1-2,3] [2-3]**	
Working status													
Yes	343	34.97±10,27	36.2 [16.2]	3.30±0,63	3,5 [1,0]	1,98±0,60	2,0 [0,8]	2,50±0,69	2,5 [1,0]	2,22±0,67	2,5 [0,5]	2,65±0,37	2,7 [0,4]
No	256	33.66±8.82	33.3 [10.9]	3.05±0,61	3,2 [0,8]	1,96±0,49	2,0 [0,5]	2,42±0,72	2,5 [1,0]	1,99±0,70	2,0 [1,0]	2,49±0,36	2,5 [0,5]
Statistical analysis*		Z=-1.976		Z=-5.366		Z=-0.795		Z=-1.549		Z=-4.093		Z=-5.484	
Probability		**p=0.048**		**p=0.000**		p=0.427		p=0.121		**p=0.000**		**p=0.000**	
Level of income													
Income<expenses[1]	197	32.01±10,44	32.3 [15.0]	3.07±0,62	3.0 [0.8]	1.96±0,49	2.0 [0.5]	2.43±0,66	2.5 [1.0]	2.04±0,65	2.0 [1.0]	2.51±0,36	2.5 [0.5]
Income=expenses [2]	352	35.40±9.16	35.4 [14.1]	3.24±0,64	3.3 [0.8]	1.96±0,58	2.0 [0.8]	2.50±0,70	2.5 [1.0]	2.16±0,71	2.0 [1.0]	2.62±0,37	2.7 [0.5]
Income>expenses [3]	50	36.93±8.53	39.3 [10.8]	3.34±0,62	3.5 [0.8]	2.06±0,60	2.3 [0.6]	2.30±0,81	2,0 [1.5]	2.23±0,69	2.5 [1.1]	2.67±0,41	2.8 [0.2]
Statistical analysis*		χ^2=17.271		χ^2=16.018		χ^2=1.992		χ^2=5.449		χ^2=5.242		χ^2=14.464	
Probability		**p=0.000**		**p=0.000**		p=0.369		p=0.066		p=0.073		**p=0.000**	
Difference		**[1-2,3]**		**[1-2,3]**								**[1-2,3]**	

*"Mann-Whitney U" test (Z-table value) was used for the comparison of measurement values of two independent groups in data not having normal distribution; "Kruskal-Wallis H" test statistics (χ2-table value) were used to compare three or more independent groups.
***THLS-32:** Turkey Health Literacy Scale – 32, **CHIAS:** Carolina HPV Vaccination Attitudes and Beliefs Scale

Table 3. Comparison of Parents' Scores from the Turkish Health Literacy Scale-32and the Carolina HPV Immunization Attitudes with Beliefs Scale According to Their Family History of Cervical Cancer and Findings of HPV/HPV Vaccine(n=599)

Variable	n	THLS-32		CHIAS									
				Harms		*Perceived barriers*		*Effectiveness*		*Uncertainty*		**Total**	
		$\bar{X} \pm S.D.$	**Median [IQR]**	**$\bar{X} \pm S.D.$**	**Median [IQR]**	**$\bar{X} \pm S.D.$**	**Median [IQR]**	**$\bar{X} \pm S.D.$**	**Median [IQR]**	**$\bar{X} \pm S.D.$**	**Median [IQR]**	**$\bar{X} \pm S.D.$**	**Median [IQR]**
History of cervical cancer in the mother	48	35.35±11.45	39.6	3.28±0.62	3.5 [1.1]	2.18±0.48	2.3 [0.3]	2.51±0.85	2.8 [1.5]	2.19±0.62	2,0 [0,5]	2.70±0.38	2.7 [0.6]
	551	34.33±9.53	[15.6]	3.19±0.64	3.3 [0.8]	1.95±0.56	2.0 [0.8]	2.46±0.69	2.5 [1.0]	2.12±0.70	2,0 [1.0]	2.57±0.37	2.6 [0.4]
Yes			33.9										
No			[12.5]										
Statistical analysis*		Z=-1.287		Z=-0.998		Z=-2.914		Z=-0.212		Z=-0.806		Z=-1.658	
Probability		p=0.198		p=0.323		**p=0.004**		p=0.832		p=0.420		p=0.097	
History of cervical cancer in the family	61	35.46±10.92	39.6	3.20±0.62	3.3 [1.0]	2.19±0.44	2.3 [0.4]	2.48±0.84	2.5 [1.3]	2.19±0.63	2.0 [0.8]	2.67±0.39	2.7 [0.5]
	538	34.29±9.55	[15.9]	3.19±0.64	3.3 [0.8]	1.94±0.56	2.0 [0.8]	2.46±0.69	2.5 [1.0]	2.12±0.70	2.0 [1.0]	2.58±0.37	2.6 [0.4]
Yes			33.9										
No			[12.5]										
Statistical analysis*		Z=-1.372		Z=-0.084		Z=-3.646		Z=-0.099		Z=-0.809		Z=-1.342	
Probability		p=0.170		p=0.933		**p=0.000**		p=0.921		p=0.419		p=0.180	
Having the child get the HPV vaccine	47	37.59±7.45	39.1	3.24±0.50	3.3 [0.5]	2.23±0.46	2.3 [0.3]	2.31±0.94	2.0 [1.5]	2.38±0.54	2.5 [1.0]	2.70±0.37	2.7 [0.4]
Yes	552	34.14±9.82	[9.9]	3.19±0.65	3.3 [1.0]	1.95±0.56	2.0 [0.8]	2.47±0.68	2.5 [1.0]	2.10±0.70	2.0 [1.0]	2.58±0.38	2.6 [0.4]
No			33.9										
			[13.4]										
Statistical analysis*		Z=-2.338		Z=-0.207		Z=-3.492		Z=-2,100		Z=-2,737		Z=-1,713	
Probability		**p=0.019**		p=0.836		**p=0.000**		**p=0.036**		**p=0.006**		p=0.087	
Status of receiving information about the HPV vaccine	294	34.52±9.97	34.4	3.35±0.60	3.5 [0.8]	1.91±0.62	2.0 [1.0]	2.58±0.70	3.0 [1.0]	2.23±0.66	2.5 [0.5]	2.67±0.35	2.7 [0.4]
	305	34.31±9.43	[16.1]	3.04±0.63	3.0 [0.8]	2.02±0.47	2.5 [0.5]	2.35±0.69	2.0 [1.0]	2.02±0.71	2.0 [1.0]	2.50±0.38	2.5 [0.5]
Yes			34.4										
No			[11.9]										
Statistical analysis*		Z=-0.184		Z=-6.513		Z=-2.069		Z=-4.597		Z=-3.598		Z=-5.323	
Probability		p=0.854		**p=0.000**		**p=0.039**		**p=0.000**		**p=0.000**		**p=0.000**	

*"Mann-Whitney U" test (Z-table value) was used for the comparison of measurement values of two independent groups in data not having normal distribution.

***THLS-32:** Turkey Health Literacy Scale – 32, **CHIAS:** Carolina HPV Vaccination Attitudes and Beliefs Scale

Table 4. Examination the Cronbach-α Coefficient of the Turkish Health Literacy Scale-32 and the Carolina HPV Immunization Attitudes with Beliefs Scale

Scale (n=599)		**Madde sayısı**	**Cronbach-α coefficient**
THLS-32		32	0.971
CHIAS	*Harms*	6	0.835
	Perceived barriers	4	0.834
	Effectiveness	2	0.784
	Uncertainty	2	0.754

***THLS-32:** Turkey Health Literacy Scale – 32, **CHIAS:** Carolina HPV Vaccination Attitudes and Beliefs Scale

Table 5. Examination of the Relationship Between the Turkish Healthy Literacy-32 Scale and the Carolina HPV Immunization Attitudes with Beliefs Scale Scores Correlation* (n=599)

	THLS-32	
	r	*p*
CHIAS		
Harms	**0.128**	**0.002**
Perceived barriers	**0.435**	**0.000**
Effectiveness	**-0.143**	**0.000**
Uncertainty	0.050	0.220
Total	**0.250**	**0.000**

*"Spearman" correlation coefficient was used to examine the relationships of two quantitative variables that do not have a normal distribution

DISCUSSION

This is the first study on the examination of the relationship between parental attitudes and beliefs about the HPV vaccine and HL. Parents play an important role in deciding whether children will get the HPV vaccine. At the individual level, the decision is based on attitudes, beliefs, knowledge, norms, socio-demographic characteristics, and cultural characteristics. However, government policies

and access to adequate health services (such as health checks and vaccination and screening programs) are other important factors affecting the decision process.

Considering that midwives have the main responsibility for immunization, they should determine parents' knowledge levels, beliefs, and attitudes towards HPV and HPV vaccine, and provide them with accurate evidence-based information. In this way, a significant contribution can be made to reducing the incidence of cervical cancer, which is one of the preventable cancers, and the resulting death rates. As a matter of fact, in our study, it was determined that most of the parents had received information about the HPV vaccine and that their primary source was the health personnel.

In a cross-sectional study conducted in Thailand, a significant relationship was found between parents' socio-demographic characteristics and their knowledge, beliefs, and acceptance of the HPV vaccine (21). Various studies on the subject have shown that parents' attitude towards the HPV vaccine is significantly correlated to socio-demographic characteristics, such as age, gender, education level, and income status, and knowledge levels (22-26). Similar to these studies, the results of our study also indicated that parents' attitudes and beliefs toward the HPV vaccine differed according to their socio-demographic characteristics and status of obtaining information. In a study conducted to evaluate the relationship between maternal HPV experiences and having children get the HPV vaccine, it was determined that children with a family history of cervical cancer were more likely to be vaccinated against HPV (27). In a cross-sectional study conducted in China on HPV knowledge and the acceptability of the HPV vaccine, getting the HPV vaccine was found to be associated with a family history of cervical cancer (28). Similar to the literature, it was found in our study that parents who had a family history of cervical cancer were more likely to have their children vaccinated against HPV than those who did not.

In a study conducted to evaluate the relationship between HPV vaccination in adolescents and parental attitudes by using the CHIAS, it was found that with each 1-point decrease on uncertainty sub-dimension of the CHIAS, the probability of getting the next vaccine dose increased by 4.9, and that a higher score on the harms sub-dimension of the CHIAS was the only significant predictor of the lower probability of completing the vaccine dose (29). In our study, it was determined that with each 1-point increase in the perceived barriers sub-dimension score of the CHIAS, the risk of not having the child vaccinated would decrease by 0.569 times and that with each 1-point increase in the uncertainty sub-dimension score, this risk would decrease by 0.473 times.

One of the main factors affecting the low level of knowledge about the HPV vaccine is the low level of HL. In a study, it was shown that there was a positive and significant relationship between knowledge of HPV vaccination and HL in young adults (30). A high level of HL enables individuals to obtain information from the right sources about vaccination and thus plays an important role in reaching herd immunity and fighting diseases (31). In another study on HPV knowledge, vaccination status, and HL in university students, a positive correlation was found between HL and HPV knowledge (32). In a cross-sectional study conducted by Faluca et al. (2022) to determine the factors affecting the acceptance of HPV vaccine among university students; It was concluded that HL level directly affects the acceptability of HPV vaccine (33).As a result of the cross-sectional study conducted by McCaffery et al. (2020) to examine the change in HL level and COVID-19 knowledge, attitude, behavior and psychosocial behaviors; It has been determined that there are significant differences in knowledge, attitudes and behaviors related to COVID-19 according to the HL level of individuals (34).In a study conducted to examine the relationship between parents' HL levels and their attitudes and behaviors towards childhood vaccines, it was determined that, unlike other studies, there was no relationship between parents' HL levels and their attitudes and behaviors towards childhood vaccines (35). A review of the literature conducted to evaluate the relationship between HL and knowledge of vaccines indicated the results of studies were not consistent and that there were both positive and negative relationships (36, 37). It is thought that the different results obtained from the studies may have originated from the different measurement tools used, the small number of studies published so far, and sample characteristics. Our study results showed that as HL levels increased, parents' attitudes and beliefs and the rate of having their children get the HPV vaccine increased significantly. The European Center for Disease Prevention and Control (ECDC) has defined the role that HL can play in relation to infectious diseases (38). Infectious diseases pose one of the most pressing problems for health systems. However, the impact of a crucial social determinant such as HL on clinical and social outcomes related to infectious diseases has not been satisfactorily investigated. Since parents have a primary role in the vaccination decision of their children and HPV vaccine is not included in the vaccination calendar of the Ministry of Health, there is a need for further research, increasing awareness and providing individuals with access to reliable information sources. Adopting readability calculators and examining readability of materials will help develop evidence-based and up-to-date content

for HPV vaccine in light of science, potentially increasing health literacy and vaccine uptake.

Limitations

This study has some limitations. First, due to the cross-sectional nature of the study, temporality and causal inferences could not be made. In this case, no definite conclusions could be drawn regarding the direction of the relationships between the different results regarding HPV and its determinants. Second, the sample was selected from a single geographic region. This may limit the generalizability of the findings and therefore these results may not reflect the relationship between the knowledge, attitudes, and behaviors of parents and health literacy across the country. Third, there may be a recall bias as the data were derived from participants' responses and not from medical records.

