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EDITORIAL

Dear Followers,

We are proud to have published the 4th volume 2nd issue of the Journal of Health Sciences and Medicine in March 2021. We are here with eighteen interesting original articles and two case reports on this issue.

We know that our journal, which increases its scientific quality with each new issue, is also eagerly awaited by you, our valuable readers. To further increase the scientific quality of our journal, we work hard, including those on the editorial board.

We thank all the authors and our valuable readers who contributed with their international and national articles and remind you that we need you more than ever for our journal to take place on the scientific platforms we desire.

We would like to express our gratitude to the healthcare professionals who worked devotedly during the COVID-19 outbreak. We commemorate our colleagues who lost their lives in this process with respect and longing.

Yours truly

Assoc. Prof. Dr. Ercan YUVANÇ
Assoc. Editor-in-Chief

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Stroke in long-term intensive care unit

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ABSTRACT

Aim: Long-term acute care hospitals (LTACH) provide specialized care for patients recovering from severe acute diseases and for chronic and critically ill patients; who need long-term ventilatory support. This study aimed to investigate the factors affecting the length of stay of stroke patients in our long-term intensive care unit (LTICU).

Material and Method: This retrospective study included 200 stroke patients; who were followed up in the LTICU. Demographic characteristics, comorbid diseases, and culture results of patients were examined as variables and the effects of these variables on the length of intensive care unit stay were analyzed.

Result: Of the patients; the mean age was 79 (41-99) years, 99 (49.5%) were males, and the mean length of stay was 46 (7-463) days. The length of stay in LTICU was significantly long in stroke patients with a tracheostomy and positive growth in urine cultures ($p=0.013$, $p=0.018$). The length of stay was significantly short in patients; who received total parenteral nutrition (TPN) ($p<0.01$).

Conclusion: Our study has demonstrated that presence of a tracheostomy and positive growth in urine cultures increased but TPN significantly decreased the length of stay of stroke patients in LTICU. To optimize LTACH facilities for stroke patients; we suggest that the overall pattern of care in such centers should be better known, further integration with acute care units should be established, and further studies should be conducted.

Keywords: Long-term intensive care unit, stroke, tracheostomy, urine cultures

INTRODUCTION

Long-term acute care hospitals (LTACH) provide specialized care for patients recovering from severe acute diseases and for patients with chronic and critical illnesses; who need long-term ventilatory support (1,2). Stroke is the main cause of severe disability. Despite advances in prevention and treatment, stroke has been the second most common cause of death for the last two decades (3). However, the percentage of stroke patients admitted to intensive care units for monitorization and management of post-stroke complications continues to grow (4).

Chronic and critical diseases such as stroke place a huge burden on the healthcare system (5). Clinicians; who wish to develop innovative care strategies for such patients, transfer these patients from traditional short-term care hospitals to LTACH (1). The Centers for Medicare and Medicaid Services describe LTACH as acute care facilities with a mean length of hospital stay of 25 days or longer (6). Typically; LTACH provide care to patients, who do not need full-fledged services of a short-term care facility but who need ongoing care considerably (7).

Despite the growing role of long-term acute care, population-based data about overall patterns of use of LTACH is very limited and little is known about the evolution of the characteristics of patients transferred to long-term acute care. In the literature, no studies are available about long-term intensive care of stroke patients. The aim of this study was to evaluate the demographic characteristics, comorbid diseases, and culture test results of stroke patients and to analyze the effects of these factors on the length of stay in the long-term intensive care unit (LTICU).

MATERIAL AND METHOD

The study was approved by Ankara Numune Training and Research Hospital's Ethics Committee (date: September 06, 2018; Approval No: E-18-2200). All study procedures were conducted in compliance with the principles of the Declaration of Helsinki. Files of 200 patients; who were followed up in LTICU for stroke in the period from January 1, 2015 to January 1, 2020,

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were reviewed retrospectively. Stroke patients were categorized into the ischemic stroke (IS), intracerebral hematoma (ICH), and subarachnoid hemorrhage (SAH) groups. Age, gender, Glasgow Coma Scale (GCS) scores, the Acute Physiology and Chronic Health Evaluation II (APACHE II) scores of the study patients; percentages of mechanical ventilation (MV) use, tracheostomies, percutaneous endoscopic gastrostomies (PEG), pressure ulcers (PU), enteral nutrition (EN) (via PEG and nasogastric tube), and total parenteral nutrition (TPN) use; comorbid diseases including hypertension (HT), diabetes mellitus (DM), heart disease (HD), and the effects of these variables on the length of intensive care unit stay were investigated. In LTICU, stroke patients were primarily tried to be fed enterally. TPN was used when EN was not possible (GIS problems, PEG infections, etc). Calorie and protein needs were calculated for each patient by taking age, height, weight, gender, and body temperature into account. The culture test results of the study patients in the blood, urine, wound, rectal, and tracheal aspirate samples, and the effects of these culture test results on the length of intensive care unit stay were examined. Bacteria growing in culture samples were grouped as *Escherichia coli* (*E. coli*), Methicillin-resistant *Staphylococcus aureus* (*MRSA*), *Acinetobacter* spp., *Pseudomonas* spp., *Proteus* spp., and other bacterial spp (*Enterococcus*, *Candida* spp., etc.).

GCS comprises criteria to assess the general neurological status including the level of consciousness of the patient. GCS scores are determined based on the verbal response, motor response, and eye-opening response of the patient. A score of 15 points is assigned for the best response and a score of 3 points is assigned for the worst response (8).Total APACHE II scores can range from 0 to 71, with increasing scores representing higher disease severity (9).

Statistical Analysis

The study data collected from 200 patients were transferred to the IBM SPSS Statistics 23 package software for statistical analyses. Categorical data were presented via frequency distributions (in numbers and percentages). Numerical data were summarized by descriptive statistics (mean, standard deviation, median, and minimum and maximum values). The numerical variables were tested by the Kolmogorov-Smirnov normality test; which revealed a non-normal distribution ($p<0.05$). Therefore, non-parametric statistical tests were used in the statistical analysis of the study data. Differences between two independent groups were tested by the Mann-Whitney U test. Differences across more than two groups were analyzed by the Kruskal-Wallis test.

RESULTS

Of 217 patients followed up in LTICU in the period from 2015 to 2020, 17 were excluded from the study because of missing data and recurrent admissions. Therefore, a total of 200 patients were included in the study for statistical analysis. Ninety-nine (49.5%) patients were men; 101 (50.5%) patients were women, and the mean age of the patients was 79 (41-99) years. While 43 (21.5%) patients were discharged, 157 (78.5%) died. The mean GCS and APACHE-II scores were 7 and 24, respectively. The mean length of stay was 46 (7-463) days (**Table 1**).

