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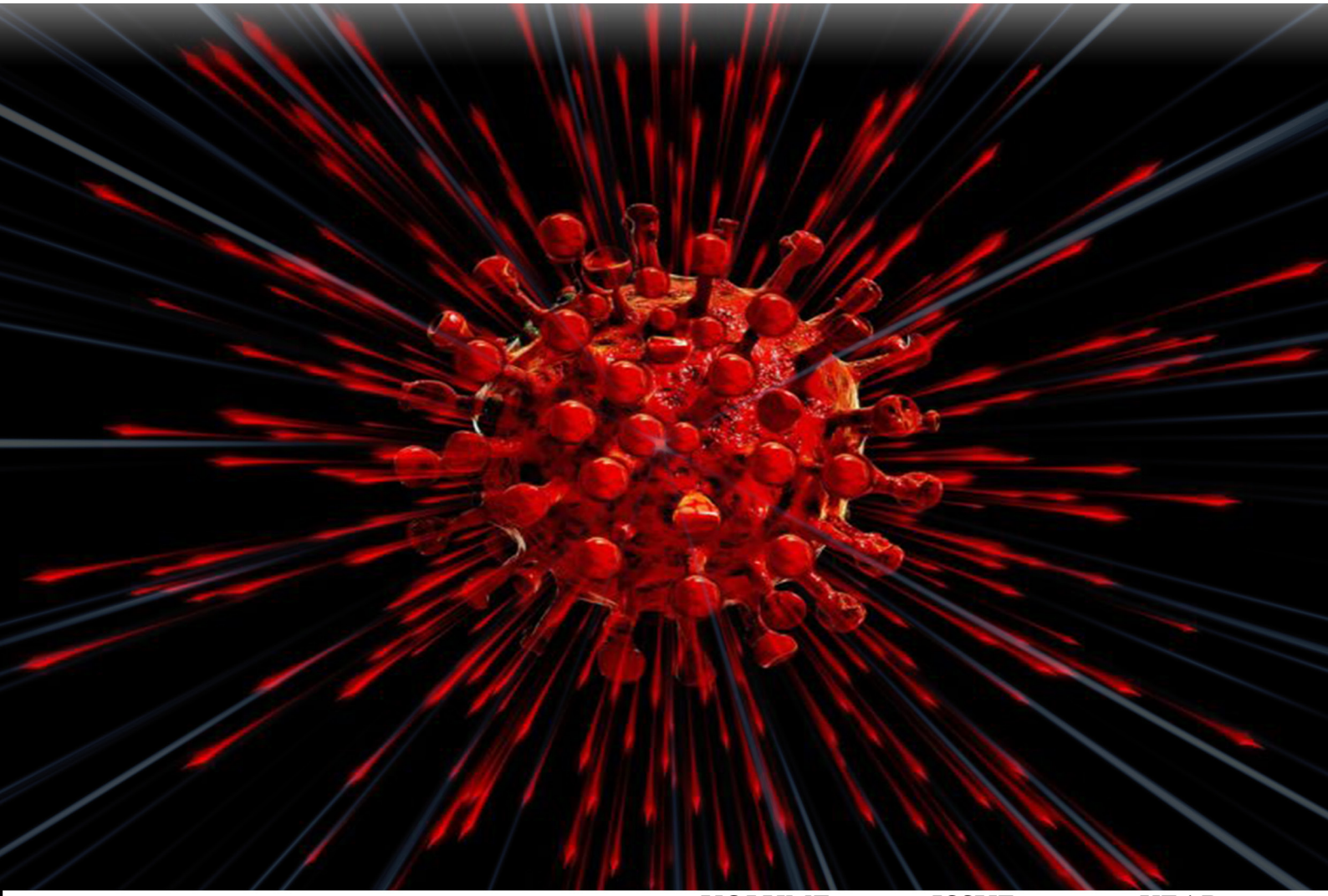

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ANATOLIAN CURRENT MEDICAL

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Evaluation of alcohol, substance and antidepressant drug use of university students during the COVID-19 pandemic

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ABSTRACT

Aim: This study aimed to investigate how the mental tension and environmental conditions caused by the COVID-19 pandemic affected the alcohol and substance use habits of university students and the changes in the rates of antidepressant drug use for mood disorders due to the pandemic.

Material and Method: This cross-sectional survey study was conducted using an electronic survey completed by university students in Turkey between the years 2021-2022. Our study consisted of a questionnaire prepared to collect the demographic information of the participants and the Bapirt-Alcohol/Substance scale used to determine alcohol/substance addiction level. The survey, which took 5 minutes to complete, was randomly distributed to university students.

Results: A total of 641 participants, approximately 66% female and 34% male, between the ages of 18 and 41 years were included in the study. The mean alcohol dependence scores before and during the COVID-19 pandemic were 0.91 and 1.35 and substance addiction scores were found to be 0.17 and 0.33. There was a significant difference in the average alcohol and substance addiction scores of the students during the pandemic compared to the pre-pandemic period (for alcohol addiction: p=0.000; for substance addiction: p=0.007). Students who perceived their income level as mostly low had significantly higher alcohol addiction scores during the pandemic period compared to pre-COVID-19 (p=0.014). Compared to the pre-pandemic period, the average alcohol and substance addiction scores were found to be significantly higher in students residing in dormitories/student houses during the pandemic period (p=0.014 for alcohol; p=0.001 for substances). Antidepressant drug use rates of the participants were found to be higher during the pandemic period compared to the pre-pandemic period.

Conclusion: As a result of this study, when the addiction levels for alcohol and substance use in university students before the COVID-19 pandemic and during the pandemic were compared, addiction increased in general. The prevalence of depression increased due to changing lifestyles and disruptions as a result of the pandemic, and accordingly, serious increases were observed in the use of antidepressant drugs.

Keywords: COVID-19, alcohol, substance, addiction, antidepressant

INTRODUCTION

Experimental studies showing that alcohol and substance use seriously affect the immune system and thus increase susceptibility of the host to infections reported that chronic consumption paves the way for bacterial and viral diseases (1). In addition, extensive evidence highlights the negative impact of alcohol and substance use on lung health and its causal relationship with many respiratory diseases. Alcohol consumption was associated with many lung diseases, including tuberculosis, respiratory syncytial virus, and acute respiratory stress syndromes (2). Other stimulants, opioids, depressants, hallucinogens and cannabis, apart from alcohol, on the other hand, were associated with many conditions such as coronary artery occlusion, cerebrovascular diseases, severe hypertension, speech disorder, fatigue, drowsiness, respiratory disorder, depressed mood and hallucinations (3). Considering these effects of alcohol and substances, their chronic consumption is a risk factor for immune system diseases and increased likelihood of fatal COVID-19 complications (4).

The global COVID-19 pandemic, which started in the first quarter of 2020, caused many people to die due to lung and other target organ problems (5). Due to the pandemic, unexpected and major changes occurred in the lifestyle of the society, along with an extreme decline in socialization. As a result of this phenomenon, many

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factors such as the transfer of education and work to online systems, the long-term isolation of people with the disease, the reduction of social activities to reduce the contagiousness of the disease, etc. had a traumatic effect on young people. In this period, the use of inappropriate substances by individuals whose coping skills were insufficient increased their risk of addiction (6). Additionally, the impaired substance-related immune systems of individuals receiving addiction treatment, injected drug use and efforts to access substances in unhygienic environments increased the risk of contracting COVID-19 (7).

Our study aimed to evaluate the evidence of changes in alcohol and substance usage behavior among Turkish university students during the COVID-19 pandemic, to understand the rate of antidepressant drug levels used due to mood disorders and how demographic factors affect these behaviors.

MATERIAL AND METHOD

The study was carried out with the permission of Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 50). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Survey Instrument and Dissemination

A web-based, cross-sectional study was conducted using a questionnaire tool after obtaining written informed consent from university students in Turkey between the years 2021-2022. This cross-sectional survey was conducted from 13 December 2021 to 2 March 2022 by means of an electronic survey. The study was carried out with a questionnaire prepared from the Bapirt-Alcohol/Substance Scale and questions to determine the demographic information of the participants and the level of alcohol/substance addiction. The questionnaire was randomly distributed to university students. A total of 641 participants, between the ages of 18 and 41 were included in the study. By using the Bapirt-Alcohol, Bapirt-Item Scales and demographic data, a 26-item questionnaire tool was developed, including details about COVID-19. Demographic data, alcohol and substance addictions, addiction levels before and during COVID-19 were questioned. The developed draft survey tool was made accessible via a link and distributed to 10 randomly-selected faculty members from different regions for a comprehensive assessment of the content areas of the survey. The final version of the questionnaire was revised based on the responses. The final version of the questionnaire was applied to participants using the e-mail databases of universities in Turkey.

Statistical Analysis

Evaluation of research data was done using SPSS 20.0. Frequency, percentage, mean and standard deviation values were used for descriptive analysis. In the research, after checking the normality assumption for the data, Pearson Correlation Analysis, cross tables and chi-square statistics were used to evaluate the correlations among variables. In comparisons of two groups, the t-test was used for independent groups and One-Way ANOVA was used for comparisons for more than two groups. A p value of 0.05 or less was considered significant in all tests.

RESULTS

Descriptive statistics

The sample for the study consisted of 641 university students. The study data for students from universities in different provinces in Turkey were gathered online. The socio-demographic characteristics of the participant students are shown in **Table 1**.

Table 1. The evaluation of demographic characteristics of the students

	Frequency (n)	Percentage (%)
Gender		
Male	213	33.2
Female	428	66.8
Living place		
With parent	358	55.9
Dormitory/student house	283	44.1
Mother education status		
No formal education	42	6.6
Primary school	208	32.4
Middle school	109	17.0
High school	162	25.3
University	108	16.8
Master's	8	1.2
PhD	4	0.6
Father education status		
No formal education	9	1.4
Primary school	165	25.7
Middle school	115	17.9
High school	167	26.1
University	158	24.6
Master's	21	3.3
PhD	6	0.9
Economical situation		
Mostly insufficient	198	30.9
Mostly sufficient	396	61.8
Always sufficient	47	7.3

Table 2 contains the descriptive statistics of university students. Before the COVID-19 pandemic, the mean scores for alcohol and substance abuse were 0.91 and 0.17, respectively. The mean scores for alcohol and substance abuse during the COVID-19 pandemic were determined as 1.35 and 0.33, respectively.

Table 2: Addiction score averages before and during the COVID-19 pandemic

	Average	Standard Deviation
Alcohol addiction score before the COVID-19 outbreak	0.91	1.740
Alcohol addiction score during the COVID-19 pandemic	1.35	2.391
Substance abuse score before the COVID-19 outbreak	0.17	0.886
Substance abuse score during the COVID-19 pandemic	0.33	1.246

There was a significant difference in the average alcohol and substance addiction scores of the students during the pandemic compared to the pre-pandemic period (for alcohol: p=0.000; for substance: p=0.007).

Students who thought their income level was mostly insufficient had significantly higher alcohol addiction scores during the pandemic period compared to pre-COVID-19 (p=0.014). Compared to the pre-pandemic period, the average alcohol and substance addiction scores were significantly higher for students residing in dormitories/student houses during the pandemic period (p=0.014 for alcohol; p=0.001 for substance) (**Table 3**).

Table 4 shows the change in antidepressant drug use before and during the COVID-19 pandemic. According to the table, the antidepressant drug use rates of the participants varied significantly from the pre-COVID-19 and COVID-19 period (p<0.05). According to the data obtained, the use of antidepressant drugs increased during the pandemic compared to the pre-pandemic period. Some of the participants also stated that they considered using antidepressant medication during the pandemic (**Table 4**).

Table 3: Comparison of alcohol and substance addiction scores before and during the pandemic

	Alcohol		Substance	
	Pre COVID-19	During COVID-19	Pre COVID-19	During COVID-19
Gender				
Male	1.5070±2.23	2.6009±3.15	0.3709±1.35	0.8263±1.92
Female	0.6192±1.34	0.7220±1.57	0.0631±0.49	0.0818±0.56
Test Statistics	t=6.264	t=10.081	t=4.196	t=7.421
p	0.000	0.000	0.000	0.000
Living Place				
With Parent	0.7207±1.44	0.9078±1.79	0.0950±0.56	0.1844±0.89
Dormitory/Student H.	1.1590±2.04	1.9011±2.89	0.2544±1.17	0.5124±1.57
Test Statistics	t=-3.189	t=-5.333	t=-3.189	t=-5.333
p	0.001	0.000	0.024	0.001
Economical Situation				
Mostly insufficient	0.9899±1.62	1.6717±2.60	0.1465±0.64	0.4141±1.31
Mostly sufficient	0.9167±1.85	1.2702±2.36	0.1894±1.03	0.3157±1.28
Always sufficient	0.5745±1.16	0.6170±1.24	0.0426±0.29	0.0851±0.41
Test Statistics	F=1.084	F=4.263	F=0.641	F=1.387
p	0.339	0.014	0.527	0.251
Mother Education Status				
No formal education	0.7381±1.56	0.9762±1.91	0.2143±0.87	0.5476±1.78
Primary School	0.6442±1.49	0.8125±1.91	0.1058±0.89	0.1971±1.02
Middle School	1.2018±1.96	1.5321±2.50	0.1193±0.50	0.2018±0.79
High School	0.9074±1.66	1.4074±2.30	0.1543±0.92	0.3951±1.38
University	1.0370±1.78	2.1481±2.98	0.2963±1.10	0.5278±1.54
Master's	1.2500±1.58	0.7500±1.16	0.2500±0.71	0.2500±0.71
PhD	5.2500±4.57	5.0000±4.76	0.7500±1.50	0.5000±1.00
Test Statistics	F=5.943	F=5.950	F=0.926	F=1.350
p	0.000	0.000	0.475	0.233
Father Education Status				
No formal education	1.4444±2.30	1.0000±1.58	0.0000±0.00	0.0000±0.00
Primary School	0.8303±1.81	1.0121±2.17	0.1091±0.98	0.2667±1.32
Middle School	0.8435±1.42	1.0957±2.01	0.1217±0.64	0.1478±0.67
High School	0.8443±1.69	1.3054±2.28	0.1617±0.79	0.4251±1.47
University	0.9810±1.72	1.7532±2.69	0.2025±0.90	0.3797±1.14
Master's	1.0952±1.45	2.1905±3.25	0.2381±0.77	0.5238±1.60
PhD	3.3333±4.46	3.3333±4.46	1.6667±2.86	1.3333±2.42
Test Statistics	F=2.317	F=2.720	F=3.216	F=1.531
p	0.032	0.013	0.004	0.165

Table 4. Antidepressant drug use rates before and during the COVID-19 outbreak

	Pre-COVID-19		During COVID-19	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Never used	571	89.1	518	80.8
I use it when I need it	34	5.3	28	4.4
I use it regularly with a prescription	25	3.9	48	7.5
I use it regularly without a prescription	3	0.5	20	3.1
I intend to use	8	1.2	27	4.2

DISCUSSION

Addiction is among the most important diseases that societies struggle with. This disease, which is especially common in adolescents and young adult groups, threatens the future of societies (8). Studies show that substance use mostly begins during adolescence (9-11) and that young people who are introduced to substances in this period develop addiction in later years (12). A person who starts to use alcohol and drugs, especially in their 20s, has a very high risk of developing addiction in later years. The average age of the students participating in our study was determined as 20.9 years. The alcohol addiction mean score of participants was 0.91 before the COVID-19 pandemic and 1.35 during the COVID-19 period. The mean substance addiction scores were found to be 0.17 before the COVID-19 pandemic and 0.33 during the COVID-19 period. Looking at the mean scores in both cases, both alcohol and substance addiction scores increased during the pandemic.

Psychological mood swings caused by the COVID-19 pandemic can affect alcohol and substance use habits of young adults. Studies conducted during the pandemic period showed that restrictions cause an increase in stress in a person and accordingly trigger the desire to take drugs or alcohol more frequently and then lead to relapse (13,15). In a study by Wu P et al. (16) in June 2020 including 1074 Chinese people, they found results showing an increased risk of severe psychiatric disorders with higher anxiety, depression, dangerous and harmful alcohol consumption, and lower mental well-being due to the COVID-19 pandemic and mass isolation. In another study conducted in China, alcohol consumption during the COVID-19 pandemic was examined using the Alcohol Use Disorder Identification Test (AUDIT), and there was a 29.1% increase in risky consumption, 9.5% in harmful use and 1.6% increase in alcohol dependence (17). As a result of our study, the mean addiction scores of female and male students before the COVID-19 pandemic ($p<0.005$) and during the COVID-19 pandemic ($p<0.005$) increased in line with the studies in the literature. In addition, it was observed that male students had higher alcohol and substance addiction scores than female students before and during the pandemic.

Differences in physiological stress and poor decision-making abilities increase the risk of stress-induced alcohol consumption. In a meta-analysis study evaluating gender differences in risk taking, risk-taking behavior in adolescence was found to be more common in boys compared to girls, although it is valid for both genders (18). In the study by Ahmed MZ et al. (17), in which they investigated the psychological problems associated with the COVID-19 pandemic, the rate of harmful users and dependent users was found to be six times higher in men than in women for alcohol and substance use by gender. In accordance with these results, in our study, male students had higher alcohol and substance addiction scores than female students before and during the pandemic.

Stressful events are powerful negative environmental factors which can predispose individuals to psychiatric disorders, especially depression (19,20). Research has indicated that people experience negative emotional reactions such as anxiety and depression symptoms during epidemics (21). During the current COVID-19 pandemic, too, the practice of social distancing reduced or changed people's daily activities to reduce interactions between people and thus reduce the possibility of new infections. These factors can lead to different levels of psychological pressure, which can trigger feelings of loneliness and helplessness or a variety of dysphoric emotional states such as stress, irritability, hopelessness, physical and mental fatigue. The rate of antidepressant drug use may increase in individuals who experience such an affective period. As a matter of fact, it was observed that antidepressant consumption increased worldwide with a significant increase in the prevalence of depression associated with COVID-19. A recent study in the United States showed a 21% increase in the number of antidepressant, anti-anxiety, and anti-insomnia prescriptions during the first period of the pandemic between February and March 2020 (22,23). Rabeea et al. (22) studied antidepressant prescription trends during the pandemic (January-August 2020) compared to a similar period in the last three years before the pandemic to assess the impact of the COVID-19 pandemic and associated quarantines on antidepressant use. Overall consumption of antidepressants was observed to be higher throughout the pandemic compared to consumption in 2019. According to the data from our survey study, the

use of antidepressant drugs increased more during the pandemic period than before the pandemic, and some of the participants stated that they considered using antidepressant drugs during the pandemic period.

However, despite the relevant findings and strengths (such as a large population and diversity of variables), our study has some limitations. The use of self-reported questionnaires leads to well-known biases, such as self-representation biases, introspective limits, or social desirability. Other assessment methods, such as qualitative methods, should also be considered. It is clear that people suffering from alcohol use disorder, characterized by a range of mental, physical and behavioral symptoms, belong to a marginalized community and are always more susceptible to infection during the COVID-19 pandemic. This population should be included in future research. Also, as the sample is over-represented by women, subsequent studies should aim to reach more male participants.

CONCLUSION

In our study it was observed that alcohol and substance use among university students increased before and during the COVID-19 pandemic. In parallel to this, it was observed that the prevalence of depression increased due to the changing life style and disruptions as a result of the pandemic, and accordingly, there was a serious increase in the use of antidepressant drugs. According to these results, the young population needs comprehensive management for the use of alcohol and addictive substances, which pose a great risk for the COVID-19 epidemic, and for the consumption of antidepressant drugs. For this reason, necessary plans should be made by the relevant health institutions in order to end alcohol and substance use habits by taking advantage of the pandemic and about the conscious use of antidepressant drugs.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Çukurova University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 50).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Determination of the risk factors and delirium in the intensive care unit

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ABSTRACT

Aim: The objective of the present study is to increase nurses' awareness of delirium risk factors, make nurses gain competence in using Nursing Delirium Screening Scale (Nu-DESC), and improve the quality of care by detecting delirium early in intensive care unit patients.

Material and Method: The research is a descriptive and correlational study. The sample consisted of 55 patients in an intensive care unit. Data of the study was collected with the Personal Information Form, the Richmond Agitation and Sedation Scale, the Glasgow Coma Scale, and the Nu-DESC.

Results: The majority of patients (89%) demonstrated the symptoms of anxiety and agitation. There was a significant correlation between age and the day delirium was detected and the Nu-DESC. In the study, isolation need, ventilator support, and pain were determined as risk factors.

Conclusion: The results of the study revealed the necessity of using measurement tools for the early detection of delirium in clinical practice by nurses.

Keywords: Delirium, intensive care unit, nursing, risk factors

INTRODUCTION

Delirium is defined as a disorder of consciousness, cognition, attention, and perception (1). According to the International Classification of Diseases and the Diagnostic and Statistical Manual of Mental Disorders, delirium is an acute disorder of attention, cognition, and awareness (2). These symptoms are usually of sudden onset, and they fluctuate transiently throughout the day (1). Delirium is frequently encountered in intensive care units (ICU) due to reasons such as multiple invasive interventions applied in the ICU, mechanical ventilation, physical restriction, lack of day and night concept due to the physical conditions of the ICU, alarm and monitor sounds (3). For this reason, it is also known as 'intensive care psychosis' and 'intensive care syndrome' (4,5). The prevalence of delirium in ICU varies between 30% and 75.6% (6,7). The hospital stay and the length of stay in the ICU of patients with delirium are prolonged, resulting in an increase in hospital costs and mortality rates after discharge (8). Therefore, early diagnosis of delirium is important (9). Increasing awareness about the diagnosis and types of delirium can affect the rates of early detection; thus, delirium can be generally prevented (10). Although delirium is an important problem frequently encountered among patients in the ICU, it is usually not noticed by healthcare team members in the early period (11). Increasing the nurses' awareness of delirium and minimizing the negative consequences of delirium can be possible with the knowledge of delirium management (12,13). In the intensive care unit, preventive approaches and treatment for patients at risk for delirium can be realized with the cooperation of healthcare team members (14-16). The nurse, one of the healthcare professionals in the ICU, plays a primary role in patient communication and care (17). Intensive care nurses are team members who easily notice every physiological and behavioral change, as they constantly observe the patients. In this sense, nurses play an important role in the evaluation and early diagnosis and treatment of patients at risk of delirium (14-16). It is necessary for nurses to have knowledge and competence in this regard for the effective

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prevention of delirium. When the nursing literature in Turkey is examined, it is striking that there is a limited number of studies aimed at identifying patients at risk of delirium among nurses working in the ICU. Our aim is to increase nurses' awareness of delirium risk factors, make nurses gain competence in the use of Nu-DESC, and improve the quality of care by detecting delirium early in ICU patients.

MATERIAL AND METHOD

The study was carried out with the permission of Gaziantep University Clinical Researches Ethics Committee (Date: 07.02.2022, Decision No: 2022/03). Before the data were collected, the participants were informed about the research and their written consents were obtained. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The research is a descriptive and relation-seeking research. This research was conducted with patients in the General Intensive Care Unit of a patient. The sample of the study included the patients who were older than 18 years of age, who were not in a coma (The Richmond Agitation and Sedation Scale (RASS): between -3 and +4, the GCS: 10 or higher), and who did not have a psychiatric diagnosis. The sample size's confidence interval was α=0.05, the power of the test (1-β) was 0.95, and a total of 45 patients were calculated. During this study, 55 patients were reached and the study was completed in 55 patients.

Data Collection Tools

Personal information form: This form consists of questions that include sociodemographic and disease-related information.

Richmond agitation and sedation scale (RASS): RASS aims at a good observational evaluation. First of all, the sedation status of the patient is evaluated. If the patient is under deep sedation, unconscious, or if the RASS scores are between -4 and -5, an evaluation cannot be made. In this situation, which is called coma or stupor, it would not be appropriate to evaluate for delirium, as the patient cannot react to anything. However, after the patient wakes up (RASS> -3), he/she can be evaluated for delirium. It is based on a scoring system between +4 and -5 (7). 4 levels (+1 to +4) are used for anxiety and agitation, the level (0) is used to indicate alertness and calmness, and 5 levels (-1 to -5) are used to indicate sedation (18).

Glasgow coma scale (GCS): It is one of the physiological scoring systems used to monitor the clinical progress of patients from the moment they are admitted to the hospital and during their stay. It is a simple, objective scoring system that is frequently used to determine the previous state of the patient, define the state of consciousness, express the change in the level of consciousness, and measure the degree of coma in the most reliable way. The GCS's maximum point value is 15. Those with a GCS score of 8 or lower are considered to be in a coma (19). A GCS score of 10 or higher was taken for delirium evaluation in the present study.

Nursing delirium screening scale (Nu-DESC): Since delirium is a difficult situation to diagnose, Gaudreau et al. (20), who aimed to develop a scale with a low workload but sufficient to diagnose it, developed the Nu-DESC in 2005. The Turkish adaptation was made by Karataş (2019), and it was found to be valid and reliable in diagnosing delirium in the intensive care patients (21). It is a tool that can be defined very easily and quickly in the clinical practices of nurses. In addition to providing an easy evaluation of delirium at the bedside, it is recommended for routine use of patients in ICU, as characteristic fluctuations can be witnessed (22). A score between 0 and 2 is given for each item, and a maximum of 10 points can be obtained from the scale (23). It is reported that the threshold value for delirium is two (21). The Nu-DESC used in many countries is based on observation only and requires an estimated time of 1 minute. Also, no training is required for the use of this scale.

Research Process

The personal information form to identify the sociodemographic characteristics of patients, the RASS to determine the state and severity of agitation, the GCS to evaluate the consciousness-unconsciousness, and the Nu-DESC to evaluate the status of delirium were applied twice a day as day and night shifts.

The RASS and Glasgow coma scale were first applied to the patient who came to the ICU. The Nursing-Delirium Screening Scale was applied to those who received RASS -3 or higher and those who were not in a coma. If delirium was detected in the patient at the initial evaluation, follow-up was terminated. Those who were in a coma or who had the RASS between -4 and -5 were included in the study when their condition improved. Patients who were not found to have delirium in the initial evaluation were followed up for delirium during their stay in the ICU. When delirium was detected in the follow-up, the process was terminated.

Data Analysis

Descriptive statistics, mean and standard deviation, t-test, Anova, X^2 test, and correlation analysis were used in the analysis of the data. A value of $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the patients in our study was 65.4±17.6, and most of them (88.9%) were over 41 years old. 69.1% of the patients were male, and 90.9% of them were married. When the educational level was examined, it was found that the majority of the patients (30.9%) were primary school graduates, their income was mostly at a moderate level (80%), and 69.1% of them were unemployed. 63.6% of the patients had a surgery. 83.6 of the patients had a chronic disease, with the most common high blood pressure and heart failure. 14 patients received ventilator support due to respiratory problems. 10 patients needed isolation. 60% of patients in the ICU had a diagnosis of delirium on days 2 and 3. The majority of patients (89%) demonstrated the symptoms of anxiety and agitation. More than half of the patients (59.3%) in the ICU were hospitalized for 1-7 days. The patients' discharge from the ICU was in the form of discharge/referral at a rate of 72.7% (**Table 1**).

Table 2 presents the GCS and RASS scores of the patients in the ICU. For the evaluation of delirium, the GCS score was taken as 10 or higher, and it was determined that it concentrated on 13 (21.8%), 14 (30.9%), and 15 (29.1%) points. When the RASS was evaluated, it was determined that the patients had high anxiety-agitation rates (from +1 to +4).

Table 2. The GCS and RASS scores of the patients in ICU

GCS	n	%	RASS	n	%
10 points	2	3.6	+4	21	38.2
11 points	5	9.1	+3	17	30.9
12 points	3	5.5	+2	10	18.2
13 points	12	21.8	+1	1	1.8
14 points	17	30.9	0	3	5.5
15 points	16	29.1	-1	1	1.8
			-2	2	3.6
Total	55	100.0	Total	55	100.0

GCS: Glasgow Coma Scale, RASS: Richmond Agitation and Sedation Scale

The distribution of the conditions of patients in the ICU according to Nu-DESC is presented in **Table 3**. In the first 24 hours, the rate of disorientation was 18.2%, the rate of inappropriate behavior was 47.3%, the rate of inappropriate communication was 16.4%, the rate of illusion/hallucination was 9.1%, and the rate of psychomotor retardation was 12.7%. In the following days, the increasing rates in these areas attracted attention. Between the 4th and 7th days in the ICU, these rates were 58.2%, 96.4%, 67.3%, 30.9%, and 36.4%, respectively.

In the correlation analysis, there was a weak and significant positive correlation between age and the Nu-DESC ($p<0.05$), and a moderate to very significant negative correlation was determined between the day when delirium was detected and the Nu-DESC ($p<0.05$) (**Table 4**).

Table 1. Distribution of the characteristics that identify patients

	n	%
Age		
40 years old or younger	6	11.1
41 years old or older	49	88.9
Gender		
Female	17	30.9
Male	38	69.1
Marital Status		
Married	50	90.9
Single	5	9.1
Educational Level		
Illiterate	14	25.5
Literate	16	29.1
Primary School	17	30.9
Middle School-High School	8	14.5
Employment Status		
Employed	17	30.9
Unemployed	38	69.1
Income Level		
Low	8	14.5
Moderate	44	80.0
Good	3	5.5
Ventilator Support (Respiratory Distress)		
Present	14	25.5
Absent	41	74.5
Isolation Need		
Present	10	18.2
Absent	45	81.8
Surgery		
Present	35	63.6
Absent	20	36.4
Chronic Diseases		
Absent	9	16.4
Diabetes Mellitus (DM)	10	18.2
High Blood Pressure (HBP, Heart Failure)	19	34.5
Metabolic Syndrome (DM, HBP, kidney failure)	6	10.9
Sepsis	11	20
Delirium Detection		
During admission to intensive care	9	16.4
On the 2nd or 3rd day	33	60.0
Between the 4th or 7th day	12	21.8
Absent	1	1.8
Number of Days in ICU		
Between 1 and 7 days	33	59.3
8 days or more	22	40.7
Discharge from the ICU		
Discharge/Referral	40	72.7
Death	15	27.3
General Condition in ICU		
Anxiety-Agitation	49	89
Calm/Awake	3	5.5
Sedation	3	5.5
TOTAL	55	100.0

Table 3: Distribution of the conditions of intensive care patients by Nu-DESC

Variables	On the 1st day		On the 2nd or 3rd day		Between the 4th and 7th days	
	Yes n/%	No n/%	Yes n/%	No n/%	Yes n/%	No n/%
Disorientation	10/18.2	45/81.8	17/30.9	38/69.1	32/58.2	23/41.8
Inappropriate behavior	26/47.3	29/52.7	41/74.5	14/25.5	53/96.4	2/3.6
Inappropriate communication	9/16.4	46/83.6	19/34.5	36/65.5	37/67.3	18/32.7
Illusion/hallucination	5/9.1	50/90.9	7/12.7	48/87.3	17/30.9	38/69.1
Psychomotor retardation	7/12.7	48/87.3	53/96.4	2/3.6	20/36.4	35/63.6

Nu-DESK: Nursing Delirium Screening Scale

Table 4. The relationship between patients' Nu-DESC mean scores and some characteristics

Variables	Nu-DESC
Age	r= 0,361 * p=0.007
The day when delirium was detected	r= -0,591 p=0.001

Nu-DESK: nursing delirium screening scale, *r: correlation analysis

In **Table 5**, some characteristics of the patients are compared according to the Nu-DESC mean scores of the patients. As a result of the statistical analysis, a statistically significant difference was found between isolation need, ventilator support, pain, and Nu-DESC ($p<0.05$).

DISCUSSION

Delirium is a common neurological and behavioral problem in hospitalized patients (24,25). Delirium can cause adverse outcomes such as prolonged mechanical ventilation, longer ICU stay, high mortality, and higher cost of care (1). Therefore, early detection and intervention gain importance in handling delirium.