CONCLUSION

In conclusion, the researchers determined that nearly half of the parents had received information about the HPV vaccine and that the health personnel were the first among their sources of information. The risk of not having children vaccinated decreased among those who had received information about the HPV vaccine compared to those who had not. However, although a good number of parents had received information, only 7.8% of them had their children vaccinated against HPV. Our results point to the urgent need for education and intervention to increase awareness about the relationship between HPV and cervical cancer. Such an educational activity can encourage people and increase their desire to get the HPV vaccine even if they do not have prior knowledge about HPV. Studies should be conducted to examine the effects of this type of education on women's psychology and health-seeking behaviors, and hence the incidence of cervical cancer. It is thought that the dissemination of plain, understandable, and evidence-based scientific knowledge about HPV and HPV vaccines by public healthcare midwives will have an impact on parents' attitudes, beliefs, and awareness about HPV and HPV vaccines.

In addition to raising knowledge and awareness in increasing vaccination rates, it may be beneficial to include the HPV vaccine in the national immunization program in Turkey, as in many European countries. One of the main elements is to ensure that individuals can afford to pay for vaccinations. With the Global Vaccine Action Plan, millions of deaths can be prevented by achieving equitable access to vaccines. Turkey has a free and well-functioning vaccination program with high coverage. Including vaccination against HPV in the national immunization program for children will be consistent with the policies of the Ministry of Health for the protection of the population against vaccine-preventable diseases.

In addition, as the parents' level of HL

increased, the rate of getting their children vaccinated against HPV increased significantly. Considering this situation, it is thought that knowledge of vaccines can be increased, attitudes and beliefs can be improved, and vaccination rates can be increased by targeting HL in the interventions to be implemented.

Ethics Committee Approval: Ethics committee approval was obtained for this study from the Non-Invasive Ethics Committee of Çukurova University Faculty of Medicine (date: 21.05.2021 and issue: 111/114).

Peer-review: Externally peer-reviewed.

Author Contributions:

Concept, Design, Literature search, Data Collection and Processing, Analysis or Interpretation, Writing – FNT, ED.

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Investigation of Senior Nursing Students' Views on Internship Program

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Abstract

Objective: The information obtained from this study may contribute to the improvement of the clinical qualifications and learning outcomes of intern nurses. This study was conducted to determine the views of fourth-year nursing students about the internship program

Methods: The population of the descriptive study was 97 students in the 4th grade of the nursing department of a state university in the Ordu. The sample of the descriptive study was 96 nursing students. The data were collected Nightingale Intern Program Evaluation Scale (NIPES).

Results: In this study, nursing students' NIPES total scale score was 3.81±0.75, sub-dimension of the scale mean scores were 3.81±0.75 for "Professional Development", 3.83±0.78 for "Personal Development", and 3.96±0.89 for "Guidance/Counseling", 4.13±0.88 for "General Features of the Program", 4.03±0.87 for "Application Field Features", 3.80±0.97 for "Communication with Patients and their Relatives". According to the presence of nurses in their first-degree relatives, the mean scores of the sub-dimensions "Professional Development", "Personal Development", "Guidance / Counseling", "General Characteristics of the Program", "Patient and Relatives" and "Communication" of NIPES were were statistically significantly different between the groups ($p<0.05$).

Conclusion: Nursing students had a positive opinion about the internship program. However, it is important for program managers to take precautions to increase the success of the internship program.

Key Words: Nursing student, internship, clinical education

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INTRODUCTION

Nursing education aims to prepare students for the nursing profession by supporting students' emotional, cognitive, and psychomotor development, in which theoretical and practical teaching methods are carried out together. Due to the increasing use of technology in health care, nurses have to adapt to developing technology, and healthcare needs have become very complex, it has been necessary for nurses to develop their skills in making correct decisions and critical thinking (1, 2). To develop students' skills, skill laboratories alone are not sufficient, in addition to skill laboratories, practical training in clinical settings should be combined.

Students' clinical practice at every stage of the education process will enable them to communicate professionally with healthy/sick individuals, to develop the knowledge, skills, attitudes, and values that will be necessary for their professional life, and to develop problem-solving, critical thinking and decision-making skills (1,3-4).

Recently, new educational approaches have been implemented in universities in our country within the scope of the Bologna Process and the Turkish Higher Education Qualifications Framework. In this context, the internship program has started to be implemented in the nursing programs of the universities. Internship practice aims to prepare students more effectively for the profession and to provide professional care by integrating all the knowledge, skills, attitudes, behaviors, and values they have learned in the real practice area (5-6).

As a result of the updating of the curriculum of the nursing department of the faculty of health sciences of the university where this study was conducted in 2016, intern education was included for two semesters in the fourth year, and this program started to be implemented as of the 2019-2020 academic year. Nursing students take 32 hours of practice and two hours of theoretical intern courses each semester. Within the scope of this course, senior nursing students practice under the guidance of clinical nurses and under the supervision of lecturers in clinics that include internal diseases, surgical diseases, obstetrics and gynecology, pediatric health and diseases, mental health, psychiatry diseases, and public health units.

This study is the first study on the views of nursing students about the intern program at Ordu University. The information obtained from this study may contribute to the improvement of the clinical qualifications and learning outcomes of intern nurses. This study was conducted to determine the views of fourth-year nursing students about the intern program.

Research questions;

- What are the students' views on the internship program?

• Is there a difference between the students' views on the internship program according to their socio-demographic characteristics?

METHODS

Study design and participants

The population of the descriptive study was 97 students in the 4th grade of the nursing department of a state university in the Ordu. It was aimed to reach the entire population without selecting the sample and 96 nursing students who agreed to participate in the study were included in the sample. These students are the students who performed the internship application in the fall semester of the 2019-2020 academic year. Due to the COVID-19 epidemic, the 4th-year students of the nursing department of our faculty were able to practice as an intern in the clinic for 14 weeks in the first semester and only 4 weeks in the second semester.

Data Collection

The data were collected with face-to-face method via the personal information form prepared by the researchers and the "Nightingale Intern Program Evaluation Scale (NIPES).

Questionnaire form

The questionnaire consists of questions about the student's age, gender, type of high school graduated, success score, working status as a nurse, willingness to choose the profession, the order of choosing this university, the situation of finding nursing suitable, the status of a first-degree relative of a nurse, and place of intern application.

Nightingale Internship Program Evaluation Scale

Nightingale Intern Program Evaluation Scale developed by Şahin et al. in 2016 (1). This scale consists of Professional Development (11 statements), Personal Development (9 statements), Guidance/Counseling (5 statements), General Features of the Program (7 statements), Features of the Application Area (4 statements), Communication with Patients and their Relatives (3 statements) sub-dimensions. Responses to the statements in the scale are scored as “strongly disagree (1 point), disagree (2 points), no idea (3 points), agree (4 points), totally agree (5 points)”. While evaluating the scores obtained from the scale; It is calculated by dividing the total score from each sub-dimension by the number of items in the sub-dimension and taking the arithmetic average. Cronbach's alpha values of the total and sub-dimensions of NIPES were found 0.95 for total NIPES, 0.94 for “Professional Development”, 0.92 for “Personal Development”, 0.91 for “Guidance/Counseling”, 0.85 for “General Features of the Program”, 0.72 for “Application Area”, “Patient and their Relatives” 0.89 for “Communication” (1). In this study, Cronbach's alpha values were found to be 0.98, 0.92, 0.92, 0.94, 0.95, 0.87, and 0.93, respectively.

Analysis of data

The analysis of the data was done with SPSS 24 demo version. The conformity to the normal distribution of the variables was evaluated with the Kolmogorov-Smirnov test. It was determined that the data did not conform to the normal distribution. In the evaluation of the study data was used descriptive statistical methods included frequency, percentage, mean, standard deviation, median, minimum, and maximum. Also, the Mann-Whitney U test was used to evaluate the quantitative data between two groups that did not show normal distribution, and the Kruskal-Wallis test was used for the evaluation of quantitative data between more than two groups. Significance was evaluated at the $p<0.05$ level.

Ethical considerations

Permission to use the Nightingale Internship Program Evaluation Scale was obtained by e-mail from the author. Written permission was obtained from the institution where the research would be conducted. Ethics committee approval was obtained from the clinical research ethics committee of Ordu University (09.07.2020/147). The purpose and benefits of the study were explained to the nursing students in the sample, it was stated that they should not write their names on the data collection forms, and their informed written consent was obtained by attention to the voluntariness and willingness to participate in the research.

RESULTS

It was determined that 82.3% of the nursing students were female, 58.3% of them chose the nursing profession voluntarily, 45.8% of them found the nursing profession suitable for themselves, and their academic success average was 2.83±0.36. It was found that 53.1% of nursing students were interns in internal diseases, obstetrics and gynecology, and psychiatry in the fall, and 46.9% of them were interns in surgical diseases, pediatric health and diseases, and public health nursing (see Table 1).

Students' Nightingale Internship Program Evaluation Scale Scores

In this study, nursing students' NIPES total scale score was 3.81±0.75 (min 1.05, max 4), sub-dimension of the scale mean scores were 3.81±0.75 for "Professional Development", 3.83±0.78 for "Personal Development", and 3.96±0.89 for "Guidance/Counseling", 4.13±0.88 for "General Features of the Program", 4.03±0.87 for "Application Field Features", 3.80±0.97 for "Communication with Patients and their Relatives" (see Table 2).

Comparison of Students' characteristics and NIPES Scores

While there was a statistically negative significant relationship ($r=-0.251$; $p<0.05$) between the NIPES "professional development" sub-dimension point average and the faculty achievement score of the students, there was no statistically significant

relationship between "personal development", "guidance/counseling", "general features of the program", "features of the application area" and "communication with patients and their relatives" sub-dimensions (p>0.05). The mean scores of the NIPES sub-dimensions were not statistically different according to the gender of the students, their working status as a nurse, their willingness to choose a profession, and the units they worked for (p>0.05). According to the presence of nurses in their first-degree relatives, the mean scores of the sub-dimensions "Professional Development", "Personal Development", "Guidance / Counseling", "General Characteristics of the Program", "Patient and Relatives" and "Communication" of NIPES were were statistically significantly different between the groups (p<0.05), (see Table 3).

Characteristics	**Min-Max**	**Mean±SD (Median)**
Age (years)	19-24	21.40±0.68 (21.00)
Faculty achievement score	1.00-4.24	2.83±0.36 (2.80)
Gender	**n**	**%**
Female	79	82.3
Male	17	17.7
Working as a nurse		
Yes	2	2.1
No	94	97.6
Willingness to choose the profession		
I chose voluntarily	56	58.3
I chose it at the request of my family.	32	33.3
I randomly chose	8	8.3
Order of choosing nursing in university		
1 st	16	16.7
2nd	22	22.9
3rd	23	24.0
4 or more	35	36.5
Finding a suitable job		
Yes	44	45.8
Partially suitable	44	45.8
Not available	7	7.3
Status of being a nurse in a first-degree relative		
Yes	16	16.7
No	80	83.3

Table 1. Distribution of students' socio-demographic characteristics (n=96)

Table 2. Students' Nightingale Intern Program Evaluation Scale Values

NIPES sub-dimensions	**Min-Max.**	**Mean±SD (Median)**	**Cronbach Alpha**
Professional development	1.18-5	3.81±0.75 (3.90)	0.925
Personal Development	1-5	3.83±0.78 (3.88)	0.920
Guidance / Counseling	1-5	3.96±0.89 (4.00)	0.941
General features of the program	1-5	4.13±0.88 (4.14)	0.957
Features of the application area	1-5	4.03±0.87 (4.00)	0.872
Communication with patients and their relatives	1-5	3.80±0.97 (4.00)	0.931
NIPES Total	1.05-4.87	3.81±0.75 (3.87)	0.982

Table 3. Comparison of NIPES subdimensions scores according to the characteristics of the students