Table 1. Patient characteristics

Variables	Median	Min.-Max.
Age (years)	79.0	41-99
GCS	7.0	3-12
APACHE II	24.0	12-40
LOS in LTICU (days)	46.0	7-463
	N	%
Gender		
Female	101	50.5
Male	99	49.5

GCS: Glasgow coma scale, APACHE II: Acute physiology and chronic health evaluation II, LOS: Length of stay, LTICU: Long-term intensive care unit,

The diagnoses were IS in 134 (67%) patients, ICH in 42 (21%) patients, and SAH in 24 (12%) patients. HT was present in 194 (97%) patients; HD was present in 99 (49.5%) patients, and DM was present in 49 (24.5%) patients (**Table 2**).

Table 2. Patient conditions/comorbidities

Conditions/comorbidities	N	%
IS	134	67.0
ICH	42	21.0
SAH	24	12.0
HT		
Present	194	97.0
Absent	6	3.0
DM		
Present	49	24.5
Absent	151	75.5
HD		
Present	99	49.5
Absent	101	50.5

IS: Ischemic stroke, ICH: Intracerebral hematoma, SAH: Subarachnoid hemorrhage, HT: Hypertension, DM: Diabetes mellitus, HD: Heart disease

Mechanical ventilation support was provided to 162 (81%) patients. A tracheostomy and a PEG were present in 172 (86%) and 174 (87%) patients, respectively. Eighty-five (42.5%) patients were administered TPN and 196 (98%) patients received EN (PEG and nasogastric tube). A PU was found in 167 (83.5%) patients (**Table 3**).

Table 3. Patient characteristics

Concomitant problems	N	%
MV		
Present	162	81.0
Absent	38	19.0
Tracheostomy		
Present	172	86.0
Absent	28	14.0
PEG		
Present	174	87.0
Absent	26	13.0
EN		
Present	196	98.0
Absent	4	2.0
NG		
Present	22	11.0
Absent	178	89.0
PU		
Present	167	83.5
Absent	33	16.5
TPN		
Present	85	42.5
Absent	115	57.5
Discharge Condition		
Alive	43	21.5
Exitus	157	78.5

MV: Mechanical ventilation, PEG: Percutaneous endoscopic gastrostomy, EN: Enteral nutrition, NG: nasogastric tube TPN: Total parenteral nutrition, PU: Pressure ulcer

Table 4. Examination of the relationship between the length of stay and the bacteria type

Type of Culture	N	Length of Stay Median (Min.-Max.)	K.W.	P
Blood				
E. coli	23	53 (10-280)	1.016	0.797
Acinetobacter spp.	16	59.5 (11-305)		
MRSA	85	45 (7-249)		
Others	11	36 (13-247)		
Urine				
E. coli	57	44 (10-463)	3.138	0.535
Pseudomonas spp.	16	61.5 (12-142)		
Acinetobacter spp.	8	70 (13-247)		
Proteus spp.	7	90 (10-280)		
Others	8	118 (12-198)		
Wound				
E. coli	30	42 (9-375)	3.637	0.457
Pseudomonas spp.	20	52.5 (13-305)		
Acinetobacter spp.	15	60 (17-283)		
MRSA	10	53.5 (41-249)		
Others	9	36 (10-280)		
Tracheal Aspirate				
E. coli	22	43 (9-164)	2.093	0.553
Pseudomonas spp.	18	64.5 (12-463)		
Acinetobacter spp.	17	44 (10-247)		
Others	13	62 (10-280)		

KW: Kruskal Wallis, *E. coli*: *Escherichia coli*, *MRSA*: Methicillin-resistant *Staphylococcus aureus*

When culture test results were examined, no statistically significant differences were observed in the length of LTICU stay by the species of bacteria isolated from culture samples (p>0.05). Methicillin Resistant *Staphylococcus aureus* (*MRSA*) and *E. coli* growth occurred in the blood culture tests of 85 (42.5%) and 23 (11.5%) patients, respectively. *E. coli* and *Pseudomonas* growth occurred in urine culture tests of 57 (28.5%) and 16(8%) patients; in wound culture tests of 30 (15%) and 20 (10%) patients, and in tracheal culture tests of 22 (11%) and 18 (9%) patients, respectively (**Table 4**).

The length of LTICU stay was statistically significantly different by the presence of a tracheostomy, TPN use, and bacterial growth in urine culture tests (p<0.05). The length of LTICU stay was significantly longer in patients with a tracheostomy and in patients with positive bacterial growth in urine culture tests (p=0.013 and p=0.018, respectively). The length of LTICU stay was significantly shorter in the patients; who received TPN (p<0.01). However; age, gender, GCS or APACHE II scores, diagnosis; presence of comorbidities, MV, PEG, or PU, and the culture test results excluding urine cultures did not act on the length of LTICU stay (**Table 5**).

Table 5. Variables related to the length of stay in a long-term intensive care unit

	N	Length of Stay Median (Min.-Max.)	Z	P
Tracheostomy				
Present	172	48.5 (7-463)	-2.477	0.013*
Absent	28	33.5 (10-375)		
TPN				
Present	85	43 (9-305)	-2.768	0.006*
Absent	115	53 (7-463)		
Urine Culture				
Presence of growth	96	54.5 (10-463)	-2.356	0.018*
No growth	104	44.5 (7-375)		

Z: Mann-Whitney U, *:p<0.05 (Statistically significant), TPN: Total parenteral nutrition

DISCUSSION

Acute stroke is one of the leading reasons for admission to neurological ICUs. Minor progress that was achieved so far in stroke management approaches continues to be the cause of current high mortality and morbidity rates (10,11). Stroke patients are first seen in an emergency room then admitted to an acute care hospital in an average of 4-5 days. This process is followed by admission to an inpatient rehabilitation facility, a long-term acute care hospital (LTACH), a skilled nursing facility, home healthcare, outpatient

clinic care, or a combination of such facilities (12). Acute stroke patients may need a long time of hospital stay due to a need for MV, intravenous antibiotic therapy, or wound care (13). Of the 200 stroke patients admitted to our LTICU from acute ICUs; 134 (67%) had a diagnosis of IS, 42 (21%) had ICH, and 24 (12%) had SAH. Of these patients; 81% needed MV and 83.5% had PU requiring care.

Kahn et al. (14) have demonstrated that mortality rates range from 8.4% to 48.1% in LTACH. Scheinhorn et al. (2) observed in their multi-center study that the mean age was 71.8 years and the median length of stay was 40 days in LTACH patients, and one in three survived 12 months after admission. We found out that the mean age was 79 years, the median length of stay was 46 days, and the mortality rate was 78.5% in our stroke patients followed up in LTICU. We have found no similar studies in the literature on stroke patients treated in LTICU.

A tracheostomy is a common procedure used in patients with acute respiratory distress, especially when clinicians predict the need for long-term MV in a patient (15). Tracheostomy use in stroke patients has increased steadily over the past two decades. Chatterjee et al. (16) observed that increased use of tracheostomy in stroke patients was associated with earlier placement, reduced in-hospital mortality, and a shorter average length of stay. However; they demonstrated that discharge rates to non-acute care centers increased but rates of discharge to home decreased in stroke patients, who underwent a tracheostomy procedure. Consistent with that study; a tracheostomy was present in 86% of the stroke patients admitted to our hospital's LTICU in our study. However; we found out that the length of stay in the LTICU was significantly longer in stroke patients with a tracheostomy contrary to that of acute-care ICUs. Similarly; Turcotte et al. (17) demonstrated an increased length of stay in LTACH patients, who need dialysis, tracheostomy care, ventilator support, and the placement of a feeding tube.