Although delirium is preventable and reversible, it predisposes patients to longer hospital stays and reduced quality of life (26,27). In the present study, more than half (59.3%) of the patients stayed in the ICU between 1 and 7 days. The Nu-DESC mean scores of those who stayed in the ICU for longer than one week were found to be higher; however, there was no statistical significance. Delirium symptoms are characterized by blurred or fluctuating levels of consciousness, limited attention, and disorientation (28). In the current study, when disorientation, inappropriate behavior, inappropriate communication, illusion/hallucination, and psychomotor retardation were evaluated according to Nu-DESC, it was found that the rate of deterioration in these areas increased as the length of hospital stay. A long hospital stay may have increased the risk of delirium by affecting the physiological and cognitive statuses, and patients may have stayed in the ICU for longer as a result of developing delirium.

It has been reported in the studies that delirium starts on average between the 2nd and 3rd day after admission to the ICU (29). In the present study, delirium was detected in the majority of patients (60%) on the 2nd and 3rd days. It was determined that the majority of intensive care patients in the current study demonstrated the symptoms of anxiety and agitation. The Nu-DESC mean scores of agitated patients were high. It was found that patients who needed isolation presented high delirium symptoms. Early detection of delirium with delirium screening tools for intensive care patients can minimize

Table 5: Comparison of the Nu-DESC mean scores and some characteristics of patients

Variables		n	X±SD	Test - p values
Age	40 years old or younger 41 years old or older	6 49	3.81±1.83 4.00±2.00	t=0.230* p>0.05
Gender	Male Female	38 17	4.07±1.83 3.29±1.75	t=1.483 p>0.05
Chronic Diseases	Present Absent	46 9	4.00±2.06 3.80±1.80	t=0.290 p>0.05
General Condition	Calm/Awake Anxiety-Agitation Sedation	3 49 3	3.33±2.5 3.87±1.86 3.66±0.57	F=0.134** p>0.05
Isolation Need	Present Absent	10 45	4.90±2.23 3.60±1.67	t=2.090 p=0.041
Days in ICU	Between 1 and 7 days 8 days or more	33 22	3.75±1.86 3.95±1.80	t= -0.387 p>0.05
Ventilator Support	Present Absent	14 41	5.57±1.91 3.24±1.39	t=4.896 p=0.001
Pain	Present Absent	30 25	5.06±1.57 2.36±0.63	t=8.054 p=0.001
Discharge from ICU	Discharge/Referral Death	40 15	3.67±1.68 4.26±2.18	t= - 1.067 p>0.05

Nu-DESK: nursing delirium screening scale, *t test: independent sample t test, **F test: one way anova

the use of sedative drugs, which are commonly used in the treatment of agitation among the patients (26). The routine use of reliable delirium evaluation tools such as Nu-DESC by nurses may be beneficial in the early detection of delirium.

Advanced age is the leading risk factor for delirium (30). In the present study, a positive, weakly significant correlation was determined between age and the Nu-DESC mean scores. As the age progresses, The Nu-DESC mean scores of the patients also increase. The fact that patients in the ICU are older is important in terms of the risk of delirium. The majority (88.9%) of the intensive care patients in the current study were over the age of 40. Individuals are more likely to have serious illnesses with advancing age, and this may also result in a longer hospital stay.

Medical conditions such as sepsis, respiratory failure, metabolic imbalances are among the predisposing risk factors. The increased burden of comorbid disease further increases the risk (31). It was reported that adding delirium to chronic diseases would increase mortality (32). In the present study, although the Nu-DESC mean scores of those with chronic diseases were higher, a statistically significant difference was not found. The small number of patients included in the study may have affected the results.

In the current study, the Nu-DESC mean scores of patients with respiratory distress and ventilator support were found to be significantly higher. Delirium may have caused longer mechanical ventilation and adversely affected the patients. In the literature, it has been reported that critical patients requiring mechanical ventilation are at high risk for delirium (33), and approximately 80-87% of patients have delirium (1,34). The results of the present study are in harmony with the studies.

More than half of the patients in the ICU were postoperative. In addition, the Nu-DESC mean scores of the patients experiencing pain were found to be very significantly high. Increased pain levels have been shown to increase delirium due to increased stress response, especially in the postoperative setting (31). The result of the current study supports the literature.

The presence of delirium is also a sign of poor prognosis (26) and an independent predictor of mortality (35). It has been estimated that the mortality rates of patients with delirium during hospitalization are 25-75% during their hospitalization (26). In the present study, the mortality rate was 27.3%. Although there was no statistical significance, the Nu-DESC mean scores of the patients were higher than those who were discharged. Our finding is consistent with studies reporting no relationship between delirium and mortality (1,36).

Prevention of delirium is possible by non-pharmacological means (e.g. orientation, landscaping) (28,37). Current guidelines recommend routine screening for delirium in all ICU patients. Within 24 hours of ICU admission, it is recommended to evaluate individuals at risk for clinical factors contributing to the occurrence of delirium and provide an intervention tailored to the individual needs of patients and the setting of care based on the results of the evaluation (38). Nursing practices can be effective in preventing delirium and shortening its duration in intensive care. The most important aspect of patient-centered care is preventing delirium (35). In a meta-analysis by Liang et al. (39), studies involving delirium prevention interventions among intensive care patients were examined, and physical environment regulation, daily observation of patients in terms of behavioral changes and fluctuations, and evaluation with approved measurement tools (38) were found to be effective in preventing delirium (39). Similarly, according to the Guidelines of the Society of Critical Care Medicine (SCCM), updated in 2018, and the Evidence and Opinion-Based Guidelines for the Management of Analgesia, Sedation, and Delirium in Intensive Care (2015), applications such as providing physiological support and person-space-time orientation, early mobilization, cognitive stimulation, reducing noise and light, establishing sleep patterns, and visitations in the ICU reduce the risk of delirium, the length of stay in the hospital, and increase the patients' satisfaction (40,41). At this point, nurses should know and apply current methods that may minimize the risk of delirium.

Limitations

The fact that the study was conducted in a single ICU and the number of patients was low caused limitations in determining the risk factors. In addition, the fact that the drugs taken by the patients (especially sedatives and steroids) were not recorded in the study prevented us from revealing the dose relationships and the correlation between the excessive use of these drugs and the risk of delirium.

CONCLUSION

In the present study, delirium and risk factors that may cause delirium were investigated by using the Nu-DESC, which was accepted as a delirium screening scale for intensive care patients. Of the 55 patients included in the study, 54 demonstrated delirium symptoms during the ICU. Patients who develop delirium after admission to the ICU may present different characteristics from the patients who already have delirium at the time of admission to the ICU. Considering that the cause of delirium is multifactorial, non-pharmacological

environmental interventions such as early mobilization, regular sleep, effective treatment of pain, and regulation of noise and light may be promising for the prevention of delirium. In the current study, advanced age, mechanical ventilator, isolation, and presence of pain were determined as risk factors for delirium. It was concluded that the identification and follow-up of patients with risk factors for delirium by the nurses working in the ICU would contribute to the early diagnosis and treatment of delirium.

The results of the study revealed the necessity of using measurement tools for the early detection of delirium in clinical practice by nurses. Enabling nurses to evaluate delirium with scales in order to increase the quality of care for patients may be effective in preventing complications that may develop. We believe that it would be useful to add standard rating scales to daily routine evaluation forms in order to record delirium systematically. In addition, this study may shed light on future studies on delirium nursing.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziantep University Clinical Researches Ethics Committee (Date: 07.02.2022, Decision No: 2022/03).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Can tubal reversal be an alternative to IVF? Cohort study

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ABSTRACT

Introduction: Tubal reversal is the surgery done after tubal sterilization. Mostly, sterilization at a younger age or a new partner makes women request tubal reanastomosis. In the literature, pregnancy and ectopic pregnancy rates after tubal reversal is about 65% and 5.6%, respectively.

Material and Method: In our study, data from the files of patients who had tubal reversal operations between 2015-2021 years in Sanliurfa Training and Research Hospital were collected retrospectively. Demographic features, surgical and pregnancy outcome data of patients were collected. This study investigated the pregnancy rates and associated factors with pregnancy rates after tubal reanastomosis operations.

Results: In our study, 112 patients with tubal reversal operations were recorded. 25 out of 112 patients had spontaneous pregnancy after the tubal reversal operation. Age at a tubal reversal was a significantly important factor between a pregnant and non-pregnant group. According to age, below 40 years seems an ideal age factor for pregnancy. In our study, pregnancy rates were lower than in the literature.

Conclusion: Tubal reversal operation can be an alternative to IVF below 40 years of age.

Keywords: Pregnancy, sterilization, tubal reversal

INTRODUCTION

According to the authority of World Health Organization data, the percentage of tubal sterilization according to various countries changed and is 1% for Japan, 18.7% for the UK(1). Sterilization at a younger age, a relationship with a new partner, and lower socioeconomic status are the main reasons for tubal reversal requests.

Tubal reversal operations may be performed by laparotomy, laparoscopy, or robotic approach. However, there are patients who are considering direct IVF treatment if tubal reanastomosis fails or for fear of ectopic pregnancy. In the literature; reported pregnancy rates vary between 57% to 84%, and the associated risk for ectopic pregnancy is 2%–7% (2). IVF treatment is expensive as if the patient has health insurance, she will not pay for this surgery with tubal factor indication, but if there is no health insurance, the price of tubal reversal is about 500 dollars, while IVF treatment is around 2000 dollars, but the cost-effectiveness of tubal reversal surgery is controversial.

This study investigated the pregnancy rates and associated factors with pregnancy rates after tubal reanastomosis operations, and to determine at which patient can be selected for tubal renastomosis instead of IVF treatment by the success of the surgical procedure.

MATERIAL AND METHOD

The study was carried out with the permission of Harran University Clinical Researches Ethics Committee (Date: 07.06.2021, Decision No: HRU/21.11.13). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Data for the study is collected from the files of patients who had tubal reversal operations between 2015-2021 years in Şanlıurfa Training and Research Hospital, retrospectively. In addition, patients' demographic features and surgical and pregnancy outcome data were collected. Because of the standardization of the tubal ligation technique, only the Pomeroy technique during cesarean section were selected for the study. A detailed evaluation of the fertility potential of each woman who requested tubal reanastomosis was evaluated before tubal reversal operations. Semen analysis of partners of all patients was also assessed to exclude male factor infertility. Patients

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with organic pathologies associated with female factor infertility, like myoma uteri, unexplained ovarian masses, adenomyosis, and endometriosis, have been excluded from the study. All tubal reversal operations have been performed laparoscopically.

Surgical Procedure

Under general anesthesia, the patient was prepared in the lithotomy position. A uterine manipulator was inserted for chromopertubation, a Verres needle was inserted from an infraumbilical incision, and pneumoperitoneum was created. After an adequate pneumoperitoneum for surgery, 12 mm trocar was inserted from the umbilical entrance after the output of the Verres needle. Two separate 5 mm trocars were inserted from the entrances from bilateral 2-3 cm medial incisions of anterior iliac spines. If necessary, an extra trocar was inserted from the midclavicular line, 2-3cm below the umbilicus level. A chromopertubation was created to see the ligated parts of tubes in detail, and residual tubal length was measured. The previously damaged parts of tubes were cut by laparoscopic scissors, and healthy tubal luminal tissues were expected to be seen in detail. A flexible 2 mm catheter (18G epidural catheter) was inserted from the fimbria and moved through the cornual side to perform a fixed tube sutured. For a proper alignment, reanastomosis was achieved by suturing four separate sutures at 6, 3, 9, and 12 o'clock positions by absorbable 5-0 monofilament sutures (**Figure 1**). The tubal length formed after tubal renastomosis (between cornual side and fimbria) was measured with a sterile paper meter inserted through a 10-gauge trocar. Tubal patency of both tubas was evaluated by methylene blue injection (**Figure 2**). All the surgeries were performed by the same surgeon (Esercan A). In the first month after the operation, tubal patency control was performed with hysterosalpingography in all patients, and at least one tubal passage was considered successful in the process. All the patients were discharged from the hospital on the next day of surgery.

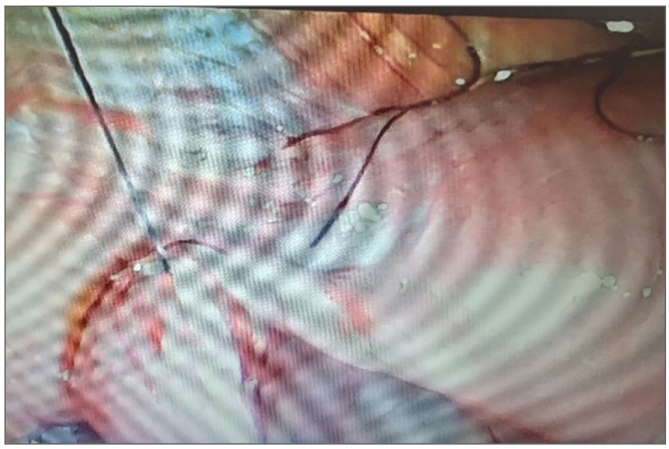

Figure 1. Proper alignment of reanastomosis was achieved by suturing separate sutures

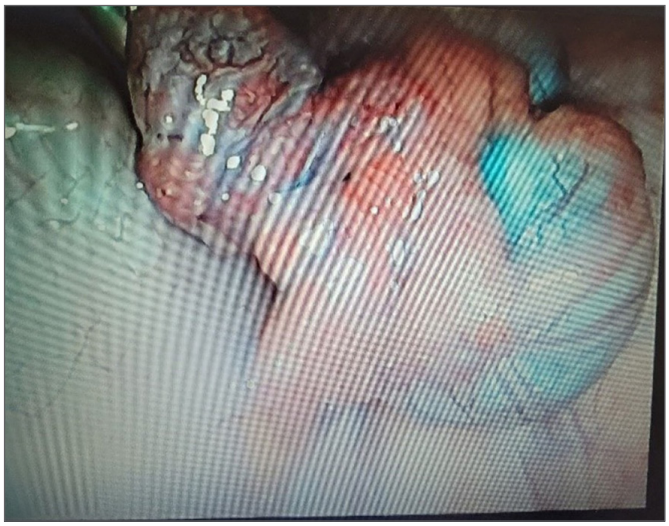

Figure 2. Control of tubal patency by methylene blue injection (methylene blue flux on the fimbria)

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS.22, IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., Armonk, NY, USA). The -Kolmogorov-Smirnov test was used to verify the normality of the distribution. Mean, or median values were used to describe the data characteristics for normal distribution. Categorical data were presented as percentages. Chi-square, and Fisher's exact tests were used to analyze categorical data. The t-test was used to calculate two independent means, and the significance level for all tests was defined as $p < 0.05$.

RESULTS

Between 2015-2021 years, 160 women had tubal anastomosis operation. 112 women out of 160 women with available data met the inclusion criteria were included in the study. In the first month after the operation, tubal patency control was performed with hysterosalpingography in all patients, and at least one tubal passage was considered successful in the process. Tubal methylene blue flow was seen in both tubes in the surgery at the final but at postoperative period at least one tubal passage was considered enough so all of the patients were included for the pregnancy follow-up. The mean age of women at tubal ligation was 33.69± 0.51(20-42) years. Mean gravida was 5 (3-10). The mean cesarean number at tubal ligation was 3(1-5). The mean FSH test level at a tubal reversal was 8.21± 0.94(1,82-15). The mean age at a tubal reversal was 36.5± 0.53 (22-46) years. The mean time between tubal ligation and reversal was 2.86±0.16(1-9) years. These data were given in **Table 1**. The operation time ranged from 90 to 160 minutes, and the mean operation time was 120 minutes. No postoperative surgical complication has been experienced.

Table 1. Means and standard deviations for study variables

Studyvariables	Pregnant group (n=25)		Non-pregnant group(n=87)	
	M	SD	M	SD
Age at tubal ligation	31.48	0.97	34.37	0.57
Age at tubal reversal	34.08	1.05	37.19	0.60
FSH level	5.64	0.55	5.72	0.84
Number of cesareans	2.56	0.19	2.80	0.23
Gravida	5.4	0.27	5.2	0.61
Residual tubal length (cm)	5.6	0.34	5.2	0.45

25 out of 112 (22%) women had spontaneous pregnancies after tubal reversal operations. After tubal reversal operations, the meantime to have pregnancy after tubal reversal operations was 22.72±3.16(6-60) months. Of these pregnancies, one woman (1/22 pregnancy) had an ectopic pregnancy and could be treated successfully medically without the need for surgery.

In our study, according to age groups; pregnancy rates were 30%, 29%, and 2% in the 22-34 age, 35-39 age, and 40-46 age groups, respectively (**Figure 3**). There was no significant difference in FSH levels, cesarean number and gravida between pregnant and non-pregnant group. There was a significant difference and decline in pregnancy rates after tubal reversal after age 40 ($p<0.05$).

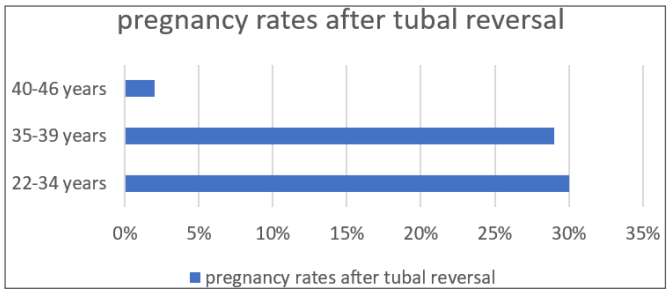

Figure 3. Pregnancy rates according to age groups after tubal reversal

DISCUSSION

No statistical difference was reported for tubal reversal by Elci et al. (3) between laparoscopy, laparotomy, and robotic surgery groups in terms of pregnancy rates; in their study, Gomel et al. (4) reported that; age at the time of tubal reversal was the most critical factor in the outcome of tubal reanastomosis Women who were younger than 35 years of age at the time of reversal can anticipate an intrauterine pregnancy rate of greater than 70% . In these women, most pregnancies have occurred within the 18 months after surgery. Our study showed no difference between pregnancy and non-pregnancy groups according to residual tubal length($p>0.05$). However, some studies have reported that the residual tubal length is effective in the operation's success. The remaining total tubal length > 4 cm appears to be one of the positive prognostic factors of tubal reanastomosis success. Because the total length of the fallopian tube is 11-12 cm, and 2/3 of the total length is the ampulla of the fallopian tube, this 4 cm definition seems to be associated with this. According to a systematic review of 15 studies, pregnancy rates after laparoscopic reversal ranges from 25% to 83%, with a pooled pregnancy rate of 65% (95%CI: 61%-74%). The mean pooled ectopic pregnancy rate was 5.6% (95%CI: 3%-9%)(5). In our study, according to age groups, in the 22-34 age group, 35-39 age group, and 40-46 age group, the pregnancy rate was 30%, 29%, and 2%, respectively. There was a significant difference and decline in pregnancy rates after age 40($p<0.05$).

According to the American Society for Reproductive Medicine committee opinion in 2015, pregnancy rates are not suitable compared with IVF because success is defined as pregnancy rates per patient in surgery. In contrast, IVF success rates are defined per cycle. The tubal reversal has significantly higher cumulative pregnancy rates than IVF, and it has more advantages without considering complications of multiple pregnancies and ovarian hyperstimulation syndrome (6).

Same as our study results; Messinger et al. (7) reported that tubal reversal surgery is more cost-effective in patients younger than 41 years. At the same time, IVF is more cost-effective in patients older than age 41. Van De Water et al. (8) in 2015 published an excellent series of 88 patients in favor of laparoscopic reversal with a pregnancy rate of 73% for women < 40 years.

In the literature, 37 seems to be an essential criterion. According to Boeckxstaens et al. (9) cumulative pregnancy rates are higher for tubal reversal in patients below 37 years old and higher for IVF in patients over 37, even though Godin et al. (10) did not reach a statistical difference.

Although, patients who had organic factors were excluded from the study; in our study, pregnancy rates were lower than in the literature. Our residual tubal length is about 5 centimeters it may cause lower pregnancy rates.

The one-stitch technique (at 12 o'clock), two-stitch technique (at 6 and 12 o'clock), three-stitch technique, and four-stitch technique (at 3, 6, 9, and 12 o'clock) were reported for tubal anastomosis. We used a single-layer surgical technique. To keep firm alignation and maintain blood flow, the four-stitch technique was considered the most reasonable method. In our opinion, too many or too few sutures would not be optimal, and we, therefore, used the four-stitch technique.

Our study has some limitations. First, this is a retrospective design, and the results may be affected due to retrospective design. Also, we have only included the patients who have undergone tubal sterilization by the

Pomeroy technique in the study. The patients that have undergone tubal sterilization by other techniques were omitted. However, the primary strength of our study is that all tubal reversal operations have been performed by the same physician that, excludes the surgical experience factor of the surgeon.

CONCLUSION

The most important factor of pregnancy success rate is age; especially less than 40 years old.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Harran University Clinical Researches Ethics Committee (Date: 07.06.2021, Decision No: HRU/21.11.13).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Alexithymia; an often missed condition prevelant in the practice of nephrology

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ABSTRACT

Aim: Alexithymia is a disorder associated with difficulty in recognizing and expressing feelings, which can lead to an increased susceptibility to disease development that makes alexithymia a possible risk factor for chronic kidney disease (CKD). This study was performed to evaluate for alexithymia in pre-dialysis CKD and end-stage renal disease patients undergoing hemodialysis.

Material and Method: The study was conducted in the Nephrology Outpatient Department and Hemodialysis unit of Ümraniye Research and Training Hospital. The patients had to have a diagnosis of CKD. Demographic data form, routine biochemical follow up data of patients, Toronto Alexithymia Scale (TAS), and Hospital Depression and Anxiety Scale (HADS) were used in this study. The statistical significance level was determined as $p<0.05$.

Results: A total of 111 patients (mean age 58.10±13.48 years, 61% female) were included in the study, 83.9% of whom were found to have alexithymia. The incidence of alexithymia in CDK patients was significantly higher than that in the control group ($p=0.004$); however, no statistically significant difference was detected in its incidence between the hemodialysis and pre-dialysis groups ($p>0.05$). The results showed that increased incidence of depression resulted in a 4.47-fold ($p=0.035$) increase in the incidence of alexithymia.

Conclusion: Alexithymia has been found to be high in patients with chronic kidney disease. Therefore, it is essential that we be aware of patients' emotional stress and assess them for depression, anxiety, and alexithymia.

Keywords: Alexithymia, chronic kidney disease, hemodialysis

INTRODUCTION

Chronic kidney disease (CKD) is defined as a state of decreased glomerular function with creatinine clearance lower than 60 mL/min/1.73 m2, which is ongoing for at least 3 months. (1). Chronic hemodialysis is initiated in stage 5 CKD, when the glomerular filtration rate (GFR) is under 10 mL/min, taking other clinical indicators into account, such as presence of hypervolemia, hyperkalemia, metabolic acidosis, and patient's comorbidities (2). Prolonged inactivity, dependence on the dialysis machines, continuous medication, intensive diet, and fluid restriction cause physical and physiological pathologies in these patients (3). Alexithymia is a concept that describes the inability to differentiate between bodily sensations and emotions as well as difficulty in describing emotions The main characteristics of this disorder are dysfunction in social attachment, emotional awareness and relationship with other people.In additon people with alexithymia have difficulty in appreciating the emotions of other people and they seem to be unemphatic.. Alexithymia may be neurological, which may be caused by disruptions in the neuronal pathways that process emotions, and can also develop as a self defense measure against stressful situations such as terminal illness, which is then called psychological alexithymia (4). Severe alexithymia is a predisposing factor for many psychiatric and somatic disorders (5), and studies have shown that there is a high incidence of alexithymia in patients undergoing hemodialysis (4-6). In addition, it has been shown that higher psychological distress contributes to increased comorbidity and earlier mortality in patients with CKD (7).

This study aimed to evaluate the prevalence of alexithymia and its relation with depression and anxiety in CKD patients in the predialysis stage as well as in those receiving hemodialysis.

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MATERIAL AND METHOD

The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.04.2022, Decision No: B.10.1.TKH.4.34. H.GPO.0.01/156). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 111 participants, including CKD patients undergoing hemodialysis (n:72) and patients in pre-dialysis stage of CKD (n:39), were included in this study, and all were informed about data privacy and provided written informed consent. The patients who were in pre-hemodialysis stage randomly selected among those who came to the outpatient clinic within 2 months. The inclusion criterias were; 1) ability to understand and speak Turkish, 2) age between 18 and 75 years, 3) patients receiving hemodialysis for at least 3 months and patients with diagnosis of CKD and a GFR of <60 ml/min/day, 4) signed informed consent for the study. Exclusion criterias were; 1) use of anti-stress and anxiety medications 2) a diagnosis of any mental disorder. 33 subjects between the ages of 18-65 without a psychiatric or physiological disorder were included in the control group.

This study lasted for 8 weeks, during which blood samples were obtained from the pre-dialysis patients in the morning after an overnight fast of at least 12 hours. In the hemodialysis patients, blood samples were taken before and after hemodialysis, while the urea, creatinine, sodium, potassium, c-reactive protein, albumin, ferritin, and hemoglobin levels of all patients were also measured. In addition, Kt/v and URR values were noted for the hemodialysis patients. The patients' demographic data were obtained using a data collection form prepared by the researchers, requesting for patients' age, gender, duration of dialysis/disease, etiology of CKD, education status, and marital status. The patients were asked to complete two questionnaires: 1) the Toronto Alexithymia Scale (TAS-20) and 2) the Hospital Anxiety and Depression Scale (HADS). Patients undergoing hemodialysis filled out the forms during hemodialysis sessions. Patients in pre-dialysis stage forms were given to them to complete and return. Finally the patients were divided into two groups according tothe development of alexithymia and the datas were compared according to these groups.

The HADS is a 14-item self-report questionnaire that includes an anxiety (HADS-A) and a depression score (HADS-D) for assessing symptoms of anxiety and depression. HADS-A and HADS-D can be combined to give HADS-total, and the use of HADS has been confirmed for evaluating patients between the ages of 18 and 79 years with an appropriate average internal consistency for the depression (Cronbach's α=.75) and anxiety (Cronbach's α=.80) subscales. HADS-A (anxiety) and HADS-D (depression) were included as continuous sum scores in the regression models (7).

TAS-20 is a scale that investigates alexithymia. It is a Likert-type self-assessment scale that consists of three subscales with a total of twenty items. The subscale for difficulty recognizing emotions (TAS-1) is defined as difficulty identifying emotions and differentiating them from bodily sensations; the subscale for difficulty expressing feelings (TAS-2) is defined as hardness of transmitting emotions to others; while the subscale for extroverted thinking (TAS-3) is defined as the disabilty of introverted thinking and imagination power. High scores indicate a high level of alexithymia, while the cutoff value for TAS-20 is 51 or less, with 52–60 indicating possible alexithymia and a score of >61 confirming alexithymia (8).

Statistical Package for Social Science (IBM SPSS Statistics New York, USA) version 20.0 was used to perform statistical analyses. Descriptive statistics are reported as means±SDs for continuous variables and as number and frequencies for binary and categorical variables. The normality of continuous variables was investigated using the Shapiro-Wilk test. Descriptive statistics are presented as mean and standard derivation for normally distributed variables and median (and minimum maximum) for non-normally distributed groups. Student t test was used. Non -parametric statistical methods were used for values with skewed distribution the comparison of 2 non-normally distributed groups, the Mann-Whitney U test was used. The Chi-square (x2) test was used for categorical variables and expressed as observation counts (and percentages). The logistic regression was used to analyze the alexithymia in chronic kidney disease. Variables were entered into a univariate (UV) logistic regression model. In addition, parameters that we thought would be significant such as age, gender, disease duration, anxiety and depression were included in the model. A multivariate (MV) logistic regression model was then constructed using an enter procedure among those candidate variables with the significance level p. p<0,05 was set as statistical significance.

RESULTS

A total of 111 patients (mean age 58.10±13.48 years, 61% female) were included in the study, 72 (64.9%) of whom were CKD patients on hemodialysis and the remaining 39 CKD patients in the pre-dialysis stage. The control group included 33 subjects.

Table 1 represents the demographic and clinical data of the overall study population and the pre-dialysis (PG) and hemodialysis subgroups (HG).

Table 1. Demographic and clinical data

	Pre-dialysis group	Hemodialysis group	p value	Overall
	(n:39) Median, range	(n:72) Median, range		(n=111) Median, range
Female/Male (n)	17/22	34/38	0.512	51/60
Age (years)	56.77±2.182 (19-77)	58.82±1.584 (20-75)	0.496	55,27±2.085 (35-82)
Disease duration (month)	71.33±5.590 (12-154)	68.80±9.200 (4-360)	0.424	69.71±64.93 (4-360)
CKD ethology				
Hypertension (n)	10	33	0.037	43
Diabetes Mellitus (n)	12	19	0.623	30
Glomerulonephritis (n)	2	8	0.293	10
Polycystic kidney disease (n)	1	1	0.657	2
Others (n)	11	10	0,066	21
Literacy (n)	35	66	0.735	101
Education (n)			<0.024	
Illiterate (n)	5	6		11
Primary education (n)	9	38		47
Middle school (n)	12	10		22
High school (n)	9	9		18
University (n)	4	9		13
Alexithymia (n)	35	58	0.210	93
Possible alexithymia	16	29		45
Alexithymia	19	29		48
Total alexithymia score	60.82±1.295 (38-76)	58.26±0.981 (36-75)	0.118	53.00±10.592 (27-73)
Depression (n)	18	30	0.693	48
Depression score	7.54±0.698 (0-17)	6.80±0.511 (0-17)	0.443	7.09±0.710 (1-16)
Anxiety (n)	17	25	0.387	42
Anxiety score	7.51±0.868 (0-19)	6.21±0.522 (0-19)	0.234	6.70±0.716 (1-17)

P<0.05, CKD: Chronic kidney disease

The mean age of patients was 55.27±2.085 years. There was no significant difference between PG and HG according to mean ages (p: =.496)

Hypertension (43) and diabetes (30) were the most common etiology of chronic kidney disease in our patients.

Mean duration of hemodialysis of patients was 69.71±64.93 months. There wasn't statistically difference between two groups according to hemodialysis duration (p: 0.424).

Up to 83.9% of the patients were found to have alexithymia, while 60.6% of the control group had alexithymia. The mean TAS score of the patients was 57.75±9.209, average depression score according to HADS was 7.07±4.234, while anxiety score was 6.68±4.638. The prevalence of alexithymia in CDK patients was significantly higher than that in the control group (p=0.004), and comparing the pre-dialysis and control groups, the prevalence incidence of alexithymia was determined to be higher in the former than that in the control group (p=0.004). The prevalence of alexithymia in the HG was also found to be higher than the control group (p=0.030). However, no statistically significant difference in the prevalence of alexithymia was detected between the HG and PG groups (p>0.05). Furthermore, there was no statistically significant difference between the TAS scores of the HG and those of the PG patients (p=0.118).

Table 2 shows the comparisons between the patients have alexithymia (AG) and patients don't have alexithymia (NAG). No difference was detected between the educational statuses of AG and NAG (p>0.05).

The TAS results of 40.45% of the CKD patients indicated possible alexithymia, while 43.45% of the patients were confirmed to have alexithymia.

Table 3 shows the logistic regression analysis made to predict alexithymia in CKD patients. The results show that increased prevalence of depression resulted in a 4.47-fold increase in the prevalence of alexithymia (p=0.035). However, no difference in the prevalence of alexithymia was detected based on the patients' age, sex, duration of disease, and treatment type (conservative or hemodialysis).