Students' characteristics	n	%	Professional Development	Personal Development	Guidance/Counseling	General Features of the Program	Features of the Application Area	Communication with Patients and their Relatives
	Mean (Median) (Min-Max)		Mean (Median) (Min-Max)	Mean (Median) (Min-Max)	Mean (Median) (Min-Max)	Mean (Median) (Min-Max)	Mean (Median) (Min-Max)	Mean (Median) (Min-Max)
Faculty achievement score	2.83±0.36 (2.80) (2-4.20)		2.83±0.36 (2.80) (2-4.20)	2.83±0.36 (2.80)(2-4.20)	2.83±0.36 (2.80) (2-4.20)	2.83±0.36 (2.80) (2-4.20)	2.83±0.36 (2.80) (2-4.20)	2.83±0.36 (2.80)(2-4.20)
r ve p values			r=-0.251 p=**0.020**	r=-0.176 p=0.104	r=-0.189 p=0.081	r=-0.124 p=0.256	r=-0.110 p=0.315	r=0.024 p=0.824
Gender								
Female	79	82.3	4.13 (1-5)	4.00 (1-5)	4.00 (1-5)	4.00 (1-5)	3.50 (1.25-5)	4.00 (1-5)
Male	17	17.7	4.09 (1.55-5)	3.66 (1.11-5)	4.20 (1-5)	3.85 (1.14-5)	3.62 (1-5)	4.00 (1-5)
MWU and p values			460.000 p=0.691	436.500 p=0.065	663.500 p=0.561	529.500 p=0.376	621.000 p=0.976	640.000 p=0.812
Working as a nurse								
Yes	2	2.1	4.18 (4.18-4.18)	4.38 (4.11-4.67)	4.70 (4.60-4.80)	4.21 (3.71-4.71)	2.87 (2.25-3.50)	3.83 (3.67-4.00)
No	94	97.6	4.09 (1-5)	4.00(1-5)	4.00(1-5)	4.00(1-5)	3.50(1-5)	4.00(1-5)
MWU and p values			42.500 p=0.966	48.000 p=0.292	29.500 p=0.115	73.500 p=0.657	129.500 p=0.359	140.000 p=0.258
Willingness to choose the profession								
I chose voluntarily	56	58.3	4.45 (1-5)	4.00 (1-5)	4.00 (1-5)	4.00 (1-5)	3.50 (1-5)	4.00 (1-5)
I chose it at the request of my family	32	33.3	4.00(2-5)	4.00(2-5)	4.00(2.20-5)	3.85 (2.43-5)	3.50(2-5)	4.00(2-5)
I randomly chose	8	8.3	4.00 (3.64-4.64)	4.00 (3.44-4.67)	4.00 (2-4.60)	3.85 (2.71-4.71)	3.50 (2.50-4.25)	4.00 (4-4.33)
KW and p values			2.190 p=0.335	0.291 p=0.865	0.13 p=0.937	0.35 p=0.839	0.354 p=0.838	0.588 p= 0.745
Order of choosing nursing in university								
1st	16	16.7	4.45 (1.36-5)	4.00 (1-5)	4.10 (1.60-5)	3.71 (1-5)	3.37 (1.50-4.75)	4.0 (1.33-5.0)
2nd	22	22.9	4.31 (2.82-5)	4.05 (1.33-5)	4.10 (1-5)	4.00 (1.14-5)	3.50 (1-5)	4.16 (1-5)
3nd	23	24.0	4.27 (1-5)	4.00 (1.22-5)	4.00 (1-5)	4.00 (1-5)	3.50 (1.25-5)	4.00 (1-5)
4 or more	35	36.5	4.00 (1.55-5)	4.00 (1.11-5)	4.00 (1.40-5)	3.78 (1.14-5)	3.50 (1.25-5)	4.00 (1-5)
KW and p values			1.918 p=0.590	1.286 p=0.732	1.995 p=0.574	3.535 p=0.316	1.454 p=0.693	3.169 p=0.366
Finding a suitable job								
Yes	44	45.8	4.63 (1-5)[ab]	4.22 (1-5)[a]	4.20 (1-5)	4.00 (1-5)	3.50 (1.25-5)	4.33 (1-5)
Partially suitable	44	45.8	4.00 (2-5)[a]	4.00 (1.33-5)[b]	4.00 (1-5)	3.85 (1.14-5)	3.50 (1-5)	4.00 (1-5)
Not available	7	7.3	4.00 (3.64-4.73)[b]	3.55 (3.22-4)[bc]	4.00 (3.40-4.20)	3.71 (3.29-4)	3.25 (2-3.50)	4.00 (3.67-5)
KW and p values			10.617 p=**0.021**	10.718 p=**0.031**	5.902 p=0.052	6.016 p=**0.049**	2.241 p=0.326	5.572 p=0.062
Status of being a nurse in a first-degree relative								
Yes	16	16.7	3.90 (1.36-4.73)	3.66 (1-4.78)	3.40 (1.60-5)	4.00 (1.14-4.86)	3.50 (1.50-4)	4.00 (1.33-5)
No	80	83.3	4.31 (1-5)	4.00 (1.11-5)	4.00 (1-5)	4.14 (1-5)	3.50 (1-5)	4.00 (1-5)
MWU and p values			728.500 p=**0.006**	861.000 p=**0.004**	795.000 p=**0.006**	783.500 p=**0.037**	684.000 p=0.341	787.500 p=**0.038**
Department								
Internal medicine, obstetrics, and gyn., psychiatry	51	53.1	4.13 (1-5)	4.00 (1.22-5)	4.00 (1-5)	4.00 (1-5)	3.50 (1.25-5)	4.00(1-5)
Surgıcal, child diseases and public health	45	46.9	4.09 (1.36-5)	4.00 (1-5)	4.00 (1-5)	4.00 (1-5)	3.50 (1-5)	4.00 (1-5)
MWU and p values			1.042.00 p=0.631	1.070.00 p=0.954	1089.00 p=0.778	1.046.50 p=0.807	1.276.00 p=0.179	1.152.00 p=0.824

DISCUSSION

Internship practice is very important for nursing students in the transition to their professional life. Internship practice in nursing education enables them to gain experience to be able to use and develop the professional knowledge, skills, and attitudes they have learned in the education-learning processes, to be able to fulfill the care needs of healthy/sick individuals, families, and society in line with the nursing process, to communicate effectively with other individuals in the health care team, in order to this communication and to apply professional regulations in the field of work (3,5) .

The clinical environment is known to be extremely beneficial for professional nurse candidates for reasons such as identifying their mistakes (7), increasing critical thinking skills (8), providing holistic care to patients (9), clinical judgment, and ethical decision-making skills (9-10).

In this study, it was found that the NIPES mean score of the students was 3.81±0.75, and the mean of its sub-dimensions was between 3.80 and 4.13. The subscale scores were respectively "General features of the program" (4.13±0.88), "Characteristics of the practice area" (4.03±0.87), "Guidance/counseling" (3.96±0.89), "Personal development" (3.83±0.78), "Professional development" (3.81±0.75), “Communication with patients and their relatives” (3.80±0.97). Considering that the highest "5 points" can be obtained in the scale items, it was observed that the senior nurse students were more than moderately satisfied with the intern program. Yılmaz-Karabulutlu et al. (2020) indicated that the students’ NIPES total mean score was 3.88±0.74, while the highest mean score was on the sub-scale of communication with patients and their relatives (4.02± 0.87), followed by the sub-scales of professional development (3.96±0.80), guidance/counseling (3.93±0.95), personal development (3.86 ± 0.82), application area properties (3.76 ± 1.02) and general features of the program (3.75±0.93), (11). Similar findings were found in the other study (12). In a previous study, Mohammed and Ahmet (2020) found that the hospital was the most effective factor in the satisfaction of trainee nurses during the clinical internship and that it was associated with the nurse interns' perception of autonomy role, perception of education, and perception of social support (13). The same study indicated that the presence of mentoring in the hospital, the support of the hospital team, and the perception of the autonomous role of the nurse interns are important in nursing education (13).

Leufer and Cleary-Holdforth (2020) determined that senior nursing students were prepared for the profession with internship practice, and they improved themselves in drug management and case management (14). Ateş et al. (2017) determined that the vast majority

of students gave positive feedback about the intern practice (3). Sabancıoğulları et al. (2012) found that the majority of intern students contributed to the development of the knowledge and skills of the program after the application and positively affected their readiness for the profession (5). This study's results, which are similar to the results of the studies in the literature, have been interpreted as contributing to the student's professional knowledge, skills, attitudes, and values as well as their personal development in line with the objectives of the intern program.

It was determined that there was a statistically significant negative correlation between the students' faculty achievement score average and the NIPES professional development sub-dimension score. With the internship program, it is expected that students will be able to develop their nursing care skills, provide care following the nursing process, use the knowledge, skills, and attitudes related to the profession while performing the roles and functions specific to the nursing profession, and develop their skills to effectively manage patient care and emergencies. At the same time, a negative correlation was found between the academic success score of the student and the sub-dimensions of "personal development", "guidance/counseling", "general features of the program" and "features of the application area" of NIPES. This result suggested that students with lower academic success scores might have developed these aspects better.

In this study, there was no statistically significant relationship between the genders of the students and their views on the internship program. Keshk et al. (2018) found that there was a statistically significant relationship between gender and gaining advanced skills in the study in which they examined the effect of the intern program on the acquisition of advanced skills by nursing students (15). This finding, which is not similar to the literature, made us think that both male and female students agreed about the intern practice program.

The significant relationship between the students' finding the profession suitable for themselves and the sub-dimensions of NIPES including "professional development", "personal development" and "guidance/counseling" was interpreted as that the students were able to realize many aspects of the nursing profession during intern practice and that they might have given more positive feedback about the intern practice program.

It was determined that the students' opinions about the internship program were different according to the presence of a nurse among their first-degree relatives. This finding of the study suggested that students whose relatives were nurses might have been positively affected by the internship program and their readiness for the profession.

Limitations of the study

This study has some limitations. These limitations are the small sample size and the fact that it was conducted in only one faculty. Further studies with larger sample sizes are needed using the random sampling technique.

CONCLUSION

As a result of this study, it was found that nursing students had a positive opinion about the internship program. There were statistically significant differences in the students' opinion about the intern program, according to their professional development characteristics, faculty achievement score, finding the profession suitable for themselves, and having a nurse in their first-degree relatives. Also, there were statistically significant differences between the personal development characteristics of the students and the situation of finding the profession suitable for themselves and having a nurse in their first-degree relatives; between guidance/counseling and presence of nurses in first-degree relatives; between the general features of the program and the situation of finding the profession suitable for oneself and having a nurse in their first degree relatives; between communication with patients and their relatives and the presence of a nurse in their first-degree relatives.

In line with these results, it can be suggested that similar studies should be carried out to evaluate the program in nursing departments where the internship program is applied so that nursing students can be evaluated in different universities within the scope of the internship program.

Practical importance to the educational field

Determining nursing students' satisfaction with the internship program can enable nursing faculty members to make important decisions that can help students benefit from the internship period. Examining the factors that affect the views of nursing students about the internship program and taking precautions for the factors that affect the nursing students' views by the faculty members who are in charge of nursing education will positively affect the success of the program and contribute positively to the preparation of the students for professional life.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University (09.07.2020/147).

Author Contributions:

Concept: N.E, Y.K.U, H.D, NB, Design N.E, Y.K.U, H.D, N.B, Literature search: N.E, Y.K.U, H.D, N.B, H.G.U, Data Collection and Processing: Y.K.U, H.D, H.G.U, N.B, F.B.T,Ö.B, A.Ç, N.G, A.N.K, E.Ş, Analysis or Interpretation: N.E, Y.K.U, H.D, Writing: N.E, Y.K.U, H.D, N.B.

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Reduced levels of plasma strong antioxidant uric acid in children with Autism Spectrum Disorder and Attention deficit-hyperactivity disorder

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Abstract

Objective: Uric acid (UA) is one of the most powerful antioxidants in human body fluids, as well as being the end product of purine metabolism. UA alone constitutes half of the scavenging effect of oxidant substances in the plasma.It has proinflammatory and metal chelation effects. In this study, UA levels and UA situation according to the normal range were investigated in patients with Autism Spectrum Disorder (ASD), and Attention deficit-hyperactivity disorder (ADHD).

Methods: Eighty-two ASD, 28 ADHD patients and 66 healthy control subjects were compared and serum UA levels were measured. ASD and ADHD severity were determined by CARS and Atilla Turgay ADHD scale. K-SADS-PL was administered to all subjects over the age of 6 years.

Results: UA levels were found to be significantly lower in the ASD and ADHD groups. UA levels were found to be lower than the normal range in nearly half of the ASD and ADHD groups. This rate was found to be 18.2% in the control group.

Conclusion: it was determined that UA levels were low in ASD and ADHD patients. It can be suggested that it may play a role in the pathophysiology of ASD and ADHD. UA may be a potential treatment target.

Key Words: uric acid, antioxidant capacity, oxidative stress, autism, attention deficit-hyperactivity disorder

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INTRODUCTION

Uric acid (UA) emerges as the final metabolic product of adenine and guanine purines from animal proteins, destroyed, damaged and dying cells. Adenine and Guanine are converted to inosine and guanosine by deamination and dephosphorylation. These are then converted to hypoxanthine and guanine by purine nucleoside phosphorylase. These two molecules are converted to Xanthine and then to UA by Xanthine oxidase. UA is mainly produced in the liver, intestine, and vascular endothelium and excreted by the kidneys (1). Although UA excess is associated with some pathological conditions, UA has been shown to have extremely important physiological functions. In fact, some hypotheses have suggested that there is relative hyperuricemia only high ape and humans in mammals, which, besides its many advantages such as increase of life span and decrease cancer rates, stimulates the cerebral cortex and may affect the formation of superior intellectual powers (2-4).

UA accounts for at least half of the antioxidant capacity in plasma. UA has strong antioxidant properties with its reactive oxygen specifies (ROS) and peroxynitrite scavenging effect. Peroxynitrite is formed by the reaction of nitric oxide with superoxide radicals and is associated with many pathologies (5). However, UA contributes to tissue repair and healing by initiating inflammatory processes, scavenging free oxygen radicals, and activating progenitor endothelial cells (6).

UA has been found to be effective in the pathogenesis of some neurodegenerative diseases. For example, UA contributes to the protection of myelin by scavenging peroxynitriteand ROS, which in crease the destruction of myelin. Therefore, Multiple Sclerosis (MS) is almost never seen in gout patients. UA was also found to be low in MS patients (7-10).Similarly, UA levels were found to be generally low in Alzheimer and Parkinson diseases. It has been found that high UA levels are associated with decreased Alzheimer risk (11). Similarly, high UA prevents cognitive decline in Parkinson's patients, and low UA levels are associated with worse cognitive performance (12). Experimental cerebral ischemia studies have shown that UA protects neurons against excitotoxic and metabolic damage (13).