Artificial nutrition support has become the primary therapeutic intervention for preventing metabolic impairments and loss of lean body mass in order to improve outcomes in critically ill patients (18). Although enteral feeding is physiological and is beneficial for the protection of microbial versatility and the structural and functional integrity of the intestines, the use of the enteral route for nutrition is associated with disadvantages especially in acute disease stages and gastrointestinal dysfunction (19,20). Almost all patients (98%) followed up in LTICU received EN in our study but 85(42.5%) patients received TPN due to gastrointestinal problems and issues with PEG. We have found out in our study that the length of stay in the LTICU was significantly shorter in stroke patients; who received TPN support, compared to the other patients. It has been shown that TPN is a favorable source for protein and calorie support when EN is not possible or used as a supplementary method (21,22).

Healthcare-associated infections constitute an important health problem and act on morbidity and mortality significantly (23). Bogason et al. (24) demonstrated that urinary tract infections of any type were associated with an increased length of stay in hospitalized stroke patients. Similar to the findings of the current study; it has been reported that infections resistant to antibiotics and HIV infections increased the length of stay in long-term care hospitals (17). Consistent with the findings of that study, we have found out in our study that the length of stay was significantly long in patients with positive growth in urine culturww

CONCLUSION

We observed that the presence of a tracheostomy and positive growth in urine cultures increased but TPN administration reduced the length of stay of stroke patients followed up in LTICU. In parallel with the aging population and advances in critical care, LTACHs are becoming increasingly and significantly involved in the provision of care for critical chronic diseases including stroke. We think that further information is needed about the overall LTACH use patterns of stroke patients; that integration with acute care units should be improved, and that further studies are required in order to optimize the use of LTACH by stroke patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Research Ethics Committee of Ankara Numune Training and Research Hospital (date: September 06, 2018; Approval No: E-18-2200)

Informed Consent: Because the study design was 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

HEALTH SCIENCES
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Evaluation of seasonality in the diagnosis of diffuse large B cell lymphoma in Turkey

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ABSTRACT

Introduction: Within subtypes of non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma (DLBCL) is most commonly diagnosed, with an incidence of 6/100.000 in Turkey. Aetiology of DLBCL is unknown: several factors such as immunosuppression, AIDS, transplantation, autoimmunity, UV radiation, pesticides, hair dyes and dietary intake are hypothesized to be related with increased risk. Here, we aimed to determine the relationship between the diagnosis time of DLBCL and seasons.

Material and Method: A total of 369 DLBCL patients, diagnosed in our centre were included in the study. Data related to gender, age and time of diagnosis were analysed retrospectively.

Results: Median age of patients with DLBCL included in the study was 61 (range 16–81). The number of female patients were 178 (48.2%) and 191 (51.8%) were male. There was no relationship between the season of diagnosis time and DLBCL incidence (p:0,805).

Conclusion: According to our literature review, this is the first study that sort for a relationship between DLBCL diagnosis frequency and seasons in Turkey. We could not find a relationship between diagnosis time of DLBCL and seasons. This can be explained by the fact that the diagnosis of DLBCL displays a homogeneous distribution throughout the year due to a number of factors playing roles in the ethiopathogenesis of DLBCL.

Keywords: Non-Hodgkin lymphoma, diffuse large B cell lymphoma, seasonality

INTRODUCTION

Within subtypes of non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma (DLBCL) is most commonly diagnosed, with an incidence of 6/100.000 in Turkey (1,2). Most of the patients with DLBCL are over the age of 60 years, men are more frequently affected than women (3,4). Aetiology of DLBCL is unknown: several factors such as immunosuppression, AIDS, transplantation, autoimmunity, UV radiation, pesticides, hair dyes and dietary intake are hypothesized to be related with increased risk (5). Primary central nervous system lymphoma is highly associated with Epstein-Barr virus (EBV) (6). Vitamin D has an important role in immune system functioning and can act as an anti-proliferative in various haematological cancers (7,8). Other factors associated with sunlight exposure may also reduce prostate cancer and NHL risk. Sunlight exposure modulates subclinical both local and systemic inflammation on a cellular basis (12). Serum levels of the vitamin D is season dependant, dietary intake and vacations in sunny regions are main factors (13-15). Turkey is located between 36°–42° North parallels and 26°–45° East meridians. Months between December to February are winter; March to May are spring, June to August are summer and September to November are the autumn months (16,17).

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Although several studies aimed to show the effect of between sunlight and lymphoma or solid tumours; data regarding seasonal variation of DLBCL diagnosis is scarce (18-20). Since vitamin D modulates proliferation and differentiation of cancer cells, we aimed to determine the relationship between the diagnosis time of DLBCL and seasons.

MATERIAL AND METHOD

The study was approved by Health Sciences University, Ankara Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Clinical Researches Ethics Committee (decision no: 2020-06/677; date: 24.06.2020). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

A total of 369 DLBCL patients, diagnosed in our centre were included in the study. Data related to gender, age and time of diagnosis were analysed retrospectively. Patients over 18 years of age who were diagnosed with DLBCL by examining tissue biopsy by immune histochemical analysis were included in the study. Patients who were diagnosed in another centre or those whose diagnosis date could not be reached were not included in the study.

Data analysis was performed using IBM SPSS v26 software. Descriptive statistics were utilized to summarize data. Categorical data were presented as number-percentages, and numerical data were presented as median, minimum, and maximum. Differences between categorical variables were analysed with Chi-Square tests. A p value of ≤0.05 was considered statistically significant. The study was approved by the local ethics committee.

RESULTS

Median age of patients with DLBCL included in the study was 61 (range 16–81). The number of female patients were 178 (48.2%) and 191 (51.8%) were male. The months when patients were diagnosed with DLBCL are shown in **Table 1**, and seasons are shown in **Table 2**. There was no relationship between the season of diagnosis time and DLBCL incidence (p=0.805).