Table 2. Correlation between alexithymia and non-alexithymia group

	Alexithymia Group	Non alexithymia Group	p value
	(n:93)	(n:18)	0.040
Female/Male (n)	44/49	7/11	0.512
Age (years) (Median,Range)	58.12±12.49 (19-82)	55.00±15.34 (20-77)	0.496
Disease duration (month) (Median, Range)	72.78±68.91 (5-360)	54.17±36.55 (4-120)	0.424
Etiology			
Hypertension (n)	37	6	0.584
Diabetes Mellitus (n)	26	4	0.599
Glomerulonephritis (n)	8	2	0.744
Polycystic kidney disease (n)	1	1	0.194
Others (n)	16	5	0.305
Depression	45	3	0.012
Anxiety	39	3	0.040
Hemodialysis group (n:72)			
Kt/V (n)	42	8	0.266
URR (n)	48	12	0.790
Hemoglobin (g/dl)	10.47±1.23	11.34±1.28	0.049
Albumin	3.98±3.57	4.07±3.67	0.630
C-reactive protein (mg/L)	13.32±19.35	7.24±8.74	0.757

P<0.05 URR: Urea reduction ratio

Table 3. Logistic regression analysis for factors associated with alexithymia, Model-B

	B	S.E.	Wald	Exp (B)	Model B %95 CI		P
					Lower	Upper	
Gender	-0.321	0.626	0.262	0.726	0.213	2.477	0.609
Age	0.027	0.020	1.865	1.028	0.988	1.069	0.172
Duration of disease	0.005	0.006	0.738	1.005	0.994	1.016	0.390
Depression	1.499	0.713	4.423	4.477	1.107	18.101	0.035
Anxiety	1.104	0.760	2.108	3.015	0.680	13.378	0.147
Group	-0.824	0.650	1.608	0.439	0,439	0.123	0.205

Nagelkerke Model B: 0.218

DISCUSSION

In this study, we found that patients with CKD had significantly higher scores for alexithymia, depression, and anxiety than those in the control group, and there was no statistically significant difference in terms of alexithymia development between the patients on hemodialysis and patients undergoing conservative therapy. Furthermore, no link was obsreved between the age of the patients and development of alexithymia. The results have also shown that depression was increased in CKD patients with alexithymia.

According to several studies prevelance of alexithymia is between 10%-23% in geneal population (9-11). In a study conducted with 300 healthy individuals, most of whom (77%) were university students and graduates in our country, it was reported that 20% of individuals showed alexithymic symptoms (12).

In our study, we found the prevalence of alexithymia in control group to be significantly higher than these studies. We think that this difference is related to the education levels, ethnic origins and socioeconomic conditions of the population participating in the studies and the time intervals in which the studies were conducted.

The prevalence of emotional disorders is higher in CKD patients compared to the general public due to restricted diet and physical activity, intensive medical treatments, and fear of death, and the emotional disorders and stress CKD patients suffer are major contributing factors to the development of alexithymia. This study has found that 13–83% of CKD patients undergoing hemodialysis suffer from alexithymia (13). These results are consistent with those from previous literature such as the study by Jardonova et al. (4) in which it was found that half of the patients on dialysis manifested alexithymia.

Lai et al. (14) reported that pre-dialysis CKD patients had a higher prevelance of alexithymia than that of dialysis patients, although the difference was not statistically significant, and was predicted to be due to the fact that the pre-dialysis patients did not want to face their feelings about being chronically ill as an escape mechanism, meanwhile, the dialysis patients could not ignore the reality of the situation, since they received invasive treatments. Similarly, the results from this study show that the pre-dialysis CKD patients had a higher prevelance of alexithymia than the control group; however, no statistically significant difference was observed in the prevelance of alexithymia between the pre-dialysis and hemodialysis patient groups.

Advanced age is related to higher grades of alexithymia in the general population, and a potential reason for the brain's frontal lobe's functional disruption.12 Individuals over 65 years with no comorbidities or signs of dementia show significantly higher prevelance of alexithymia compared to younger individuals (15), and several studies have established a higher prevalence of alexithymia in HD patients above the age of 60 (16). Similar to the findings from this study, Pojatic et al. (17) did not observe a correlation between the age of hemodialysis patients and development of alexithymia. Therefore, it is possible that patients undergoing chronic hemodialysis develophigh levels of alexithymia through other mechanisms, and alexithymia serves as a defense mechanism against physical stressors (18).

Although some studies have directly correlated the duration of dialysis with alexithymia score (19), we found no significant relationship between duration of dialysis and duration of disease and development of alexithymia, perhaps because the sample size was small and mean age of patients was <60 years.

Furthermore, anxiety and depression are seen in patients with chronic illnesses, and depression among CKD patients, including those not on hemodialysis, may cause rapid decline in physical health, resulting in adverse outcome (20). Anxiety in pre-dialysis CKD patients is associated with poor clinical outcome such as depression (21), and in the study by Duan et al. (22), and Wang et al. (23) no significant difference in depression traits across CKD stages was reported, whereas other studies reported that CKD patients in stages 4 and 5 were more likely to have depression than those in the early stages. From the present study, no significant differences in the development of anxiety and depression was observed between the pre-dialysis and hemodialysis groups. However, the patients who were alexithymic had increased depression and anxiety scores.

Only depression was determined as a risk factor for alexithymia in the multivariable regression analysis, and whereas older studies have reported negative correlations between depression and alexithymia, more recent studies that used the TAS-20 found positive correlations similar to those from this study (24). This difference may be due to the fact that the older studies utilized the MMPI alexithymia scale, which lacks certain psychometrics that TAS-20 includes.

The results showed a negative correlation between hemoglobin levels and alexithymia scores of CKD patients, and no correlations were detected between the development of alexithymia and high CRP, ferritin, and albumin levels, all of which increase morbidity in hemodialysis patients. Meanwhile, a statistically significant negative correlation was found between development of alexithymia and hemoglobin levels among the hemodialysis patients. Although these findings were statistically significant, it does not suffice to claim that low hemoglobin levels increase the prevelance of alexithymia. A study conducted by Aktyz et al. (6) on 51 hemodialysis patients reported no correlation between hemoglobin levels and alexithymia development. Therefore, the relationship between alexithymia and hemoglobin levels in CKD patients should be further investigated using larger sample sizes for a bmore accurate claim.

One of the limitations of this study is the fact that it was not multicentric, and the pre-dialysis patients were not categorized into subgroups based on their CKD stageing. Exclusion of peritoneal dialysis and renal transplant patients are also some of the limitations that should be mentioned.

Although the incidence alexithymia has been previously studied in CKD patients on hemodialysis, only few studies have been conducted on the prevelance of alexithymia in pre-dialysis CKD patients. Therefore, the results from the present study are highly valuable regardless of the limitations of the study. Furthermore, the fact that the pre-dialysis CKD patients also had increased alexithymia scores is of importance, since this affects the emotional state, treatment compliance, and disease progression of these patients.

CONCLUSION

The study results showed that the prevelance of alexithymia in CKD patients is higher than that in the control group; however, no difference in the prevelance of alexithymia was observed between hemodialysis and conservative treatments for CKD patients. Also, it was found that depression increased the prevelance of alexithymia in CKD patients, and that the prevelance of alexithymia also increased when the patients had a GFR of <60mL/min/day.

The overall results of this study indicate that healthcare workers should be wary of patients' emotional states alongside their physical states while under their care, and that patients with chronic diseases such as CKD especially require close attention due to their increased depression, anxiety as well as incidence of alexithymia.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ümraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 21.04.2022, Decision No: B.10.1.TKH.4.34. H.GPO.0.01/156).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Prognosis prediction of the mean tracheal air column area in COVID-19 patients

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ABSTRACT

Aim: SARS-CoV-2 infection frequently affects the lungs, it can also cause severe inflammation in the lower respiratory tract, leading to tracheal damage. We aimed to investigate the relationship between the mean tracheal air column and COVID-19.

Material and Method: Chest computed tomography scans of COVID-19 patients treated in an intensive care unit between June 1st, 2020 and October 1st 2022 were retrospectively evaluated. The air column area of the trachea was measured and the effect of the values obtained on mortality and length of stay in the intensive care unit for patients COVID-19 was examined.

Results: We found that an increase in the mean tracheal air column increased mortality by 1.218 times. We also determined that an increase in the mean area of the tracheal air column increased the length of stay in the intensive care unit. Furthermore, we showed that advanced age and an increase in the length of stay in the intensive care unit were factors that increased mortality.

Conclusion: Tracheomegaly is a poor prognostic factor in COVID-19 disease and is easily diagnosed with CT.

Keywords: COVID-19, computed tomography, trachea, prognosis

INTRODUCTION

COVID-19 is caused by the SARS-CoV-2 virus and mostly affects the respiratory system (1). This agent may be asymptomatic in patients or may cause mild, moderate,or severe infection, leading to acute respiratory syndrome and multisystem organ failure (2,3). The diagnosis of COVID-19 is made by real-time-reverse transcription polymerase chain reaction (RTR-PCR) amplification of a nasopharyngeal swab taken from individuals witha suspected disease (4). This virus binds to the angiotensin-converting enzyme 2 receptor, which is found specifically in the epithelium of the lungs, distal airways, and trachea. Therefore, COVID-19 is expected to cause more damage to these organs (5). Studies have shown that the SARS-CoV-2 virus can cause severe inflammation in the trachea (6).

Tracheomegaly is a rare and often missed diagnosis, and it is diagnosed when its diameter exceeds the upper limit in both males and females (7, 8). Chest radiography, computed tomography, and bronchoscopy methods are used for the diagnosis of tracheomegaly (9). Among these methods, computed tomography is the best noninvasive method that can show tracheal changes and is considered the gold standard (10). Tracheomegaly is frequently congenital but may also occur in connective tissue diseases, because of prolonged inflammation, and because of smoking or the use of mechanical ventilation (11, 12). Several studies in the literature (6, 13-16) have examined characteristics of tracheomegaly and COVID-19, such as their relationship to each other, incidence rate, and management. However, as far as we are aware, there is no existing study examining the effect of tracheal dilatation on prognosis in COVID-19 patients by measuring the tracheal air column area. Therefore, we believe that this study will make important contributions to the literature.

MATERIAL AND METHOD

Patient Selection

In our study, designed as a cross-sectional observational analysis, a retrospective evaluation of patients treated for SARS-CoV-2 infections in an intensive care unit in our hospital between June 1st, 2020 and October 1st, 2022 was performed. This study was approved by Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 15.11.2022, Decision No: 2022/8-28).

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All procedures were carried out under the ethical rules and the principles of the Declaration of Helsinki. The effect of the mean tracheal air column area of the observed patients on prognosis of COVID-19 was examined. Patients over 18 years of age were included in the study; patients with positive RT-PCR for SARS-CoV-2 RNA and those whose test results showed compatibility with COVID-19 pneumonia on thorax CT imaging were also included in the study. Patients with negative RT-PCR for SARS-CoV-2 RNA and whose test results showed no compatibility with COVID on thorax CT examination were excluded from the study. Thoracic CT examinations comprised thoracic CT scans performed at the time of initial admission to the hospital. Patients with a history of intubation were excluded. Patients included in the study were those treated in the intensive care unit of our hospital. The criteria for treatment in the intensive care unit were based on criteria specified in the Republic of the Turkey Ministry of Health's COVID-19 adult patient treatment guideline (17).

Exclusion Criteria

- Chronic obstructive pulmonary disease,
- Tracheal compression lesion (thyroid nodule, enlarged lymph node, malignancy, vascular variations, and so on)
- Thoracic kyphoscoliosis
- Calcific trachea
- Not having been monitored properly (motion artifacts, images that do not include Level 1, and so on)
- Tracheas with dense secretions
- Intubated patients
- Poorly performed examinations

Imaging

All thorax CT images used in our study were performed using a 16-detector CT scanner (MX16, Philips Medical Systems, Koninklijke, The Netherlands). Images were acquired using parameters of 0.75's rotation time, 16 × 0.75-mm beam collimation, 1-mm slice thickness, 1-mm slice reconstructions, 90–120 kV tube voltage, and 50–110 mAs effective tube current. Thorax CT images were analyzed using the Oracle Database program, version 1.10.48.299, to measure the tracheal air column area. Axial and coronal reformat images were used for all measurements. To measure the mean tracheal air column area, two separate area measurements were made from the proximal and distal levels measured by Breysem et al. (18), and the mean value was subsequently obtained. For the proximal tracheal area, the tracheal air column area was measured at the cylindrical-shaped 1st tracheal level just below the larynx; for the distal tracheal area, the tracheal air column area was measured 6 mm above the tracheal bifurcation (Figure). The mean tracheal air column area was obtained by taking the arithmetic mean of the measured tracheal air area in both regions. All evaluations and measurements were performed by a radiology specialist (M.Ç.) with 12 years of experience in the field.

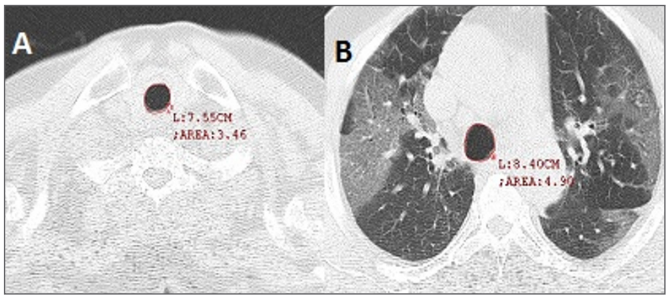

Figure. Axial thorax CT images of the measurements made from level 1 (A) and bifurcation superior (B) (mean tracheal air column area= (3.46+4.90)/2: 4.18 cm2).

Statistical Analysis

In the statistical analysis phase of the study, the effects of tracheal air column area on survival were examined. Frequency analysis of categorical variables and descriptive statistics of numerical variables were then performed. An independent samples t-test was used for independent group comparison tests, and a Pearson correlation test was applied to evaluate the direction and severity of the correlation between numerical measurements. The effect of the mean tracheal air column area on survival was examined in the survival analysis performed by applying the Cox regression model. The margin of error in the study was 5%, and the entire application was performed with the R-Project program (R Core Team, 2022) (19).

RESULTS

Among the patients included in the study, 59.9% were male and 40.1% were female. Approximately 63.6% of these patients died and 36.4% survived. The mean age of the patients was 64.44±12.26 years, the mean pneumonia severity score was 13.88±4.88, the mean length of stay in the intensive care unit (days) was 6.64±6.50, and the mean tracheal air column area was 2.76±0.79 (**Table 1**).

Table 1. Descriptive statistical values of the distribution of gender and living status of the patients and age, length of stay in the intensive care unit (days), and tracheal air column area variables.

Variable	n	%
Gender		
Male	290	59.9
Female	194	40.1
Living status		
No	308	63.6
Yes	176	36.4
	X±SD	**Median (min–max)**
Age	64.44±12.26	71.00 (27.00–95.00)
LSICU	6.64±6.50	4.00 (1.00–40.00)
MTACA	2.76±0.79	2.75 (1.26–5.50)

X±SD: mean±standard deviation, min: minimum, max: maximum, LSICU: length of stay in ICU (days), MTACA: mean tracheal air column area

Table 2. Comparison of each patient's age, length of stay in the intensive care unit (days), and mean tracheal air column area variables according to gender and living status.

Variable	Gender*				Living status*			
	Male	Female	t	p	No	Yes	t	p
Age	68.58±12.44	70.73±11.91	–1.897	0.058	71.14±11.48	66.48±13.04	4.085	<0.001
LSICU	6.21±5.50	7.28±7.74	–1.769	0.078	7.38±6.56	5.35±6.21	3.329	0.001
MTACA	3.13±0.69	2.21±0.60	15.135	<0.001	2.90±0.80	2.53±0.72	4.963	<0.001

*mean±standard deviation, LSICU: length of stay in ICU (days), MTACA: mean tracheal air column area

In **Table 2**, the t-test results of the age, length of stay in the intensive care unit (days), and average tracheal air column area variables of the patients participating in the study according to gender and living status are shown. It was observed that the variables related to age and length of stay in the intensive care unit (days) did not differ significantly according to gender ($p>0.05$). However, it was determined that the mean tracheal air column area of the patients differed significantly according to gender ($p<0.05$). The mean tracheal air column area of male patients was higher than that of female patients. It was determined that the variables of age, length of stay in the intensive care unit (days), and mean tracheal air column area of the patients differed significantly according to their living status ($p<0.05$). Age, length of stay in the intensive care unit (days), and the mean tracheal air column area values of the patients who survived were lower than those of the patients who died (**Table 2**).

In **Table 3**, the results of the Pearson correlation test showing the correlation between age, length of stay in the intensive care unit (days), and tracheal air column area variables of the patients included in the study are shown. It was determined that there was a low level of positive correlation between the length of stay in the intensive care unit (days) and age, respectively ($r=0.173$, $p<0.05$). There was a statistically significant positive correlation between the tracheal air column area and the length of stay in the intensive care unit ($p<0.05$) (**Table 3**).

Table 3. Correlation between age, length of stay in the intensive care unit (days), and mean tracheal air column area variables.

	1	2	3
Age	1		
LSICU	–0.119*	1	
MTACA	–0.012	0.001	1

*p<0.05; LSICU: length of stay in ICU (days); MTACA: mean tracheal air column area

The effects of the meantracheal air column area on survival time were statistically significant ($p<0.05$). A 1-unit increase in the mean tracheal air column area increases the probability of mortality by 1.218 (95% CI: 1.064–1.395) times (**Table 4**).

Table 4. Cox regression analysis performed on the effect of mean tracheal air column area on survival time

Variable	OR	95% CI		p
		Lower limit	Upper limit	
MTACA	1.218	1.064	1.395	0.004

OR: odds rate, CI: confidence interval

DISCUSSION

In this study, we revealed that an increase in the mean tracheal air column area increases mortality and length of stay in the intensive care unit in COVID-19 patients. We showed every 1unit increase in the mean tracheal air column increased mortality by 1.218 times. Our study showed that tracheal air column width is a poor prognostic factor in COVID-19 disease.

As far as we are aware, there is no existing study in the literature examining the prognosis prediction in COVID-19 patients by measuring the mean tracheal air column area. In a study conducted by Ünlü et al. (13), the anterior–posterior and transverse diameters of the trachea were measured and their effect on prognosis in patients with COVID-19 was examined. The tracheal diameter measurement method used in this study only considers the measurement of the length of the trachea in the linear direction in the axial section. Therefore, we believe it would be more accurate to examine the effect of tracheal dilation on prognosis by measuring the mean air column area. A study conducted by Ünlü et al. (13) similarly revealed that an increase in mean tracheal diameters resulted in poor prognosis in COVID-19 patients. However, our study also showed that the mean tracheal air column area increased the length of stay in an intensive care unit. Therefore, the present study is more comprehensive one compared to other studies because of its method and results.

Oliver et al. (20) revealed in their work that COVID-19 causes inflammation in the bronchi, trachea, and larynx, as well as in the lungs, causing damage to these tissues. Tissue destruction resulting from inflammation caused by the cytokine storm leads to organ deterioration (21). The membranous posterior part of the trachea, which is continuous with the cartilage component forming its anterior part, allows dilatation in the tracheal air column

area because of tissue damage caused by inflammation (22). Therefore, we think that the dilatation of the tracheal air column area is directly proportional to the severity of inflammation in the lower respiratory tract as a result of COVID-19. According to the results of our study, we interpret this to mean that an increase in the mean tracheal air column area is an indirect indicator of an increase in inflammation due to COVID-19.

Tracheomegalyis divided into four groups according to its etiology. These groups are the following: patients with previous tracheal intervention; patients with recurrent infection and pulmonary fibrosis; patients with extrapulmonary elastolysis; and patients without a clear predisposing factor (23). It has been demonstrated in the literature that tracheomegaly develops in patients undergoing mechanical ventilation due to COVID-19 (16). It has also been hypothesized that high-pressure ventilation causes dilation of the trachea (14). In the study conducted by Tarle et al. (15), it was shown that tracheomegaly occurred due to severe damage to the trachea as a result of long-term mechanical ventilation in COVID-19 patients.

Although the diagnosis of tracheomegaly is initially made with chest radiographs, the method has its limitations (24,25). Tracheomegaly is often missed on chest radiography, leading to misdiagnosis and underdiagnosis. Tracheomegaly may not be visible on chest radiography until the trachea's diameter exceeds the vertebral column (26). Jaiswal et al. (27) showed that tracheal diameter measured above 30 mm on chest radiography was compatible with tracheomegaly. The gold standard method used in the diagnosis of tracheomegaly in other studies in the literature is CT (28,29). Smaller diameters are used as limit values for tracheomegaly for females, while higher values are used for males (9). The findings of our study also show that the mean air column area is higher in males than in females. Although the upper limit for tracheomegaly was above 30 mm in some studies (25, 27), Krustins et al. (30) found that 36.1 mm was the upper limit. The aim of our study was not to examine the presence of tracheomegaly in patients, but rather to investigate the effect of tracheal enlargement on prognosis. Therefore, a prognosis derived from grouping patients according to the presence or absence of tracheomegaly can be a useful subject for future studies.

In our study, instead of examining tracheomegaly, we thought we could make a more specific contribution to the literature using the mean tracheal air column, which we believed to be a better indicator of tracheal dilation. As far as we are aware, there is no existing study in the literature measuring the tracheal air column area. Therefore, we could not show the presence of tracheomegaly because the normal values were not known. Although this seems like a limitation, it makes our study unique because of its method, which has a unique structure. In our research, we believe the values obtained by CT images, accepted as the gold standard for the diagnosis of tracheomegaly, are more objective in demonstrating tracheal dilation. Moreover, biases were eliminated by measuring the area from the levels that Breysem et al. (18) measured to diagnose tracheomegaly.

Limitations

Our study had several limitations. First, it was a single-center study and was designed retrospectively. Other limitations include the patient's history of smoking and previous tracheal intervention; the presence of additional diseases, such as frequent recurrent infections and connective tissue disease; and the unknown status of the drug used for the previously mentioned ailments. Considering the findings in our study, more research is needed to evaluate the condition of the trachea in the long term after COVID-19 diagnosis. Will tracheomegaly develop because of relaxation in the elastic fibers that form the trachea, especially after inflammation—and damage the muscles? Will tracheal stenosis occur due to tracheal calcification as a result of inflammation? The answers to these questions will be obtained as a result of more comprehensive studies possibly carried out in the future.

CONCLUSION

COVID-19, a multisystemic disease, damages the trachea, as well as the lungs. An increase in the diameter of the air column in the trachea increases mortality and prolongs the length of stay in intensive care units. Our study revealed that the tracheal air column area, which can be easily evaluated with CT, can lead to better, more precise predictions of COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 15.11.2022, Decision No: 2022/8-28).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Investigating clinical and laboratory findings and mortality rates among vaccinated and unvaccinated COVID-19 inpatients

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ABSTRACT

Aim: COVID-19 is an important public health problem in world and Turkey. The present study aimed to compare the clinical and laboratory findings and mortality rates among vaccinated and unvaccinated COVID-19 inpatients.

Material and Method: We included patients receiving inpatient treatment in COVID-19 wards of our hospital between April 25 and October 22, 2021. The patients were divided into two groups: those with and without the COVID-19 vaccine. We extracted patient information from anamnesis files and the hospital information system. Then, we recorded the patients' epidemiological and laboratory findings and vaccination status. Patients with at least two doses of the COVID-19 vaccine were considered "vaccinated." We performed Fisher's exact test and Chi-square test to analyze the data. All statistical analyses were performed in SPSS, and a p-value <0.05 was accepted as statistically significant.

Results: The study included 63 vaccinated and 83 unvaccinated patients. With a mean age of 71.4±12.3 years, thirty (47.6%) of the vaccinated patients were females, and 33 (52.3%) were males. Of the unvaccinated ones, 40 (48.1%) were females, while 43 (51.8%) were males (mean age=52.2±14.4 years). The mean age was significantly higher in the vaccinated group than in the unvaccinated group (p<0.01). While 82.5% of the vaccinated patients received two doses, 17.5% received three doses of the COVID vaccine. Besides, 95.3% of the patients received their first dose of inactivated vaccine (Sinovac, China) and 4.7% of an mRNA vaccine (BioNTech, Germany). We found that comorbidities were significantly more prevalent in the vaccinated group than in the unvaccinated group (44 (69.8%) vaccinated and 34 (40.9%) unvaccinated patients had a comorbid disease, p<0.01). Among the accompanying diseases, hypertension was significantly more prevalent in the vaccinated group than in the unvaccinated group (p<0.01). Considering their laboratory findings, the vaccinated patients had significantly higher leukocyte, troponin, and ferritin values than the unvaccinated patients (p=0.008). Consequently, five (57.9) of the vaccinated patients and 4 (4.8%) of the unvaccinated patients died (p=0.05).

Conclusion: Similar mortality rates between our vaccinated and unvaccinated patients may be attributed to the fact that the vaccinated group was relatively older, had more comorbid diseases, and received their second dose after an average of 100.6 days following their first dose of inactivated vaccine. In conclusion, further clinical research involving more cases that received different COVID-19 vaccines is needed to uncover the factors affecting mortality and morbidity among vaccinated patients.

Keywords: COVID-19, vaccine, mortality, morbidity

Poster presentation at the 10th Turkey EKMUD Scientific Congress on May 25-29, 2022 in Antalya

INTRODUCTION

The causative agent of the novel Coronavirus disease-2019 (COVID-19) is SARS-CoV-2, an enveloped RNA virus leading to a pandemic worldwide. As of July 1, the World Health Organization (WHO) reported the total number of confirmed COVID-19 cases as 545,226,550, the number of COVID-19 deaths as 6,334,728, and the number of doses administered all over the world as 11,986,040,938. As of the same date, Turkey reported the total number of COVID-19 cases to be 15,123,331, the total number of deaths to be 99,032, the number of new cases in the last one week to be 26,635, and the number of deaths in the last one week to be 17. Accordingly, Turkey ranked 10th in the number of total cases and mortality rates (1). COVID-19 disease may follow diverse clinical courses from asymptomatic infection to mild upper respiratory tract infection and from mild to severe viral pneumonia that may result in death (2, 3). Major risk factors for severe COVID-19 disease are age, male sex, obesity, smoking, and comorbid chronic conditions such as hypertension (HT) and diabetes mellitus (DM). A

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substantial body of research revealed that advanced age appears as the most significant risk factor for the severe COVID-19 disease (2,3-5).

The most efficient methods to control the pandemic are shown as non-drug infection control measures such as vaccination, mask wearing, phyisical distance, and hand washing. A plethora of studies documented that COVID-19 vaccines protect against diseases with a severe course and significantly reduce hospitalization rates in intensive care units (6).

Apart from approved and frequently used vaccines against COVID-19, there are also ongoing scientific trials to develop new vaccines. The available vaccines in clinical use are mRNA vaccines (e.g., BNT162b2 vaccine, Pfizer–BioNTech), recombinant adenovirus vector vaccines (e.g., ChAdOx1-S vaccine, AstraZeneca–Oxford University), and inactivated vaccines (e.g., CoronaVac vaccine, Sinovac Biotech). BNT162b2 and CoronaVac are widely used in Turkey to protect against COVID-19.

The present study aimed to compare the clinical and laboratory findings, mortality rates, and mortality-affecting risk factors between the vaccinated and unvaccinated patients receiving inpatient treatment in the COVID-19 wards of Ankara Training and Research Hospital.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara Training and Research Hospital Clinical Researches Ethics Committee (Date: 10.20.2021, Decision No: E-765/2021). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We included patients receiving inpatient treatment in COVID-19 wards of Ankara Training and Research Hospital between April 25 and October 22, 2021. The patients were divided into two groups: those with and without the COVID-19 vaccine. Then, we recorded the patients' epidemiological and laboratory findings and vaccination status. While we extracted patient information from anamnesis files, their epidemiological, clinical (tachypnea, oxygen saturation), laboratory (lymphocyte, C-reactive protein, troponin, D-dimer, ferritin), and radiological data were obtained from the hospital information management system. Patients with at least two doses of the COVID-19 vaccine were considered "vaccinated."

We performed Fisher's Exact test and Chi-square test to analyze the data. All statistical analyses were performed in SPSS a p-value < 0.05 was accepted as statistically significant.

RESULTS

The study included 63 vaccinated and 83 unvaccinated patients. With a mean age of 71.4 ±12.3 years, thirty (47.6%) of the vaccinated patients were females, and 33 (52.3%) were males. Of the unvaccinated ones, 40 (48.1%) were females, while 43 (51.8%) were males (Mage=52.2 ±14.4 years). The mean age was significantly higher in the vaccinated group than in the unvaccinated group (p< 0.01). While 82.5% of the vaccinated patients received two doses, 17.5% received three doses of the COVID vaccine. Besides, 95.3% of the patients received their first dose of inactivated vaccine (Sinovac, China) and 4.7% of an mRNA vaccine (BioNTech, Germany). We found that comorbidities were significantly more prevalent in the vaccinated group than in the unvaccinated group (44 (69.8%) vaccinated and 34 (40.9%) unvaccinated patients had a comorbid disease, p< 0.01). Among the accompanying diseases, HT was significantly more prevalent in the vaccinated group than in the unvaccinated group (p< 0.01). Considering their laboratory findings, the vaccinated patients had significantly higher leukocyte, troponin, and ferritin values than the unvaccinated patients (p=0.008). Consequently, five (5.79%) of the

Table. Demographic and laboratory findings of the vaccinated and unvaccinated patients having died due to COVID-19

Patient no.	Sex	Age (years)	Concomitant disease	Type of vaccine/number of doses administered/ time since the last dose	Leukocytes (ref.: 4000-10000/mm³)	Lymphocyte count (ref.: 800-4000 mm³)	Troponin (ref.: 0-14ng/mL)	Ferritin (ref.: 13-150 micg/mL)
Dead unvaccinated patients								
1	Female	80	HT,DM, COPD	None	6,640	1,200	25.6	377
2	Female	75	HT, Asthma	None	6,120	370	20.93	173
3	Female	75	COPD	None	9,070	520	41.69	328
4	Female	49	None	None	3,340	610	6.72	608
Dead vaccinated patients								
1	Male	77	HT	CoronaVac/2/104days	10,930	410	29.74	989
2	Female	73	HT	CoronaVac/2/64days	5,500	910	32.6	89.3
3	Male	67	HT,DM	CoronaVac/2/67days	11,170	2,090	39.78	134
4	Female	84	HT, DM, COPD	CoronaVac/ 2/131 days	7,650	2,140	3	82.6
5	Female	70	HT, DM	CoronaVac/ 2/137 days	6,530	900	9.25	507

vaccinated patients and 4 (4.8%) of the unvaccinated patients died (p=0.05). Logistic regression analysis, which was run to reveal risk factors of mortality among the patients, yielded no significant finding, which may be due to the low number of patients having died in the groups. Table shows the demographic and laboratory findings of the vaccinated and unvaccinated patients having died due to COVID-19.

DISCUSSION

The causative agent of the novel type of coronavirus, also known as coronavirus infectious disease 2019 (COVID-19), is SARS-CoV-2, one of the coronaviruses that appeared in Wuhan, China first, and then caused a worldwide pandemic. COVID-19 is a contagious infectious disease caused by SARS-CoV-2, characterized by atypical pneumonia. Most patients with COVID-19 have a mild or moderate course, but 5-10% may experience severe or even a life-threatening course. Disease-related mortality rates are reported as about 2% (7).

Apart from currently available COVID-19 vaccines deployed in the effective fight against the COVID-19 pandemic, scientific trials to develop new vaccines are still ongoing. Vaccines have a vital role in the control of the COVID-19 pandemic. There has been a significant decrease in mortality and morbidity rates thanks to the introduction of COVID-19 vaccines. Yet, underdeveloped countries have experienced problems in the supply of vaccines. In Turkey, the rate of persons fully vaccinated with the last dose of primary series per 100 population has been 63.84 (1). Despite no major difficulties accessing the COVID-19 vaccine, vaccination rates have remained under the desired level worldwide and in Turkey (1, 8).

The relevant research showed that unvaccinated persons have a 13.9 times higher risk of infection and 53.2 times higher risk of COVID-19-related death compared to fully vaccinated persons having received a single booster dose while 4.0 times higher risk of infection and 12.7 times higher risk of death compared to fully vaccinated persons not having received a booster dose (9).

In a cohort study with 19,625 nursing home residents in the United States, individuals vaccinated with the mRNA vaccine (Moderna or Pfizer-BioNTech) were reported to have a lower all-cause mortality rate than unvaccinated individuals. The same study also documented that the risk of mortality did not increase among those receiving the COVID-19 vaccine (10).

In vitro studies on the efficacy and neutralizing antibody levels of COVID-19 vaccines indicated that mRNA and Novavax protein subunit vaccines lead to higher antibody responses than viral vector and inactivated vaccines. Although efficacy rates between 60% and 94% were reported for vaccines used against COVID-19, it was noted that these rates are susceptible to research design, the population studied, and the prevalence of SARS-CoV-2 variants during the study; therefore, a one-to-one comparison between vaccines is not always convenient (6).