The brain is more exposed to oxidative stress and more susceptible to damage due to its high metabolic activities and using half of the oxygen consumed daily. Especially the high lipid content of the brain makes it sensitive to lipid peroxidation. Mechanisms to prevent lipid peroxidation are of great importance. UA against lipid peroxidation with its strong antioxidant effect reflects the neuroprotective effect of UA (14). Apart from these effects, UA acts as a chelator of metal ions such as iron and copper and transforms them into poorly

reactive forms, making them unable to catalyze free radical reactions (6,15,16).

There are publications that low UA plays a role in the pathogenesis of schizophrenia (17). In first episode schizophrenia patients, UA was found to be low as an antioxidant (18). In a recent meta-analysis study, it was suggested that increased oxidative stress response accompanied by first-episode Sch patients and decreased UA may be a risk factor for schizophrenia, especially in males (19). In patients with depression, low UA is a characteristic of depression and UA returns to normal with antidepressant treatment (20).

Considering the above-mentioned physiological effects of UA and its effects on the pathogenesis of some neuropsychiatric-neurodegenerative diseases, a hypothesis was formed in this study that UA levels may play a role in the pathogenesis of Autism Spectrum Disorder (ASD) and Attention Deficit-Hyperactivity Disorder (ADHD).

METHODS

Participants

Participants of the patient group were formed from those who applied to the Child and Adolescent Psychiatry Department of Ordu University Medical Faculty Training and Research Hospital. Patient groups were formed from subjects diagnosed with ASD and ADHD according to DSM 5 criteria. The subjects were examined by a specialist child psychiatrist and detailed information was obtained from their families. While the severity of the disease in the ASD group was measured with the Childhood Autism Rating Scale (CARS), the severity of the disease in the ADHD group was determined with the AtillaTurgay (AT) DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale.The healthy group consisted of volunteers who applied to the general pediatric outpatient clinic of our hospital during the well-child visit, did not have any psychiatric diagnosis and did not have a systemic disease in their medical history. K-SADS-PL, a structured psychiatric interview, was applied to all subjects over the age of 6 and a sociodemographic form created by the authors was filled (21). All subjects' medical conditions and histories were evaluated by a specialist pediatrician (3th author). The study was approved by the Ethics Committee of Ordu University Faculty of Medicine (Decision no: 2019/02). Biochemical tests including hemogram, thyroid function tests, kidney and liver function tests were applied to the subjects for general medical screening. These biochemical parameters were measured between 08:00 and 11:00 in the morning after overnight fasting. During these routine measurements, uric acid levels were also measured routinely. Exclusion criteria were systemic diseases, neurological deficits, epilepsy, inflammatory diseases, hypertension, thyroid disorders, gout, obesity, acute or

chronic infections, and those taking psychiatric medication in the last 6 month.

Evaluation Tools

Sociodemographic Data form

Subjects' age, disease duration, birth history, birth weight, duration of breastfeeding, medical disease history, height, weight, BMI, medication history were available.According to the normal range of UA values, groups were divided into three categories as low, normal and high.

Childhood Autism Rating Scale (CARS)

The scale, which consists of 15 items, is graded from 1 to 4 in each item. If the total score is below 30, it is considered normal, 30-36 as mild-moderate autism, 37 and above as severe autism. Turkish validity and reliability were established (22).

Atilla Turgay (AT) DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale

This scale was developed by Atilla Turgay (AT) according to DSM-IV criteria. This scale consists of 41 items. While 9 of these items measure inattention, 9 evaluate hyperactivity and impulsivity. While the remaining 8 items evaluate oppositionality, 15 items evaluate Conduct Disorder. The Turkish reliability and validity of the AT scale was established (23).

Uric acid measurement

It was routinely measured spectrophotometrically with Roche 701 device from the blood taken from the subjects in the morning after fasting overnight. According to the KIT package insert, the normal range is accepted as 3.3-7.0 mg/dL.

Statistical Analysis

SPSS 22.0 (IBM Corporation, Armonk, New York, USA) was used for data analysis. Whether the numerical variables were normally distributed or not was determined by the Kolmogorov-Smirnov test. Normally distributed variables are indicated as mean±SD. Data that do not show normal distribution are shown as median and IQR values. Categorical variables were expressed as percentages. One-way ANOVA test was used when examining the differences between the three groups of normally distributed numerical variables. Homogeneity of variances was evaluated with Levene's test. In cases where there was significant difference between groups (for UA), pairwise post-hoc comparisons were made using the Tukey test.The Kruskal Wallis test was used when examining the difference between the three groups of numerical variables that did not show normal distribution.Chi-square test was used to determine the difference in categorical variables between the groups. Pearson and Spearman tests were performed in the correlation analyzes between the variables. Since there was a significant correlation between BMI and uric acid levels, the effect of BMI had to be purged. ANCOVA test was performed upon providing the necessary assumptions. Uric acid differences between the

groups were re-evaluated, with BMI as a cofactor. The P value was below 0.05 were considered statistically significant.

RESULTS

Our study included 82 ASD patients, 28 ADHD patients and 66 healthy subjects. There was a gender distribution of 24 females (29.3%) and 58 males (70.7%) in the ASD group, 5 females (17.9%) and 23 males (82.1%) in the ADHD group. The gender distribution in the healthy control group was 20 females (30.3%) and 46 males (67.7%). There was no difference between the groups in terms of gender (p=0.434). Since the age distribution did not show normal distribution, the median value was 6 in the ASD group, 7 in the ADHD group and 5 in the control group.Since the BMI distribution was also not normal, the median value was 17.16 in the ASD group, 17.06 in the ADHD group and 16.72 in the healthy control group. There was no difference between the groups in age and BMI distribution (p values 0.069 and 0.413, respectively). The median value for the CARS score, which indicates the severity of the disease in the ASD group, was 52, and the median value for the Atilla Turgay ADHD scale score was 55.5 in the ADHD group. The distribution of these parameters between the groups is given in Table 1 in detail.

UA values showed a normal distribution among the groups. Therefore, it was shown as mean±SD value between groups. The distribution of UA as mg/dL was found to be 3.42±0.75 in the ASD group, 3.25±0.49 in the ADHD group and 3.73±0.85 in the healthy control group. A significant difference was found in the distribution of UA between the groups (p=0.008). When the groups were compared in pairs in pot-hoc analyzes, a significant difference was found between the ASD and control groups and ADHD and control groups (p values 0.036 and 0.016, respectively). There was no significant difference between the ASD and ADHD groups (p=0.535). When UA values were divided into three categories as low, normal and high according to the normal range, no high value (above 7 mg/dL) was found in all groups.Therefore, the groups were divided into two categories as low and normal. While UA was found to be low (below 3.3 mg/dL) in 38 (46.3%) of the patients in the ASD group, UA was found to be low in 13 (46.4%) of the patients in the ADHD group and in 12 (18.2%) of the subjects in the healthy control group. When the low UA between the groups was evaluated categorically, a significant difference was found between the three groups (p=0.001). When the percentage values were taken into account, it was seen that the low UA percentages of both the ASD and ADHD groups were found to be significantly higher than the healthy control group. It was observed that the percentage values were very close to each other between the ASD and ADHD groups. The distribution of UA values among

the groups and their low-normal percentages are presented in detail in Table 2. In addition, the distribution of UA values between the groups is shown in Figure 1.

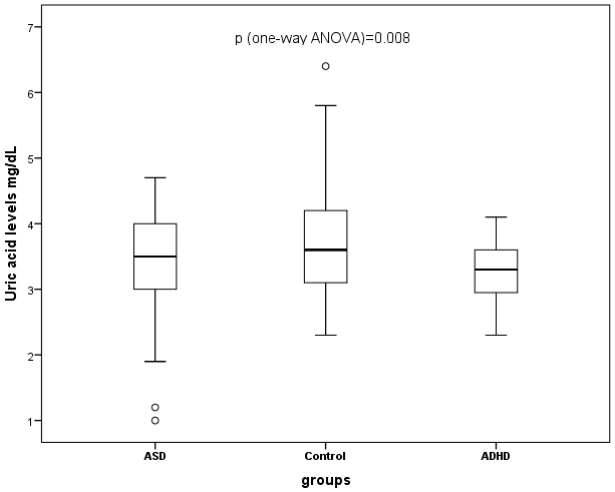

Figure 1. Distribution of Uric acid levels

When the correlation analyzes were examined, no significant results were found. No statistically significant results were found between the CARS score, which indicates the severity of the disease, and UA in the ASD group, and between the Atilla Turgay ADHD scale score and UA in the ADHD group (p values>0.05). When it was examined whether there was any correlation between UA and age and BMI values in all subjects, there was no significant correlation between age and UA, but a significant correlation was found between UA and BMI (r=0.347 and p<0.001).

Since the relationship between UA and BMI has been demonstrated, we wanted to refine the effect of BMI on UA between groups. For this reason, as a result of the evaluation of BMI as a cofactor (ANCOVA), UA values were again found to be significant between the groups (p<0.05).

Table 1. Distribution of the characteristic features of the groups

		ASD group (n=82)	ADHD group (n=28)	Healthy control group (n=66)	P value
Gender	F	24(29.3%)	5(17.9%)	20(30.3%)	0.434[a]
	M	58(70.1%)	23(82.1%)	46(69.7%)	
Age (years)	median (IQR)	6 (3.23)	7 (1,5)	5 (3.11)	0.069[b]
BMI	median (IQR)	17.16 (3.15)	17.06 (1.69)	16.72 (4.31)	0.413[b]
CARS score	Median (IQR)	52 (9.50)	-	-	-
AT score	Median (IQR)	-	55.5 (21.50)	-	-

BMI=Body Mass Index, CARS=Childhood Autism Rating Scale

AT=Atilla Turgay DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale

IQR=Inter quartile range

a=Chi-square test, b=Kruskal-Wallis test

Table 2. Distrubition of Uric acid values

	ASD group (n=82)	ADHD group (n=28)	Healthy control group (n=66)	P value
Uric acid mg/dL mean±SD	3.42±0.75	3.25±0.49	3.73±0.85	0.008[a]
Uric acid High	-	-	-	0.001[b]
Normal	44 (53.7%)	15 (53.6%)	54 (81.8%)	
Low	38 (46.3%)	13 (46.4%)	12 (18.2%)	

a=One-Way ANOVA; b= Chi-square test

DISCUSSION

In this study, UA levels compared in neurodevelopmental disorders such as ASD and ADHD with a healthy control group. According to the results we obtained, UA levels differed remarkably between ASD, ADHD and healthy control groups, which did not differ significantly in terms of age, BMI and gender, that is, they had similar characteristics. On the other hand, when UA levels were evaluated as low, normal and high compared to the normal range, UA elevation could not be found in all groups, but significantly lower UA percentage was found in the ASD and ADHD groups compared to the healthy control group. In other words, almost half of the subjects in the ASD and ADHD groups show lower UA levels than the normal range. The importance of these findings should be evaluated in the light of the information we have mentioned in the introduction.

Besides being the end product of purine metabolism, its most important physiological function of UA is to constitute half of the antioxidant capacity in human biological fluids. However, there areevidences that UA has pro-inflammatory effects and stimulates the synthesis of some cytokines (6). There is evidence that the oxidation load is high in the pathogenesis of both ASD and ADHD. Numerous studies have associated both diseases with increased oxidative stress and insufficient defensive antioxidant systems (24-25).

It has been shown that especially ASD patients are under oxidative stress. These patients are more prone to neurotoxicity due to oxidative stress (26). Due to the main reasons such as the brain metabolism rate is very high and it uses a high amount of O2, the neuron membrane contains high polyunsaturated fatty acids, high membrane surface area and the release of iron ions that catalyze free radical reactions in the whole brain, Manivasagam et al. (2020) explained in detail in seventeen items that neurons are much more sensitive to oxidative stress than other tissues (27). UA's chelation of metal ions such as iron and copper keeps these ions away from free radical-forming reactions.Therefore, with these effects

of UA, it can be thought that it protects the brain tissue (6, 15, 16).

Oxidative stress is mainly referred to as an imbalance between prooxidants and antioxidants, resulting in reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are composed of superoxide (O2−), hydroxyl, peroxyl, alkoxy, hydrogen peroxide, and peroxynitrite free radicals. This balance is disrupted in the pathogenesis of ASD. As a result, increased lipid peroxidation, mitochondrial damage, neuroinflammation and brain tissue damage, protein and DNA oxidation, altered immune response occur. In addition, with the decrease of methylation capacity, DNA methylation decreases and epigenetic dysregulation occurs. All these factors contribute to the pathogenesis and clinical development of ASD (28). It has been shown that oxidative stress and neuroinflammation coexist in the pathogenesis of ADHD, and antioxidant capacity is decreased (29).

Considering that UA is the most important scavenging antioxidant in body fluids, it can be suggested that the decrease in UA may play a role in the pathogenesis of many neurodegenerative and neuropsychiatric disorders, as well as in the pathogenesis of ASD and ADHD. This situation makes UA a potential treatment target as well as the fact that UA may play an important role in the pathogenesis of ASD and ADHD. A randomized placebo-controlled double-blind study of UA administration was conducted on healthy volunteers. Accordingly, a significant increase in serum free radical scavenging effect was observed in the group receiving UA. Moreover, no side effects were reported (30). It has been reported that adding UA to the treatment in acute stroke patients is beneficial, reduces lipid peroxidation, and increases the effect of recombinant tissue plasminogen activator (rt-PA) treatment (31). In another study, it was reported that administration of UA during acute aerobic physical exercise in healthy individuals was associated with an increase in serum antioxidant capacity and decreased oxidative stress (32). In another clinical study, a negative relationship was found between UA levels and oxidative stress during exhaustive exercise (33). As a result, the additive effect of UA on treatment should be investigated in ASD and ADHD patients and experimental studies in future. This study may be a trigger in this regard. Perhaps trying to keep UA in the upper limits of normal range or normalizing the level at low UA levels in patients with ASD and ADHD without causing urolithiasis, hypertension, and gout may work.