Table 1. The distribution of diffuse large B cell lymphoma diagnosis times

Months	DLBCL (n, %)	p value
January	24 (6.5%)	
February	23 (6.2%)	
March	40 (10.8%)	
April	26 (7%)	
May	30 (8.1%)	
June	29 (7.8%)	
July	34 (9.2%)	p=0.337
August	28 (7.6%)	
September	26 (7%)	
October	32 (8.6 %)	
November	39 (10.5%)	
December	38 (10.5%)	
Total	369 (100%)	

DLBCL, diffuse large B cell lymphoma

Table 2. The distribution of diffuse large B cell lymphoma diagnosis times

Seasons	DLBCL (n, %)	p value
Winter	85 (23.2%)	
Spring	96 (25.9%)	
Summer	91 (24.6%)	p=0.805
Autumn	97 (26.2%)	
Total	369 (100%)	

DLBCL, diffuse large B cell lymphoma

DISCUSSION

In the majority of patients, the aetiology of DLBCL is unknown. Analysing seasonal differences of incidence can improve understanding of pathogenesis and risk factors of different diseases. Hodgkin's disease and Burkitt's lymphoma have been associated with EBV. Because of this infectious aetiology, researchers investigated to find out the relationship between the diagnosis time and seasons (21,22). Some previous reports have shown significant seasonal differences in Burkitt's lymphoma diagnosis, based on the time of the first symptom (23-25). However, other studies have found no relation between seasons and Hodgkin's disease and Burkitt's lymphoma. In addition, other previous studies have reported Burkitt's lymphoma endemicity to coincide with rainfall, low altitude, as well as malaria endemicity (26-30). It has been postulated that an increase in the incidence of Burkitt's lymphoma seen during the rainy seasons may be due to increased mosquitoes that breed during the season, yet they are vectors for EBV. Furthermore, the rainy seasons also come with an increase in malaria infections, which is suspected to compromise the immunity, leading to increased susceptibility to Burkitt's lymphoma (31,32). Williams et al. (33) and Oguonu et al. (34) reported a higher but not statistically significant difference in prevalence of Burkitt's lymphoma in the dry season as compared to the wet season, in Uganda and Nigeria, respectively. Researchers had previously observed a significantly higher occurrence of Burkitt's lymphoma in the wet season as compared to the dry one in South Africa and Malawi, respectively (32,35). Similarly, a seasonal variation is demonstrated in HL; a peak around March and a drop around September in the northern hemisphere is observed (36). Moreover, Porojnicu

et al. (37) defined season of diagnosis as a prognostic factor in HL, where a lower case fatality was observed during autumn, which may be due to a higher serum level of vitamin D. In a recent review by van der Rhee et al. (38) it was stated that epidemiological data suggests chronic but not intermittent sun exposure is associated with a reduced risk of colorectal, breast, prostate cancer and NHL, however, higher vitamin D levels were only associated with a reduced risk of colorectal and breast cancer. Low serum 25-hydroxyvitamin levels were not associated with the overall risk of lymphoid cancer in two prospective studies (39,40); as well as Cohort Consortium Vitamin D Pooling Project of Rarer Cancer failed to show an elevated vitamin D level is associated with a reduced risk of NHL (41). Soni et al. (42) demonstrated an inverse association between sun exposure and risk of DLBCL. However, Swedish Lymphoma Register study failed to show a significant change in cases diagnosed per month (43). In our study, we could not demonstrate any significant seasonal variation of DLBCL diagnosis.

CONCLUSION

There are limited number of studies about the relationship between DLBCL and seasons. Among the studies examining this relationship in various geographical regions of the world, some studies found a relationship between lymphoma diagnosis frequency and seasons, whereas some other did not reveal such a relationship. According to our literature review, this is the first study that sort for a relationship between DLBCL diagnosis frequency and seasons in Turkey. We could not find a relationship between diagnosis time of DLBCL and seasons. This can be explained by the fact that the diagnosis of DLBCL displays a homogeneous distribution throughout the year due to a number of factors playing roles in the ethiopathogenesis of DLBCL.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Health Sciences University, Ankara Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital, Clinical Researches Ethics Committee (decision no: 2020-06/677; date: 24.06.2020).

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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Investigating the relation between upper extremity function and trunk control, balance and functional mobility in individuals with stroke

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ABSTRACT

Aim: The purpose of our study was to examine the relation between upper extremity function and trunk control, balance and functional mobility and to compare trunk control, balance, and mobility with respect to upper extremity motor function level in individuals with stroke.

Material and Method: This study included a total of 39 stroke patients (age 63.87±9.03 years, post stroke 19.18±16.38 month). Upper extremity motor functions were evaluated with the upper extremity sub-scale of the Stroke Rehabilitation Assessment of Movement (STREAM) Scale and Brunnstrom stages of motor recovery; trunk control, balance, and functional mobility were evaluated with Trunk Impairment Scale (TIS), Berg Balance Scale (BBS), and Timed Up and Go Test (TUG) respectively.

Result: A moderate relation was determined between the trunk control, balance and functional mobility and upper extremity functions ($p<0.05$). When the trunk control, balance and mobility performances of the individuals were compared according to Brunnstrom arm stages, it was seen that those with worse upper extremity motor recovery had poor trunk control, balance and mobility ($p<0.05$)

Conclusion: As a result of our study, a relation was detected between upper extremity function and trunk control, balance and mobility. For this reason, it is important to focus on the upper extremity as well as trunk control to improve balance and mobility in physiotherapy and rehabilitation practices.

Keywords: Stroke, postural balance, upper extremity function

INTRODUCTION

Stroke is a central nervous system disease that has a great impact on public health as the cause of long-term disability. Upper Extremity dysfunction, which occurs after stroke, is one of the most common motor problems. Upper extremity problems greatly affect functional disability in approximately 80% of the patients (1). Many studies in the literature reported the importance of upper extremity disorder in people and the extent to which this affects the quality of performance in daily work activities (2,3).

As well as upper extremity problems, another important problem in individuals with stroke is the use of atypical body characterized by weakness and abnormal compensatory strategies. Trunk plays important roles as a central axis in stabilizing the proximal movements for functional movements of the extremities and smooth performance of distal movements. For this reason, trunk stability is important for motor performance, postural balance, and coordinated extremity use in daily functional activities in recovery process (4-6). For this reason, it is very important to acquire trunk control at an early stage in the rehabilitation process (7).

When the literature was reviewed, many studies (8,9) evaluating trunk control, balance, and lower extremity motor functions were detected; however, fewer studies were seen examining the effect of upper extremity functions on postural control and balance (10). As a matter of fact, decreased arm oscillations as a result of upper extremity problems affect postural balance negatively, and increase the risk of falls (11). Hyndman et al. (12) conducted a study, and reported worse upper extremity functions of

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individuals with a history of fall when they compared individuals with fall history and individuals without a history of fall. Arm movements help keep the center of gravity within the support surface during walking (13).

For this reason, it is very important to evaluate the upper extremity functions with trunk control and balance for the planning of an effective rehabilitation program in individuals with stroke. In addition, no study comparing trunk control and balance in stroke individuals with different upper extremity motor levels has been found in the literature. The purpose of our study was to examine the relation between upper extremity motor function and trunk control, balance and functional mobility in individuals with stroke and to compare trunk control, balance, and mobility with respect to upper extremity motor function level.

MATERIAL AND METHOD

Participants and Design

The study was conducted between January 2020 and November 2020. Individuals between the ages of 18-75 years who applied to Physiotherapy and Rehabilitation clinic of Kırıkkale University Faculty of Medicine, diagnosed with ischemic or hemorrhagic stroke, who had no communication problems and who could walk independently with or without an assistive device were included.

Aside from stroke, patients who had another brain tumor, multiple sclerosis, etc. neurological disease, or orthopedic problem, cardiopulmonary disease, agnosia or visual impairment, cooperative and communication problems that would affect functionality, balance, and upper extremity use were not included in the study. The study was approved by Non-Interventional Ethics Committee of University of Kırıkkale (decision no: 2019.11.09; date: 18.12.2019). Informed consent forms were obtained from all participating individuals.