A UK-based study demonstrated that vaccination with a dose of BNT162b2 or ChAdOx1-S resulted in a significant reduction in symptomatic COVID-19 and greater protection against severe diseases in older adults. Both vaccines showed similar efficacy, providing protection during follow-up (>6 weeks). Furthermore, the second dose of BNT162b2 was found to be associated with greater protection against symptomatic disease. In addition, both vaccines were reported to have significant efficacy against the B.1.1.7 variant (11).

A national mRNA BNT162b2 (Pfizer-BioNTech) vaccine surveillance study in Israel reported that the COVID-19-related mortality rate decreased by 96.7% in vaccinated individuals. In that study, two doses of the BNT162b2 vaccine were reported to be quite effective in preventing infections (including symptomatic and asymptomatic SARS-CoV-2 and B.1.1.7 SARS-CoV-2 variants), COVID-19-related hospitalizations, severe diseases, and death in all age groups, including adults over 16 years and older adults over 85 years) (12).

Another study reported that receiving the mRNA COVID-19 vaccine significantly reduces the possibility of disease-related mortality or being connected to mechanical ventilation among patients hospitalized for COVID-19 (13).

Similar mortality rates between our vaccinated and unvaccinated patients may be attributed to the fact that the vaccinated group was relatively older and received inactivated vaccine. Ersan et al. (3) reported no significant difference in morbidity and mortality rates between the vaccinated (inactive vaccine) cases over 65 years with a definite diagnosis of COVID-19 compared to their unvaccinated counterparts. Multinational studies also highlighted that advanced age is a significant risk factor for mortality in COVID-19 disease (3, 14, 15). An Italy-based study reported COVID-19-related mortality rates to be 0.4% at the age of 40 and below, 1% between the ages of 50-60, 3.5% between the ages of 60-70, 12.8% between the ages of 70-80, and 20.2% at the age of 80 and above (14). In China, Wu et al. (15) reported mortality rates among COVID-19 patients as 0.4% among those aged 40 years and below, 3.6% among 60-70- year-olds, 8% among 70-80-year-olds, and 14.8% among those aged 80 years and older. In present study, vaccinated patients had significantly higher leukocyte, troponin,

and ferritin levels than the unvaccinated patients.In our study, vaccinated patients have older age and have more concomitant disease than unvaccinated patients These situation may explain higher level leukocyte, troponin, and ferritin levels

Risk factors other than age for a severe course and mortality in COVID-19 disease were previously reported to be male sex, obesity, smoking, and chronic underlying diseases (e.g., HT and DM) (3,16,17). In their study with vaccinated (inactivated vaccine) and unvaccinated patients aged 65 years and above, Ersan et al. (3) found no significant difference between the patients' ages (75.8±7.4 years and 73.8±8.0 years, respectively). In the same study, the overall mortality rate was 21.9% (33 patients), and it was reported that all patients who died were those who were followed up in the intensive care unit. While 19 (57.6%) of these patients were vaccinated, 14 (42.4%) were unvaccinated. The authors also found no significant difference between the mortality rates of the groups. Yet, there was a significant, positive correlation between the number of comorbidities and mortality. In our study, the rate of concomitant diseases was statistically higher in the vaccinated group (44; 69.8%) than in the unvaccinated group (34; 40.9%). Moreover, HT was significantly more prevalent in the vaccinated group than in the unvaccinated group (**Table**).

Ersan et al. (3) did not report significant differences in lymphopenia, CRP, ferritin, and D-dimer levels between vaccinated and unvaccinated patients. Considering the laboratory findings in this study, we found that the vaccinated group had significantly higher leukocyte, troponin, and ferritin levels than the unvaccinated patients.

In their placebo-controlled study with CoronaVac (inactivated vaccine), Tanrıöver et al. (17) reported the effectiveness of the vaccine as 83.5% after 14 or more days following the second dose among 10,029 participants (6,559 participants in the two-dose vaccine group and 3,470 people in the placebo group). In the same study, the frequency of vaccine-related adverse events was reported to be 18.9%. In both groups, while the most frequently reported systemic side effect was fatigue, the local side effect was pain at the injection site.

In another study comparing CoronaVac with BNT162b2 (mRNA vaccine), there was a negative correlation between age and neutralizing antibody formation. Accordingly, the neutralizing antibody titer was significantly lower in the group vaccinated with CoronaVac than those receiving BNT162b2 (3,18,19).

Some other studies revealed that mRNA and vector-based vaccines provide more efficient protection in the older adult population (11,20). Another study compared the severity of the COVID-19 disease between vaccinated (at least one dose of Pfizer-BioNTech or Oxford-AstraZeneca) and unvaccinated patients hospitalized in an intensive care unit in Saudi Arabia. The findings demonstrated that at least one dose significantly reduces the severity of the disease and is significantly associated with a reduction in 30-day all-cause mortality (21).

In a case-control study in the United States, Olson et al. (22) found the efficacy of the BNT162b2 vaccine to be 94% in preventing hospitalization for COVID-19, 95% among test-negative controls, 98% against admission to the intensive care unit, and 98% directly against COVID-19. In the study, it was reported that seven patients who died due to COVID-19 were unvaccinated. Overall, it was concluded that two doses of the BNT162b2 vaccine are highly effective against COVID-19-related hospitalization, admission to the intensive care unit, and the receipt of life support in the intensive care unit among hospitalized adult patients.

In their study in India, Muthukrishnan et al. (23) explored the efficacy of recombinant adenovirus vector vaccines on mortality associated with COVID-19. The study included 266 partially vaccinated patients (2 weeks after single-dose vaccination) and 184 full-dose vaccinated patients (2 weeks after 2 doses of vaccination). The researchers concluded mortality of 12.5% (23/184) among those who were fully vaccinated and 31.45% (309/984) among those who were not vaccinated. Thus, the mortality rate in unvaccinated patients was found to be statistically higher. Logistic regression analysis yielded that full vaccination and younger age were associated with survival.

Ersan et al. (3) studied 151 COVID-19 patients (78 patients who received two doses of inactivated vaccine and became ill 15 days after vaccination and 73 patients who were not vaccinated, received a single dose, or became ill within 15 days after two doses of vaccine) but could not detect significant differences between the vaccinated and unvaccinated groups by intensive care admission and intensive care mortality rate.

Similar mortality rates between our vaccinated and unvaccinated patients may be attributed to the fact that the vaccinated group was relatively older, had more comorbid diseases, and had their second dose after an average of 100.6 days following their first dose of inactivated vaccine.

Limitations

The present study is not free of a few limitations. This was a retrospective and single-center study, and we only considered hospitalized cases. Moreover, the number of patients who died in the groups was too low. Finally, all vaccinated patients received no vaccine other than an inactivated vaccine.

CONCLUSION

In conclusion, further clinical research involving more cases that received different COVID-19 vaccines is needed to uncover the factors affecting mortality and morbidity among vaccinated patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Training and Research Hospital Clinical Researches Ethics Committee (Date: 10.20.2021, Decision No: E-765/2021).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Comparison of different endometrial preparation protocols in frozen-thawed embryo transfer cycles in women with polycystic ovary syndrome

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ABSTRACT

Aim: This study aimed to evaluate the most suitable endometrial preparation protocols such as hormone replacement therapy (HRT) with gonadotropin releasing hormone analogue (GnRH-a) suppression, HRT without GnRH-a suppression and mild ovarian stimulation (OS) for women with polycystic ovary syndrome (PCOS) undergoing frozen-thawed embryo transfer (FET).

Material and Method: We conducted a historical cohort analysis of 161 women with PCOS who underwent the "freeze-all" strategy between December 2018 and August 2020 because of their high risk for ovarian hyperstimulation syndrome. Three endometrial preparation protocols were used: HRT with GnRH-a suppression (n=43); HRT without GnRH-a suppression (n=86); mild-OS (n=32).

Results: The biochemical pregnancy results (55.8 % vs 54.65 % vs 53, p=0.900), ongoing pregnancy rates (44.2 % vs 43 % vs 40.62, p=0.572), and abort rates (20.8 % vs 21.3 % vs 23.52, p=0.900) were similar between the HRT with GnRH-a suppression, without GnRH-a suppression and mild-OS, respectively. This study showed no statistically significant difference between the three protocols in laboratory parameters (p>0.05).

Conclusion: There was no statistically difference between three groups in terms of pregnancy outcomes. Dependent on clinical experience and facility, one of these protocols could be deployed for FET in women with PCOS.

Keywords: Frozen-thawed embryo, GnRH-a, implantation, polycystic ovary syndrome

INTRODUCTION

Frozen embryo transfer (FET) is generally employed in assisted reproductive medicine due to its ability to lower the risk of ovarian hyperstimulation syndrome (OHSS) improving the reproductive outcomes (1). FET as an alternative to fresh cycle transfer has been suggested to be applied for women with polycystic ovary syndrome (PCOS) because the significantly increasing risk of OHSS under this condition (2). In fact, there is much proof for a considerable advantage of this method for women with PCOS (1).

Depending on the diagnostic criteria, PCOS affects 5%-18% of reproductive-aged women worldwide (3). PCOS, as a common disorder, has a relationship with infertility (4,5). It is essential to identify the importance of the factors such as types of endometrial preparation protocols that affecting the success of assisted reproductive methods in women with PCOS.

The ideal endometrial preparation protocol should be considered for women with PCOS. Different strategies for endometrial preparation have been described, including a natural modified cycle (NMC) where hCG is administered to design embryo transfer (ET) rather than measuring luteinizing hormone (LH), a purely natural cycle (NC) with detection of LH in blood or urine, artificial cycle with progesterone (P4), and estradiol (E2), hormone replacement therapy (HRT) with or without gonadotropin-releasing hormone (GnRH) analogs and stimulated cycles with low doses

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of gonadotropins (6,7). In the latest meta-analysis, the use of one strategy over others is not supported, but using the pure NC over the NMC or a NC with progesterone over the NC have been supported by other authors to report better results (8,9). Several approaches in artificial or natural preparation has also been shown in surveys, including 179 centers in the world. One can find several different approaches in answers about preparation of FET and in questions such as if its timing in an artificial or a natural cycle shows various responses and if P4 is needed (6).

The comparison of the method of endometrial preparation in reproductive-aged women has been evaluated in many studies, and different results have been reported (10-13). Infertility is more prevalent in women with PCOS, and they need more assisted reproduction technology (14,15). However, the comparison of these methods in women with PCOS is less studied, and there is a need for serious research in this field.

The prominent importance of endometrial preparation protocols in the favorable pregnancy outcomes is known. However, the best protocol for women with PCOS who experience FET cycles is still uncertain. Women with PCOS do generally not have a regular menstrual cycle, so the NC or NMC protocol will not be the most suitable choice for women with PCOS (16). For this reason, this protocol was not examined in this research. This study aimed to evaluate the suitable endometrial preparation protocols such as HRT with GnRH-a suppression, HRT without GnRH-a suppression, and mild ovarian stimulation (OS) for women with PCOS undergoing FET.

MATERIAL AND METHOD

This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

One hundred sixty one women participated in this study between December 2018 and August 2020. In this period, 851 patients who underwent FET were examined, and 161 women with PCOS aged between 20 and 35 were included. In 43 patients, it was employed the HRT with GnRH-a suppression (Group:1), in 86 patients, it was employed the HRT without GnRH-a suppression (Group:2), and in 32 patients, it was employed the Mild ovarian stimulation (Group:3).

The exclusion criteria were as follows: 1) known chronic disease, 2) over 35 years of age, 3) body mass index (BMI)>30,4) Having additional infertility factors other than PCOS, and 5) 2 or more failed attempts.

The inclusion criteria were as follows: 1) 20-35 years old, 2) Anti- mullerian hormone (AMH)>5 ng/ml, 3) Women with PCOS according to Rotterdam criteria, 4) Those who have no previous attempts or at most one attempt, and 5) Top-Good Quality (5AA-5AB-4AA-4AB) single blastocyst transfer.

Endometrium Preparation Protocols

The three primary endometrial priming protocols for FET were HRT, mild-OS, and NC. Among these protocols, mild-OS is employed less than the other two (7). HRT cycles can be used with or without GnRH-a for pituitary suppression (17).

Statistical Analysis

The Kolmogorov-Smirnov test performed to check the normality, and the nonparametric tests performed given the non-normality of the groups before the statistical analyses. Mean and standard deviations (SD) measured to check each continuous variable, including age, BMI, total oocytes, MII oocytes, , multi-pronuclei (PN), AMH, prolactin, Free T4 (FT4), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), E2, and endometrial thickness. The Kruskal Wallis-H test deployed to examine the difference between the three endometrial preparation protocols. Chi-square tests were applied to describe the relationship between proportions of categorical variables such as pregnancy results, ongoing pregnancy rate and abort rate. SPSS v24 used for statistical analyses. A value of p-value < 0.05 was accepted as statistically significant.

We utilized the G-Power 3.1 program to calculate the example size. The two groups' total mean was calculated based on the Mann-Whitney test with a power of 90%, an effect size of 50%, and a 0.05 type 1 error for at least 146 patients (18).

RESULTS

This study included One hundred sixty one age-matched (30.75±3.39) and BMI-matched (23.78±2.28) women. 43 patient in the first group with the mean age (30.34±3.90), 86 patient in the second group with the mean age (30.39±3.64), and 32 patient in the third group with the mean age (30.37±4.30) were compared with each other. **Table 1** shown information about the descriptive statistics of maternal characteristics and laboratory parameters. We compared laboratory parameters between three groups and assessed the capability of those parameters to differentiate between groups.

Table 1. Descriptive statistics of study parameters in women with PCOS (n=161)

Study parameters	median (range) mean ± SD
Maternal characteristics	
Age	32(20-35)30.38±3.83
BMI	24(19.8-29)24±2.2
Laboratory parameters	
Total oocytes	0(0-1)0.34±0.48
MII oocytes	5(5-7)5.19±0.44
PN	9(7-16)10.25±2.49
AMH	8(6-15)9.07±2.38
Prolactin	8(6-14)8.51±2.07
FT4	15(8.48-25)16.76±5.51
TSH	1(0.31-1.62)1.04±0.27
FSH	1.21(0.63-2.46)1.49±0.53
LH	7.42(4-12)7.56±1.26
E2	9(3.52-17)8.68±2.54
Endometrial thickness	41(30-51.2)40.05±6.5

SD, standard deviation; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol.

As stated in **Table 2**, a Kruskal Wallis-H test did not find a statistically significant association between the three treatment groups in regard to age and BMI (p>0.05). AMH of first group (mean = 5.21) were comparable than the second group (mean = 5.17) and the third group (mean = 5.19). A Kruskal Wallis-H test indicated that this difference was not statistically significant (p>0.05). No significant difference was observed between the three groups regarding total oocytes, MII oocytes, PN, prolactin and FT4 (p>0.05). TSH, LH and FSH levels were similar between the three groups (p>0.05). There was no statistically significant difference between groups in terms of E2 and endometrial thickness (p>0.05).

Table 2. Comparison of study parameters between three groups

Study parameters	first group (n=43) mean ± SD	second group (n=86) mean ± SD	third group (n=32) mean ± SD	p
Age	30.35±3.9	30.4±3.65	30.38±4.31	0.776
BMI	24.16±2.07	23.85±2.22	24.16±2.39	0.453
AMH	5.21±0.52	5.17±0.41	5.19±0.4	0.797
Total oocytes	10.23±2.48	10.24±2.41	10.28±2.79	0.792
MII oocytes	9.05±2.5	9.09±2.35	9.03±2.36	0.925
PN	8.51±2.11	8.5±1.97	8.53±2.34	0.934
Prolactin	16.61±5.55	16.85±5.47	16.7±5.72	0.940
FT4	1.05±0.25	1.04±0.28	1.05±0.27	0.970
TSH	1.51±0.53	1.49±0.54	1.46±0.51	0.973
FSH	7.52±0.97	7.55±1.4	7.61±1.26	0.814
LH	8.72±2.65	8.67±2.23	8.67±3.18	0.941
E2	40.02±6	40.03±6.69	40.16±6.83	0.997
Endometrial thickness	9.95±1.05	9.92±1.05	9.94±1.08	0.975

M, Mean; N, number of subjects; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, Estradiol; All variables tested by a Mann-Whitney U test.

As stated in **Table 3**, a chi square test found that there was not a statistically significant association between the pregnancy results (biochemical and ongoing) and the three treatment groups (HRT with GnRH-a suppression, HRT without GnRH-a suppression, and mild-OS) (p>0.05).

Table 3. The relationship between pregnancy (biochemical and ongoing) results and the three groups

Variables		First group (n=43) n(%)	Second group (n=86) n(%)	Third group (n=32) n(%)	p
Pregnancy results Bhcg(+)(%)	Yes	24 (55.8)	47 (54.65)	17 (53)	0.900*
	No	19 (44.2)	39 (45.35)	15 (47)	
Ongoing pregnancy rate(%)	Yes	19 (44.2)	37 (43)	13 (40.62)	0.572*
	No	24 (55.8)	49 (57)	19 (59.38)	

*A Chi-square test

As stated in **Table 4**, a chi square test found that there was not a statistically significant association between the abort rate and the three treatment groups (p>0.05).

Table 4. The relationship between abortion rate and the three groups

Variable		First group (n=24) n(%)	Second group (n=47) n(%)	Third group (n=17) n(%)	p
Abortion rate (%)	Yes	5(20.8)	10(21.3)	4(23.52)	0.249*
	No	19(79.2)	37(78.7)	13(76.48)	

*A Chi-square test.

DISCUSSION

The present study investigated whether biochemical pregnancy results, ongoing pregnancy rates, and abort rates varied when three different endometrial preparation protocols were employed for FET in women with PCOS. Therefore, we retrospectively examined our data of FET cycles and included three endometrial preparation protocols in this study: HRT with GnRH-a suppression, without, and mild-OS. Our results indicate that overall, patients with programmed HRT with or without GnRH-a suppression did not have higher biochemical pregnancy results, ongoing pregnancy rates, and abort rates compared with patients with mild-OS.

In our study, biochemical pregnancy results (55.8 % vs 54.65 % vs 53, p=0.900), ongoing pregnancy rates (44.2 % vs 43 % vs 40.62, p=0.572), and abort rates (20.8 % vs 21.3 % vs 23.52, p=0.900) were similar between the HRT with GnRH-a suppression, without GnRH-a suppression and mild-OS, respectively. The ongoing pregnancy rates and abort rates was relatively low in the mild-OS compared to the HRT protocols. This study showed no statistically significant difference between the three protocols in laboratory parameters.

By studying the literature, we found that many studies have been performed comparing women's fertility outcomes of different endometrial preparation protocols. However, studies that have studied women with PCOS are limited. The comparison of endometrial preparation protocols in women with PCOS is a new topic, and a few existing studies have not reached a general conclusion about the ideal protocol. Some of the studies (19-22) have documented no significant differences between the different endometrial preparation protocols, and some studies (23-27) have indicated better pregnancy results for one protocol over the other.

According to Li et al. (19), HRT protocols had pregnancy outcomes similar to stimulated cycles (STC) for endometrial preparation. The available evidence shows that HRT may be a reasonable choice for the PCOS young women prepared for FET, who do not accept injections. On the contrary, STC may lead to reduced operational costs and unnecessary anxiety, and increased flexibility for patients. Najarkolaei et al. (20) reported no difference in abort rates and pregnancy outcomes between the mild-OS and the HRT protocols.

Peigne et al. (21) concluded that HRT and mild-OS groups showed comparable clinical pregnancy rates (20.8% vs. 24.4%). This study included women with PCOS i.e., about 20% of the patients.

The retrospective study by Yu et al. (22) showed similar endometrial thickness in the mildly stimulated cycle and HRT which resulted in non-statistically different rates of clinical and ongoing pregnancy and live birth. However, there was a significantly higher abortion rate in the mild-OS.

According to Man et al. (23), there is a significantly higher live birth rate in the PCOS women undergoing endometrial preparation during their initial FET cycle using the OS and NC methods using HRT. Nevertheless, there is a significantly higher rate of cycle cancelation in the NC group than in the other groups. the different groups do not show a significant difference in the rate of adverse events, such as preterm delivery, ectopic pregnancy, etc. This study has special significance since it is the first study on PCOS women.

Wang et al. (24) reported the better outcomes to HRT protocols. According to Niu et al. (25), both letrozole and Human Menopozal Gonadotropin (HMG) ovulation induction regimen had an association with more acceptable pregnancy results than the HRT regimen, such as a lower pregnancy loss rate, and a higher livebirth rate among the PCOS patients undergoing frozen single-blastocyst transfer.

Guan et al. (26) showed a higher live birth rate of the mild-OS and abort rates lower than the HRT in obese women with PCOS. This paper along with the available proof demonstrated superior pregnancy outcomes in the mild-OS than in the HRT. In a large retrospective study, Zhang et al. (27) reported significantly lower pregnancy loss rates of letrozole-stimulated cycles and higher live birth rates than the HRT protocol.

In this study, we acknowledge that we did not collect data about pregnancy difficulties, such as preeclampsia, gestational hypertension, and diabetes as the potential risk factors for adverse neonatal outcomes leading to a confounding effect on the results. The bias potential of medical records and the retrospective study design are the main limitations. The present study has the main strength which is the comparison of the most commonly used protocols for endometrial preparation in a large cohort of patients undergoing FET cycles, and the similarly increasing transferred good quality embryos in both HRT and mild-OS by the experienced clinicians and the verification method application in all embryos in a single center, affecting desirable outcomes of pregnancy.

CONCLUSION

This study revealed that pregnancy results, ongoing pregnancy rate and abort rate were similar among natural and artificial endometrial preparation protocols performed for FET cycles. Optimal endometrial preparation is necessary to receive successful pregnancy rates. Nevertheless, no statistical significance was found in the protocols. Dependent on clinical experience and facility, one of these protocols could be deployed for FET.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Comparison of clinical outcomes of intensive care patients with COVID-19 pneumonia receiving and not receiving tocilizumab treatment

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ABSTRACT

Aim: In patients with Coronavirus disease 2019 (COVID-19) infection, a situation called cytokine storm and an increase in proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1 (IL-1) in the blood has been observed and it has been found that this is clinically related to the development of severe disease. Therefore, tocilizumab (TCZ) therapy that blocks IL-6 will reduce the immunological response and thus potentially harm caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The aim of this study is to determine the effect of TCZ treatment on length of hospital stay, need for invasive mechanical ventilation and mortality in COVID-19 patients followed in the tertiary intensive care unit.

Material and Method: This retrospective cross-sectional study was conducted among patients hospitalized with the diagnosis of COVID-19 pneumonia between 01.09.2020 and 01.01.21 in intensive care units. Data were analyzed and evaluated separately in patients who received and did not receive TCZ treatment. Patients older than 18 years of age, who were hospitalized for at least 24 hours with the diagnosis of COVID-19 pneumonia and needed ≥36 hours of oxygen therapy, were not referred to another health center, were included in this study. Pregnant and lactating women were not included in the study. Patients with missing at least one data in the parameters to be evaluated were excluded from the study. Patients treated with an IL-6 inhibitor other than TCZ were excluded.

Results: After excluding patients who did not meet the inclusion criteria, 565 patients were included in the study. It was found that patients who received TCZ treatment after propensity score matching (PSM) had a significantly higher mean age (P<0.001) and lower obesity rates (P=0.002). There was no significant difference between the patients who received and did not receive TCZ treatment in terms of mechanical ventilation need, length of hospital stay and mortality (P=0.505, P=0.661, P=0.834).

Conclusion: As a result of our research, it was seen that TCZ treatment did not affect the need for invasive mechanical ventilation, hospital and intensive care unit stay, and mortality.

Keywords: COVID-19, pneumonia, tocilizumab, intensive care unit

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with high morbidity and mortality and impairing quality of life (1,2). The first case of pneumonia due to COVID-19 emerged in December 2019 in Wuhan city of China's Hubei province (3). The World Health Organization (WHO) declared a pandemic on March 11, 2020, after the disease spread rapidly around the world and cases emerged on all continents (4).

In COVID-19 infection, the emergence of an uncontrolled and excessive host immune response called cytokine storm syndrome is thought to be associated with the development of severe disease (5). It has been shown that the levels of proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-8 (IL-8), especially interleukin-6 (IL-6), increase in the circulation in cytokine storm syndrome (6).

Treatments for COVID-19 infection, for which there is no definitive cure yet, are usually in the form of

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supportive treatments. Anti-cytokine treatments targeting suppression of proinflammatory cytokines have been started to be applied in order to control cytokine storm syndrome, especially in people with severe or critical illness. For this purpose, IL-6 inhibitors, IL-1 inhibitors and kinase inhibitors, which are frequently used in COVID-19 patients, especially in autoimmune and autoinflammatory diseases, are applied (7).

IL-6 is a proinflammatory cytokine that plays a major role in the pathogenesis of COVID-19 associated cytokine storm syndrome. IL-6 is produced by nearly all stromal cells and immune system cells (T lymphocytes, B lymphocytes, macrophage, dendritic cell, monocytes, mast cells) (8). IL-6 plays a role in B cell differentiation, acute phase response, increase and activation of T cell population, and angiogenesis (9). While circulating IL-6 levels in healthy individuals are extremely low (in the range of 1–5 pg/mL), IL-6 levels have been found to be increased in many inflammatory conditions associated with cytokine release (10).

Tocilizumab (TCZ), sarilumab, siltuximab are IL-6 antagonists with different pharmacological properties. TCZ is an IgG1 type recombinant humanized monoclonal antibody against the IL-6 receptor, which binds to both membrane-bound and soluble IL-6 receptors and inhibits signal transduction mediated by these receptors (8). TCZ is approved for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, giant cell arteritis (11). It has been shown that IL-6 production increases with the stimulation of immune cells by SARS-CoV-2, and especially high plasma IL-6 levels in COVID-19 patients with severe disease presentation (8). Establishing the relationship between the course of proinflammatory responses, associated disease symptoms and clinical pictures is an important algorithm in treatment approaches. Therefore, TCZ therapy that blocks IL-6 will reduce the immunological response and thus potentially harm caused by SARS-CoV-2.

The underlying hypothesis of our study was that IL-6 receptor blocking TCZ treatment would prevent serious disease outcomes by disrupting the cytokine storm associated with COVID-19 in patients hospitalized in the intensive care unit for COVID-19 pneumonia who do not need invasive mechanical ventilation yet. The aim of this study is to evaluate and compare the length of stay in the intensive care unit, length of stay in inpatient services, need for invasive mechanical ventilator support, complications and mortality rates of patients who received and did not receive TCZ treatment for COVID-19 pneumonia in intensive care units.

MATERIAL AND METHOD

Ethical Approval

This retrospective cross-sectional study was carried out in Uşak University Medical Faculty Clinical Researches Ethics Committee (Date: 15.06.2022, Decision No: 108-108-06). This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was waived due to the retrospective nature of the study.

Study Design and Population

A retrospective observational analysis of electronic medical records of COVID-19 patients admitted to a public tertiary hospital was performed. This study was conducted among patients hospitalized with the diagnosis of COVID-19 pneumonia between 01.09.2020 and 01.01.21 in intensive care units.

Consecutive adults aged 18 and over years admitted with X ray confirmed pneumonia caused by laboratory confirmed COVID-19 infection were suitable for the study. Data concerning demographic, medical study, laboratory findings and treatment during hospital stay, admissions and outcomes were extracted from electronic medical records. Patients with TCZ were identified from pharmacy records of all patients to whom TCZ was dispensed. The observation period ended at final discharge.

In this study, patients younger than 18 years of age, pregnant and lactating women, patients referred to another health center, and patients with at least one missing data on the parameters to be evaluated were excluded from the study. Patients hospitalized for <24 h or those with no need of oxygen therapy for >36 h were excluded. Patients treated with an IL-6 inhibitor other than TCZ were excluded from the study. All patients included in the study meet the TCZ treatment criteria for the treatment of COVID-19 infection in accordance with the Turkish Ministry of Health guidance during the study period (12).

The TCZ administration criteria were severe pneumonia caused by COVID-19 and presence of one of the following laboratory parameters IL-6 >40 uL/L, D-dimer >1500 mcg/mL or if patient exhibited persistently rising D-dimer parameter. Patients with liver enzymes five times over the upper limit of normality or concomitant severe bacterial infection were not eligible for TCZ treatment (12). The final decision to use TCZ was at the discretion of the treating clinician.

All patients admitted in the hospital with confirmed COVID-19 infection were treated with a standard pharmacological protocol including antiviral drugs, antibiotic prophylaxis. Tocilizumab was initialy administered at a dosage of 8 mg/kg max 800 mg by two

consecutive administrations 12 h apart (12).

Data Collection

Demographic data, comorbidities, mechanical ventilator treatment process, complications (such as pneumothorax pneumomediastinium, emphysema, pulmonary embolism), length of hospital stay, length of stay in intensive care unit (ICU), and survival data of all patients included in the study were analyzed. These data were analyzed and evaluated separately in patients who received and did not receive TCZ treatment.

Outcomes

The primary end point forthis analysis was all cause mortality. Death was assessed as occurring during hospitalization. Secondary outcomes included in lenght of hospital and ICU stay. Lenght of hospital stay was calculated from day of admission to the day discharge alive.

Statistical Analysis

This study was designed to investigate the effect of TCZ administration on the outcomes of these patients, using the propensity score matching (PSM) method to eliminate the influence of other confounding factors. To minimize indication bias patients to be included in each analysis were selected by matching their individual propensity for receiving therapy with TCZ, conditional on their demographic and clinical variables. The following variables were fitted to a logistic regression model to derive their propensity score: age, sex, asthma/chronic obstructive pulmonary disease (COPD), obesity, hypertension, diabetes, cancer, chronic renal failure and a ratio of arterial oxygen saturation measured by pulse oximetry to fraction of inspired oxygen (SpO_2/FiO_2). The variable was the worst SpO_2/FiO_2 within the first 72 h after admission. PSM were performed to account for counfounding by indication bias in the logistic regression model analysis. Other characteristics and outcomes of the patients compared using Students' t test and chi square test. Statistical analysis were performed using IBM SPSS Statistics for Windows, version 27.0 (Armonk, NY) and statistical significance was set as $P < 0.05$.

Tocilizumab Treatment

TCZ treatment is applied in accordance with the guideline published by our Ministry of Health in patients who do not respond to systemic corticosteroid treatment in severe disease course or cytokine storm syndrome in COVID-19 infection (12). TCZ can be administered at a dose of 8 mg/kg and depending on the severity of the disease, it can be administered as 400 mg or 800 mg IV (maximum 800 mg) at a time. After the application, the clinical and laboratory parameters of the patient are followed. If necessary, a repeat dose of 200-400 mg can be administered within 24 hours after the first administration.

TCZ treatment should not be applied to pregnant women, patients with active tuberculosis and active hepatitis B, hepatitis C, patients with neutropenia (<500/mm3) in laboratory results, and patients with allergy and hypersensitivity reaction (12). Patients with a history of diverticulitis should be followed closely for the development of gastrointestinal perforation during TCZ treatment. During the TCZ treatment process, laboratory findings such as platelet count and liver function tests of the patients should be followed closely and TCZ treatment should not be applied to patients whose liver function tests are above five times the normal value (12).

A volume equal to the TCZ concentration to be applied to the patient is withdrawn from a sterile 100 ml SF (0.9% isotonic sodium chloride) solution. For example, 10 ml for 200 mg, 20 ml for 400 mg, 40 ml for 800 mg. The amount of TCZ to be applied to the patient is withdrawn from the vial and added to the 100 ml infusion bag. The final volume of the infusion fluid should be 100 ml. The solution is mixed slowly without foaming and infusion therapy is applied to the patient at a rate of 100 cc/h.

RESULTS

A total of 589 patients, 565 of whom were admitted to the hospital between 01.09.20 and 01.01.21, were included in the analysis. After exclusion of patients hospitalized for <24 h (n=12) for no need of oxygen therapy >36 h (n=12) or other exclusion reasons. Among the included individuals 517 (91.5%) patients were included in the control group, 48 (8.5%) were treated with TCZ. Characteristics of the patients included in the study are listed in **Table 1**.