So far, UA levels have been evaluated in ASD patients treated with risperidone. In this study, ASD patients who received and did not receive risperidone were compared. Accordingly, it has been suggested that UA levels are higher in ASD patients receiving

risperidone, and this may be effect on the metabolic side effects of risperidone. However, in this study, ASD patients were not compared with the healthy control group (34).

This study has some strengths and weaknesses. First of all, the greater the number of subjects will allow us to reach more accurate results. Especially in the ADHD group, the number of subjects is quite insufficient. However, the fact that the subjects did not receive any treatment in the last 6 months, which is one of the strengths of this study, prevented us from recruiting a sufficient number of subjects. Because drugs can affect UA levels. It is also a positive value that the two most important neurodevelopmental disorders, ASD and ADHD, were evaluated together. In addition, the acceptance of medical conditions that may affect UA levels as exclusion criteria strengthens the study. In ASD and ADHD, there is a need for further studies such as evaluating it together with other oxidative stress and antioxidant capacity parameters besides UA.

As a result, it was determined that UA levels were low in ASD and ADHD patients. In addition, the percentage of low UA according to normal range was found to be higher in ASD and ADHD patients.

CONCLUSION

Accordingly, it can be suggested that UA may play a role in the pathophysiology of ASD and ADHD, especially through oxidative stress-antioxidant capacity. In addition, metabolic pathways related to UA may be a new target in the treatment of ASD and ADHD.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University (21/02/2019- 2019-40)

Author Contributions:

Concept: E.E, Design: E.E; Literature search: E.E,E.Y.E, Data Collection and Processing: E.E, E.Y.E; Analysis and/or Interpretation: EE, EYE; Literature Review: E.E,E.Y.E; Writing: E.E, E.Y.E.

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Effectiveness of Curcumin on Tracheal Anastomosis Created in the Rat Model

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Abstract

Objective: We aimed to investigate the effect of curcumin on tracheal wound healing in the anastomosis model following tracheal incision in rats.

Methods: Twenty-one healthy male Wistar Albino rats were included in the study. A horizontal incision was made between the 2nd-3th rings of the rat trachea and damage was created in the inner tracheal mucosa. Tracheal incision area was sutured and anastomosis was performed. Rats were divided into three groups. Ringer was given to the first group, corticosteroids to the 2nd group, and curcumin to the 3rd group for 28 days. The rats were sacrificed on the 28th day and the tracheal anastomosis line samples were sent for histological examination. Wound healing parameters, tracheal lumen width, wall thickness, and stenosis index were evaluated.

Results: Statistically significant difference was detected in tracheal lumen width and wall thickness in the curcumin group ($p<0.05$). Statistically significant differences were observed in the curcumin group in parameters of inflammation, collagen production and fibrosis. ($p<0.05$).

Conclusion: It is important to prevent the formation of tracheal stenosis, which is difficult to treat in clinical practice. Desired results cannot be achieved in the side-effect profile of some medical drugs used in this regard. We found the positive effect of Curcumin in the prevention of stenosis following tracheal injury.

Key Words: Rat, Trachea, Stenosis

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INTRODUCTION

Tracheal stenosis is one of the difficult pathologies that usually develops as a result of prolonged endotracheal intubation or tracheotomy. Stenosis can also develop as a result of trauma, some autoimmune diseases and neoplasia. It has been reported that stenosis developed in 66% after intubation and 26% after tracheotomy (1). Factors affecting the development of tracheal stenosis include the timing and mode of mechanical ventilation, mucosal damage caused by endotracheal tube cuff pressure, radiotherapy and recurrent upper respiratory tract infections (2,3). Today, despite many studies, the pathological mechanism and risk factors of tracheal stenosis have not been fully understood (4). There are different treatment modalities such as endoscopic methods, open surgical procedures, mitomycin-c, steroid therapy in its treatment. Among surgical treatments, resection of the stenotic area and end-to-end anastomosis stand out as the best method (5). Restenosis can be seen even after the most appropriate intervention. The uncontrolled excess of granulation tissue seen during wound healing as a result of tracheal mucosal damage is accepted as a precursor lesion for contraction and stenosis in wound healing (6).

Curcumin is a natural polyphenol compound and has been used in traditional medicine for centuries in Far East Asian countries. It is known that curcumin has antioxidant, anti-inflammatory activity and positive effects on wound healing (7). Although there are many studies on the effectiveness of curcumin on many inflammatory diseases, studies on tracheal stenosis have not been conducted. The aim of our study was to determine the efficacy of curcumin in preventing stenosis secondary to experimentally induced tracheal injury. In order to evaluate the efficacy of curcumin, granulation tissue secondary to tracheal damage performed on rat models was formed and histological parameters were determined.

METHODS

The study was approved by the animal experiments ethics committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine, with the date/ protocol number 27.02.2020/13 and the decision number 06, and was carried out at Kahramanmaraş Sütçü İmam University Experimental Animals Application and Research Center.

Animals

The minimum sample size was calculated as 7 for each group, with a 95% confidence interval and 5% tolerable error assumption, based on the study by Mirapoglu et al. (8). This study was carried out using 21 healthy and adult male Wistar-Albino type rats, weighing 250-350 grams, obtained from Kahramanmaraş Sütçü İmam University Medical Faculty Experimental Animal Laboratory. The rats were kept in groups of seven in 3 separate cages at 22 ± 2 °C, in a humidity-adjusted

environment suitable for a 12-hour light and dark cycle, and were fed ad libitum with standard feed and tap water.

Drugs

Curcumin preparation Sigma-Aldrich (Product number; C1386-10G, St Louis, MO, USA), Methylprednisolone drug (Prednol-L 40 mg. ampoule, Mustafa Nevzat) and Ringer's solution (Bioflex Ringer Lactate 500 ml solution, Osel) were used in the study.

Study Design

The rats were divided into three groups of seven as control group and two study groups. The 1st group was the control group, the 2nd group was the curcumin group, and the 3rd group was the steroid group.

Surgery

Anesthesia was achieved by administering 75-100 mg/kg ketamine hydrochloride (Ketalar 10 ml vial, E.Warner Lambert) and 0.5 mg/kg chlorpromazine (Rompun 50 ml 2% flacon, Bayer) intraperitoneally to the rats in all groups, and they were allowed to breathe spontaneously. A milliliter of epinephrine lidocaine hydrochloride (Jetokaine ampul, Adeka) was administered subcutaneously to the anterior neck of the rats in the supine position as a local anesthetic. The anterior neck of the rats was shaved and cleaned with povidone iodine (Poviiodeks ®,1000 ml). The operation area was covered sterile. Then, surgical procedures were performed under the operating microscope and with a microsurgery set. Strap muscles were reached by starting with a vertical two-centimeter incision made from the upper part of the thyroid cartilage to the incisura jugularis level in the midline. The submandibular gland was removed superiorly and the strep muscles were retracted laterally, the pretracheal fascia was passed, and the trachea was exposed. Inferior thyroid vein and recurrent laryngeal nerves were identified in the tracheoesophageal groove. The tracheal lumen was reached by making a horizontal incision with the help of the number 11 scalpel between the second and third rings of the trachea, based on the inferior thyroid veins laterally. Tracheal inner mucosa was damaged with the help of microcurette. Open tracheal ends were anastomosed end-to-end with 6/0 vicryl (Ethicon, Diegem, Belgium) starting from the lateral side. Approximately two sutures were placed, leaving the knots outside the trachea (Fig.1). After the bleeding control, the layers were duly closed, the operation was terminated, and the rats were placed in their cages after awakening. All rats in three groups were kept in the same environment and fed the same way. Peroperative intraperitoneal 50 mg/kg cefazolin sodium (Cefazol 250 mg, Mustafa Nevzat) was administered. The rats, who were followed up for respiratory distress after the operation, were taken to their cages when they woke up.

Treatment Modalities

All rats were followed for 28 days, which is known as sufficient time for stenosis

development after tracheal injury (9). Meanwhile, 1 mg/kg saline solution i.p. was administered to the control group, 100 mg/kg curcumin per oral to the 2nd group (curcumin group), and 1 mg/kg methylprednisolone i.m. to the 3rd group (steroid group) once a day. The administered drug doses were applied with reference to literature studies (10,11).

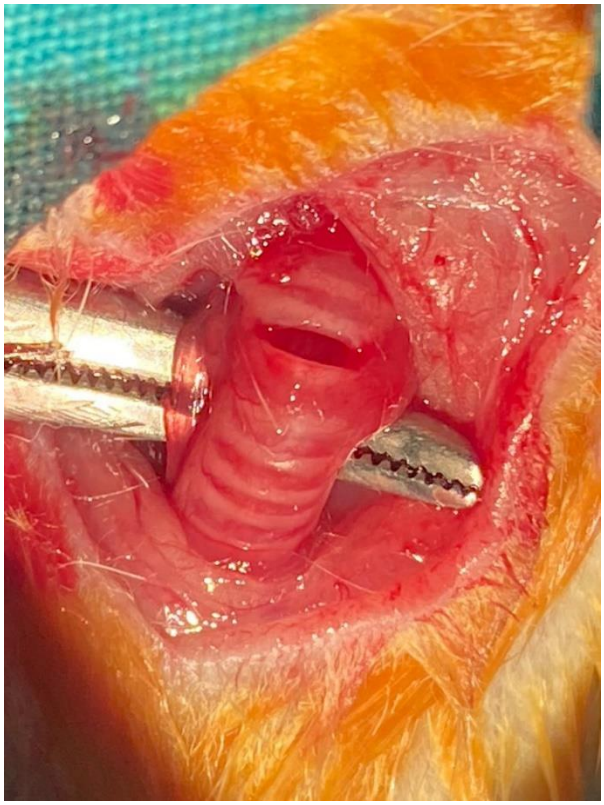


Figure 1. Incision and suturation of Trachea

Histological Evaluation

At the end of the follow-up periods, the rats in the study and control groups were sacrificed with high-dose intraperitoneal pentothal on the same day, and tracheal segments of five millimeters in length including the anastomosis line were removed.

The cross-sectional areas of the trachea were measured histometrically and compared. These excised tracheal segments were placed in 10% formaldehyde and turned into paraffin blocks, and then 4 micron thick sections were taken. Cross-sections of 4 microns were created using a Shandon FINESSE-ME microtome (Thermo Fisher Scientific, Waltham, MA). These sections were stained with hematoxylin and eosin and evaluated for inflammatory cell infiltration, epithelial degeneration and regeneration. The mean of these two diameters was determined by measuring the largest diameter and the diameter perpendicular to this diameter in the sample selected for the average lumen width. For the wall thickness, the mean value of the maximum and minimum distance between the luminal surface of the mucosal surface epithelium and the outer radial border of the wall was determined. Tracheal cartilage tissue was included in the wall thickness. The following formula was used to calculate the stenosis index; stenosis index = (wall thickness/2)/(lumen diameter). n addition, Masson trichrome staining was performed to evaluate collagen deposition by collagen fiber density. A semi-automated stereology workstation stage for stereological analysis consisting of a digital camera (Nicon Coolpix E 4500, Tokyo, Japan), image capture card (Flash Point 3D, Integral Technologies, Indianapolis, IN, USA), personal computer and computer-controlled motorized sample (Proscan II, Prior,

Rockland, MA, USA), a microcamera (Heidenhein, Traunreut, Germany) and a light microscope (Nicon, Eclips E 600, Tokyo, Japan) were used. In order to evaluate wound healing, the degrees of inflammation (polymorphonuclear leukocytes (PMNL), lymphocytes, histiocytes, macrophages), fibrosis, neovascularization and epithelialization were examined. In all parameters; mild, moderate and severe characterizations were made as follows: 0-10 inflammatory (PMNL, lymphocyte, histiocyte, macrophage) cells (-), 11-100 inflammatory cells mild or (+), 101-1000 inflammatory cells moderate or (++), more than 1000 inflammatory cells were evaluated as severe or (+++) inflammation. If the thickness of the collagen fibers was normal, it was evaluated as (-), minimal thickening as mild or (+), moderate thickening as moderate or (++), intense thickening as severe or (+++) fibrosis. In terms of neovascularization, if there is no neovascularization in the x 400 magnification field (-), less than 3 neovascularization is mild or (+), 4-10 neovascularization is moderate or (++), more than 10 neovascularization is severe or (+++). These parameters were digitized for statistical evaluation, and (-) evaluations were scored as 1, (+) evaluations as 2, (++) evaluations as 3, (+++) evaluations as 4 (12).