Data Collection Tools

The socio-demographic characteristics of all individuals (age, height, body weight, body mass index, exercise and smoking habits, dominant side, lesion side, stroke type and onset, comorbid states, etc.) were recorded in the scope of the study. Upper extremity functions were evaluated with the upper extremity sub-scale of the Stroke Rehabilitation Assessment of Movement (STREAM) instrument; trunk control, balance, and functional mobility were evaluated with Trunk Impairment Scale (TIS), Berg Balance Scale (BBS), and Timed Up and Go Test (TUG) respectively. The upper extremity motor recovery level of the patients was clinically evaluated with the Brunnstrom stages of recovery.

Stroke Rehabilitation Assessment of Movement

It is used for clinical motor evaluation in stroke patients. The scale consists of a total of 3 parts as Upper Extremity (UE) voluntary act, lower extremity involuntary act, and basic mobility. Each part consists of 10 items, and is scored separately. The extremity movements are scored between 0 and 2 in a 3-point scale. The total score in STREAM UE is between 0 and 20. The scores are given according to the quality of the movement and the amount of doing it (14).

Trunk Impairment Scale

It was developed to evaluate the trunk balance (control) in patients who had neurological impairments. TIS consists of 17 parameters evaluating static (3 parameters, 7 points in total), and dynamic (10 parameters, 10 points in total) sitting balance, and trunk coordination (4 parameters, 6 points in total). TIS items are scored over 2 and 3 scores. The total score is 0-23. Higher scores indicate better performance (15,16).

Berg Balance Scale

It was designed to evaluate the static and dynamic balance, and identify the risk of falls. BBS consists of 14 items aimed to observe the protection of trunk balance during performance directly. Each item is scored between 0 and 4. The test measures the level of dependency and/or independency during positions like standing without sitting, standing with feet adjacent, standing in tandem position, balance on one leg, and the ability of the person to make position changes. According to the scores obtained from this test, individuals are divided into "high risk of falling (0-20 points)", "medium risk of falling (21-40 points)", "low risk of falling (41-56 points)", and the highest score of 56 is considered to show the best balance (17).

Timed Up and Go Test

It is applied to evaluate the risk of functional mobility and fall of patients. A standard chair is used for this test. Firstly, the patient is asked to sit on the chair. Then, the individual is asked to stand up from the chair, walk regularly at a distance of predetermined 3 meters, return at the end of 3 meters, and sit in the chair again. In the test, the patient's walking time is recorded in seconds with a stopwatch. The test is repeated 3 times, and the mean value is recorded (18).

Brunnstrom stages of motor recovery

Brunnstrom motor recovery evaluation consists of 6 stages. Higher Brunnstrom stages indicate better motor recovery (19).

Statistical Analysis

The SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA) program was used for the analysis of the data. The agreement of the variables to normal distribution was checked with Shapiro-Wilk Test. The homogeneity of the group variables was checked with the Levine Test. $P<0.05$ level was considered to be statistically significant.

The descriptive statistics were given as mean±standard deviation (mean±SD). The Spearman Correlation Test was used in the relation measurements between the variables. Correlation coefficients were interpreted as 0-0.19=very low, 0.20-0.39=low, 0.40-0.69=moderate, 0.70-0.89=high, 0.90-1.0=very high correlation (20). The post-hoc power analysis with G*Power program (version 3.0.10 Universität Düsseldorf, Düsseldorf, Germany) was also used. In the post-hoc power analysis, when the statistical significance of alpha was found to be 5%, and the confidence interval was taken as 95%, the power (1-β) of the study was found to be 96%. The primary outcome was determined as TIS and STREAM upper extremity score. Effect size was calculated as 0.524.

RESULTS

This study included a total of 39 stroke patients (age 63.87±9.03 years, post stroke 19.18±16.38 month). The sociodemographic and clinical data of the individuals are given in **Table 1.**

Table 1. Socio-Demographic characteristics of the participants (N: 39)

	Participants
Gender	
Female, n (%)	11 (28.2)
Male, n (%)	28 (71.8)
Age, (years) median (minimum-maximum)	64 (41-81)
BMI, (kg/m^2) median (minimum-maximum)	26.57 (20.20-36.33)
Stroke duration (month) median (minimum-maximum)	12 (1-48)
Brunnstrom stage-arm, n (%)	
Brunnstrom-arm≤3	20 (51.3)
Brunnstrom-arm>3	19 (48.7)
Stroke type, n (%)	
Hemorrhagic	11 (28.2)
Ischemic	28 (71.8)
Dominant side, n (%)	
Right	35 (89.7)
Left	4 (10.3)
Affected side n (%)	
Right	14 (35.9)
Left	25 (64.1)
Falling history n (%)	
Nonfaller	28 (71.3)
Faller	11 (28.2)
Stream upper extremity score, median (minimum-maximum)	7 (0-20)
BBS score, median (minimum-maximum)	46 (7-56)
TUG (second), median (minimum-maximum)	19 (6.01-90)
TIS score, median (minimum-maximum)	14 (3-23)

BMI: Body mass index; STREAM: The stroke rehabilitation assessment of movement scale. BBS: Berg balance scale; TUG: Timed up and go test; TIS: Trunk impairment scale

A moderate relation was determined between the trunk control, balance and functional mobility and upper extremity functions (p<0.05, **Table 2**).

When the trunk control, balance and mobility performances of the individuals were compared according to Brunnstrom arm stages, it was seen that those with worse upper extremity motor recovery had poor trunk control, balance and mobility (p<0.05, **Table 3**).

Table 2. The correlations between upper extremity function, trunk control, balance and functional mobility in patients with stroke

	Stream-UE	TIS	BBS	TUG	
Stream-UE	-	-	-	-	-
TIS	r=0.803* p=0.001	-	-	-	-
BBS	r=0.524* p=0.001	r=0.672* p=0.001	-	-	-
TUG	r=0.394* p=0.013	r=0.499* p=0.001	r=-0.809* p=0.001	-	-

*p<0.05; STREAM-UE: The Stroke Rehabilitation Assessment of Movement Scale-Upper Extremity; TIS: Trunk Impairment Scale; BBS: Berg Balance Scale; TUG: Timed Up and Go Test

Table 3. Comparison of upper extremity motor functions, balance, trunk control and functional mobility according to the level of Brunnstrom stages in stroke individuals

	Brunnstrom-arm≤3 (n=20)	Brunnstrom-arm>3 (n=19)	p value
Stream-UE	4.78±3.90	13.15±6.34	0.001
TIS	10.90±4.17	16.94±4.46	0.001
BBS	34.25±15.45	45.31±10.72	0.007
TUG	41.35±29.09	19.90±15.69	0.011

*p<0.05; STREAM-UE: The Stroke Rehabilitation Assessment of Movement Scale-Upper Extremity; TIS: Trunk Impairment Scale; BBS: Berg Balance Scale; TUG: Timed Up and Go Test

DISCUSSION

As a result of our study, it was found that there is a relation between upper extremity functions and trunk control, balance, and mobility; and it was seen that patients with worse upper extremity functions had more disrupted trunk control, balance and mobility. Accordingly that upper extremity motor dysfunction as well as trunk control may be a factor adversely affecting the balance and mobility in individuals with stroke. When the literature was examined, the effect of lower extremity functions on mobility and balance was investigated (21), or the relations between upper extremity functions and trunk control, and the effects of trunk control on upper extremity performance were examined in current studies (22). The focus was also on the effect of shoulder sling or orthosis use on trunk control or on balance and gait in patients with upper extremity affected, or the effect of rehabilitation programs on upper extremity functions (23-26).