In unmatched analysis the ICU stay was longer in patients treated with TCZ (9.3 vs. 7.2 days respectively $P<0.05$). Overall mortality in the patients was 71.5% and no differences were found in mortality between the groups (60.4% vs. 72.5%). Mean outcomes differences before matching are showed in **Table 2**.

TCZ and control group were well matched after propensity score (PS), comparison of PS distributions is shown in **Table 3**.

After matching the results showed no tendency towards the association between TCZ use and hospital lenght of stay. After PS analysis no difference in overall hospital mortality was noted between TCZ and control group. Mean outcomes differences after matching are listed in **Table 4**.

Table 1. Characteristics of the patients at cohort entry

Characteristics	Total (n = 565)	TCZ (n= 48)	Control (n =517)	P value*
Age (years ±Sd)	**71.2±12.2**	**71.8±12.1**	**64.8±11.6**	**<0.001**
Male sex	336 (59.5%)	28 (58.3%)	308 (59.6%)	0.989
Obesity	**141 (25.0%)**	**21 (43.8%)**	**120 (23.2%)**	**0.003**
Asthma/COPD	174(30.8%)	15 (31.2%)	159 (30.8%)	0.943
Cancer	19 (3.4%)	1 (2.1%)	18 (3.5%)	1.000
Hypertension	368 (65.1%)	22 (54.2%)	342 (66.2%)	0.131
Diabetes	255 (45.1%)	23 (47.9%)	232 (44.9%)	0.800
Coronary artery disease	174 (30.8%)	14 (29.2%)	160 (30.9%)	0.926
Chronic renal failure	15 (2.7%)	0 (0.0%)	15 (2.9%)	0.629
SpO_2/FiO_2 ratio ±Sd	77.0±39.2	71.9±37.6	77.4±39.0	0.350

*P value obtained comparing tcz group with control group. List of abbreviations: COPD=Chronic Obstructive Pulmonary Disease, TCZ=Tocilizumab

Table 2. Mean outcomes differences before matching

All cohort outcomes	Total (n=565)	TCZ (n=48)	Control (n=517)	P value*
Mechanical ventilation	413 (73.1%)	35 (72.9%)	378 (73.1%)	0.976
Pneumothorax, Pe, Pnmed, emphysema	48 (8.5%)	4 (8.3%)	44 (8.5%)	1.000
In hospital stay	13.3±7.7	15.2±6.7	13.1±7.7	0.066
ICU stay	**7.4±4.7**	**9.3±5.4**	**7.2±4.6**	**0.011**
In hospital mortality	404 (71.5%)	29 (60.4%)	375 (72.5%)	0.107

*P value obtained comparing tcz group with control group. Data are presented as n (%) unless vontinious variables (days) presented as mean ±Sd, List of abbreviations: Pnx=Pneumothorax, Pe=Pulmonary embolism, Pnmed=Pneumomediastinium, TCZ=Tocilizumab, ICU=Intensive care unit

Table 3. Patients characteristics after matching

Characteristics	Total (n = 96)	TCZ (n= 48)	Control (n =48)	P value*
Age (years ±Sd)	**58.3±14.7**	**64.8±11.6**	**52.0±14.8**	**<0.001**
Male sex	55 (57.3%)	28 (58.3%)	27 (56.2%)	0.837
Obesity	**57 (59.4%)**	**21 (43.8%)**	**36 (75.0%)**	**0.002**
Asthma/COPD	30 (31.2%)	15 (31.2%)	15 (31.2%)	1.000
Cancer	2 (2.1%)	1 (2.1%)	1 (2.1%)	1.000
Hypertension	48 (50.0%)	26 (54.2%)	22 (45.8%)	0.414
Diabetes	48 (50.0%)	23 (47.9%)	25 (52.1%)	0.683
Coronary artery disease	27 (28.1%)	14 (29.2%)	13 (27.1%)	0.820
SpO_2/FiO_2 ratio ±Sd	71.5±32.3	71.9±37.5	71.1±26.5	0.906

*P value obtained comparing tcz group with control group. List of abbreviations: COPD=Chronic Obstructive Pulmonary Disease, TCZ=Tocilizumab

Table 4. Mean outcomes differences after matching

All cohort outcomes	Total n=(96)	TCZ (n=48)	Control (n=48)	P value*
Mechanical ventilation	67 (69.8%)	35 (72.9%)	32 (66.7%)	0.505
Pnx, Pe, Pnmed, emphysema	8 (8.3%)	4 (8.3%)	4 (8.3%)	1.000
In hospital stay	14.9±7.8	15.2±6.7	14.5±8.9	0.661
ICU stay	8.6±5.3	9.3±5.4	7.9±5.1	0.198
In hospital mortality	59 (61.5%)	29 (60.4%)	30 (62.5%)	0.834

*P value obtained comparing tcz group with control group. Data are presented as n (%) unless vontinious variables (days) presented as mean ±Sd, List of abbreviations: Pnx=Pneumothorax, Pe=Pulmonary embolism, Pnmed=Pneumomediastinium, ICU=Intensive care unit, TCZ=Tocilizumab

DISCUSSION

In this study, we examined the effects of TCZ treatment on length of hospital stay, length of ICU stay, and mortality in patients with COVID-19 infection. We found that the mean age of the patients who underwent TCZ treatment after PSM was significantly higher than the control group. Cytokine storm is a condition that occurs with the uncontrolled proliferation of proinflammatory markers in the human body. These proinflammatory markers such as IL-6 and IL-8 increase in circulation when cell death is about to occur. In elderly patients, the inflammatory signaling process is faster in these patients because of aging-related changes in cells and cellular changes caused by comorbidities (13). Therefore, these patients are more prone to cytokine storms because of the weaker adaptive immune response and the faster rise of proinflammatory markers in their circulation (13). We think that this is the reason for the higher average age of the patients who received TCZ treatment in our study. Severe anorexia and adipsia due to cytokine increase are common in elderly COVID-19 patients (14). A

meta-analysis concluded that obesity increases the risk of severe COVID-19 infection and increases in-hospital mortality rates in COVID-19 infection (15). Chronic diseases such as diabetes and coronary artery disease increase the risk of severe COVID-19 infection (13). Since obesity increases the risk of developing chronic diseases (such as diabetes, coronary artery disease), obesity is also thought to have a high risk of developing severe COVID-19 infection (16). The results of our study showed that the mean body mass index (BMI) of patients who received TCZ treatment because of severe disease was lower than those who did not receive TCZ treatment.

In the study of Shcherbak et al. (17), male gender and being over 40 years of age were found to be among the predisposing factors for cytokine storm in COVID-19 patients. In our study, no significant difference was found between the genders of the patients who received and did not receive TCZ treatment. Although male gender is among the predisposing factors for cytokine storm, it did not reveal a significant difference between female gender in the development of severe disease and the application of TCZ treatment due to cytokine storm.

Cytokine storm syndrome was more common during COVID-19 infection in patients with comorbidities such as diabetes, hypertension, chronic renal failure, asthma, chronic arterial disease, and cancer (13,18). In the study of Guaraldi et al. (18), patients receiving TCZ treatment were more likely to have comorbid diseases such as diabetes, hypertension, and chronic kidney failure, whereas in our study, no significant difference was found between patients who received TCZ treatment and those who did not.

In the randomized clinical study of Salvarani et al. (19), patients with COVID-19 pneumonia with a PaO_2/FiO_2 ratio of 200-300 mmHg were selected. In other words, research has been done on the effectiveness of TCZ treatment at an earlier stage, but it has not been seen that early TCZ treatment has a significant effect in reducing the need for intubation or mortality. Campochiaro et al. (20), no significant difference was found between patients who received and did not receive TCZ treatment in terms of clinical improvement and mortality, but it was observed that TCZ treatment initiated in patients with a PaO_2/FiO_2 ratio above 100 provided higher clinical improvement. In our study, the mean values of the patients whose SpO_2/FiO_2 ratios were recorded were found to be approximately 71 mmHg after PSM in both groups who received and did not receive TCZ treatment, and there was no statistically significant difference between the two groups. There are studies that suggest that the use of SpO_2/FiO_2 ratio may also be reliable in predicting early invasive mechanical ventilation (21). Different studies have tried to decide on threshold values (21,22). When we examined, values of 100 mmHg and below are indicative of the development of severe disease and these patients are likely to need early mechanical ventilation. In our study, no significant difference was found between the patients who received and did not receive TCZ treatment in terms of the need for invasive mechanical ventilation. In other words, in our study, there was no additional benefit of TCZ treatment in reducing the need for invasive mechanical ventilation in patients with severe acute respiratory distress syndrome (ARDS). Klopfenstein et al. (23) showed that patients receiving TCZ treatment needed less invasive mechanical ventilation. In the study of Salama et al. (24) among patients with moderate and severe COVID-19 pneumonia, patients who received TCZ treatment combined with antiviral and glucocorticoids needed less mechanical ventilation than those who received placebo and combined antiviral, glucocorticoid. According to the results of the same study, there was no difference in mortality from any cause between patients who received and did not receive TCZ treatment. In a study conducted in patients who developed ARDS due to COVID-19 infection, complications such as pneumothorax, pneumomediastinium, emphysema, and hemothorax due to invasive mechanical ventilation were examined (25). No significant difference in mortality was reported between the group with and without mechanical ventilation-induced barotrauma. (25). It has been observed that these complications may occur due to the barotraumatic effect of mechanical ventilation, as well as non-barotraumatic due to inflammation, consolidation and necrosis in the lung tissue due to COVID-19 infection (26-28). In our study, no significant difference was found between the patients who received and did not receive TCZ treatment in terms of complications such as pneumothorax, pneumomediastinum, pulmonary embolism, and emphysema.

In our study, although the patients who received TCZ treatment had longer stays in the ICU and hospital, there was no statistically significant difference compared to the patients who did not receive TCZ treatment. There was no significant difference in in-hospital mortality rates between patients who received and did not receive TCZ therapy. Klopfenstein et al. (23), it was observed that TCZ treatment had no effect on hospitalization and length of stay, but significantly reduced mortality rates. In the study of Rosotti et al. (29) it was observed that TCZ treatment prolongs the length of hospital stay but reduces the mortality rate.

In a retrospective cohort study by Colaneri et al. (30) in Italy, no significant difference was found between the length of stay in the ICU and the seven-day mortality rates in patients who received and did not receive TCZ therapy. In a retrospective observational study, it was found that TCZ treatment shortened the length of stay and was associated with a decreased mortality rate (31). In a multicenter observational study, it was found that TCZ treatment reduced the mortality rate in COVID-19 patients hospitalized in the ICU (32). Rossi et al. (33) in a study conducted in Italy, mortality rates were found to be significantly lower in patients who received TCZ treatment.

In the placebo-controlled randomized study of Rosas et al. (34), no significant difference was observed in the 28-day mortality in COVID-19 patients who were started on early TCZ treatment compared to the placebo group. Hermine et al. (35) reported that as a result of their randomized clinical trial, TCZ treatment did not make a significant difference in the need for mechanical ventilation and 28-day mortality rate in COVID-19 patients. In the randomized, double-blind, placebo-controlled study of Stone et al. (36) it was also seen that TCZ treatment did not have an effect on 28-day mortality. In the study of Veiga et al. (37) they reported that they could not find a beneficial effect of TCZ treatment on clinical outcomes in patients with moderate-to-severe COVID-19 pneumonia. In addition, as a result of their analysis, they found that starting TCZ treatment early or late did not change the effect of treatment on clinical outcomes. A meta-analysis investigating the efficacy of TCZ therapy in COVID-19 patients found that TCZ therapy did not add any additional benefit to clinical outcomes (38).

As a result of the researches, we saw that there was no consensus on the results of TCZ treatment, and we conducted this cross-sectional retrospective study. Between the dates examined by the study, access to TCZ was not easy, and patients could be provided and treated within at least two days after they were admitted to the ICU. Patients hospitalized in the ICU had the development of severe ARDS. Although we cannot clearly determine the reason why TCZ treatment does not reduce the need for invasive mechanical ventilation and the mortality rate, we think that this may be related to the time we start treatment in patients with severe disease. Considering that the severity and mortality of COVID-19 is related to IL-6, it was expected that TCZ treatment, which is an IL-6 antagonist, would increase clinical recovery, shorten the length of stay and reduce mortality rates. As a result of a retrospective analysis, it was observed that there was a slight decrease in the mortality rate when TCZ was started in patients with low IL-6 levels, but a higher decrease in mortality rate was observed when it was started in patients with high IL-6 levels (39). If this treatment was applied to patients with low IL-6 levels, this may explain the results of our study. In our study, IL-6 levels were not measured before starting TCZ therapy. We cannot reveal data on this. In addition, the patients in our study were patients with severe COVID-19 infection, so we cannot make inferences about the clinical results of TCZ treatment before ICU admission or in the early stage of the disease.

Our study has limitations as it is a retrospective and single-center study. Mortality rates were evaluated during the hospitalization of the patients, and a long-term evaluation could not be made after discharge. The PSM model allowed us to reduce the resulting bias as it provided randomization. This model, which is used to match patients, cannot control the effect of variables that are not included in the matching (40).

CONCLUSION

A clear consensus has not yet been established on the effect of TCZ treatment on IL-6 receptor blockade and reducing inflammation. Although the COVID-19 pandemic has reduced its severity, its effects continue today and the disease has not been completely eradicated. We conducted our study in the hope of guiding clinicians in the treatment of the disease and scientists who will conduct a placebo-controlled randomized study in case the COVID-19 epidemic worsens or similar viral pandemics occur. The population that will benefit most from TCZ treatment, the timing, dose, regimen, and complications of treatment are still unclear. There is no consensus on treatments (anti-inflammatory, glucocorticoid, antiviral, etc.) combined with TCZ. Therefore, randomized controlled studies are needed to determine which patients will have the beneficial effect of TCZ treatment on the possible clinical outcome. At the same time, there is a need for randomized controlled clinical studies for the treatment of TCZ by creating a patient cytokine profile such as IL-6 level. This retrospective cross-sectional study showed that TCZ therapy had no beneficial or significant effect on any of the outcomes of invasive mechanical ventilation need, length of stay in hospital and ICU, and mortality in patients with severe disease development and hospitalized in the ICU. The results of larger, randomized, placebo-controlled studies are needed to understand the efficacy of tocilizumab in the treatment of COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Uşak University Medical Faculty Clinical Researches Ethics Committee (Date: 15.06.2022, Decision No: 108-108-06).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Evaluation of carotid intima-media thickness of female fibromyalgia patients and determination of their relationship with disease activity, severity of fibromyalgia, anxiety and depression levels

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ABSTRACT

Aim: Carotid artery intima-media thickness is thought strong predictor of cardiovascular diseases. To evaluate the common carotid artery intima-media thickness (CCIMT) in female patients with fibromiyalgia (FM) and determine its relationship with disease activity, severity of fibromyalgia, anxiety and depression levels.

Material and Method: Thirty nine patients who had fibromyalgia syndrome according to 2016 American College of Rheumatology (ACR) classification criteria and 41 healthy controls were included. Pain level and disease activities were assessed with Numerical Rating Scale (NRS) and Fibromyalgia Impact Questionnairre (FIQ) respectively. According to ACR 2016 classification criteria, Widespread Pain Index (WPI), Symptom Severity Score and Hospital Anxiety and Depression Scale (HADS) were performed. The LDL , HDL and Triglyceride levels were evaluated. Bilateral common carotid artery intima-media thicknesses (CCIMT) were performed by a blind radiologist to the groups of participants.

Results: Age, weight and height were similar between groups ($p>0.05$). Triglyceride level was significant higher in patients with FM ($p=0.001$). HDL and LDL levels were similar between groups ($p=0.297$, $p=0.061$). Bilateral CCIMT was significantly higher in patients with FM ($p<0.001$). Bilateral CCIMT were found positively correlated with age in FM group ($r=0.390$, $p=0.014$, $r=0.404$, $p=0.011$ respectively). There were found no correlations between bilateral CCIMT, height, weight, triglyseride level, LDL level, FIQ, NRS, WPI, Symptom Severity Score, HADS scores. .

Conclusion: In FM patients, bilateral CCIMTs were found increased compared to healthy controls. No associations were found between CCIMT, LDL, triglyceride levels, disease activity and pain level, anxiety and depression level in patients with FM.

Keywords: Fibromiyalgia, common carotid artery intima-media thickness, disease activity, anxiety, depression

INTRODUCTION

Fibromyalgia syndrome (FM) is a chronic, painful musculoskeletal disorder of unknown etiology. Fatigue, depression, cognitive dysfunction and headache were shown as symptoms in patients with FM (1,2). Various studies show that oxidative stress may play a role in the pathogenesis of FM (3,4). Increased oxidative stress, which is characterized by protein and lipid oxidation in the vascular wall, plays a role in the development of atherosclerosis (5).

Paraoxonase-1 enzyme is a high-density-lypoprotein (HDL) related antioxidant enzyme which is synthesized in the liver (6). It is thought that decreased paraoxonase activity plays a role in the development of coronary artery disease (7). Oxidative stress causes an increase in carotid intima-media thickness as well as coronary artery disease by making endothelial remodelling (8,9). There are some studies showing that patients with FM are exposed to oxidative stress and their paraoxonase and arylesterase activities are reduced (5). Therefore, it is thought that patients with FM are prone to the development of atherosclerosis(5). Also in FM patients sympathetic activity was thought increased because of pain and stress (10-12). It may cause endothelial damage and cardiovascular diseases (13).

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In literature, carotid intima-media thickness measurement was thought strong predictor of cardiovascular diseases (14). Although there are few studies evaluating bilateral carotid intima media thicknesses in patients with FM, there is no study evaluating the relationship between carotid-intima media thicknesses and disease activity, severity of FM, anxiety and depression in patients with FM. We aimed to evaluate the common carotid artery intima-media thickness (CCIMT) in female patients with FM by using ultrasonography and to determine its relationship with disease activity, severity of FM , anxiety and depression levels.

MATERIAL AND METHOD

This study was carried out with the permission of Hitit University Clinical Researches Ethics Committee (Date: 08.09.2020, Decision No: 330). A well written informed consent was obtained from all participants according to the principles of the Helsinki Declaration.

Thirty-nine patients were admitted to our clinic with a diagnosis of fibromyalgia according to the 2016 ACR classification criteria for fibromyalgia syndrome and 41 healthy controls were included in the study (Group 1: patients with FM, Group 2: healthy controls). Participants with concomitant rheumatic disease, neurological disease; history of other systemic diseases such as hypothyroidism/hyperthyroidism, diabetes mellitus; previous history of overt trauma, history of coronary artery disease, and other cardiac diseases and hypertension or related family history were excluded.

Number of participants was determined assuming a 0.66 mm mean difference and 0.24 mm SD of thickness at common carotid artery intima-media (CCIMT) with 80% power and 5% significance and 35±4 participants were planned to invite the study for each group (15).

Demographic and clinical characteristics were recorded. Pain level and disease activities were assessed with the Numerical Rating Scale (NRS) and Fibromyalgia Impact Questionnaire (FIQ) respectively. The LDL , HDL and Triglyceride levels of participants were evaluated. Venous blood samples were obtained at least after a 12 hour overnight fast and all samples were collected between 07:30 and 09:30 AM. Bilateral CCIMTs were performed by a blind radiologist to the groups of participants.

Fibromyalgia Impact Questionnaire (FIQ): The validity and reliability of the Fibromyalgia Impact Questionnaire (FIQ) for Turkey was assessed by Sarmer et al. (16,17). This scale is composed of 10 items. Physical functioning, well-being, missed work days, difficulty in work, pain, fatigue, morning tiredness, stiffness, anxiety, and depression are measured in this scale. Evaluation was realized over a total of 100 points, including 10 points for each subheading. Low score indicates low severity of disease and high score indicates high severity of disease(16).

Numerical Rating Scale (NRS): is a subjective measurement and pain levels of participants were evaluated on an 11-point numerical scale. It is composed of 0 (no pain) to 10 (worst pain)(18).

Hospital Anxiety and Depression Scale (HADS): It is used to determine anxiety and depression levels (19). It consists of 14 questions and anxiety and depression levels are evaluated with seven questions each. Higher scores indicate increased severity of anxiety or depression. The reliability and validity of the Turkish language version were examined (20). Cut-off scores for Turkish society have been determined as 7 for anxiety and 10 for depression (20).

The measurement of common carotis artery intima-media thickness (CCIMT): While the participant was sitting in the supine position, the measurement was taken by rotating the neck to the left for the right common carotid artery and by rotating the neck to the right for the left common carotid artery. For common carotid artery orientation, first transversal imaging was made from the base of the neck to the carotid bifurcation. The vessel wall was viewed longitudinally, approximately 1 cm below the bifurcation. At least 3 carotid intima-media thickness measurements were made and the averages were determined. If plaque was seen, the presence of plaque was noted. With the optimal B mode setting, gain, dept and focal zones were adjusted to obtain the best image for carotid intima-media thickness and measure (21).

Statistical Analyses

All data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) 15.0 program for Windows. Visual and analytical methods were used for the variables for determining whether or not they are normally distributed. Continuous variables were expressed as mean ± SD and nonparametric variables were expressed as median (quartiles). Independent sample t test was used to compared FIQ score, Triglyseride level, HDL level, LDL level, Symptom Severity Score, weight, BMI and left CCIMT. Man Whiney U test was used to compared age, height, NRS, Widespread Pain Index, HADS anxiety and depression scores and right CCIMT. Pearson and Spearman correlation coefficients were used to evaluate the linear relationship between predictive variables. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Thirty-nine patients with a diagnosis of fibromyalgia according to 2016 ACR classification criteria for fibromyalgia syndrome and forty healthy participants were included. All participants were female. There were 16 (41%) patients who used duloxetine and 17 (43.5%) patients who used pregabalin in FM group. Age, height, weight and BMI were similar between groups (**Table 1**) Triglyceride level was significant higher in patients with FM compared to healthy controls (p=0.001). HDL and LDL levels were similar between groups (p=0.297, p=0.061). FIQ score was 67.10±11.56, NRS score was 8 (6-8) in patients with fibromyalgia. Bilateral common carotid artery intima-media thicknesses were significantly higher in patients with fibromyalgia compared to healthy controls (p<0.001). In fibromiyalgia group, there were 1 patient with left common carotid artery plaque and 2 patients with right common carotid artery plaque (p=0.481, p=0.741, respectively). There were no participants with common carotid artery plaque in healthy controls. In FM group, there was no significant difference in right and left CCIMT between patients who used duloxetine and pregabalin (p=0.845 and p=0.822 respectively).

Table 1. Demographic and clinical features of patients with fibromyalgia and healthy controls

	Patients with fibromyalgia n=39	Healthy controls n= 41	p value
Age (year)	44 (37-49)	40 (30-45)	0.051
Height (cm)	164 (160-167)	165 (158.5-168)	0.873
Weight (kg)	71.08 ± 8.6	67.24± 12.6	0.115
BMI (kg/m²)	26.85± 3.74	25.27± 4.74	0.104
Disease duration (month)	36 (12-60)		
FIQ (score)	67.10±11.56		
NRS (score)	8 (6-8)		
Widespread pain index (WPI)	8 (6-11)		
Symptom severity score	8.1± 1.79		
Triglyceride (mg/dL)	193.05±148.55	99.9±46.9	0.001
HDL (mg/dL)	54.66±12.01	52.12±8.94	0.297
LDL (mg/dL)	128.68±46.33	110.131±31.82	0.061
HADS anxiety score	6 (4-10)	2 (2-3)	<0.001
HADS depression score	6 (4-9)	2 (2-3)	<0.001
Right carotid intima-media thickness (mm)	0.69 (0.65-0.73)	0.48 (0.44-0.56)	<0.001
Left carotid intima-media thickness (mm)	0.69±0.11	0.51±0.12	<0.001

HADS: Hospital Anxiety Depression Scale. Data are presented as mean±standard deviation or median and quartiles. p<0.05

Bilateral CCIMT were found positively correlated with age (**Table 2**). HDL level was found negatively correlated with left CCIMT (**Table 2**). There were found no correlations between bilateral CCIMT, height, weight, BMI, triglyseride level, LDL level, FIQ, NRS, Widespread Pain Index (WPI), Symptom Severity Score, HADS anxiety and depression scores (**Table 2**).

Table 2. Correlation between carotid intima-media thicknesses and clinical features in fibromyalgia patients

	Right carotid intima-media thickness		Left carotid intima-media thickness	
	r	p	r	p
Age	0.390	0.014**	0.404	0.011**
Height	-0.029	0.863**	0.025	0.881**
Weight	0.198	0.227**	0.207	0.207**
BMI	0.208	0.204**	0.215	0.188*
Disease duration	0.008	0.961**	0.137	0.404**
FIQ score	-0.086	0.603**	-0.023	0.889*
NRS score	-0.260	0.110**	-0.239	0.142**
Triglyceride level	0.086	0.607**	0.038	0.823*
HDL level	-0.312	0.057**	-0.393	0.015*
LDL level	0.135	0.462**	0.235	0.196*
Widespread pain index (WPI)	0.067	0.684**	0.055	0.740**
Symptom severity score	0.036	0.827**	-0.043	0.795*
HADS anxiety score	-0.146	0.375**	-0.184	0.262**
HADS depression score	-0.165	0.317**	-0.199	0.224**

*: Pearson correlation, **: Spearman correlation

DISCUSSION

Our results demonstrated that common carotid artery intima-media thicknesses were increased bilaterally in patients with fibromyalgia. We found that there was an association between age and bilateral common carotid artery intima-media thicknesses. Also there was an association between blood HDL level and left common carotid intima- media thickness. In the literature there are a few studies evaluating common carotid intima-media thicknesses in patients with FM. One study was done Bölük et al. (22) and they found that carotid intima-media thicknesses were increased in patients with FM. But they did not describe how they measured carotid intima-media thicknesses or which carotid artery intima-media thicknesses (internal carotid artery, external carotid artery or common carotid artery) were measured. Low number of patients participated to their study. We evaluated the drug usage and disease duration and found that CCIMTs were similar in both FM patients who used duloxetine and pregabalin in our study. But they did not evaluated the relationship between disease duration or using drug and carotid intima-media thicknesses. Also differently from our study, they did not evaluate the association between carotid intima-media thicknesses and anxiety and depression levels. In a study which evaluated the relationship between endocan levels and carotid intima-media thickness in patients with FM, serum endocan levels and carotid intima-media thickness were found

significantly higher in patients with FM (23). This study supports to our results but we did not evaluate serum endocan level or other inflammatory marker with blood labratory test in our study. Although these two studies examined carotid intima-media thickness in patients with FM, there is no study in the literature designed as comprehensive as our study.

In FM patients, stress and pain were known related with increased activity of sympathetic nervous system (10-12). Sympathetic hyperactivity may contribute to endothelial damage and cause cardiovascular diseases (13). So increased chronic pain may cause endothelial dysfuction and increased common carotid artery intima-media thickness. In the U.S. National Health Interview Survey, myocardial infarction was found more common in patients with FM than in patients without FM (24). Also, in a Taiwanian study, the risk of coronary heart disease was found increased in patients with FM (25). The results of our study support to the increased risk of cardiovascular diseases. In many studies, carotid intima-media thickness measurement was found successful for assessment of cardiovascular disease risk (26-30). Carotid intima-media thickness measurement was shown independently predictive measurement for old and young subjects in future vascular events (14). Also, brain infarction was found associated with increased common carotid artery intima-media thickness (29). However most participants were middle-aged in our study and we found that bilateral CCIMTs were higher in patients with FM. But we did not find any association between bilateral CCIMT, disease activity, severity of FM, pain, anxiety and depression levels. According to our study, it can be concluded that increased disease activity, anxiety or depression levels do not increase CCIMT in patients with fibromyalgia. Our results support the predisposition to atherosclerosis diseases in patients with FM. Therefore, stroke and cardiovascular diseases are expected to be more common in patients with FM. So we could predict cardiovascular disease performing ultrasonography for measuring CCIMT.

Tseng CH et al. (31) found that diabetes mellitus, hypertension, hyperlipidemia and coronary artery disease were more prevalent in fibromyalgia patients than healthy controls. Also they showed an increased risk of cumulative stroke in patients with fibromyalgia in a 3-year follow up cohort study. Age is an important risk factor for stroke (31). We found that there was an association between age and bilateral common carotid artery intima-media thicknesses. In their study, the stroke weight was found relatively higher in the younger population in patients with FM. Because stroke-related comorbidities were found more common in the patients with FM (31). Similar to this study, we found that triglyceride level was higher in patients with FM. But we found that HDL and LDL level were similar in patients with FM and healthy controls. In this and our study most participants were middle-aged. We did not evaluate the presence of hypertension and diabetes mellitus in patients with FM in our study. Because we excluded the participants with hypertension and diabetes mellitus from our study. The lack of difference in LDL levels between patients and healthy controls in our study may be due to the exclusion of patients with diabetes mellitus and hypertension from our study.

Headache and impaired sleep quality are common known symptoms in patients with FM. Tatar IG et al. (32) said that carotid intima-media thickness was increased in patients with migraine. Brutto OH et al. (33) showed that there was an association between sleep quality and increased carotid intima media thickness. So one of the causes of headache and impaired sleep quality in patients with FM may be the increased CCIMT. But we did not evalute the sleep quality in our study.

To the best our knowledge, this is the first study evaluating the relationship between common carotid artery intima media thicknesses and disease activity, disease duration, severity of FM, pain, anxiety and depression levels in patients with FM. Inclusion of sufficients number of patients, making the ultrasonographic measurements by a blinded radiologist, addition to disease activity evaluation of pain level, severity of FM, anxiety and depression levels are the superior aspects of our study. We think that this study will provide valuable contributions to the literature and clinical practice in terms of early recognition and prevention of cardiovascular diseases in patients with FM. But there are some limitations in our study. Exclusion of male patients with FM and measurement of only common carotid artery intima-media thicknesses, single-site location of the investigation may be some limitations of our study.

CONCLUSION

This study shows that in fibromiyalgia patients, bilateral carotid artery intima media thicknesses were found increased compared to healthy controls. If increased carotid artery intima-media thickness was thought a strong predictor of cardiovascular events, patients with fibromylagia should be followed up more carefully in terms of cardiovascular events in long term. No association were found between carotid intima-media thickness, LDL, triglyceride levels, disease activity, severity of fibromyalgia, anxiety, depression and pain level in patients with fibromiyalgia.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Hitit University Clinical Researches Ethics Committee (Date: 08.09.2020, Decision No: 330).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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The relationship between systemic immune-inflammation indexes and treatment response in locally advanced esophageal cancer

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ABSTRACT

Aim: Systemic immune-inflammation indexes have been reported to be associated with clinical outcomes in several malignancies. Herein, we aimed to evaluate the potential relationship between prognostic nutritional index (PNI), systemic immune-inflammation index (SII), the neutrophil- to- lymphocyte ratio (NLR), the monocyte- to- lymphocyte ratio (MLR), the platelet-to-lymphocyte ratio (PLR) and the treatment response in patients with esophageal cancer who underwent neoadjuvant chemoradiotherapy (CRT).

Material and Method: Esophageal cancer (EC) patients who underwent neoadjuvant CRT were retrospectively enrolled in the study. Immune-inflammation indexes were calculated from pretreatment blood counts in samples obtained. The relationships between PNI, SII, NLR, MLR, PLR values, treatment response, and overall survival (OS) rates were examined.

Results: The data of 103 patients with EC who were referred to the Radiation Oncology Clinic of Dr Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital were retrospectively analyzed. In the univariate analysis for OS, alcohol consumption, CRT agent, NLR, MLR, PLR, SII and PNI were found as prognostic factors. Also alcohol consumption was found as an independent prognostic factor in multivariate analyzes (HR:5.201, 95% CI:1.9-14.2, p=0.01).

Conclusion: In our study, high SII and low PNI values were not found to be independent poor prognostic factors for OS, but lower OS rates were observed in patients with high SII and low PNI values.