Statistical Analysis

In the evaluation of the data, the conformity of the variables to the normal distribution was examined with the Shapiro Wilk test. The comparisons of 3 groups in the variables conforming to the normal distribution were examined with the One Way Anova test. Tukey HSD test, Tamhane T2 test and Dunnett test were used for pairwise comparisons. Comparisons of 3 groups in variables that did not conform to normal distribution were analyzed with the Kruskal Wallis H test. Dunn's test, one of the post-hoc tests, was used for pairwise comparisons. The differences in frequency distributions according to the groups in qualitative variables were examined with the Chi-Square test and Exact test. Statistical significance was accepted as $p<0.05$. Statistical parameters were expressed as Mean±SD, Median (25% quartile-75% quartile) and n(%). IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) and R.3.3.2 software were used to evaluate the data.

RESULTS

When evaluated in terms of inflammation parameters, it was found that inflammatory cell infiltration, fibrosis and collagenosis parameters in the curcumin group were statistically significantly lower than in the other groups (Fig. 2,3). There was no significant difference between the groups in neovascularization, epithelial degeneration and regeneration parameters. Histopathological findings of all groups are given in Table 1.

Table 1. Comparison of Histopathological Parameters Between Groups

		None		Mild		Moderate		Intensive		
		N	%	N	%	N	%	N	%	P
Inflammation	Curcumin	3	42,9	3	42,9	1	14,3	0	0,0	**0.032***
	Steroid	0	0,0	2	28,6	4	57,1	1	14,3	
	Control	0	0,0	2	28,6	2	28,6	3	42,9	
Fibrosis	Curcumin	2	28,6	3	42,9	2	28,6	0	0,0	**0.047***
	Steroid	1	14,3	3	42,9	2	28,6	1	14,3	
	Control	0	0,0	1	14,3	3	42,9	3	42,9	
Collagen	Curcumin	4	57,1	1	14,3	2	28,6	0	0,0	**0.028***
	Steroid	2	28,6	2	28,6	2	28,6	1	14,3	
	Control	1	12,5	3	37,5	1	14,3	2	28,6	
Neo vascularization	Curcumin	1	16,7	1	16,7	2	33,3	3	42,9	0.940
	Steroid	1	14,3	2	28,6	2	28,6	2	28,6	
	Control	0	0,0	3	42,9	2	28,6	2	28,6	
Epithelial degeneration	Curcumin	0	0,0	2	28,6	2	28,6	3	42,9	0.739
	Steroid	1	14,3	2	28,6	3	42,9	1	14,3	
	Control	2	28,6	2	28,6	2	28,6	1	14,3	
Epithelial regeneration	Curcumin	1	14,3	2	28,6	3	42,9	1	14,3	0.651
	Steroid	1	14,3	3	42,9	2	28,6	1	14,3	
	Control	2	28,6	2	28,6	2	28,6	1	14,3	

*Exact test; p< 0.05 difference statistically significant

When the histomorphometric results are examined, it is seen that the lumen width is greater in the curcumin group than in the other groups (Graph 1, p<0.001). Statistically significant wall thickness was lower in the curcumin group and steroid group compared to the control group (Graph 2, p=0.011, p=0.002). There was no significant difference between the curcumin group and the other groups in the stenosis index (Graph 3, p=0.151). There was no significant bleeding during or after the operations in any of the groups during the study period. We did not observe any wound infection in the groups. No cutaneous or esophageal fistula developed in any of the rats, and there was no rat death in any group.

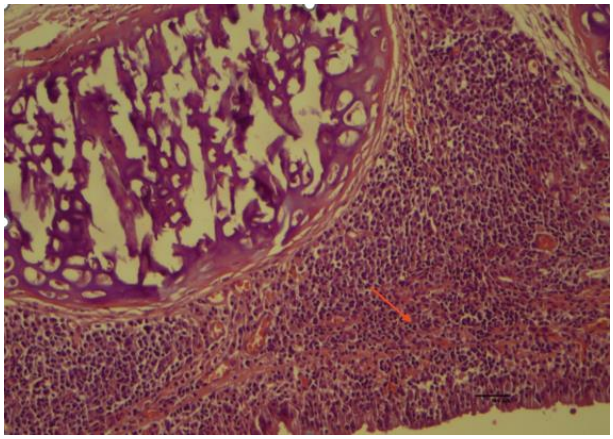


Figure 2a. Section from the tracheal injury site of a rat in the control group. Severe lymphoplasmocytic inflammation in the lamina propria (marked with an arrow) (H/EX200)

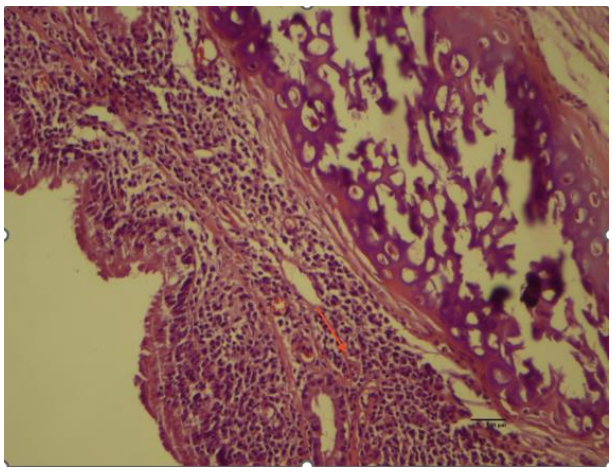


Figure 2b. Section from the tracheal injury site of a rat in the steroid group, moderate lymphoplasmocytic inflammation in the lamina propria (marked with arrow) (H/EX200)

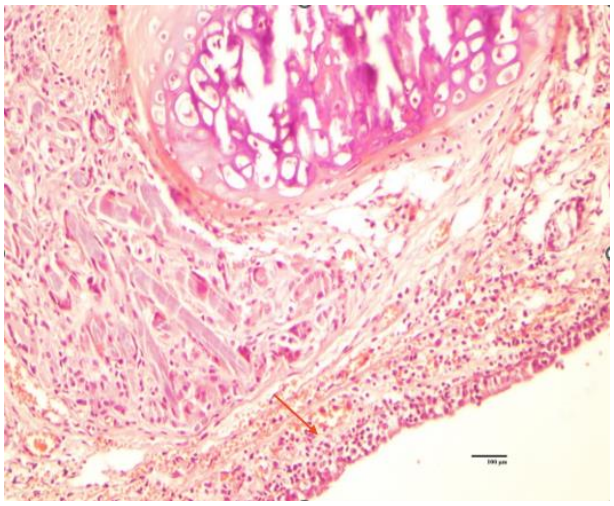


Figure 2c. Section from the tracheal injury site of a rat in the curcumin group, mild lymphoplasmocytic inflammation in the lamina propria (marked with arrow) (H/EX200)

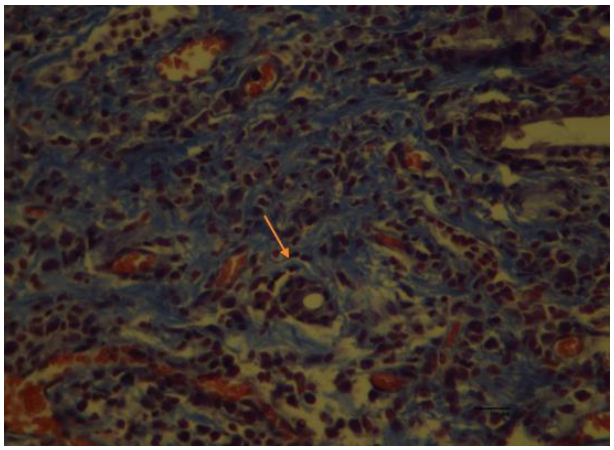


Figure 3a. Moderate fibrosis (Asterisk) with a condensed and bundled bluish appearance in the control group (MTX400)

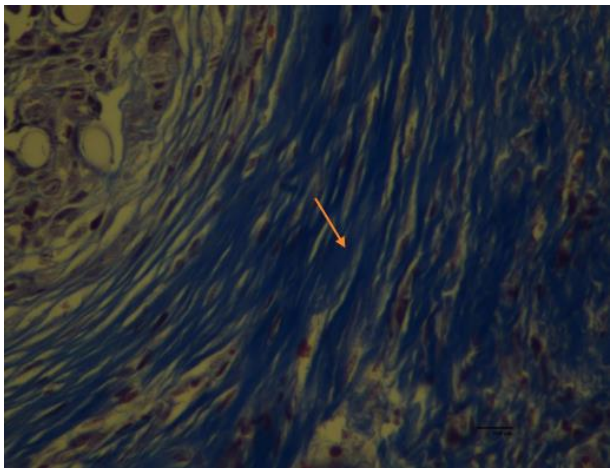


Figure 3b. Moderate fibrosis with a bluish appearance concentrated in the steroid group (marked with an asterisk) (MTX400)

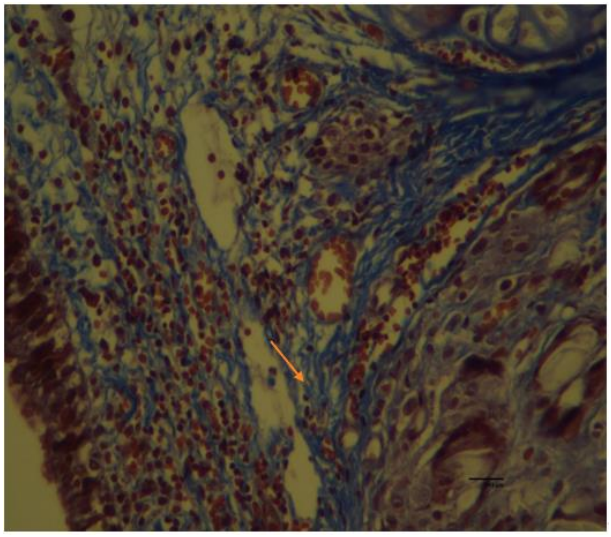
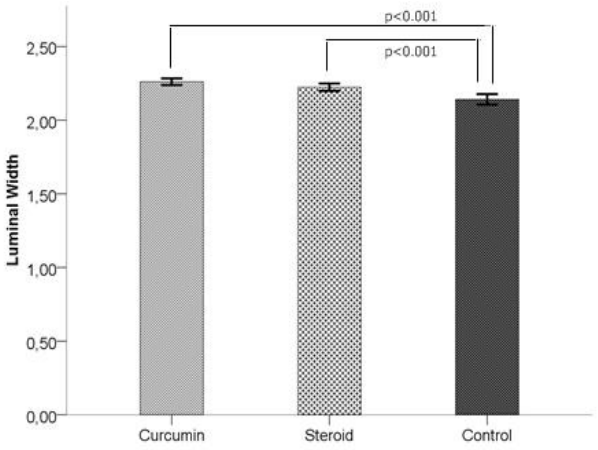
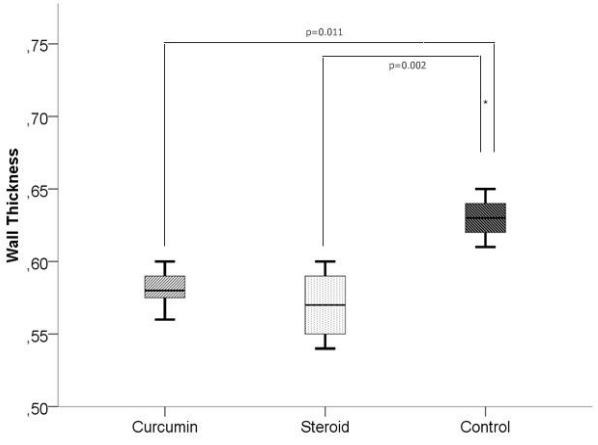


Figure 3c. Mild fibrosis (Asterisked) stained bluish in the curcumin group (MTX400)



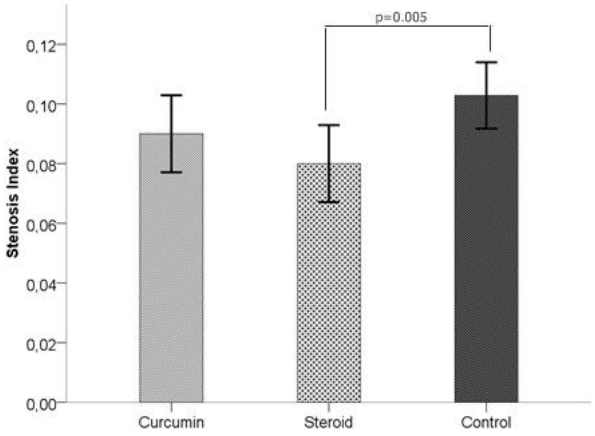
* lumen width measurement unit is mm

Graph 1. Comparison of Tracheal Lumen Width between Groups



*Tracheal Wall Thickness measurement unit is mm

Graph 2. Evaluation of tracheal wall thickness between groups



Graph 3. Evaluation of Intergroup Stenosis Index

DISCUSSION

Tracheal stenosis is narrowing of the airway caused by hypertrophic scar tissue that develops as a result of abnormal wound healing in the tracheal lumen. Damage to the mucosal tissue on the cartilage ring of the trachea leads to ischemic necrosis (13). In the acute phase of wound healing secondary to tracheal injury, polymorphonuclear leukocytes and macrophages are abundant in injured tissue. Macrophages increase the release of chemical mediators such as interleukins (IL) 1 and 6, tumor necrosis factor - α (TNF -α) and transforming growth factor - β (TGF - β), which play an important role in wound healing. These chemical mediators activate endothelial cells. With the effect of factors such as Fibroblast Growth Factor (FGF), Platelet Derived Growth Factor (PDGF) and TGF-β, fibroblasts and keratinocytes migrate to the wound to repair wound healing and initiate collagen synthesis (14). TGF-β1 overexpression, which plays a key role here, causes overproduction of matrix metalloproteinase enzyme. This situation continues with the overexpression and contraction of collagen synthesis. This pathological condition, which is observed in the tracheal mucosa, then progresses to granulation tissue and fibrosis with the involvement of deep tissues, resulting in thickening of the submucosal and mucosal layer and thus stenosis in the tracheal lumen(15). Considering this situation, reducing fibrosis by regulating wound healing in treatment will be an effective application in preventing the formation of tracheal stenosis (16). For this purpose, we used curcumin, which has a strong immunomodulatory activity in the prevention of experimental tracheal stenosis. Our histopathological and histomorphometric data showed that curcumin may be effective in preventing tracheal stenosis.