Wee et al. (27) investigated the relations between the restoration of upper extremity functions in the lower extremity function in stroke patients, and reported that the external trunk support in the lower extremity function had a significant effect on the upper extremity function. It was also reported that the recovery of upper extremity function developed in connection with the improvement of the trunk functions, and the trunk was decisive in the recovery of the upper extremity functions of the trunk in stroke patients.

For this reason, in our study, the effects of primarily upper extremity motor functions, but also trunk control, on balance and mobility in individuals with stroke were investigated.

The trunk is the most important dynamic stabilizer of the trunk, and is also the most important part of hemiplegia rehabilitation. The trunk muscles, which are active during sitting and standing, being affected after a stroke causes hemiplegic patients to experience activity limits (28).

Upper extremity function and posture are interconnected systems and are necessary to gain trunk control and improve the quality of upper extremity movements. In this sense, the development of postural control increases the function of upper extremities; and upper extremity movements are also important for the development of postural control, facilitating the trunk muscles. Ustinova et al. (29) conducted a study to determine how arm movements affect postural oscillation in hemiplegia patients, and found that while the center of pressure arm movement slid reverse in oscillation stage in healthy individuals, center of pressure and arms moved in the same direction in hemiplegic individuals. In other words, during the posture phase in healthy individuals, the trunk balanced the arm movements, the trunk moved with the affected arm in hemiplegic patients. As a result of the study, researchers concluded that stroke caused abnormal patterns in the interaction of the arm-trunk and center of gravity. Similarly, in another study, it was reported that there were highly positive relations between upper extremity functions and postural control (30). Ashburn et al. (31) evaluated individuals with stroke for the risk of falling after discharge, and reported that there were higher risks of falling in individuals with upper extremity dysfunction. For this reason, strategies to minimize the risk of falling in stroke individuals should also be given importance among the planned treatment approaches to improve upper extremity functions.

Actually, this is a vicious circle. Problems in both affect each other negatively. Based on these studies, although it is known how much trunk control affects the functional use of the upper extremity, the severity of upper extremity involvement is also important for trunk control, balance and mobility. In our study, when we compared the trunk control and balance levels of individuals with stroke according to the upper extremity Brunnstrom recovery stages, we found that individuals with better upper extremity functions had better balance and mobility of trunk control. For this reason, approaches to improve upper extremity functions should be considered among the treatment strategies planned in individuals with stroke. We believe that our study results can bring a different perspective to researchers.

Limitation

The limitation of our study was that the upper extremity functions are only considered with STREAM, there was no control group, and the affected side is not taken into account. STREAM evaluates only the quality of the movement. Fine and gross motor skills and functionality should also be evaluated in terms of upper extremity functions. Also, considering that the affected part may affect postural control, we recommend that these should be evaluated in future studies in detail.

CONCLUSION

As a result of our study, it was concluded that there is a relationship between upper extremity function and trunk control and balance and functional mobility, and those with poorer upper extremity functions and trunk control have worse balance. When we compared the trunk control, balance levels and mobility of individuals with stroke according to the upper extremity brunnstrom recovery stages, we found that individuals with better upper extremity functions had better balance, mobility and trunk control. Therefore, in physiotherapy and rehabilitation applications, it is important to focus on the upper extremity as well as trunk control to improve postural control, balance and mobility.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Non-Interventional Ethics Committee of University of Kırıkkale (decision no: 2019.11.09; date: 18.12.2019). Informed consent forms were obtained from all participating individuals.

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

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

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Original Article

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Multidimensional assessment of interoceptive awareness (MAIA 2): psychometric properties of the Turkish version

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ABSTRACT

Aim: Interoception, defined as the perception of internal bodily changes. The aim of this study is to evaluate the Turkish validity and reliability study of the Multidimensional Assessment of Interoceptive Awareness-2 (MAIA 2) scale developed by William Mehling et al. (2018).

Material and Method: The research is methodological. Research data was collected between April 2020 and May 2020 (n=400). Forward and backward translation were used to translate the MAIA 2 into Turkish. We conducted exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) to analyze the psychometrics of the MAIA 2 in a 70:30 split sample. Statistical analysis were done with R Project.

Results: The Kaiser-Meyer-Olkin value (KMO=0.910) was acceptable, and there exists a significant correlation structure with Bartlett's test of sphericity ($\chi2$=5134.120, $p<0.001$). We observed six factors with Horn's Parallel analysis. The Cronbach Alphas of these six factors were acceptable ($\alpha>0.60$). Standardized loadings were positive and >0.40 with significant results ($p<0.05$) ($r=0.71$, $p<0.05$). The RMSEA is nearly zero and SRMR is extremely low.

Conclusions: As a result, the Turkish version of the Multidimensional Assessment of Interoceptive Awareness Scale (MAIA 2) developed by Mehling et al. and originally in English is valid and reliable according to our results.

Keywords: Body awareness, body-mind, interoceptive awareness, validity, reliability

INTRODUCTION

While interoception is a term that has gained and still is gaining popularity in the academic literature since the start of the millennium, consensus on its meaning is as yet not fully established. What is generally agreed upon by most current scholars is that interoception is the perception of the state of the body (1,2).

Interoception is a sense that provides information about the internal condition of our body-how our body is feeling on the inside. Interoception allows us to experience many body sensations such as a growling stomach, dry mouth, tense muscles or racing heart.

Although several self-report measures to assess interoceptive and/or body awareness are available, they assess only very limited aspects (e.g., negative aspects) of the concept, which may not capture the complex nature of interoceptive awareness (3). The Multidimensional Assessment of Interoceptive Awareness (MAIA) questionnaire is designed to assess interoceptive awareness (4). The MAIA is a questionnaire able to distinguish between different interoceptive attentional styles that can be adaptive (referred to as a receptive, mindful awareness attitude according to Kabat-Zinn (5) or maladaptive (e.g., anxiety-driven) in processing interoceptive sensations to regulate emotions and behavior. Additionally, the MAIA has been used to assess changes in interoceptive awareness in interventional studies (3-6).

This article presents the adaptation into the Turkish language of the multidimensional assessment of interoceptive awareness-2 (MAIA 2) self report instrument developed by Mehling et al. (7) (2018), and the evaluation of its psychometric properties in the Turkish population.