Keywords: Esophageal cancer, treatment response, systemic immune-inflammation index

INTRODUCTION

Esophageal cancer (EC) is the sixth leading cause of death and 8th most common cancer worldwide (1). Most common subtype is squamous cell cancer (SCC). Use of tobacco products, alcohol and nitrosamines are risk factors for esophageal SCC. Obesity, Barrett's esophagus, gastroesophageal reflux disease and use of tobacco products are important risk factors for esophageal adenocarcinoma (EAC) (2). Diagnosis at an early stage is very important. While surgery alone is sufficient for the treatment of early-stage tumors, multidisciplinary treatment options are preferred in locally advanced disease. Prognosis of advanced disease is poor. Although modalities such as radiotherapy (RT), chemotherapy (CT) and surgery are being used in combination in the treatment of esophageal cancer, its prognosis is unfortunately poor with five-year overall survival (OS) rates ranging between 15% and 25% (3). In patients with borderline resectable locally advanced esophageal cancer, preoperative chemoradiotherapy (CRT) followed by surgery is the most commonly used treatment modality. In the ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study (CROSS), patients were randomized to preoperative RT and concurrent weekly CT followed by surgery or surgery alone, and it was reported that OS was significantly increased in the CRT arm with a median 7-year follow-up with similar complication rates (4). In the study of Donohoe at al. (5) pathological complete response was observed in less than 30 % of the patients with EC who underwent neoadjuvant CRT. In another study, survival benefit was reported in patients who had complete response to treatment (6).

In recent years, increasing evidence has shown that inflammatory biomarkers are significantly associated with poor prognosis in EC. However, the detailed mechanisms still remain unclear. There are possible explanations for the

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association between inflammatory biomarkers and poor prognosis in patients with solid tumors. Firstly, neutrophils promote proliferation of tumor cells by producing proteolytic enzymes including matrix metalloproteinases (MMPs) and serine proteases, and stimulate both tumor angiogenesis by releasing proangiogenic factors including MMP 9 and vascular endothelial growth factor (VEGF). Neutrophils cause local immunosuppression by disrupting T-cell responses and inducing T-cell death (7-10). Secondly, there is increasing evidence that T-lymphocytes play a critical anti-tumor role by inhibiting tumor cell proliferation and metastasis, inducing cytotoxic cell death, and promoting antitumor immune responses (11). Thirdly, platelets interact directly with tumor cells and release factors that promote tumor growth, invasion and angiogenesis (12). Platelets can contribute to metastasis by stabilizing tumor cell arrest in the vasculer system, stimulating tumor cell proliferation, and promoting extravasation of tumor cells (13). Recently, it has become important for clinicians to foresee patients that will respond to treatment and to devise an individualized treatment plan.

In our study, we aimed to investigate the relationship between pretreatment inflammatory parameters such as NLR, MLR, PLR, SII and PNI, and response to treatment and survival in patients with esophageal cancer who had undergone neoadjuvant chemoradiotherapy.

MATERIAL AND METHOD

This study was carried out with the permission of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date:26.05.2022, Decision No: 2022-05/109). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This analysis was conducted in 103 patients with EC who underwent neoadjuvant chemoradiotherapy (CRT) in our clinic from January 2014 to January 2022. Staging was done using the 8th Edition American Joint Committee on Cancer TNM Staging Manual guidelines (14). Histologically confirmed EC patients with Eastern Cooperative Oncology Group (ECOG) performance status scores between 0 and 2 were included in the study. Patients with active concomitant infection, distant metastases at the time of diagnosis, autoimmune diseases, hematological diseases, missing baseline blood test results and corticosteroid users during treatment were not included in the study. Data related to clinicopathological variables such as gender, age, tumor localization,and pathology, smoking status, alcohol consumption, were obtained by retrieving medical records. All patients had neoadjuvant concurrent CRT (chemotherapy including paclitaxel (50 mg/m²)-carboplatin (AUC 2) and fluorouracil (750-1000 mg/m²) and cisplatin (75-100 mg/m²) and a daily radiotherapy dose of 1.8/2 Gy amounting to a total dose of 41.4-54 Gy delivered using intensity modulated radiotherapy (IMRT). IMRT was planned using Eclipse (ver. 11: Varian Medical Systems, Inc. Palo Alto, CA, USA) planning software. To evaluate the response to neoadjuvant treatment all patients underwent restaging 4-6 weeks after the completion of CRT. Immune-inflammatory factors were obtained from pretreatment values and immune-inflammation indexes were calculated. The PNI was calculated using the formula:serum albumin (g/dl) + 5 x absolute lymphocyte count. The neutrophil (N; $\times10^9$/l) to lymphocyte (L; $\times10^9$/l) ratio (NLR) was calculated by dividing the absolute neutrophil counts by absolute lymphocyte counts. The monocyte (M; $\times10^9$/l) lymphocyte ratio (MLR) was calculated by dividing the absolute monocyte counts by absolute lymphocyte counts. The platelet (P; $\times10^9$/l) lymphocyte ratio (PLR) was calculated by dividing the absolute platelet counts by absolute lymphocyte counts. SII was estimated using the formula: platelet counts x neutrophil counts/lymphocyte counts (15-17).

Treatment response was evaluated using histopathology reports in patients who were surgically treated and using Response Evaluation Criteria in Solid Tumors (RECIST) criteria in patients who were not surgically treated. The categories were defined as complete response, partial response, stable disease and progresive disease (18). The relationship between PNI, NLR, MLR, PLR, SII values and treatment response-OS were evaluated statistically. We defined OS as the time from the histopathologic diagnosis of EC to the last follow-up or death.

Statistical Analysis

The statistical analysis was performed using SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) Version 22 software package. The descriptive categorical data were expressed as numbers (n) and percentage values (%) while descriptive continuous data were presented as mean±standard deviation (mean±SD). Chi-Square test was applied for intergroup comparisons of categorical variables. The fitness of continuous variables to normal distribution was evaluated by Kolmogorov-Smirnov test. In intetrgroup comparisons. Student-t test was used for normally distributed variables while Mann-Whitney U test was applied for non-normally distributed variables. In intergroup comparisons One Way ANOVA test was used for variables with normal and Kruskal -Wallis test with non-normal distribution. Receiver operating characteristic (ROC) curve analysis was performed to analyze the area under the (ROC) curve and to determine optimal cut-off values. Kaplan-Meier method was used for univariate analysis of local control and survival. Multivariate Cox regression analysis that contained all the factors of univariate analysis was performed. The statistical significance level of the analyses was set at $p<0.05$.

RESULTS

A total of 103 patients with a median age of 60 years (range: 19-84 years) including 37 female (35.9 %) and 66 male (64.1%) cases diagnosed as esophageal carcinoma enrolled in this study, Most patients had a history of smoking (60.4%) and a few (5.1%) patients were alcohol users The indicated number of patients had ECOG performance status scores of 0 (n=27), 1 (n=72), and 3 (n=4) points.

Tumors were located proximally in 15, thoracically in 29 and distally in 59 patients. The majority of the cases (88.3%) had squamous cell carcinoma, and the rest adenocarcinoma. In addition, 87 (84.5%) patients had received paclitaxel-carboplatin, the others fluorouracil and cisplatin as concurrent chemotherapy. Fifty patients (48.5%) underwent surgery, 27 patients (26.2%) didn't undergo surgery and 26 patients (25.2%) didn't want to undergo surgery after neoadjuvant chemoradiotherapy. All patients had locally advanced disease. Patients were staged with PET-CT before initation of the treatment. Neoadjuvant treatment was planned for all patients. All patients received neoadjuvant concurrent chemoradiotherapy. Fifty patients (48.5%) underwent surgery 6-8 weeks after the completion of neoadjuvant treatment but 53 patients did not want surgery so had no operation.

The response rate was evaluated histopathologically in patients who had and radiologically in patients who had not unergone surgery . Complete, and partial response rates were observed in 54 (52.4%), and 28 (27.2%) patients, respectively. Twelve patients had no response and evaluated as stable disease. Nine patients had progressive disease. The relationship between PNI, NLR, MLR, PLR and SII values and treatment response was not statistically difference (p>0.005) (**Table 1**). In the univariate analysis for OS, alcohol consumption, CRT agent, NLR, MLR, PLR, SII and PNI were found that as prognostic factors. Also alcohol consumption was revealed as an independent prognostic factor in multivariate analyzes (HR:5.201, 95% CI:1.9-14.2, p=0.01). Although the p value was significant, the SII value was not considered significant because the confidence interval included 1.0. Gender, comorbidity, pathology, tumor localization, ECOG scores were not found to be associated with survival (**Table 2**).

In the univariate analysis 5 -year survival rates were %29.8 in patients with SII ≤ 604 and %9.7 in patients with SII>604 (p=0,003); 7.4% in patients with PNI ≤ 40 and 28.9% in patients with PNI > 40 (p=0.014) without any statistically significant difference as for 5-year survival rates. The findings are shown in **Figures 1a-1b**. When all patients were evaluated, the median follow up time, and average survival time were 15.5, and 31.5 months, respectively. While 2- year and 5- year survival rates were 38.8% and 16.9%, respectively.

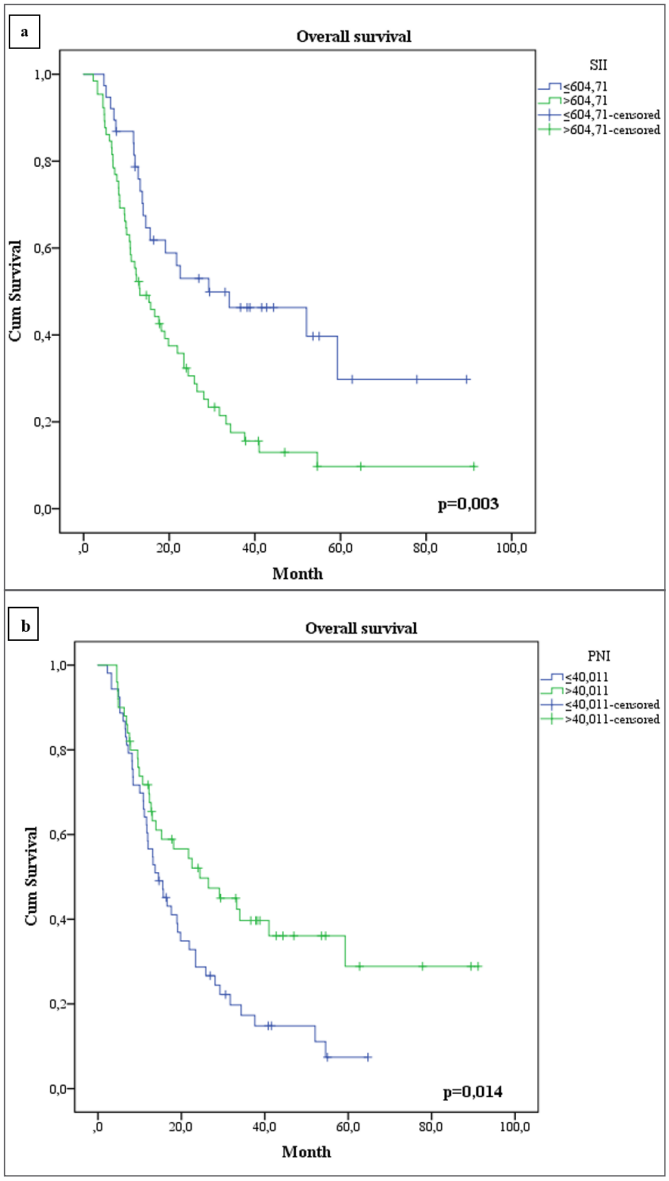

Figure 1. Kaplan-Meier survival curves for a SII and b PNI on survival overall.

Abbreviations: SII: Systemic immune-inflammation index, PNI: Prognostic nutritional index

Table 1. The relationship between NLR, MLR, PLR, SII and PNI values and. treatment response.

	Complete response	Partial response	Stable disease	Progressive disease	P value
NLR, mean±SD	3.4±3.1	3.4±1.5	2.9±.9	4.9±6.7	0.801*
MLR, mean±SD	.3±.2	.3±.1	.3±.1	.5±.6	0.561*
PLR, mean±SD	170.9±122.1	205.2±97.0	161.2±71.6	209.6±111.9	0.063*
SII, mean±SD	904.5±1182.2	1021.1±511.1	841.8±397.3	1200.3±1352.5	0.295*
PNI, mean±SD	38.6±55.3	37.0±54.3	39.9±58.9	36.6±68.4	0.558**

*Kruskal- Wallis analysis, **One Way ANOVA analysis Abbreviations: NLR: neutrophil- to- lymphocyte ratio, MLR: monocyte- to- lymphocyte ratio, PLR: platelet- to- lymphocyte ratio, SII: systemic immune-inflammation index, PNI: prognostic nutritional index.

Table 2: Univariate and multivariate analysis of the prognostic factors affecting OS.

Univariate Analysis		**Patients n (%)**	**5-year OS (%)**	**Mean**	**Median**	**p-value**
Gender						0.131
	Female	37 (35.9)	24.3	38.3±6.0	19.1	
	Male	66 (64.1)	14.7	28.0±3.9	15.5	
Alcohol consumption						0.002
	Present	5 (5.1)	N/A	8.7±3.9	4.8	
	Absent	93 (94.9)	17.9	32.3±3.5	16.6	
Comorbidity						0.732
	Absent	36 (35.0)	11.7	31.7±5.5	18.9	
	Present	67 (65.0)	18.9	30.8±4.0	16.6	
Pathology						0.752
	SCC	91 (88.3)	16	30.8±3.5	17.6	
	Adenocarcinoma	12 (11.7)	20	34.3±10.3	7.6	
Tumor localization						0.270
	Proximal location	15 (14.6)	14.8	25.6±6.1	18.9	
	Thoracic location	29 (28.2)	NR	22.9±3.9	15.2	
	Distal location	59 (57.3)	23.3	35.9±4.7	21.9	
ECOG						0.137
	0	27 (26.2)	12.8	25.7±5.7	12.7	
	1	72 (69.9)	12.5	28.9±3.2	18.1	
	2	4 (3.9)	50	42.9±11.2	29.1	
CRT agent						0.048
	paclitaxel-carboplatin	87 (84.5)	23	35.1±3.9	21.7	
	fluorouracil and cisplatin	16 (15.5)	NR	18.1±3.5	12.3	
NLR						0.023
	≤2.99	53 (51.5)	23.7	38.2±5.1	21.9	
	>2.99	50 (48.5)	11	24.8±3.9	13	
MLR						0.018
	≤0.32	69 (67.0)	21.3	35.9±4.2	22.5	
	>0.32	34 (33.0)	7.7	19.2±3.1	11.1	
PLR						0.022
	≤121.66	23 (22.3)	NR	34.9±4.2	34	
	>121.66	80 (77.7)	14.5	27.8±3.4	14.5	
SII						0.003
	≤604.71	38 (36.9)	29.8	44.1±6.2	29.2	
	>604.71	65 (63.1)	9.7	24.1±3.4	13.1	
PNI						0.014
	≤40.011	53 (51.5)	7.4	21.5±2.6	14.6	
	>40.011	50 (48.5)	28.9	40.9±5.5	24.4	
Multivariate Analysis		**Hazard ratio**		**95% CI**		**p-value**
Alcohol consumption	Present vs Absent	5.201		1.900-14.238		0.001
CRT agent	Paclitaxel-carboplatin vs fluorouracil and cisplatin	1.738		.969-3.117		0.064
NLR	≤2.99 vs >2.99	.717		.349-1.475		0.366
MLR	≤0.32 vs >0.32	1.428		.818-2.494		0.210
PLR	≤121.66 vs >121.66	1.273		.589-2.749		0.539
SII	≤604.71 vs >604.71	1.900		.846-4.267		0.120
PNI	≤40.011 vs >40.011	.636		.379-1.067		0.087

Abbreviations: OS: overall survival, ECOG: Eastern Cooperative Oncology Group, CRT: chemoradiotherapy, NLR: neutrophil- to- lymphocyte ratio, MLR: monocyte- to-lymphocyte ratio, PLR: platelet- to- lymphocyte ratio, SII: systemic immune-inflammation index, PNI: prognostic nutritional index, CI: confidence interval, N/A: not available.

DISCUSSION

The varied response in EC patients after neoadjuvant CRT is a serious challenge for administration of appropriate treatment to these patients. The prediction of the prognosis is very important for the management of treatment for EC. There are various pre- and post -treatment parameters in the literature. Chen et al. (16) determined a systemic inflammation parameter, namely systemic immune-inflammation index (SII), which is a predictor for OS and recurrence of colorectal cancer. Gao et al. (17) reported that the SII is an independent prognostic factor in patients with surgically resected esophageal SCC. Prognostic nutritional index (PNI) was used by Buzby et al. (19) in 1980 to estimate operative risk after gastrointestinal surgery. Onodera et al. (20) developed PNI to predict postoperative morbidity and mortality in patients undergoing gastrointestinal surgery.

In recent studies, a relationship between inflammation and disease survival with parameters as NLR, PLR, SII and PNI has been shown, but there is no consensus on the cut-off values of these parameters yet. Fu et al (21) evaluated the prognostic significance of preoperative systemic inflammation index score (SIS), calculated by a composite score of the lymphocyte-to-monocyte ratio and the albumin content in serum, in patients with esophageal SCC and reported that the optimal cut-off values for preoperative NLR, LMR and albumin were 2.27, 3.79 and 36.55, respectively. Univariate analyses found that NLR, LMR, albumin and SIS were significantly associated with OS. The authors found that SIS was an independent prognostic factor. Cai et al. (22) aimed to analyze the association of hematologic markers with prognosis and toxicities in patients with locally advanced esophageal SCC who underwent neoadjuvant CRT. They also reported that patients with high SII (≥583.45), PLR (≥142.17) and NLR (≥2.77) had significantly worse prognosis and severe adverse events. One of the inflammation indexes is platelet/lymphocyte ratio (PLR). Asher et al. (23) found that median OS in patients with a PLR of <300 was 37.4 months (95% CI 26.1-48.7) and 14.5 months (95% CI 11.7-17.2) in patients with a PLR of > 300. They have shown that PLR is a novel independent prognostic marker in patients with ovarian cancer. Hirahara et al. (24) retrospectively analyzed data from 169 patients who underwent radical esophagectomy and found that patients with low PNI had significantly worse OS than that of the patients with a high PNI (HR 2.612; 95% CI 1.600–4.405). In this study, PNIs <49.2 (HR 3.887) were determined as independent adverse predictive factors for cancer specific-survival.

In a study, the researchers found that pre-CRT NLR, pre-CRT PLR, absolute lymphocyte counts estimated during CRT, post-CRT platelet counts and post-CRT PLR were significantly associated with complete response in esophageal SCC patients after neoadjuvant CRT. They demostrated that pre-CRT NLR, post-CRT PLR were independent predictors of complete response contrary top re-CRT PLR (25). We observed no relationship between treatment response and NLR, PLR values. Koh et al. (26) evaluated LMR, NLR and PLR before and after definitive concurrent CRT in esophageal SCC patients. They reported that post-CRT NLR predicted OS better than the other above mentioned parameters. In their study the median follow up time was 11.4 months, and the OS rates at 1 and 3 years were 48.5% and 21.6%, respectively. However, in our study, cut-off values of NLR (2.99), PLR (121.66), SII (604.71), and PNI (40) for OS were as indicated. In our study, lower survival rates were observed in patients with higer NLR, SII and lower PLR and PNI values. In recent studies varying survival rates have been reported in patients with locally advanced esophageal SCC. In a review Herskovic et al. (27) reported that 3- and 5-year- OS rates ranged between 19.2%-32 % and 33%-39% for locally advanced esophageal cancer patients treated with concurrent CRT followed by surgery, respectively. Yang et al. (28) compared the treatment efficacy of neoadjuvant CRT plus surgery with surgery alone among patients with locally advanced esophageal cancer. They reported respective 3-, and 5-year- OS rates as 65.8% and 59.9% in the neoadjuvant CRT group compared with corresponding OS rates of 57.8% and 49.1% in the surgery group. In our study 2-, and 5-year survival rates were 38.8% and 16.9%, respectively.

Our study have some limitations as being a single-center retrospective trial performed in small number of heterogeneous patient population. So conduction of multicenter prospective studies will be needed to evaluate these findings.

CONCLUSION

Pretreatment immune-inflammation indexes including PNI and SII may be potentially effective prognostic factors in locally advanced EC patients who underwent neoadjuvant chemoradiotherapy. In this study, however, higher SII and lower PNI values were not found to be independent adverse prognostic factors for OS, however lower OS rates were detected in patients with comparatively higher SII and lower PNI values.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date:26.05.2022, Decision No: 2022-05/109).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Could niaouli aromatherapy oil be an option in the treatment of urinary tract infections in hemiplegic patients?

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ABSTRACT

Aim: Stroke remains a massive public health burden, affecting approximately 795,000 individuals each year. It is the leading cause of long-term disability in adults and the third leading cause of death in developed countries. After a stroke, medical complications are common and can prolong hospital stay, worsen stroke outcomes, and increase the cost of care. The most common medical complications related to stroke are infections, including pneumonia and urinary tract infection (UTI). Intervention strategies previously investigated in these cases and other patient populations include prophylactic antibiotics, antiseptic-impregnated catheters, and quality improvement interventions to reduce inappropriate catheterization. In addition, in recent years, complementary and alternative medicine methods, such as *Melaleuca viridiflora* (also known as tea tree or Niaouli oil) have become increasingly popular. The primary uses of this oil have historically been associated with the antiseptic and anti-inflammatory effects of this plant. In this study, we investigated the efficacy of M. viridiflora (Niaouli) oil in the treatment of UTIs in stroke patients.

Material and Method: We did not find any study in the literature on the effects of Niaouli aromatherapeutic oil on UTIs, which are common in hemiplegic patients; therefore, we planned the current study. The hospital records were screened to identify patients treated at the Physical Therapy and Rehabilitation Unit of Health Sciences University Adana City Training and Research Hospital, who were diagnosed with UTIs during their follow-up and recommended Niaouli aromatherapy oil as a complementary treatment. The oil was supplied by the patients themselves. As the method of use, the patients were asked to prepare a washing solution by dripping 10 drops of Niaouli oil into 1 liter of water. The patients were recommended to wash the perineum area three times a day with this solution for 20 days.

Results: The mean age of the hemiplegic patients evaluated in the study was 51.55±19.20 (min=18, max=77) years. Of the patients, 72.7% were male, 42.4% had an American Spinal Injury Association classification of C, 54.5% had spontaneous bladder emptying, and 30.3% had stage 1, 21.1% had stage 2, and 3.0% had stage 3 spasticity. Leukocyte esterase and leukocyte in urine and sedimentation values statistically significantly decreased in the post-treatment period compared to the pre-treatment period.

Conclusion: UTI is a common complication in stroke patients. In this study, it was determined that the efficacy of the treatment of UTIs increased, and the use of antibiotics significantly decreased with the utilization of the fungicidal and bactericidal effects of M. viridiflora (Niaouli) aromatherapy oil.

Keywords: Stroke, urinary tract infection, *Melaleuca viridiflora* (Niaouli), aromatherapy

INTRODUCTION

In terms of frequency and importance, stroke ranks first among neurological diseases seen in adulthood. According to the latest World Health Organization report, stroke is the second leading cause of death across the world (1). It is also globally the most common and serious neurological problem. Stroke patients become prone to many complications, both due to the stroke itself and the disability caused by this condition (2). After a stroke, medical complications are common and can prolong hospital stay, worsen patient outcomes, and increase the cost of care (3). Among the most common medical complications related to stroke are infections, such as pneumonia and urinary tract infection (UTI) (4). UTI is a common cause of morbidity in the general population, but stroke patients are at a higher risk of infections and may have more negative significant outcomes (5).

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The use of Foley catheters is a well-defined risk factor for healthcare-associated UTIs, and their inappropriate use may be more common in stroke patients, further increasing the risk of UTIs (5). Stroke patients, whether catheterized or not, are at a particularly high risk of developing UTIs in hospital, and the incidence of UTIs among these patients is more than double compared to the general medical and surgical populations (6). Immunosuppression that develops after a stroke, urinary retention, urinary incontinence, and catheterization are the main reasons for the increased risk of UTIs during this period (6-8). However, through some interventions, the development of post-stroke UTIs can be reduced. The use of prophylactic antibiotics, antiseptic-coated catheters, silver alloy catheters, and condom use in male patients, as well as the reduction of Foley catheter use are among the methods applied to reduce the risk of UTIs that may develop after a stroke (9-10). In addition, complementary medicine methods, including aromatherapy and phytotherapy have been increasingly used for antiseptic purposes in recent years. In particular, *Melaleuca viridiflora* (also known as tea tree or Niaouli) oil has become popular. This essential oil has been used in Australia for nearly a century, but it is now also becoming available across the world both as pure oil and as an active ingredient in a number of products. The primary uses of tea tree oil (TTO) have historically been based on the antiseptic and anti-inflammatory effects of the plant. TTO contains terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and related alcohols (11).

Belonging to the Myrtaceae family, tea tree (*Melaleuca alternifolia* [*M. alternifolia*] Cheel) is an herb with yellow or purple flowers and needle-like leaves. The terpinen-4-ol chemotype of *M. alternifolia* typically contains 30% and 40% of terpinen-4-ol (12) and is used in commercial TTO production. Despite the inherent variability of commercial TTO, to date no significant difference has been reported in its bioactivity in vitro or in vivo. It has been suggested that the oil obtained from a certain *M. alternifolia* clone enhances microbicide activity (13). The antibacterial action mechanism of TTO is based on its hydrocarbon structure and accompanying lipophilic structure. The hydrocarbon structure acts by disrupting the biological membranes of microorganisms (14). There are studies investigating the bactericidal effects of TTO on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (15-17). Recent studies have also shown that *Candida albicans*, a number of yeasts, dermatophytes, and other filamentous fungi are susceptible to TTO (18,19). TTO also has antiviral and antiprotozoal activity (20,21). Although there are many studies demonstrating the antimicrobial, antiviral, antifungal, and antiprotozoal properties of TTO, we did not find any study in the literature concerning its efficacy in UTIs.

In this study, we investigated the efficacy of the aromatherapeutic oil of *Melaleuca viridiflora* (Niaouli), a TTO derivative, in the treatment of UTI, a common complication after a stroke.

MATERIAL AND METHOD

This study was carried out with the permission of Adana City Hospital Clinical Researches Ethics Committee (Date: 08.09.2022, Decision No: 2136). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

To the best of our knowledge, there is no study in the literature on the effects of the Niaouli aromatherapeutic oil on UTIs, which are common in hemiplegic patients.

Therefore, we planned the current study. Patients who were treated in the physical therapy service, who had symptoms of urinary system infection such as dysuria, urinary incontinence, fever, or who were found to have urinary system infection in the urinalysis were included in the study.

The hospital records were screened to identify patients treated at the Physical Therapy and Rehabilitation Unit of Health Sciences University Adana City Training and Research Hospital, who were diagnosed with UTIs during their follow-up and recommended Niaouli aromatherapy oil as a complementary treatment. The oil was supplied by the patients themselves. As the method of use, the patients were asked to prepare a washing solution by dripping 10 drops of Niaouli oil into 1 liter of water. The patients were recommended to wash the perineum area three times a day with this solution for 20 days. The patients' demographic characteristics, length of hospital stay, bladder emptying methods (spontaneous bladder emptying, Foley catheter use, clean intermittent catheterization, and diaper use), disease symptoms, hemogram, sedimentation, and CRP values, complete urinalysis and urine culture results before and after Niaouli oil use were recorded from the patient files. The examination, test, treatment, and clinical evaluations of the patients were undertaken by the principal researchers working at the hospital. The data of patients using standardized Niaouli oil were screened (Niaouli certifications: EEC ORGANIC certified by FR-BIO-01.N'CAS EINECS: 132940-73-9).

The chromatographic analysis of Niaouli oil was performed with the combined headspace solid-phase microextraction (HS/SPME) and gas chromatography-mass spectrometry (GC/MS) methods for the determination of the volatile compounds of the oil. The extraction of essential oils was performed by treating the oil with 5 M $CaCl_2$ on a magnetic

stirrer at 3°C for 20 minutes in a standard headspace glass bottle (Supelco, 75 mm×23 mm). Analyses were undertaken with three replications. The volatiles were absorbed by polydimethylsiloxane (PDMS) using an SPME needle (Supelco, Bellefonte, PA). PerkinElmer GC (Clarus 600) equipped with HP-5 MS (30 m×0,25 mm×0,25 µm) and a fused-silica capillary column were used for the separation of volatiles. The carrier gas was helium (0,6 ml/min). The injection temperature was set as 280°C. The initial column heating was 40°C for 2 minutes, followed by an increase of temperature to 250°C at a rate of 5°C/minute, at which the sample was kept for 20 minutes. Compounds were determined by obtaining their mass spectra and using the NIST, Wiley, and flavor libraries according to their retention time.

The following exclusion criteria were used: other neurological diseases that may cause neurogenic bladder other than hemiplegia, dementia diagnosis, history of tumors and infections, diagnosis of polyneuropathy, advanced spasticity, and history of prostate and bladder disease.

In statistical analysis, continuous variables were expressed as mean±standard deviation and/or median (minimum-maximum) values, and categorical data as numbers and percentages. The normality of the distribution continuous variables was checked using the Kolmogorov-Smirnov goodness-of-fit test. Since the data were not suitable for a normal distribution, intra-group comparisons before and after treatment were made with the Wilcoxon signed-rank test. The McNemar test was conducted to compare categorical data before and after treatment. Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA). The statistical significance level was accepted as $p < 0.05$.

RESULTS

The mean age of the hemiplegic patients evaluated within the scope of the study was 51.55±19.20 (min=18, max=77) years. Of the patients, 72.7% were male, 54.5% had spontaneous bladder emptying, and 30.3% had stage 1, 21.1% had stage 2, and 3.0% had stage 3 spasticity (**Table 1**).

Leukocyte esterase and leukocyte in urine and sedimentation values statistically significantly decreased in the post-treatment period compared to the pre-treatment period (p<0.001, p<0.001, and p=0.005, respectively). C-reactive protein and white blood cell count also decreased after treatment compared to the pre-treatment values, and the differences were close to statistical significance (p=0.079 and p=0.054, respectively) (**Table 2**).

Table 1. Demographic and clinical characteristics of the patients

	Mean±Sd
Age (years) (mean±Sd)	51.55±19.20
Length of hospital stay (day) [median (min-max)]	15 (2-82)
	N (%)
Gender (n, %)	
Female	9 (27.3%)
Male	24 (72.7%)
Spasticity	
No	15 (45.5%)
Stage 1	10 (30.3%)
Stage 2	7 (21.1%)
Stage 3	1 (3.0%)
Bladder emptying method	
Spontaneous	18 (54.5%)
Catheter	11 (33.3%)
4x1 CIC	3 (9.1%)
6x1 CIC	1 (3.0%)
Total	33 (100.0%)

CIC: clean intermittent catheterization

Table 2. Laboratory parameters before and after treatment

	Before treatment	After treatment	p
Leukocyte esterase in urine [Median (min-max)]	2 (0-3)	0 (0-3)	<0.001*
Leukocyte in urine [Median (min-max)]	10 (1-382)	4 (0-464)	<0.001*
Erythrocyte in urine [Median (min-max)]	5 (1-182)	4 (0-348)	0.175*
Sedimentation [Median (min-max)]	23 (4-60)	14 (1-60)	0.005*
C-reactive protein [Median (min-max)]	22 (3-214)	13 (1-262)	0.079*
White blood cell count [Median (min-max)]	8,400 (4,600-18,000)	7,500 (4,000-14,000)	0.054*

*Wilcoxon signed-rank test

While there was growth in the urine culture of 60.6% of the patients in the pre-treatment period, this rate decreased to 27.3% after treatment, indicating a significant difference (p=0,003). It was also determined that the rate of patients with fever significantly decreased from 28,1% in the pre-treatment period to 3.1% after treatment (p=0.008). In rate of patients with urethral discharge was 28.1% before treatment and 12.1% after treatment, and the difference was significant (p=0.021). Urinary incontinence was seen at a rate of 60.6% in the pre-treatment period, and this rate significantly decreased to 33,3% after treatment (p=0,012). Although there was a decrease in the complaints of increased reflex sweating and spasticity in the post-treatment period, the difference was not statistically significant when compared to the pre-treatment rates (p=0,063 and p=0,219, respectively) (**Table 3**)

The rate of patients that developed skin lesions after treatment was determined as 18,2% (**Figure 1**).