The treatment applied in tracheal stenosis varies according to the patient's clinic and classification of stenosis. While endoscopic methods are applied to cases with Grade I-II stenosis according to the Cotton-Myer classification, surgical treatments are prominent in cases with grade III-IV stenosis (17). Along with surgical treatment, bronchoscopic dilatation, laser ablation, electrocautery, stent application, medical treatment is also performed (18). It has been used to prevent restenosis after medical treatment, surgical or bronchoscopic intervention and to reduce the need for additional intervention. Corticosteroids are one

of the first drugs used for this purpose (19). Corticosteroids inhibit the expression of injury-induced growth factors and cytokines released by macrophages at the wound site with their immunosuppressive effects, delay wound contraction and impair the healing of epithelialization (20). In some studies, on steroids, which seem to be an effective drug in preventing stenosis, it has been reported that systemic corticosteroid administration is insufficient to prevent stenosis formation. In addition, it is seen that there is no accepted approach in the literature in terms of effective dose, application form and duration of corticosteroids in preventing tracheal stenosis (21-23). On the other hand, it has been stated that intralesional steroid administration can be an effective treatment in preventing the formation of restenosis (24). In the treatment of tracheal stenosis, drugs such as 5-Fluorouracil, carnitine, mitomycin-C are also used in addition to steroids (25-27). However, the effectiveness of these drugs is also limited. To the best of our knowledge, there is no study with curcumin in the prevention of tracheal stenosis. Our work on this issue can be considered as a pioneering work. In our study, it is noteworthy that the tracheal lumen width was wider and the wall thickness was less in the curcumin group compared to the control group. Considering that the results were similar with the group given steroid treatment, we think that curcumin may be as effective as steroids.

Curcumin (diferuloylmethane) is a natural polyphenol compound obtained from the root of turmeric (curcuma longa) in the ginger family in the South Asian region. It is used in the prevention and treatment of various diseases such as pain treatment, digestive system, skin, liver diseases, menstrual diseases and chronic wounds in Far East Asian traditional medicine practices (28). Curcumin's healing effect is due to its beneficial effects such as antioxidant, anti-inflammatory, antiproliferative, anti-infectious. In recent studies, the anti-inflammatory activity of curcumin is influenced by cytokines such as PDGF, VEDF, TGF-β, TNF – α, IL – 1,6,8, nuclear factor – κB (NF- κB), cyclooxygenase, protein kinase, Matrix Metalloproteinase (MMP). It has been shown to be associated with the regulation of the activity of mediators and enzymes that are effective in the inflammatory process (28,29). Effects such as reepithelialization, acceleration of wound healing and prevention of fibrosis formation were observed in biopsies taken from wounds treated with curcumin. This effect of curcumin is associated with its regulation of TGF-β and inducible Nitric Oxide synthetase (iNOS). While it induces TGF-β in normal wound healing, it prevents overexpression(30). At the same time, it has been observed that curcumin inhibits the overexpression of proangiogenetic growth factors VEGF, FGF, and Epidermal Growth Factor (EGF)(31). This indicates that

curcumin can prevent hypertrophic scarring and advanced fibrosis, which are extreme forms of wound healing. In our study, it was found statistically significant that the parameters of inflammation, collagenization and fibrosis were lower in the curcumin group compared to the other groups. We think that it can be an effective treatment agent in the prevention of acquired tracheal stenosis when evaluated together with factors such as tracheal lumen width and wall thickness.

The main limitations of our study are performing the tracheal intervention by making an incision on the anterior surface of the trachea and not removing the tracheal ring. Since it is a surgical procedure with a high risk of mortality and morbidity, the surgical procedure was performed on the tracheal anterior surface in order to minimize rat death and complications. In addition, the inability to perform immunohistochemical staining and electron microscopic examination are among the other limitations of the study.

CONCLUSION

It is important to prevent the formation of tracheal stenosis, which is difficult to treat in clinical practice. Desired results cannot be achieved in the side-effect profile of some medical drugs used in this regard. There is a need for preparations that have less adverse effects and are at least as effective as existing drugs in preventing the formation of stenosis. In our study, we determined that curcumin may be as effective as corticosteroids. We believe that our study will shed light on advanced studies on tracheal stenosis.

Ethics Committee Approval: The study was approved by the animal experiments ethics committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine, with the date/ protocol number 27.02.2020/13 and the decision number 06 and was carried out at Kahramanmaraş Sütçü İmam University Experimental Animals Application and Research Center.

Author Contributions:

Concept: M.G.Y, E.K, N.Y Design: M.G.Y, E.K, N.Y; Literature search: D.C, A.D, Data Collection and Processing: I.O, A.Y, Analysis or Interpretation A.D, Writing: M.G.Y, E.K, D.C

Conflict of Interest: There are no conflicts of interests related to this study.

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Weight Drop Models in Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) is the leading cause of morbidity and mortality worldwide. TBI is often seen in people with loss of motor, cognitive and sensory function. TBI causes serious health problems such as death, disability and mental disorders. TBI continues to be an increasing health problem all over the world. It is estimated that approximately 1.7 million people suffer from head trauma each year and approximately 50,000 of these individuals die. Although TBI is seen in all ages and populations, the age population with the highest incidence is children and the elderly. Falls, sports activities and motor vehicle accidents are the biggest risk factors for TBI. To develop diagnosis and treatment methods for traumatic brain injury, the molecular and cellular mechanisms underlying neuropathology should be known. Therefore, different models of mild, moderate and severe experimental traumatic brain injury models are used. Animal models of traumatic brain injury are broadly classified as focal, diffuse, and mixed injury. Fluid percussion, controlled cortical effect, weight reduction and blast wave are the most preferred models in traumatic brain injury experimental research. This review describes the strengths and weaknesses of current rodent models for traumatic brain injury.

Key words: Experimental Traumatic Brain Injury Models, Weight Drop

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INTRODUCTION

1. Traumatic Brain Injury

Traumatic brain injury (TBI) is defined as brain injury caused by external mechanic force such as rapid acceleration or deceleration, blust waves, crush, impact or penetration of a bullet (1). TBI cause temporary or permanent disfunction of cognitive, physically and psychosocial (2). TBI is the leading cause of death and disability for people 75 and older (3). Around the world 10 million death and hospitalization happening in a year and an estimated 57 million people are estimated to suffer from this type of brain injury (4). Motorcycle and sporting accidents and blast wave diffuse injury are major causes of TBI (5). Most traumatic brain injury injuries cause cognitive/behavioral impairment, chronic encephalopathy, epileptic seizure and neurodegenerative Alzheimer's disease if not treated appropriately (6). Although there is no single animal model that can fully mimic human brain injury, animal models of TBI offer the best alternative for investigating the biomechanical, cellular, and molecular mechanisms as well as time-dependent effects of injury-related neuropathological progression (6, 7). Experimental models are so critical for assessment of injury severity and efficacy of therapeutic treatments after TBI (6). There are three types of experimental models for TBI: Focal, diffuse and mixed injury (8). Focal injuries causes are blow to the head, car accidents or localized tissue injury caused by violent attacks. Diffuse injury is caused by the acceleration or deceleration effect, including head movement, in motor vehicle accidents or blast wave propagation. Mixed injuries are seen after fall down or sport accidents.

2.Pathophysiology of Traumatic Brain Injury

TBI causes cognitive, motor, and sensorimotor dysfunction. Animal models are preferred to investigate the pathophysiology and treatment modalities of human TBI. TBI has mixed pathophysiology that include primary and secondary injury mechanism. This primary injury is the conclusion of moment mechanical brain tissue injury which happen by external force’s acceleration and deceleration (8). The primary injury includes confusion, hemorrhage and neuronal axonal injury. Secondary injury develops minutes to months after the primary injury. As a result of primary damage, secondary damage occurs, including molecular, cellular, metabolic and biochemical activation processes leading to neuroinflammation, neurodegeneration and atrophy (9). Primary injury can be preventable with seat belt or helmet. However, secondary damage provides a treatment opportunity for therapeutic intervention that can prevent and/or reduce brain damage and heal the patient. The first effect that making sudden cell death is not recoverable so the treatments are focusing on secondary injury path that increase the primary

injury. However, there is no proven effective neuroprotective treatment to date (10-13). The usage reason of animal model at TBI is understand the secondary injury process and develop new treatments (10). But for now there are no success treatment at clinical result which were succeed at animal models. To date, however, promising results from preclinical studies of possible TBI treatments have not translated into successful results in clinical trials (10). Pathophysiological heterogeneity, there is not enough pharmacokinetics analyses for determine the optimal dosage and compounds given outside the therapeutic window can cause the clinical failure for TBI patients (10, 14).

TBI animal models are include biochemical mechanism, oxidative stress, inflammation, apoptosis, necrose, diffuse axonal injury, neurodegeneration and cognitive dysfunction at injury place (Figure 1.) (6). Many biochemical cascades responsible for secondary damage such as glutamate excitotoxicity, disruption of cellular calcium homeostasis, increased free radical production, lipid peroxidation, mitochondrial dysfunction, inflammation, apoptosis, and diffuse axonal damage have been identified (15, 16). Secondary injury process ends with endothelial and glial cell death and white ore degeneration (17, 18). Cell death occurs within minutes after injury and continues for days to months (17, 19). Necrotic and apoptotic cell death is defined in damaged areas, injury border area and subcortical areas (19, 20). Apoptosis after TBI is seen with gray and white matter atrophy. Acute cell death and delayed cell death have important role for functional disorder after TBI (18). With this low effect TBIs can cause diffuse axonal injury with cognitive dysfunction (16).

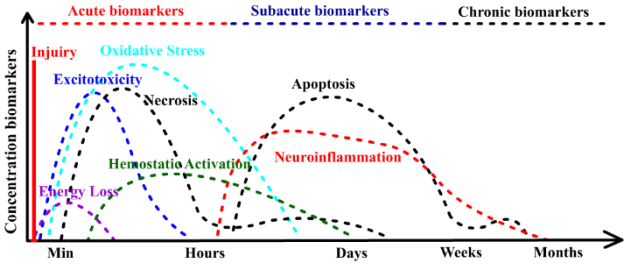

Figure 1. Pathophysiology of Traumatic Brain Injury

2. Experimental Traumatic Brain Injury Models

Pathophysiological heterogeneity at TBI patients are affected with primary injury zone, type and age, health, gender, medicine, alcohol and drugs and genetic etc. (21). TBI animal models design for well controlled and relatively homogeny parameters like age, gender, genetic and injury (10). For this reason animal model cannot summarize all part of secondary injury and this explain why at the clinical trial is not successful (22). With this animal models are necessary for develop new therapeutic treatments and make them characterize also necessary for research biomechanical, cellular and molecular sides which you cannot work at clinical trials (16). For develop new therapeutic strategies and exceed the space between

preclinic and therapeutic the animal models need to develop and change.

There are different animal models are using for understanding the TBI's pathological feature and develop possible therapeutic treatment. Animal models aim to mimic the clinical type of TBI. For the clinical heterogeneity status of TBI many animal models developed. Although larger animals are closer to humans in size and physiology, rodents are often preferred for TBI research due to their small size, low cost, and standardized outcome measures (16). At the Table 1 advantages and disadvantages of most used weight drop models in traumatic brain injury models are shown.

Table 1. Animal Weight Drop Models of Traumatic Brain Injury and Assessment of Injury Severity

Injury model	Injury	Clinical relevance	Strength	Weakness	Animals
Weight Drop Models		Hemorrhage and diffuse axonal injury. Example: falling down, motor vehicle accidents	Mechanism is similar to human TBI, severity of injury can be adjusted; well-characterized neuroscoring post-injury; inexpensive, easy and convenient	High mortality rate due to apnea and skull fractures, possibility of rebound injury, chance of inaccuracy	Mouse, Rat
Marmarou	Mainly diffuse		Damage mechanism close to human TBI; well characterized	Not highly reproducible, high mortality without ventilation	Mouse, Rat
Feeney	Mainly focal		Damage mechanism close to human TBI,	Craniotomy requirement; high death rate	Rat
Shohami	Mainly focal		Easy to use with instant neurological severity scoring in 1 hour	Not highly reproducible	Mouse, Rat
Marmarou Weight Drop Model	**Injury severity**	**Weight (gr)**	**Height (m)**	**Mortality rate**	
	Mild	450	1.0	0%	
	Middle		1.May	12.5 %	
	Severe		2.0	50%	

3.1. Weight Drop Injury Model

At the weight drop model the skull (with or without craniotomy) subjected to free falling and directed weight (23). At these models severity of damage can controlled with mass of weight and height of mass. Weight drop model is cheap, repeatable and easy to make for focal and diffuse clinical situations (6).