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MATERIAL AND METHOD

All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles. Ethical committee permission for the study was granted by Alanya Alaaddin Keykubat University Clinical Researches Ethics Committee (decision no: 20-3; date: 18.06.2020). In addition, legal permission was obtained from the hospitals to conduct the research. Informed consent was obtained from the individuals who participated in the study.

Study Design, Setting and Sample

Research data was collected between April 2020 and May 2020. The study is of a methodological type. The sample of this study includes 403 healthcare workers. For methodological research, the sample size is recommended to be at least 5–10 times more than the number of the items of the scale (8,9). The healthcare workers including physicians, dentist, nurses, midwife, health officer were evaluated as eligible to participate in the study.

All the participants were notified of the study's purpose and methods, and assured that their privacy would be protected. Once they agreed to participate by completing a written consent form, they were administered the questionnaires. Subsequently, on receiving the completed questionnaires, the research assistant checked them for missing values.

Instrument

The MAIA 2 is a self-administered instrument developed by Mehling et al. (7) (2018) to measure eight dimensions of interoceptive body awareness. It has a total of 37 items tested on a Likert scale, with six levels of ordinal response coded from 0 (never) to 5 (always). The number of items and reliability established by Cronbach's alpha (α), vary among the subscales: noticing (4 items, α=0.64), Not-Distracting (6 items, α=0.74), Not-Worrying (5 items, α=0.67), Attention Regulation (7 items, α=0.83), Emotional Awareness (5 items, α=0.79), Self-Regulation (4 items, α=0.79), Body Listening (3 items, α=0.80) and Trusting (3 items, α=0.83) (7).

Data Analysis

We implemented exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) to analyze the scale adoption process of the MAIA 2 scale items. We split the data into two parts (70:30 ratio) for exploring and validating the dimensions of the scale. In the first phase, we carried out EFA and reliability analysis based on the sub-dimensions. Then, we validated the factors using CFA. All the statistical analysis findings were obtained with R Project (10) using two R packages; psych (11) and lavaan (12).

MAIA 2 Translation into Turkish

We adapted the MAIA 2 into Turkish using a systematic translation with the aid of a focus group. Three native Turkish speakers, fluent in English and familiar with the concepts of interoception and mindfulness, independently translated the 37 items of the English MAIA 2 version into Turkish. The three Turkish versions were discussed by these experts to ensure the conceptual equivalence of the items. The consensus version was then cognitively tested in a 1-hour focus group of ten health care worker, led by two moderators who documented suggestions and comments and audio-recorded the meeting. Using the input from these focus-group participants and moderators, several items were revised for a consensual version of the questionnaire (MAIA 2 Turkish).

RESULTS

Sample Characteristics

Regarding the age distribution of research group 34.8±8.4 (20-62), 54.7% was female and 45.3% was male. The HCWs including physicians (29.6%), dentist (3.9%), nurses (29.1%), midwife (6.9%), health officer (30.5%) were evaluated as eligible to participate in the study.

Statistical Analysis

We used principal axis method for the extraction process with varimax rotation approach in EFA. Since the scale items were ordinal, we considered Polychoric correlation matrix during EFA (25).

The Kaiser-Meyer-Olkin value (KMO=0.910) was acceptable, and there existed a significant correlation structure with Bartlett's test of sphericity $\chi 2=5134.120$, $p<0.001$. We observed six factors with Horn's Parallel analysis.

Table 1 shows the rotated component matrix and communality values results for the MAIA 2 scale. Because of the low loadings in rotated component matrix, totally five items were removed. The communality values were relatively high and the remained factor loadings were positioned well. The percentage of the explained variance ratio is 82.2% and the factors successfully explain the items. The six factors were named as "Emotional Awareness" (F1), "Attention Regulation" (F2), "Body Listening" (F3), "Not-Distracting" (F4), "Trusting"(F5) and Not-Worrying (F6), respectively.

Table 2 shows the descriptive statistics of the mean factor scores and internal consistency results of the MAIA 2 scales. The Cronbach Alpha values of the six factors were acceptable since they are greater than our threshold (α>0.60).

Table 1. EFA results of the MAIA 2 scale items

Item	F1	F2	F3	F4	F5	F6	Communalities
i23	0.994						0.999
i24	0.994						0.999
i25	0.994						0.999
i26	0.994						0.999
i27	0.994						0.999
i28	0.994						0.999
i31	0.994						0.999
i30	0.994						0.999
i13		0.841					0.788
i14		0.836					0.796
i2		0.765					0.606
i3		0.763					0.615
i17		0.738					0.634
i5		0.710					0.583
i15		0.645					0.462
i16		0.562					0.426
i18		0.558					0.356
i21			0.985				0.980
i22			0.985				0.980
i33			0.985				0.980
i34			0.985				0.980
i7				0.944			0.931
i32				0.889			0.852
i10				0.889			0.852
i8				0.864			0.829
i9				0.864			0.809
i36					0.830		0.858
i37					0.830		0.858
i4					0.620		0.524
i29					0.618		0.654
i12						0.973	0.978
i11						0.971	0.977

Table 2. The internal consistency results of the MAIA 2 scale

Factors	$\bar{X}$	σ	Alpha
F1	4.721	1.335	1
F2	4.415	0.704	0.848
F3	3.802	0.951	1
F4	5.047	0.752	0.922
F5	4.678	0.340	0.631
F6	2.155	1.050	0.998

Figure 1 shows CFA results of the MAIA 2 scale factors. While implementing CFA, we excluded three items because of the insignificant and the poor loading (β<0.40) results. Then, we repeated the analysis and obtained the final CFA model. All standardized loadings were positive and greater than 0.40 with significant results (p<0.05). (r=0.71, p<0.05).

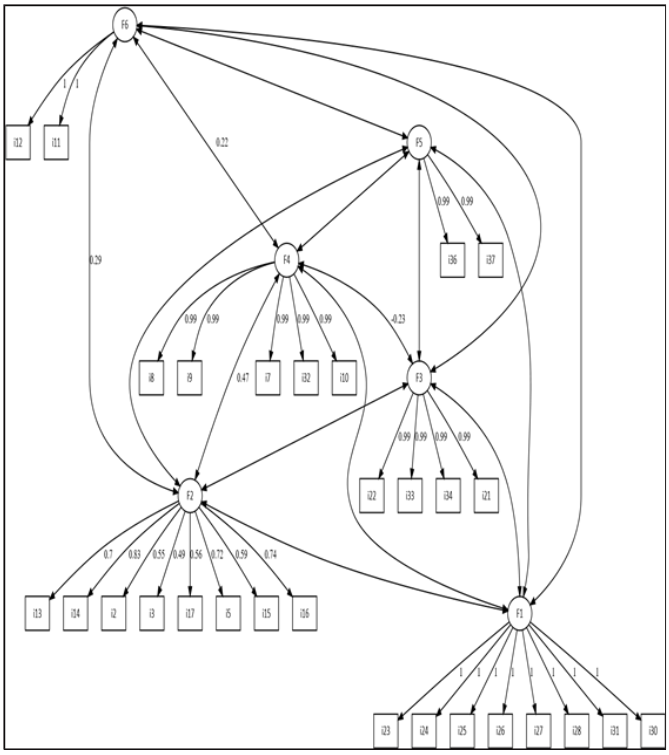

Figure 1. CFA results of MAIA 2 scale

Table 3 reports the goodness of fit index values of the CFA results for the MAIA 2 scale items. The fit index values showed that all the performance measures were perfect since GFI, AGFI, GFI, RFI, CFI were greater than 0.90 and much of them were remarkably close to 1 (Mulaik et al., 1989). The RMSEA was nearly zero and SRMR was extremely low. Lastly, χ2/sd=0.179 was lower than 2. All results pointed to the validity of the MAIA 2 scales.