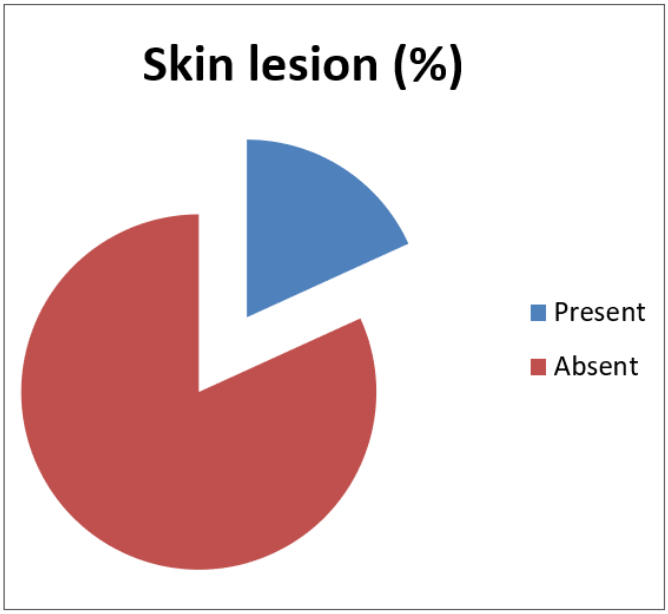

Figure 1.

Table 3. Comparison of patient symptoms before and after treatment

Growth in urine culture (post-treatment)	**Growth in urine culture (pre-treatment)**		**Total**	**p**
	Absent	**Present**		
Absent	12 (92.3%)	12 (60.0%)	24 (72.7%)	0.003*
Present	1(7.7%)	8 (40.0%)	9 (27.3%)	
Total	13 (39.4%)	20 (60.6%)	33 (100.0%)	
Fever (post-treatment)	**Fever (pre-treatment)**		**Total**	
	Absent	**Present**		
Absent	23 (100.0%)	8 (88.9%)	31 (96.9%)	0.008*
Present	0(0.0%)	1 (11.1%)	1 (3.1%)	
Total	23 (71.9%)	9 (28.1%)	32 (100.0%)	
Urethral discharge (post-treatment)	**Urethral discharge (pre-treatment)**		**Total**	
	Absent	**Present**		
Absent	20 (95.2%)	9 (75.0%)	29 (87.9)	0.021*
Present	1(4.8%)	3 (25.0%)	4 (12.1)	
Total	21 (63.6%)	12 (36.4%)	33 (100.0)	
Reflex sweating (post-treatment)	**Reflex sweating (pre-treatment)**		**Total**	
	Absent	**Present**		
Absent	24 (100.0%)	5 (55.6%)	29 (87.9)	0.063*
Present	0(0.0%)	4 (44.4%)	4 (12.1)	
Total	24 (72.7%)	9 (27.3%)	33 (100.0)	
Urinary incontinence (post-treatment)	**Urinary incontinence (pre-treatment)**		**Total**	
	Absent	**Present**		
Absent	12 (92.3%)	10 (50.0%)	22 (66.7)	0.012*
Present	1(7.7%)	10 (50.0%)	11 (33.3)	
Total	13 (39.4%)	20 (60.6%)	33 (100.0)	
Increased spasticity (post-treatment)	**Increased spasticity (pre-treatment)**		**Total**	
	Absent	**Present**		
Absent	20 (95.2%)	5 (41.7%)	25 (75.8)	0.219*
Present	1(4.8%)	7 (58.3%)	8 (24.2)	
Total	21 (63.6%)	12 (36.4%)	33 (100.0)	

*McNemar test

DISCUSSION

Infections, especially UTIs and pneumonia are among the most common complications after a stroke (4). UTIs observed after a stroke have been associated with regression in neurological status, mortality, increased disability, and prolonged hospital stay (22). In this study, the average length of hospital stay was 15 days and Leukocyte esterase and leukocyte in urine and sedimentation values statistically significantly decreased in the post-treatment period compared to the pre-treatment period ($p<0.001$, $p<0.001$, and $p=0.005$, respectively). C-reactive protein and white blood cell count also decreased after treatment compared to the pre-treatment values, and the differences were close to statistical significance ($p=0.079$ and $p=0.054$, respectively) (**Table 2**).

The typical manifestations of UTIs include dysuria, frequent urination or urgency, suprapubic pain, or flank pain often accompanied by fever, chills, and/or high peripheral leukocyte counts. Urine culture analysis is important in the diagnosis of catheter-related UTIs. Similar to the literature, in this study, fever, dysuria, and urinary incontinence complaints were predominant in patients with a diagnosis of UTIs. A significant improvement was observed in these complaints after Niaouli treatment. Urinary leukocyte esterase, urinary leukocyte count, and sedimentation rate, which are markers of UTI, were also found to significantly decrease in the post-treatment period compared to the pre-treatment values.

In order to reduce the risk of UTIs, the use of indwelling urinary catheters should be avoided as much as possible (23). In the current study, 33,3% of the patients with post-stroke UTIs were using catheters. External catheter systems (e.g., condom catheters for men and adhesive urine collection bags for women) and intermittent catheterization are alternatives that may be associated with a lower risk of UTIs compared to indwelling urethral catheters.

The use of antibiotics and preventive measures play an important role in the treatment of UTIs. Resistance to antibiotics in recent years has led to a search for other treatment alternatives. In recent years, alternative treatment methods, such as aromatherapy have been used as antimicrobials (13). However, we did not find any study in the literature on the use of aromatherapy in the treatment of UTIs. Our results revealed that while 60,6% of the patients had growth in urine culture in the pre-treatment period, this rate decreased to 27,3% after Niaouli treatment, and the difference between the two evaluations was significant (p=0,003).

Preclinical and clinical data regarding the antimicrobial activity of essential oil from Melaleuca spp. plants show

their activity against a wide range of Gram-positive and Gram-negative bacteria, fungi, and yeasts. The first clinical studies undertaken for this purpose were on acne treatment and dental applications (24,25). In this study, the growth rate in urine culture in stroke patients statistically significantly decreased after treatment. In light of these findings, we concluded that Niaouli oil could be an alternative in the treatment of UTIs.

Except for in vitro experiments, there are fewer clinical studies on skin sensitivities that may occur with the use of TTO. A few cases of contact dermatitis have been recorded during the topical use of this oil, but it should be noted that the oil was used in a concentrated form or in combination with other essential oils in these cases. In a study in which 28 individuals used a 25% TTO solution, sensitization was observed in only three participants (26). In other cases reported in the literature, reactions cannot be solely attributed to the use of TTO, since it was not used alone (27). In the current study, the rate of patients that developed skin lesions after Niaouli treatment was determined as 18,2%; however, it is not possible to conclude that this was solely related to the use of this oil. It should also be taken into consideration that the washing solution prepared with Niaouli may not have been used as recommended by some patients due to the diagnosis of hemiplegia and presence of spasticity in 54,4% of the cases, albeit at different stages.

The most important limitation of this study is the retrospective design. The lack of a control group and the number of cases are among other limitations. In addition, since the patient group was diagnosed with hemiplegia, they may not have applied the washing solution properly due to mental perception and physical activity limitations. However, the results of this study showed the statistically significant effect of the use of Niaouli oil on UTIs in stroke patients, which has not been previously investigated in the literature.

CONCLUSION

This study shows that the use of niaouli aromatherapeutic oil is beneficial in urinary tract infection in hemiplegic patients.

TTO is considered a safe antiseptic due to its natural origin and has started to be included in many pharmaceutical and cosmetic preparations in recent years. Scientific research on this oil has shown that it is effective in a wide range of microorganisms at very low concentrations. In vitro studies have proven that TTO can be used as an effective topical antimicrobial agent, and there is ongoing research to determine the possible mechanisms of action.

Although there are many in vitro studies on TTO, promising results have been obtained only from a limited number of case reports and clinical studies. Despite the proven efficacy of this oil, case studies should reach a sufficient number, and control groups should be included in clinical studies. In the treatment of UTIs, one of the serious complications in stroke patients, resistance to antibiotics has become a global problem. Our results suggest that tea tree (Niaouli) oil can be used as an alternative to medical treatment in patients with stroke that develop UTIs. It is important to increase the number of controlled clinical trials to ensure the safer use of this promising essential oil.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Adana City Hospital Clinical Researches Ethics Committee (Date: 08.09.2022, Decision No: 2136).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Total macular volume as a potential biomarker in the assessment of anti-VEGF response in patients with diabetic macular edema: real-life data analysis

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ABSTRACT

Aim: To evaluate the functional and anatomic efficacy of intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy in patients with diabetic macular edema (DME) and investigate the association between central macular thickness (CMT) and total macular volume (TMV) in real-life settings.

Material and Method: In this retrospective, observational, longitudinal study 38 eyes of 23 consecutive patients with center-involving DME were included. A loading phase of three monthly intravitreal anti-VEGF injections was initiated, followed by anti-VEGF injections if needed as per clinicians' discretion.

Results: Mean Early Treatment Diabetic Retinopathy Study (ETDRS) letters gained was 3.2 letters at month 12. The reduction in the mean of CMT and TMV were 60 μm and 1.33 mm^3 respectively at the end of 12 months. Best-corrected visual acuity (BCVA) was negatively correlated with CMT ($r=-0.573$, $p < 0.01$) and TMV ($r=-0.533$, $p < 0.01$) initially. There was a statistically significant positive correlation between the CMT and the TMV initially ($r=0.765$, $p < 0.01$) and month 12 ($r=0.937$, $p < 0.01$). Baseline TMV was found to be more predictive of treatment response at the 9th month than baseline CMT.

Conclusion: It is demonstrated that TMV may be a suitable biomarker in the assessment of treatment response of the macular region when regarded as a complete three-dimensional macular unit instead of central vertical thickness only. Although the present study contributes to a better understanding of managing DME in real-life settings, further prospective, and controlled investigations are needed.

Keywords: Aflibercept, anti-VEGF, central macular thickness, diabetic macular edema, total macular volume

INTRODUCTION

Diabetic macular edema (DME) is one of the most common ocular manifestations of diabetic retinopathy (DR). This pathology, which can be seen at any stage of DR (non-proliferative or proliferative), is also among the primary causes of vision loss (1). Although it is still not fully understood in all details, exudative fluid pooling in the intraretinal layers of the macula as a result of the blood-retinal barrier disruption is held responsible for the pathogenesis of DME (2). Also, with the emergence of the association between hypoxia-induced increased vascular endothelial growth factor (VEGF) and capillary leakage from retinal vessels in the pathogenesis; anti-VEGF treatment modalities have come into prominence plausibly.

Spectral-domain optical coherence tomography (SD-OCT) not only enables us to examine retinal structures layer by layer but also provides us with qualitative and quantitative information related to pathological alterations because of DME in the retina (3). SD-OCT has become a clinic of importance in diagnosis and classifying DME along with monitoring the treatment response (4). Previous studies showed that the assessment of central macular thickness (CMT) is a useful parameter for diagnostic sensitivity and quantitative monitoring in DME (2,3). Whereas CMT does not always accurately depict real numerical value owing to the differences in the retinal thickness of different sectors (5-7). Hence, we hypothesized that total macular volume (TMV), which can be neglected at times and offers an opportunity for a comprehensive approach, is of critical importance in clinical evaluation along with CMT.

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In clinical trials, highly determined patients are preferred to maintain timely attendance, or study criteria are strict in terms of glycemic controls, additional systemic disorders, age, etc. However, real-life settings, especially in the era of COVID-19 pandemics, may influence patient adherence and treatment response. The purpose of the current study was to investigate the association between CMT and TMV measured by SD-OCT and evaluate the functional and anatomic efficacy of intravitreal anti-VEGF therapy in patients with DME in real-life settings.

MATERIAL AND METHOD

This retrospective, observational, longitudinal study was conducted in a tertiary eye care referral center. This study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 2022, Decision No: E1-21-2179/2022). All procedures were carried out per the ethical rules and the principles of the Declaration of Helsinki. Informed written consent was obtained from all patients before receiving the anti-VEGF injections.

Study Population

Following the retrospective review of charts and intravitreal anti-VEGF logs (January 2020 - December 2021), 38 eyes of 23 consecutive patients with follow-up for at least 12 months with treatment naïve center-involving DME were included in the study. Although DME has the feature of showing bilateral involvement, it usually progresses asymmetrically. Therefore, macular findings in different eyes of the same case may be dissimilar (8, 9). That's why the second eye of some patients was also included in the study. Eighteen-year-old or older patients with severe nonproliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR), [determined by the modified Early Treatment Diabetic Retinopathy Study (ETDRS) grade] (10), at the first visit and met the following criteria were included in the study: Central subfield macular thickness of 250 μm or more on SD-OCT (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany); absence of potential causes other than DME of decreased visual acuity. Patients who met the following criteria were excluded from the study: high refractive error (≥6 diopters), posterior staphyloma, prior intraocular operation other than cataract surgery, glaucoma, history of any retinal disease other than DR, images with low picture quality less than 16 dB due to corneal opacity, dens cataract, vitreous hemorrhage, etc.

A comprehensive ophthalmological examination including detailed medical history, best-corrected visual acuity (BCVA), non-contact tonometry, dilated fundoscopy, and SD-OCT was performed initially and on the following visits for all patients. Fluorescein angiograms were done at the baseline for each patient and then as needed. HbA1c level was reported at baseline. Visual acuity readings were converted from the Snellen chart to the logarithm of the minimal angle of resolution (log MAR) units and Early Treatment Diabetic Retinopathy Study (ETDRS) letters (11).

All eyes included in the study were initiated on a loading phase of three-monthly intravitreal bevacizumab injections in accordance with the respective regulations of the ministry of health, followed by aflibercept injections if needed as per clinicians' discretion with monthly monitoring for at least 12 months. An "as needed" or in other words pro re nata (PRN) regimen was employed that was shown to have caused a reduction in the number of injections while keeping a close follow-up plan for the treatment responses(12, 13). The same retina specialist examined each patient. The criteria for reinjection were at least one of the following: A decrease in BCVA; an increase in CMT; or both. While planning the treatment, the BCVA, CMT, and TMV of each eye were evaluated independently of the fellow eyes. In addition, the response of the anti-VEGF treatment for each eye was evaluated individually in the study. For standardization, only patients that received aflibercept as an anti-VEGF treatment after the obligatory loading phase of bevacizumab were included in the study. Based on the treatment response parameters of visual acuity and SD-OCT examination stability of the patients (if there was a possibility for additional enhancement), further intravitreal injection treatment decisions were made at subsequent visits.

The loading phase of injection was restarted in a total of 12 eyes of 7 patients who did not complete their loading phase of injections and did not attend their regular patient visits initially. One of the loading doses failed in these patients because of their nonadherence due to pandemics. So, they restarted receiving three-monthly loading doses. The inclusion of these patients did not cause any statistically significant difference in the statistical analysis.

Evaluation of Macular Thickness and Volume

Retinal thickness was computed as the length between the anterior retinal boundary of the internal limiting membrane and the posterior retinal boundary of the outer border of the retinal pigment epithelium. Retinal thickness assessment generated automatically includes a map analysis with measurements as defined by ETDRS (14) for each of the 9 subfields. The retinal thickness in a 1-mm diameter circle at the fovea was used for automated CMT measurements. CMT was calculated automatically with the built-in analysis software

of the SD-OCT. Along with this, automated TMV measurements were obtained in the 6x6-mm macular area centered on the fovea using the preprogrammed "fast macular volume" setting, containing a 25-line horizontal raster scan covering 20° × 20°, fixated on the fovea.

Statistical Analysis

Statistical analyses were performed with SPSS program version 26.0 (SPSS Inc., Chicago, Illinois, USA). Results were expressed as the mean±standard deviation. The Kolmogorov-Smirnov test was performed to determine whether the data were normally distributed. Upon the distribution of data was non-normally, Wilcoxon signed-rank test and Spearman's correlation coefficient were used. Also employed is a simple linear regression model which estimates the relationship between one independent variable and one dependent variable using a straight line. Differences with a P value less than 0.05 were considered statistically significant.

RESULTS

Demographics

Data for 38 eyes (22 right eyes, 16 left eyes) of 23 patients (11 women, 12 men; mean age 60.5±9.8 years) was analyzed. All participants were Caucasian. Only one patient had type I diabetes mellitus (DM) and 22 patients had type II DM. Six patients were on oral anti-diabetic medication, one patient was on insulin medication, and the remaining were on a combination of these treatments.

The mean duration of DM was 10.5 years (standard deviation [SD]±2.2). The mean chronic HbA1c level was 8.45±2.42% at enrollment. Twenty-five (66%) eyes had NPDR, and 13 (34%) eyes had PDR. One patient had renal insufficiency secondary to diabetic nephropathy that did not necessitate dialysis. Four patients were on medication for hypertension, which was well-controlled (**Table 1**).

The mean BCVA (standard deviation (SD); Snellen) was 71.3 (0.3; 0.53) ETDRS letters (min. 0.1 - max. 0.7 Snellen), CMT was 427.9±161.8 (min. 257 - max. 880) μm, and TMV was 10.6±2.4 (min. 7.82 - max. 15.28) mm^3 before the initiation of treatment. The mean BCVA (standard deviation (SD); Snellen) was 74.5 (0.2; 0.60) ETDRS letters (min. 0.1 - max. 1.0 Snellen), CMT was 367.6±132.1(min. 235 - max. 673) μm, and TMV was 9.3±2.2 (min. 6.25 - max. 14.46) mm^3 at month 12. The mean ETDRS letters gained was 3.2 ETDRS letters at month 12. The reduction in the mean of CMT and TMV were 60.3 μm and 1.3 mm^3 respectively at the end of 12 months (**Figure 1.**). The average of total intravitreal injections was 4.4 per eye at the end of 12 months.

Table 1. Initial clinical characteristics and demographic data of patients in this study

Age, years, mean±SD	60.5±9.8
Gender, female/male	11/12
Eyes, OD/OS	22/16
Duration of DM, years, mean±SD	10.5±2.2
DM type, type 1: type 2	2:36
Other systemic conditions	
Hypertension (blood pressure ‡ 140/90 mmHg)	4 (17.3%)
Nephropathy	2 (8.6%)
Sugar control	
Oral hypoglycemic agents	10 (26.3%)
Insulin	2 (5.3%)
Combination	26 (68.4%)
HbA1c level, %	8.4±2.4
Study eye, right: left eyes	22:16
Snellen Corrected visual acuity, mean±SD, (logMAR: ETDRS)	0.53±0.30 (0.27: 74)
Lens status	
Phakic Clear	37 (97.4%)
Pseudophakia	1 (2.6%)
Diabetic retinopathy grading	
Non-proliferative diabetic retinopathy	25 (65.8%)
Proliferative diabetic retinopathy	13 (34.2%)
OCT findings, mean±SD	
Central subfield thickness, μm	427.9±161.8
Total macular volume, mm^3	7.82±2.4

SD: standard deviation; OD: right eye; OS: left eye; DM: diabetes mellitus; logMAR=logarithm of the minimum angle of resolution; OCT: optical coherence tomography.

Correlations

BCVA at baseline was negatively correlated at moderate level with CMT initially (r=-0.573, $p < 0.01$) and month 3 (r=-0.510, $p < 0.01$). BCVA at baseline was negatively correlated at moderate level with TMV initially (r=-0.533, $p < 0.05$), month 3 (r=-0.580, $p < 0.01$) and month 6 (r=-0.576, $p < 0.01$) (**Table 2.**). There was a statistically significant positive correlation between the CMT and the TMV initially (r=0.765, $p < 0.01$) and month 12 (r=0.937, $p < 0.01$). Besides, the correlation of baseline CMT was stronger compared to baseline TMV for final BCVA (r=-0.437, $p < 0.05$).

Table 2. The Spearman's ranked correlation coefficient (Spearman's rho) for BCVA, CMT and TMV (n=38).

	Baseline BCVA	BCVA at 3rd month	BCVA at 6th month	BCVA at 9th month	BCVA at 12th month
Baseline CMT	-.573**	-.539**	-.527**	-.558**	-.437*
CMT at 3rd month	-.510**	-.302	-.452*	-.506**	-.524**
CMT at 6th month	-.343	-.333	-.313	-.434*	-.360
CMT at 9th month	-.122	.010	-.044	-.273	-.310
CMT at 12th month	-.005	.111	-.010	-.089	-.134
Baseline TMV	-.533*	-.465*	-.389	-.356	-.298
TMV at 3rd month	-.580**	-.423*	-.384	-.286	-.335
TMV at 6th month	-.576**	-.389	-.222	-.229	-.203
TMV at 9th month	-.355	-.106	.049	-.060	-.054
TMV at 12th month	-.338	-.042	-.185	-.113	-.150

BCVA: best-corrected visual acuity; CMT: central macular thickness; TMV: total macular volume. * $p < .05$. ** $p < .01$.

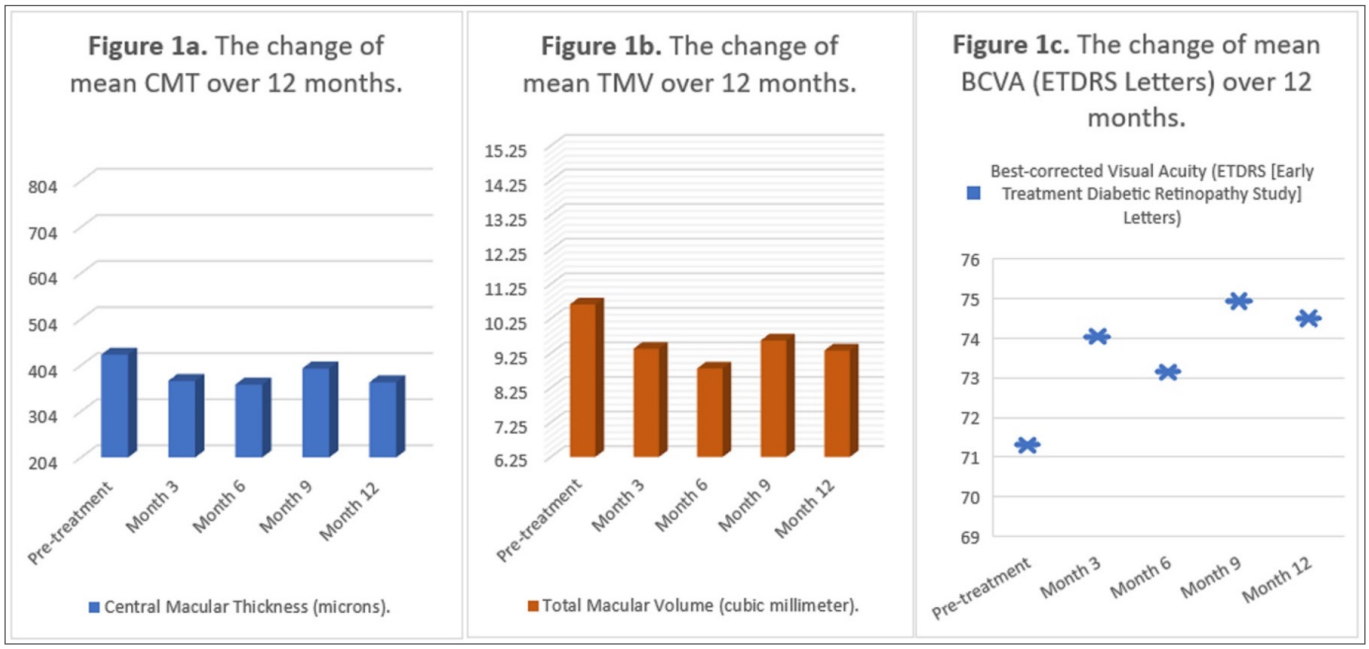

Figure 1. a. The change of mean central macular thickness (CMT) (microns) over 12 months. **b.** The change of mean total macular volume (TMV) (cubic millimeter) over 12 months. c. The change of mean best-corrected visual acuity (BCVA) in Early Treatment Diabetic Retinopathy Study (ETDRS) letters over 12 months.

Regression Analysis

Given the results (**Table 3**a.), baseline CMT significantly predicted the CMT in the 9th month (R2=0.42; Freg=12.512, p<0.01). To be more precise, CMT at baseline explained 42% of the observed variance in patients' CMT at month nine. In view of the analysis (**Table 3**b.), baseline TMV significantly predicted the CMT in the 9th month (R2=0.52; Freg=13.366, p<0.01). More precisely, the baseline TMV explained 52% of the observed variance in patients' CMT at month nine. Baseline TMV was found to be more predictive of treatment response at the 9th month than baseline CMT.

Table 3a. Simple linear regression analysis results of baseline CMT related to predict of the CMT in the 9th month

Variables	B	SHB	Beta	t	R	R2	F
Constant	69.292	97.130		0.713	0.651	0.424	12.512
Baseline CMT	0.739	0.209	0.651	3.537			

Table 3b. Simple linear regression analysis results of baseline TMV related to predict of the CMT in the 9th month

Variables	B	SHB	Beta	t	R	R2	F
Constant	-79.819	133.371		-0.598	0.726	0.52	13,366
Baseline TMV	45.986	12.578	0.726	3.656			

CMT: central macular thickness; TMV: total macular volume; B: bias; SHB: sum of squares; Beta: beta coefficient; t: t statistic; R: the multiple correlation coefficient; R2: the coefficient of determination; F: F statistic.

According to the Wilcoxon signed-rank test for related samples results, there were significant differences in BCVA [z=-2.119, p<0.05], CMT [z=-2.059, p<0.05], and TMV [z=-2.417, p<0.05] between baseline and month 12. When the mean rank and sum of ranks of the difference scores were considered, this difference seemed in favor of the positive ranks, i. e., the month 12 scores.

DISCUSSION

DR is one of the major causes of considerable visual impairment in the population of employable age. DME, on the other hand, is the most common reason for vision loss in DR (15). Since the key role of VEGF has been proven in the pathogenesis of DME, intravitreal anti-VEGF injections were established as primary treatment for patients with DME. Multiple studies have demonstrated the improvement in both anatomical and functional outcomes secondary to anti-VEGF therapy for DME (15-17). With the increasing importance of objective and reproducible OCT imaging in the diagnosis and treatment of DME, qualitative and quantitative analyzes of structural characteristics in retinal layers have gathered momentum. In OCT imaging, CMT was acknowledged as a surrogate marker for assessing the treatment effect by several experts (18). Although CMT has been the most frequently utilized biomarker in DME, TMV may be more helpful, especially in non-center involved DME studies (7, 19). In addition, TMV may provide noteworthy data about the thickness of the macular region when regarded as a complete unit (19). In the current study, CMT and TMV were investigated and compared in terms of potential relation to anti-VEGF response in real-life settings.

Previous studies have shown anatomical and functional improvements in patients treated with intravitreal aflibercept injections for DME (16, 17, 20, 21). In the DRCR.net protocol T study, the mean letters gained after the treatment of aflibercept was approximately 10 ETDRS letters with an average of 9-10 injections per year (22). However, it was approximately 3.2 ETDRS letters with an

average of 4-5 injections per year in our study, which was a perspective on real-world evidence of anti-VEGF use in the COVID-19 era. These outcomes were significantly less than observed in the DRCR.net protocol T could be explained by reduced patient adherence and injection numbers. Plus, clinical trials were presented with a highly-motivated patient profile, strict criteria of the study, increased number of injections, and timely attendance. On the other side, there were numerous impediments such as restrictions due to the pandemics, the difficulty elderly patients have in getting an appointment, lack of capacity in hospitals, increased frequency of systemic comorbidities, and financial challenges in real-life settings.

In our study, anatomical enhancement was observed at the end of 12 months. There was a limited number of studies in the literature on TMV. In line with these limited previous studies, there was a decline in both CMT and TMV following intravitreal aflibercept treatments (15-17, 20-22). DRCR.net studies up to the present have demonstrated a high correlation of macular measurements with CMT. In the present study, while the correlation between baseline CMT and BCVA was statistically significant in the 3rd, 6th, 9th, and 12th months; the correlation between baseline TMV and BCVA was statistically significant only in the 3rd and 6th months. We thought that this may be caused by the reduced initial generalized macular edema after anti-VEGF treatment, which gave place to local (especially central) macular edema gradually later on. A regional variation such as central fovea, parafoveal and perifoveal area was possible in response to macular thickening secondary to treatment.

Browning et al. (7) concluded that TMV may be preferable over CMT when macular edema is more diffuse or when it is expected that responses of CMT may be inconsistent. In parallel with this, Panozzo et al. (19) suggested that considering the macular region as a whole may provide more significant information about retinal thickness, especially in cases with a stable measurement at the fixation point but an undetected global worsening. In a nonrandomized clinical trial, Nguyen et al. (23) found a significant decrease in TMV after anti-VEGF treatment. They proposed that the large effect of reduction in thickness of the central macula was accompanied by a global reduction in edema throughout the entire macula. Although time-domain OCT was used at the time of the mentioned works of literature published, current studies with spectral-domain OCTs also support the same argument(24-26). The therapeutical effect of anti-VEGFs' occurs in the retinal layers diffusely rather than focal lesions in the center of the fovea (27, 28). In conjunction with this, our simple linear regression analysis revealed that baseline TMV had higher predictability compared to baseline CMT for treatment response in the 9th month. However, it was not found statistically significant in the 12th month. It could be explained that diffuse edema might have decreased and become more localized in the 12th month. Besides, although CMT and TMV showed a regular decrease in the 3rd and 6th months, then a relatively gradual increase was observed in the 9th and 12th months. The decreased patient adherence after the completion of the three loading doses, which are obliged to be done regularly at least one month apart by regulations of the ministry of health could be the reason.

Study Limitations

Our study has several limitations. First, the sample size of treatment naïve patients was relatively small because of the COVID-19 pandemic. Second, quantitative metrics for further analysis such as intraretinal fluid or integrity of certain retinal layers were not available. The strength of this study includes the design of a real-life setting rather than a clinical trial setting which provides practical information for clinicians treating patients in the real world during the COVID-19 pandemic and the study group had a better homogeneity with the inclusion of only aflibercept-administered cases after the obligatory loading dose.

CONCLUSION

The current study demonstrated that baseline TMV might have higher predictability compared to baseline CMT for treatment response in patients with DME. Besides, TMV may be a suitable biomarker for the assessment of therapeutic effect in the macular region when regarded as a complete unit instead of central vertical thickness only. Utilizing TMV with CMT in the management of DME may provide a more consistent and comprehensive evaluation. Although the present study contributes to a better understanding of managing DME in real-life settings, further prospective, and controlled investigations are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 2022, Decision No: E1-21-2179/2022).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Original Article

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Effects of sequential and fixed-dose estradiol valerate administration on premature progesterone rise in frozen-thawed embryo transfer cycles

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ABSTRACT

Aim: This study investigated the risk of premature progesterone (P4) rise in the fixed and sequential estradiol valerate (EV) administration for frozen embryo transfer (FET) cycles.

Material and Method: In this cross-sectional case-control study, 1272 cycles of FET were analyzed retrospectively from computer records between January 2015 to August 2020. EV was administrated in 795 patients with a fixed dose and in 477 patients with a sequential dose. P4 values were measured on the day when the endometrial thickness reached 8 mm in the patients.

Results: There were 795 patients in the fixed EV administration group with a mean age of 30.75±3.39 and 477 patients in the sequential EV administration with a mean age of 30.75±3.39. P4 of the sequential-dose group (1.05±0.31) was significantly higher than the fixed-dose group (1.01±0.33). The Pairwise Z-Tests found that the abort rate was significantly higher in the sequential-dose group (p=0.04).

Conclusion: Our results showed a higher P4 and abortion rate in the sequential-dose group. These findings show that premature P4 rise can be considered a risk factor.