Recoil affect and speeds causes different injury severity these are the disadvantages of this model (6).

3.1.1. Marmarou Weight Drop Model

Marmarou model is the most common used model for diffuse axonal TBI (24, 25). Marmarou model is used for model the fall injury and motor vehicle accidents for humans. Adult and 350-400 grams rats are prefer for this model (25). This model are designed as place a foam/sponge under the rat then fall a weight at the rat's head (24). The experimental setup consists of gravity and a freely falling metal weight column in a plexiglass tube (Figure 2.). After the animal is anesthetized, a midline incision is made to expose the skull. The stainless-steel disc is then rigidly attached to the skull with dental cement in a central position between bregma and lambda. Then the animals are placed at foam/sponge. The impact is generated by freely falling weights directly onto the cemented stainless-steel disc (26). In this model, diffuse neuronal and axonal damage occurs throughout the cerebrum and brainstem. Damage triggers a significant neuroinflammatory response in the intrathecal region. This damage leads to neurological deterioration and disruption of the blood-brain barrier (27). In the first 4 hours after the injury, a decrease in cerebral blood flow and an increase in intracranial pressure result in secondary autoregulation. Vasogenic edema occurs in the first hours, then diffuse cellular swelling occurs. Brain edema occurs in the first 20 minutes and continues for up to 24 hours. After the first 4 hours, it has been shown that blood-brain barrier permeability is impaired in relation to edema (26). Changing the mass of weight or altitude of weight causes the TBI models severity as mild/moderate/severe (28). The advantages of Marmarou's model are modeling the diffuse TBI types, usage and manipulation ease, repeatability and modelling the neuropathology very well (24). The disadvantage of Marmarou's model are strikes again (29), skull fixation (24) and the need for the use of anesthesia (3, 30).

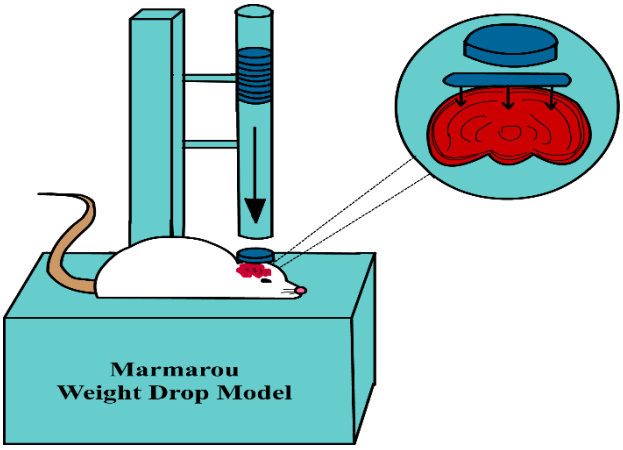

Figure 2. Marmarou weight drop experimental setup

3.1.2. Feeney's Weight Drop Model

Feeney's weight drop model happens as same Marmarou's (Figure 3.). Weight is transmitted to the dura via the craniotomy and causes cortical contusion (31). This model ends with cortical contusion which seen with hemorrhage and blood-brain barrier injury (32). Inflammatory periods cause microglia, astrocyte, neutrophil and macrophages activation (32). As morphological the injury seen in the first hours as bleedings at the white ore at 24th hour it causes necrotic cavity formation (32). Moreover this model causes delayed microcirculatory disorders and cortical spreading depression (33). Cell death after trauma depend on severity of effect (34). Primer injury is more about focal but at the cortical lesion diffuse axonal injury seen (23).

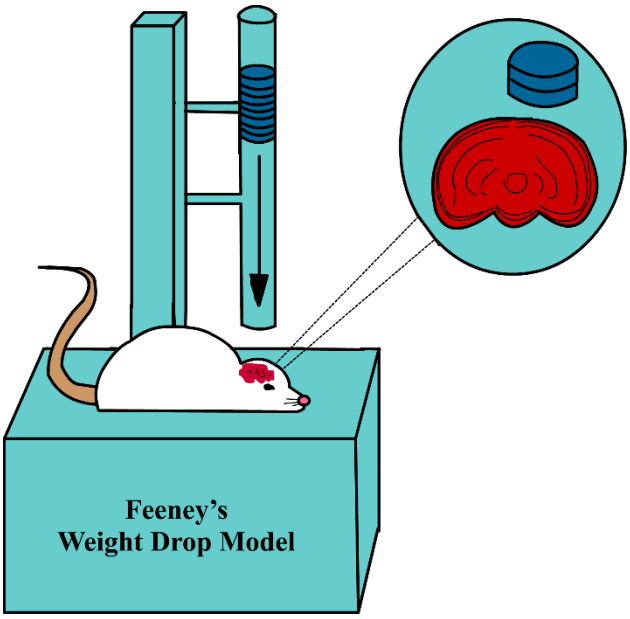

Figure 3. Feeney's weight drop experimental setup

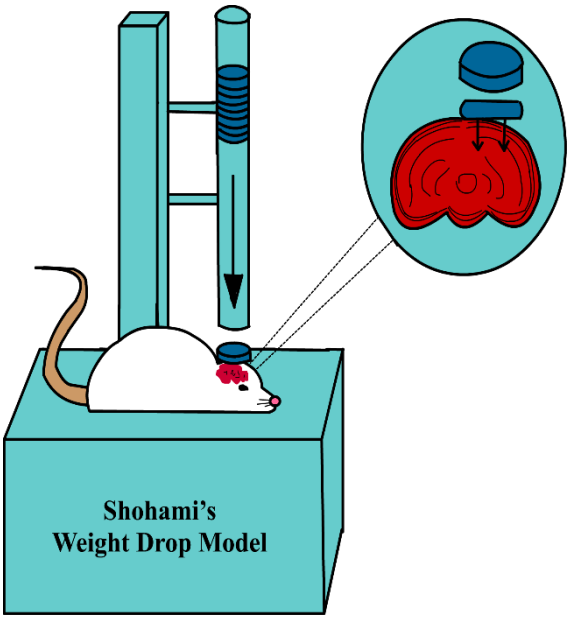

Figure 4. Shohami's weight drop experimental setup

3.1.3. Shohami's Weight Drop Model

The Shohami model is the method used in closed head injury using a weight-dropping blow to one side of the skull in rat (35) and mouse (27, 36) (Figure 4). The severity of injury in this model depends on mass of the weight and height of the fall. Therefore, heavier masses and/or increased fall height cause brain edema, cell death from the contusion site and activation of inflammatory cells over time following ipsilateral cortical brain contusion and disruption of the blood-brain barrier (32). Models using lighter masses and/or shorter fall heights show concussion-like brain injury, bilateral cell loss, short-term cerebral edema, and long-term cognitive impairment (23). Generally light weight drop injuries associates with diffuse injury beside heavy weight drop injuries causes focal contusion. This model's important advantage is under the gas anesthesia it could apply fast so shortly after the injury the neurological scoring can make (27, 36). Weight drop model disadvantage is the big differences between injury severities.

4. Limitations of Current Animal Models

4.1. Physiological differences

As physiological the human brain and other mammals brain have important similarity but as brain geometry, craniospinal angle, gyral complexity and ratio of white and gray ore there are important differences (10).

For treatment after TBI there are important differences between the gender human and animal (10). For women there are less comorbidity and complication seen after TBI (10). Experimental animal research shows us female sex hormones have neuroprotective effect (10). Current clinical evidence shows us progesterone, which female hormone is getting better the neurological results for TBI patients (10). In addition to sex hormones, many other gender differences affect outcome, including pre-injury comorbidities, brain function, and metabolism (10). Physiological variables before and after TBI, including PCO_2, PO_2, pH, blood pressure, and brain temperature, are also important in determining

pathophysiological responses to injury and treatment in TBI model studies (10).

4.2. Long- and short-term therapeutic treatments in animal TBI models

Most studies in animal models of TBI focus on short-term survival times ranging from hours to days and rarely exceeding one month after injury (37). These short-term studies provide us a lot of information about pathophysiologic and functional results at the acute time after TBI. Histological and behavioral data obtained at early post-injury time points may not provide a robust assessment of long-term outcomes and cannot be used to evaluate clinical treatments for long-term efficacy (10). More studies evaluating injury response and functional impairments over longer time periods (3 months to 1 year after TBI) are needed to confirm whether early changes can predict long-term outcome (10). Long-term functional and structural change can be happen until 1 year later from the TBI (10). These findings show therapeutic treatment is not limited with a couple hours after TBI, it could be more and more long time. Also, delayed progression of brain damage over months or even years suggests that early treatment is necessary to reduce brain damage, but not enough to support long-term recovery. Delayed treatment may benefit to TBI patients which missing the early phase of neuroprotection therapy (10). In an animal study functional recovery reported after delayed neurorestorative treatment to TBI patients which applied after 24 hours or more late. Although long-term behavioral disorders can be detected in rodent TBI models, it is known that cognitive disorders are more persistent than sensorimotor disorders (10). In animal models of TBI, it is necessary to test clinically relevant physiological parameters, long-term functional and cognitive outcomes, and the efficacy of new treatments.

4.3. TBI models comorbidities

Age is an important affect for TBI treatment. TBI is prominent death reason for children. Recovered people from the TBI when they were child is a big candidate for having behavioral disfunction (38). Clinical and experimental studies show the developing brains can be more sensitive to TBI (16, 39). TBI severity is an important risk with increasing age. The risk of TBI injury severity increases with increasing age. Elderly individuals with TBI differ from younger adults with TBI, including incidence rates, etiology of injury, nature of complications, length of hospital stay, functional outcomes, and mortality (16). Older than 75 ages adults are group with the highest hospitalization and death rates and falling is the prominent reason for TBI (16). Moreover the treatment dosage of therapeutic for youth rats may not be treat the old rats (16). This situation shows for old rats population need new treatments. Given the high incidence of TBI in the elderly population, much more preclinical research is needed in this area.

3. Neurobehavioral Assessment of Traumatic Brain Injury

5.1.Traumatic Brain Injury Behavior Experiments

At the Traumatic brain injury the motor, cognition, depression and anxiety like functional behaviors are generally evaluate. While motor tests measure the animal's motor coordination and balance, cognitive tests examine working memory and learning in rats with the help of visual cues (6). Tests that measure anxiety and depression generally examine the exploratory and anxiety behaviors of animals.

Behavioral disorders caused by TBI are mostly observed early after injury and gradually improve over time, depending on the severity of the injury. A similar pattern of improvement in cognitive impairment is observed over time in human TBI cases (40). Motor dysfunction after experimental traumatic brain injury is usually evaluated with open field, rotarod, balance beam, rod and rope grip tests (6). Cognitive dysfunctions are evaluated using Morris water maze test, radial arm maze, novel object recognition, object localization, Y and T maze tests (6).

5.2. Evaluation of Experimental Mild, Moderate and Severe Traumatic Brain Injury

With the Glasgow Coma Scale (GCS) which is a clinical evaluate method is not possible the evaluate of severity of injury because the severity of TBI in humans is assessed in the verbal response to the state of consciousness immediately after injury (6, 10). Also it is hard to evaluate an animal with anesthesia after injury. Neurological severity score (NSS) is generally used method for evaluate the injury severity for animal models (6, 10). The NSS scale evaluates loss of movement, straight walking, righting reflex, eye reflexes, limb reflexes, walking and balancing in the beam, and searching behavior (6, 10).

5.2.1. Glasgow Coma Scale (GCS)

GCS is a standard scale used for measuring the consciousness level, degree of cognitive impairment and severity of injury (10). Scoring is determined by summing up the ratings assigned to three factors based on whether and how the patient responds to certain standard stimuli by opening their eyes, verbally responding, and motor responding. A high score of 13 to 15 indicates mild brain damage, a score of 9 to 12 indicates moderate brain damage, and a score of 3 to 8 indicates severe brain damage.

5.2.2. Neurological Severity Score (NSS)

NSS is a trustworthy scale used for measuring the neurological injury, motor function and behavior after head trauma for mice and rats. NSS is combination of motor, behavior, reflex and balance tests for rats. It is rated from 0 to 18 (normal score, 0; maximum damage/impairment score, 18). A score is awarded for failure to perform tasks or tested reflex impairment: 13–18, damage severity; 7–12, medium moderate damage; 1-6, slight damage. In experimental studies, behavioral changes such as neurological severity score and motor function tests were evaluated, as well as physiological changes such as weight loss and increased intracranial pressure; Histological changes such as infarct volume and neuronal loss are also used (26, 41, 42).

CONCLUSION

Traumatic brain injury is one of the leading causes of death and disability. TBI is the result of external force causing mechanical disruption of brain tissue and delayed pathogenic events that exacerbate the damage. These pathogenic injury processes are poorly understood and thus no effective neuroprotective therapy is available so far. Experimental models, animal models are necessary to investigate the physiological and pathophysiological mechanisms of TBI, to test new therapeutic agents, and to ensure that clinical trials are safe and successful. Various rodent TBI models have been developed to model the different injury mechanisms associated with human TBI. The most commonly

used rodent models of traumatic brain injury are fluid percussion, cortical contusion effect, weight reduction and blast wave models. The design and selection of a particular model poses a major challenge for neuroscientists, as not all events that can occur in traumatic brain injury can be covered by a single rodent model. This section describes the strengths and weaknesses of current rodent models for traumatic brain injury.

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