Keywords: Estradiol valerate, progesterone, FET

INTRODUCTION

Some infertile couples use in vitro fertilization (IVF) methods to get pregnant, but the implantation rate is still low, even with the transfer of apparently healthy embryos (1). The estradiol (E2) level directly affect the maturation of the oocyte or embryo (2). A high E2 level in the follicular phase has been associated with an increase in the harvest of fertile eggs (3). The effect of high levels of E2 on the outcome of the use of assisted reproductive technologies is still debatable.

In the conducted studies, the harmful effect of high E2 on the receptivity of the endometrium has been proposed (4,5); however, in some studies, this negative effect has not been reported (6,7). The role of E2 in the follicular phase, including the proliferation of stroma and glandular epithelial vessels in the endometrial tissue, has been discussed (5,6). In addition, E2 causes the synthesis of specific proteins, growth factors, estrogen (E), and P4 receptors (3). Although the role of P4 in the implantation of the early stages of pregnancy is crucial (5), the role of E2 in the luteal phase is still not well defined.

In response to the successive release of E and P4 from the ovary, the endometrium proliferates and prepares it for embryo implantation. The changes in endometrial vessels occurs following the coordination between the actions of E and P4, which causes the adequate blood supply to the endometrium to accept the pregnancy (8). Subendometrial and endometrial vascularity has significantly decreased in women with unexplained infertility (6,7). The junction of the endometrium and myometrium is rich in blood vessels and plays a significant role in embryo implantation (9). The effects of E and P4 on the hemodynamics of this region have not been studied so far.

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Both P4 and E2 are necessary to prepare the endometrium for blastocyst implantation and successful pregnancy (6,7). In the cycles following IVF, more mature follicles are formed due to the use of ovarian stimulating drugs, so the level of these hormones can be higher than the physiological state. This increase in level can cause concern about luteal phase disorders and uterine tissue changes (7). E2 first causes hyperplasia and hypertrophy of endometrial epithelial cells (9), but its role in the luteal phase is unclear (8). Although the role of E2 in the coordination and implantation of blastocyst is not clear, the role of P4 in the luteal phase is better defined, and it has been shown that lumpectomy before the seventh week of pregnancy leads to abortion in most cases (10). P4 causes the endometrium prepared with E2 to become secreted tissue and provides an environment ready for ET to uterine tissue (11). Although research has been conducted on the effects of E2 and P4 on implantation, there is little research on their levels in the early luteal phase.

This study aimed to compare the effects of sequential and fixed-dose EV administration on P4 values measured on the day when the sufficient endometrial thickness is reached in frozen-thawed embryo transfer (FET) cycles.

MATERIAL AND METHOD

This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1). In this cross-sectional case-control study, 1272 cycles of FET were analyzed retrospectively from computer records between January 2015 to August 2020. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In the fixed dose method, 3*2 mg of estradiol valerate (known as estrofem trade name) was used. In the sequential method, 2*2 mg was used for the first 3 days and increased to 3*2 mg in the following days. Estradiol valerate support was continued until the 7th week of pregnancy.

EV was administrated in 795 patients with a fixed dose and in 477 patients with a sequential dose. Therefore, participants were divided into two groups of fixed and sequential doses of EV. Luteinizing hormone (LH) and P4 values were measured on the day when the endometrial thickness reached 8 mm in the patients. The inclusion criteria of patients were as follows: 1) be between 20-35 years old; 2) not having an endometrial factor; 3) A body mass index (BMI) between 18-30. The exclusion criteria for patients were as follows: 1) the presence of an endometrial factor; 2) the presence of other chronic diseases; 3) A BMI of more than 30. Women between the ages of 25 and 39 were included in this study.

Statistical Analysis

The Kolmogorov-Smirnov test was performed to check the normality, and the nonparametric tests were performed given the groups' non-normality before the statistical analyses. Mean and standard deviations (SD) were measured to check each continuous variable, including Age, BMI, total oocytes, MII oocytes, pronuclei (PN), anti mullerian hormone (AMH), prolactin (PRL), free T4 (FT4), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), LH, E2, endometrial thickness, and P4. The Mann-Whitney U test was performed to study the difference between the two groups. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

This study included 1272 age-matched (30.47±3.47) and BMI-matched (23.93±2.21) participants. There were 795 patients in the fixed EV administration group with a mean age of 30.75±3.39 and 477 patients in the sequential EV administration with a mean age of 30.75±3.39. **Table 1** shows the descriptive statistics of maternal characteristics and laboratory parameters.In the present study, we compared laboratory parameters between two groups. We assessed the capability of those parameters to differentiate between sequential and fixed-dose EV administration on premature P4 rise in FET cycles.

Table 1. Descriptive statistics of study parameters in women (n=1272)

Study parameters	Median (range) mean ± SD
Maternal characteristics	
Age	32 (20-35) 30.47±3.47
BMI	24 (19.8-29) 23.93±2.21
Laboratory parameters	
Total oocytes	2 (1-7) 2.76±1.43
MII oocytes	9 (7-16) 10.05±2.37
PN	8 (6-15) 8.89±2.34
AMH	8 (6-13) 8.41±1.94
PRL	15 (8.48-25) 16.92±5.54
FT4	0.99 (0.31-1.62) 1.03±0.28
TSH	1.16 (0.63-2.46) 1.47±0.54
FSH	7.83 (4-12) 7.59±1.48
LH	7 (3.52-13) 6.93±1.53
E2	40 (30-51.2) 40.12±6.66
Endometrial thickness	9 (9-12) 9.9±1.05
Progesterone	0.96 (0.31-4) 1.02±0.32

SD, standard deviation. BMI, body mass index; PN, multi-pronuclei; AMH, Anti-Mullerian hormone; PRL, prolactin ; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2; Estradiol.

Table 2 shows the comparison of the study parameters of the two groups. As stated in **Table 2**, a Mann-Whitney test did not find a statistically significant association between the two treatment groups regarding total and MII oocytes (p>0.05). No significant difference was observed between the two treatment groups regarding PN, PRL, and FT4 (p>0.05). The serum AMH, TSH, FSH, LH, and E2 levels were similar between the two treatment groups (p>0.05). There was no statistically significant difference between groups in terms of E2 and endometrial thickness (p>0.05). P4 of the fixed-dose group (Mean±SD = 1.01±0.33) was significantly lower than the sequential-dose group (Mean±SD = 1.05±0.31). The Mann-Whitney test indicated that this difference was statistically significant (p<0.05).

Table 2. Comparison of study parameters of fixed-dose and sequential-dose groups

Study parameters	Fixed-dose group (n=795) mean ± SD	Sequential-dose group (n=477) mean ± SD	p
Age	32 (20-35) 30.46±3.5	32 (20-35) 30.49±3.41	0.903
BMI	24 (19.8-29) 23.92±2.22	24 (19.8-29) 23.94±2.19	0.830
AMH	2 (1-7) 2.77±1.5	2 (1-7) 2.74±1.31	0.099
Total oocytes (n)	9 (7-16) 10.05±2.36	9 (7-16) 10.05±2.37	0.994
MII oocytes (n)	8 (6-15) 8.89±2.34	8 (6-15) 8.9±2.35	0.961
PN	8 (6-13) 8.4±1.94	8 (6-13) 8.43±1.95	0.741
Prolactin	15 (8.48-25) 16.91±5.54	15 (8.48-25) 16.93±5.54	0.919
FT4	0.99 (0.31-1.62) 1.04±0.28	0.99 (0.31-1.62) 1.03±0.28	0.862
TSH	1.21 (0.63-2.46) 1.48±0.54	1.16 (0.63-2.46) 1.47±0.53	0.761
FSH	7.83 (4-12) 7.58±1.48	7.83 (4-12) 7.61±1.49	0.827
LH	7 (3.52-9.64) 6.93±1.49	7 (3.52-13) 6.91±1.6	0.555
E2	40 (30-51.2) 40.13±6.67	39.4 (30-51.2) 40.11±6.67	0.996
Endometrial thickness	9 (9-12) 9.9±1.05	9 (9-12) 9.9±1.05	0.926
Progesterone	0.95 (0.31-4) 1.01±0.33	1 (0.31-2) 1.05±0.31	0.001

M, Mean; N, number of subjects; AMH, Anti-Mullerian hormone; PN, multi-pronuclei; FT4, Free T4; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2; Estradiol. All variables were tested by a Mann-Whitney U test.

Figure 1 shows the difference between two treatment groups (fixed-dose and sequential-dose) on premature P4.

The relationship between pregnancy results and the two treatment groups is shown in **Table 3**. As presented in **Table 3**, a chi-square test found no statistically significant association between the ongoing pregnancy rate and the two treatment groups (p>0.05). No significant difference was observed between the two treatment groups regarding cycle cancellation rate (p>0.05).

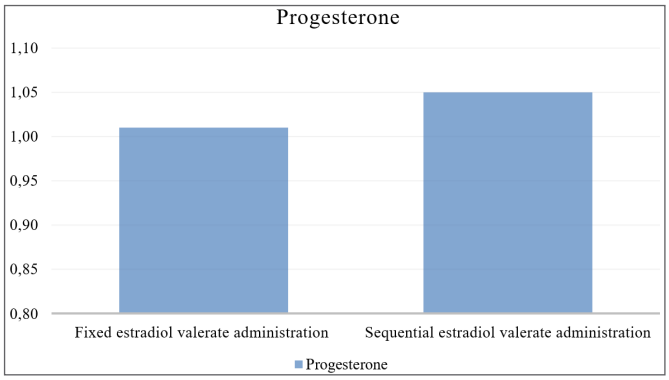

Figure 1. Progesterone of fixed-dose and sequential-dose groups

Table 3. The relationship between pregnancy results and two treatment groups

Variables		Fixed-dose group (n=795) n (%)	Sequential-dose group (n=477) n(%)	p
Pregnancy results Bhcg (+) (%)	Yes	390 (49.1)	254 (53.2)	0.148*
	No	405(50.9)	223 (46.8)	
Ongoing pregnancy rate (%)	Yes	360 (45.3)	206 (43.2)	0.392*
	No	435 (54.7)	271 (56.8)	
Cycle cancellation rate (%)	Yes	58 (7.3)	42 (8.8)	0.098*
	No	737 (92.7)	435 (91.2)	

*A Chi-square test.

The relationship between the abort rate of the two treatment groups is shown in **Table 4**. As stated in **Table 4**, a chi-square test found a statistically significant association between the abort rate and two treatment groups (p<0.05). The Pairwise Z-Tests found that the abort rate was significantly higher in the sequential-dose group (p=0.04).

Table 4. The relationship between abort rate of the two treatment groups

Variable		Fixed-dose group (n=390) n (%)	Sequential-dose group (n=254) n (%)	p-value
Abort rate (%)	Yes	30 (7.7)	48 (18.9)**	0.04*
	No	360 (92.3)	206 (81.1)	

*A Chi-square test. **The Pairwise Z-Tests.

DISCUSSION

Considering the importance of treatment for couples who suffer not only mental and emotional injuries due to infertility problems but a high cost to achieve the desired result, it is necessary to conduct more studies in this field to achieve the desired result and increase the pregnancy rate of infertile couples. In this study, we investigated the effect of EVadministration in two forms, fixed and sequential, in FET cycles. The results showed that the P4 level in the sequential-dose group was significantly higher than in the fixed-dose group (p=0.001). Also, the abortion rate in the sequential-dose group was significantly higher (p=0.04). Interesting interpretations can be made from

this result. One of the significant problems in the success of IVF methods is implantation failure, one of the critical signs of which is endometrial acceptance. Hormones also influence endometrial acceptance, and P4 is one of the essential hormones in this process (12). Since P4 and E are necessary to prepare the endometrium for blastocyst implantation and successful pregnancy, there should be more mature follicles in IVF cycles due to the use of ovarian-stimulating drugs. Therefore, the level of these hormones can be higher than the physiological state, and this increase can cause concern about the occurrence of luteal phase disorder and uterine tissue changes (13).

Out of 1272 women using the FET method in this study, 644 (50.62%) women became pregnant, and 628 (49.37%) women did not, and there was no significant difference between the two groups in terms of variables related to pregnancy. Among variables related to pregnancy, only progesterone had a significant difference between the two groups (p=0.001). This result was consistent with other studies (14,15). In Pabuccu et al. (16), which compared the ratio of E2 to P4 on the day of ET in pregnant and non-pregnant women, no significant difference was observed in the two groups of patients regarding FSH, the number of embryos transferred, and the amount of ampoule consumed. However, P4, the number of oocytes obtained, and the number of embryos formed in the two groups were significantly different from each other. It seems that demographic characteristics cannot significantly impact the outcome of pregnancy.

Pais et al. (17) showed no significant increase in P4 levels in pregnant women with the sequential-dose administration method. This result was despite the fact that the ratio of serum E2 to P4 on the day of ET was higher in pregnant women than in non-pregnant women, which was statistically significant. In Pan et al. (18), the increase in E2 levels in the transfer of one or two embryos on the day of HCG consumption had harmful effects on implantation, but this effect disappeared when three embryos were transferred. Although this relationship was not evaluated in the study, examining the level ratio between E2 and P4 on the day of embryo transfer, their lack of relationship was seen, which requires further investigation in this field.

Our results showed that the abort rate was significantly higher in the sequential-dose group. This finding is consistent with the findings of some studies (19,20). Considering the higher rate of abortion in the sequential-does group, it can be concluded that the P4 level in women whose pregnancy had an abortion was higher compared to women who had a successful pregnancy, which is consistent with the findings of previous studies (21,22). Regarding the explanation of this result, Xu et al. (23) reported that when the E2 level in the luteal phase is significantly reduced, it disrupts endometrial receptivity. It is unclear whether using E2 in these women after ovulation will improve their condition, which needs more research. In previous studies, high E2 levels had side effects on the environment of the endometrium but did not affect the quality of the fetus. Also, the high ratio of P4 to E2 level does not affect the pregnancy rate (23,24).

This study also had limitations. Due to the study's retrospective nature, E2 levels were not measured in women whose pregnancies were terminated due to miscarriage. Also, there was no measurement of the ratio of E2 to P4 in these women and its comparison with women with a successful pregnancy, which can be considered in future studies.

CONCLUSION

This study compared the risk of premature P4 rise in the fixed and sequential EV administration for FET cycles. Our results showed a higher P4 and abortion rate in the sequential-dose group. These findings show that premature P4 rise can be considered a risk factor.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of Beykoz University Research and Project Development Ethics Committee (Date: 26.10.2020, Decision No:1).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Case Report

ANATOLIAN CURRENT MEDICAL

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A rare complication of *Escherichia coli* induced urosepsis; is Guillain-Barre syndrome

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ABSTRACT

Guillain Barre syndrome (GBS) is the most common neurological cause of acute flaccid paralysis worldwide. Early diagnosis and treatment of GBS are vital due to possible deadly consequences. Awareness of the silent neurological symptoms in patients preparing for upcoming surgery may have critically crucial for a urologist. Developing GBS after relieving urosepsis is rarely addressed in the literature. Therefore, this report presents an infrequent complication of *Escherichia coli* (*E. coli*)-induced urosepsis, GBS. A 47-year-old female patient was admitted to the emergency department in a septic state. During the intensive care unit management with the preliminary diagnosis of sepsis-related hypovolemic shock, a nephrostomy catheter was placed in an obstructed left kidney due to impacted upper ureteral calculus with 1.5 cm in size. Following the improvement of the patient's condition in intensive care unit with proper management, the patient was transferred to the urology ward for definitive treatment. During the follow-up, however, the patient showed some neurological signs and symptoms considering GBS. An obtained cerebral spinal fluid analysis revealed an albumin-cytologic dissociation and examining the patient underpinned the diagnosis. The patient was treated with intravenous immunoglobulin for five days, according to the guidlines. After the treatment, the patient's condition improved rapidly following two weeks. The left obstructed ureteral stones were removed with ureteroscopy. A stone-free status was achieved the following month. GBS is the most common cause of acute flaccid paralysis worldwide, and proper management is essential due to poor prognosis. GBS after a uroseptic condition is sporadic, but any surgery on patients who experience active GBS would bode for severe consequences, so awareness of the silent neurological symptoms in patients prepared for upcoming surgery is vital for a urologist. We aimed to remind with this report of the possibility of GBS for a patient who expresses neurological symptoms following a septic state.

Keywords: Guillain Barre syndrome, urosepsis, ureteral stones, hydronephrosis

INTRODUCTION

Early diagnosis and proper management of urosepsis are vital. About one out of four adult sepsis cases worldwide are caused by urosepsis, and septic shock related to urosepsis is responsible for 20-30 % of mortality (1). From the urologist's aspect, with limited data available to evaluate the urosceptic patients' surgical outcomes, the rate of an underlying uro-surgical condition is 12-37% (1-3) In most cases, the main culprits are hydronephrosis/ pyonephrosis, benign prostatic hyperplasia, and obstructing ureteral/ renal stones (2-3). After the initial intervention with a nephrostomy tube or double j (D-J) stent placement and proper management of the condition in the Intensive care unit (ICU), these patients generally return to the urologic ward for definitive treatment after a while. However, a urologist might run into some unusual late downsides of sepsis that cause prolonged hospital stays and deter planned therapy. Therefore, this report presents an infrequent complication of *Escherichia coli* (*E. coli*)-induced urosepsis, Guillan Barre syndrome (GBS). According to our research, only two case reports (7,8) in the literature show GBS after *E. coli* infection. So, we also aimed to support the literature with this report.

CASE REPORT

A 47-year-old female patient was admitted to the emergency department in a confused state for an hour. Her initial examination revealed low blood pressure (90/50 mm/hg), increased heartbeat (114 bpm), and a low temperature (35.4°C), in addition to low O_2 saturation (82 mm Hg). Her husband reported that she had complained of left flank pain, dysuria, vomiting, and hematuria for the previous two days. She also has

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a history of bilateral renal stone surgery. An abdominal computed tomography scan showed an obstructed left upper ureteral calculus 1.5 cm in size associated with mild hydronephrosis in the left kidney and atrophic signs in the right kidney. The laboratory results showed deranged kidney functions with raised creatinine levels to 2.2, left-shifted FBC, mildly elevated liver functions, and metabolic acidosis signs in blood gas analysis, as well as leucocytosis in the urine sample (**Table 1**). After the initial management, the patient was transferred to ICU for the preliminary diagnosis of a sepsis-related hypovolemic shock. Therefore, the patient has resuscitated accordingly with intravenous fluids, O_2, and empiric antibiotic therapy. A nephrostomy catheter placement was carried out after hemodynamic stability had been ensured. According to urine and blood culture analyses, demonstrating that extended-spectrum beta-lactamase (ESBL) positive *E. coli* was the causing organism, the treatment was changed to meropenem 1 mg gr daily and gentamicin 160 mg daily for ten days.

After the patient's septic state had improved, cultures cleared up, and the vital signs returned to normal, she was transferred to the urologic ward for definitive renal stone surgery. However, the patient showed some steadily increased neurological signs and symptoms during the observation, including hypoesthesia in both hands and feet, difficulty walking, and swallowing problems. The neurological examination showed symmetrical weakness in her lower extremities, rapidly spreading to her upper extremities over the following days. Muscle strength testing revealed 2/5 strength in the arms and 2/5 in the legs. Generalized hyporeflexia was present. Mental status and cranial nerves II-XII, however, were intact. The result of the imaging study with magnetic resonance imaging of the cervical, thoracic, and lumbar spine was unremarkable.

Serum electrolytes, including calcium, magnesium, and sodium levels, were also normal. The suspicion of acute polyneuropathy related to Guillan barre syndrome leads to obtaining Cerebrospinal fluid (CSF). The CSF testing revealed albumin-cytologic dissociation with an elevated protein level (863 mg/dl) but an average glucose level (52 mg/dl). Cell counts showed no leukocyte/ mm^2 and e few dysmorphic erythrocytes. No organisms were cultured from CSF. Therefore, the diagnosis of Guillan barre syndrome was strongly suspected, and the patient was treated with a five-day course of intravenous immunoglobulin (IVIG). The patient's condition has dramatically improved following the treatment, and she regains muscle strength bilaterally over the seven days, confirming the diagnosis. Electromyography (EMG) study could not be conducted at that time due to technical problems. After two weeks, the patient recovered with oral antibiotics and physical therapy, and her muscle weakness and dyspnea gradually improved over one month.

Consequently, at her follow-up, three months after discharge, the patient's neurologıcal symptoms ultimately enhanced and did not recur. Regarding stone status, the patient's left obstructed ureteral stones were removed with ureteroscopic surgery first and followed for 15 days with a bilateral D-J stent. Finally, the stone-free state was achieved with bilateral retrograde intrarenal surgery.

Table 1. CSF, Urine and blood analyses

Parameters	Causative organisms	Antibiotic résistance +	Antibiotic susceptible +
Urine culture results	*Escherichia coli* ESBL + 50.000 CFU/mL	Cefazolin > 32 Ciprofloxacin >1 Trimethoprim/Sulplamethoxazole > 8/152 Cefixime >4 Ampicilin >16 Ceftazidime 8 Ceftriaxone >4 Levofloxacin >4 Nitrofrontain 128	Piperacilin/tazobactam 4/4 Meropenem <0.13 Gentamicin <2 Fosfomycin <16 Imıpenem 0.25
Blood Culture results	*Escherichia coli* ESBL +	Cefazolin > 32 Cefepime 8 Ciprofloxacin >1 Trimethoprim/Sulplamethoxazole > 8/152 Ampicilin >16 Ceftazidime 8 Ceftriaxone >4 Levofloxacin >4 Cefuroxime Sodium >16	Piperacilin/tazobactam 4/4 Meropenem <0.13 Gentamicin <2 Fosfomycin <16 Imıpenem 0.25 Amikasin <8 Amoxicilin/Clavulanate 8/2 Ampicilin/ sulbactam 2/8
CSF analyses	**No**	-	-
	levels of some parameters in CSF		
LDH	12 U/L		
Glucose	52 mg/dl		
Protein	**863 mg/L** (150-450 Normal ranges)		
Sodium	143 mmol/L		
Chloric	121		

CSF: cerebral spinal fluid, ESBL: extended spectrum beta lactamases, LDH: Lactate dehydrogenase

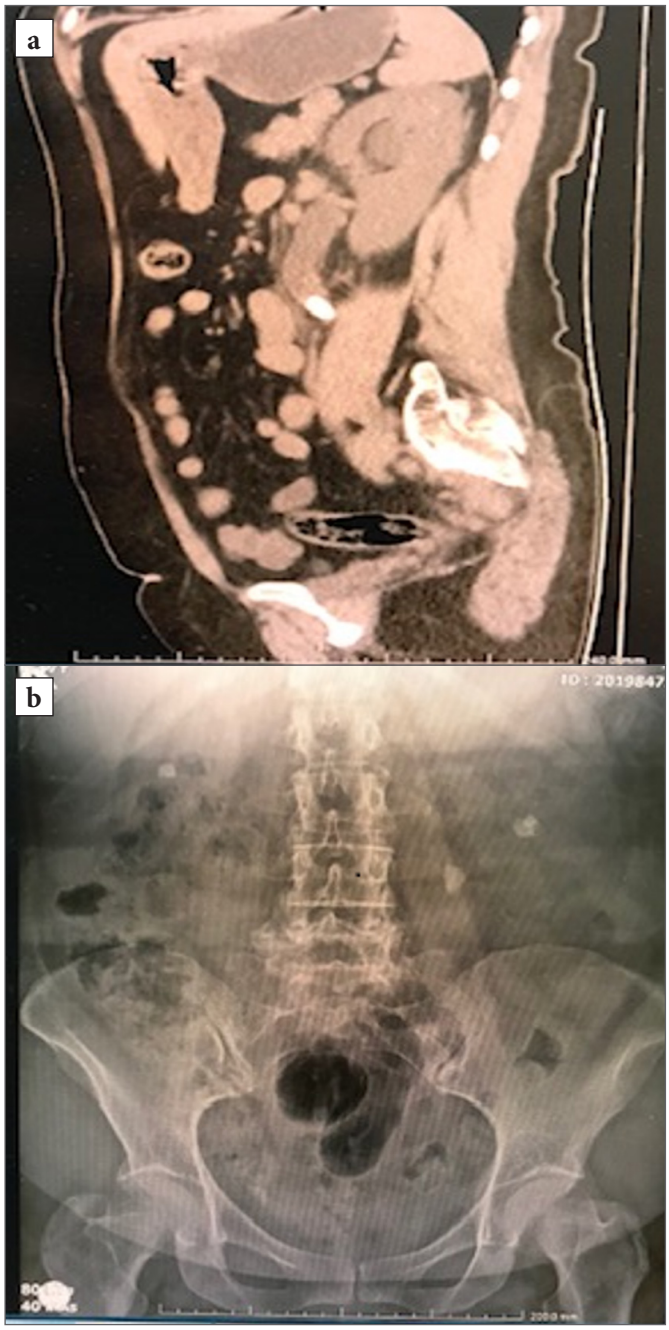

Figure 1. Stone status at first presentation. A left obstructed ureteral stone and bilateral renal stones is seen. **a)** Preoperative CT scan. **b)** Preoperative KUB film.

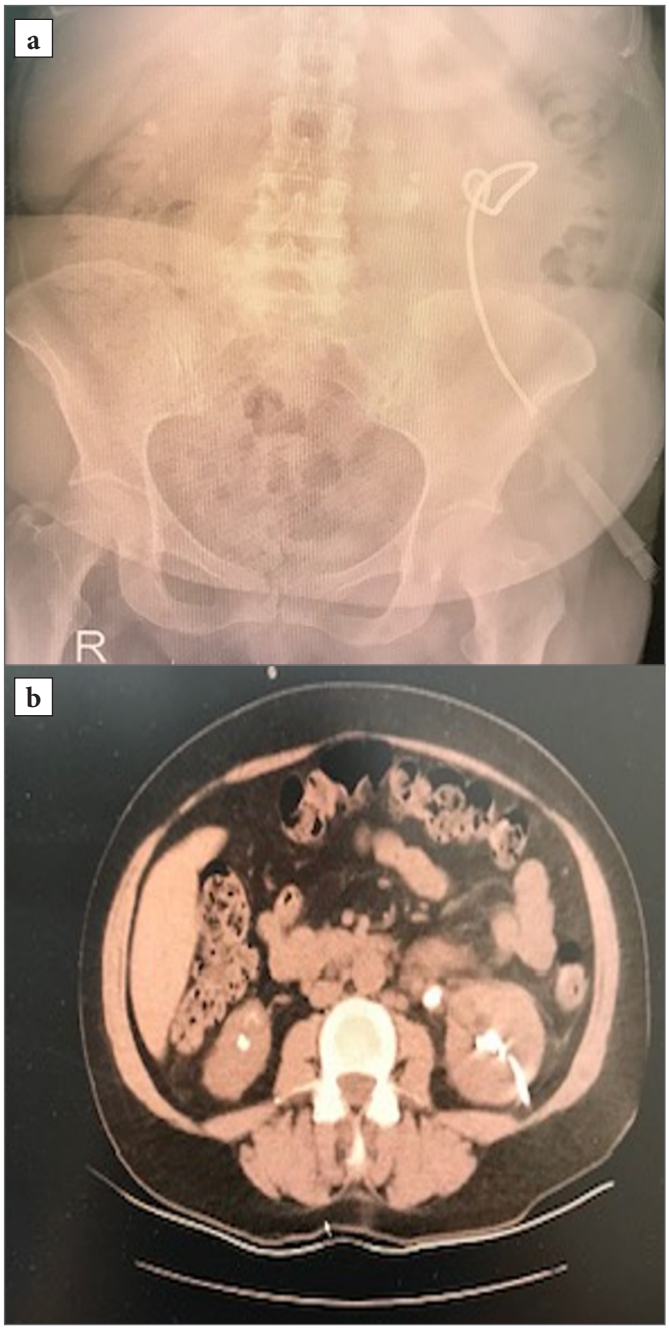

Figure 2. A nephrostomy tube was placed in the left kidney for decompression. **a)** Nephrostomy Tube on KUB, **b)** Nephrostomy Tube on CT scan

DISCUSSION

GBS is the most common cause of acute flaccid paralysis worldwide (4). This autoimmune disease is characterized by rapidly progressive and acute inflammatory features, which lead to polyradiculoneuropathy. The condition requires prompt diagnosis and treatment; otherwise, the patient's state may deteriorate quickly, and respiratory failure may develop. It is estimated that 3–10% of patients with GBS experience autonomic nervous system involvement, which may cause mortality (5). Diagnosis of GBS is based on the patient history and neurological, cerebrospinal fluid, and electrophysiological examinations. Although the exact cause of the disease is unknown, about 75 percent of patients experience preceding infections. The majority of reasons for conditions include *Campylobacter jejuni*, cytomegalovirus, hepatitis E virus, *Mycoplasma pneumonia*, Epstein–Barr, and Zika virus. Other rare infectious sources have also been reported in the literature, such as Human Immunodeficiency Virus (HIV), Haemophilus influenza, herpes simplex, rubella, and varicella-zoster (6). It has been suggested that an aberrant immune response induced by these infections may cause GBS (7). Molecular imitation is a mechanism by which infectious agents may cause an immune response against autoantigens of gangliosides.

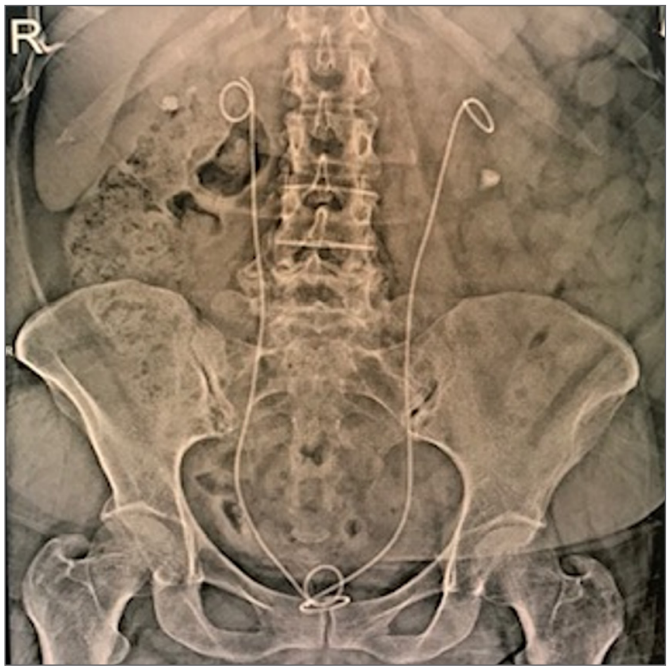

Figure 3: The left ureteral stone was removed and bilateral D-J stents were placed.

Anti-Gangliosidoz molecule 1 (GM1) IgG antibody is positive in about 30% GBS after *C. jejuni* infection (10). Because the *E. coli* capsule incorporates LPS, like all gram-negative bacilli, the most probable mechanism for developing GBS after *E. coli* infection is the same immune response as the *C. jejuni* infection. However, there is a lack of available data about the homology between *E. coli* lipopolysaccharide (LPS) and the GM1 ganglioside. More resources need to be done on this issue.

A surgery on patients who experience active GBS would bode for severe consequences, so awareness of the silent neurological symptoms in patients prepared for upcoming surgery is vital for a urologist. From that point of view, we aimed to remind the possibility of GBS for a patient who expresses neurological symptoms following a septic state.

Urosepsis is a well-known complication of GBS, but it is hard to say the reverse. Only two case reports in the literature show GBS after *E. coli* infection. One of them has been associated with *E. coli* related urinary tract infections (8) and the other one is related to a perirenal abscess formation which has not been required a surgical intervention (9). These reports are related to non-surgical causes, both of which were published in neurological journals. Our case is a unique report presenting GBS in a patient with an uro-surgical condition.

CONCLUSION

Urosepsis is a well-known complication of GBS, but it is hard to say the reverse. Only two case reports in the literature show GBS after E. coli infection. One of them has been associated with E. coli related urinary tract infections (8) and the other one is related to a perirenal abscess formation which has not been required a surgical intervention (9).

ETHICAL DECLARATIONS

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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