Table 3. The goodness of fit index values for the CFA results for the MAIA 2

χ2	sd	GFI	AGFI	RFI	CFI	SRMR	RMSEA
64.816	362	0.966	0.959	0.991	1	0.039	0.000

DISCUSSION

The aim of this study was to translate and adapt the MAIA 2 into the Turkish language, and to assess its psychometric properties in a Turkish speaking population.

The Turkish tool was tested in a sample of 403 participants aged between 20 and 62 years, from the provinces of Samsun, Turkey. This study was the first adaptation and validation of a self-report interoception assessment tool for the Turkey population.The adaptation was developed using a forward–backward translation, preserving the extension, format and the dimensional structure of the original scale.

Due to the subjective self-report nature of the information collected by the MAIA (emotions and body sensations), achieving a conceptual equivalence (14) during the linguistic translation was a challenging process. Therefore, we adapted the MAIA 2 into Turkish using a systematic translation with the aid of a focus group. Three native Turkish speakers, fluent in English and familiar with the concepts of interoception and mindfulness, independently translated the 37 items of the English MAIA 2 version into Turkish.

We implemented exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) to analyze the scale adoption process of the MAIA 2 scale items. We split the data into two parts (70:30 ratio) for exploring and validating the dimensions of the scale. In the first phase, we carried out EFA and reliability analysis based on the sub-dimensions. Then, we validated the factors using CFA. All the statistical analysis findings were obtained with R Project (10) using two R packages; psych (11) and lavaan (12).

The EFA favored a model with a factorial structure of eight dimensions with low factorial loading for items 1 and 6. Items 19 and 20 and 35 were removed because they did not contribute to the factors that they theoretically belong to. A new rotated factorial matrix was established for the 32-item scale. This matrix showed a factorial structure of six dimensions.

While the original scale includes eight factors, we found six dimensions with proper factor loadings and high communalities. Also, we observed that all the dimensions are rather reliable due to the satisfactory Cronbach's alpha values. In terms of the validity of the Turkish version of the MAIA scale, we obtained great fit indices and significant items on the sub-factors with CFA results. Consequently, Turkish version of MAIA 2 scale can be considered psychometrically valid.

In our study, unlike the original scale, different items created different scales. According to the analysis results in the study, it was determined that the items numbered 2, 3, 5, 13-18 were included in the scale of "emotional awareness". While only items 2 and 3 are located under the "noticing" in original scale, items numbered 13-15 is in "not worrying" and 16-18 is in "Attention Regulation". Therefore, considering the meanings of the items, it was decided that it would be more appropriate to collect this entire item group (2,3,13-18) under "Attention Regulation". According to the statistical analysis, the items numbered 21, 22, 33 and 34 that were included in the scale of "Attention Regulation" were gathered, in the scale "Body Listening" considering their meanings of those items.

The results suggest the necessity of making minor modifications (e.g., deletion or addition of items) to the original eight factor model to validate the MAIA scale in cross-cultural contexts (13-16). Since several studies suggested that conceptual and cultural differences may affect the construct of the MAIA (17-19). The findings imply that subjective aspects of interoceptive awareness are affected by the conceptual framework of a culture or population (20,21)

Future development of the MAIA 2 in the Turkish population should explore and provide evidence for convergent and divergent validity. Further studies with clinical and non-clinical populations, or samples with specific characteristics, are required to explore the differential performance of the items.

CONCLUSION

In this study, we attempted to adopt the MAIA 2 scale for healthcare workers in the Turkish population. Turkish version of the MAIA scale provides different structure from the original one. While the original version of the scale has eight factors, we obtained six factors. These factors were validated with CFA results with excellent model fit for six factors, namely "Emotional Awareness", "Attention Regulation", "Body Listening", "Not-Distracting", "Trusting" and Not-Worrying.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical committee permission for the study was granted by Alanya Alaaddin Keykubat University Clinical Researches Ethics Committee (decision no: 20-3; date: 18.06.2020).

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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Comparison of the efficacy of different progesterone regimens in blastocyst frozen-thawed embryo transfer cycles

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ABSTRACT

Aim: The progesterone's optimal route for luteal phase support (LPS) in frozen-thawed embryo transfer (FET) cycles is controversial. This study investigates the effect of three treatment regimens of progesterone: vaginal, oral, and subcutaneous form for LPS in FET cycles.

Material and Method: Blastocyst cryopreserved FET cycles utilizing vaginal, subcutaneous, or oral forms of progesterone between December 2018 and May 2020 were included. The main outcome was to compare clinical pregnancy rates (CPR), ongoing pregnancy rates (OPR), and live birth rates (LBR) in different progesterone groups.

Results: A total of 434 cycles were included, of which 200 utilized vaginal, 124 utilized subcutaneous, and 110 utilized oral forms. Demographic and cycle characteristics were similar between all three groups. Compared to cycles utilizing vaginal, subcutaneous, and oral forms, CPR, OPR, and LBR differ significantly between the three groups (p-value=0.000). Miscarriage was calculated according to the number of days after embryo transfer, and it was shown that the subcutaneous group had the lowest rate of miscarriage with 11 cases (8.8%). The highest OPR was associated with the subcutaneous group with 67 (54%), followed by the vaginal group with 92 (46%). The highest LBR was in the subcutaneous group, with 59 (47.6%) cases. The oral group was significantly less successful 29 (26.4%) than the subcutaneous and vaginal groups.

Conclusion: Our study results showed that subcutaneous and vaginal progesterone performed better than oral progesterone for LPS in patients undergoing FET. All three forms of progesterone administration were safe and well-tolerated.

Keywords: Frozen-thawed embryo transfer, luteal phase support, progesterone, in vitro fertilization

INTRODUCTION

Frozen-thawed embryo transfer (FET) is one of the most important advances in assisted reproductive techniques (ART) that has many benefits for patients (1). Using this method and transferring fewer embryos reduces the risk of multiple pregnancies and increases the patient's chances of getting pregnant using multiple embryos without additional stimulation with stimulated cycles (2). Also, by FET in patients at risk for ovarian hyperstimulation syndrome, this syndrome's risk is greatly reduced (3).

Progesterone is usually used as luteal support for frozen embryo transfer due to increasing implantation and pregnancy rate (1,4,5). The FET process's success depends on the progesterone support secreted by the corpus luteum, known as luteal phase support (LPS) (6). This support increases the rate of pregnancy after ART procedures (7). Today, LPS in ovulation induction and FET cycles by progesterone products have attracted a lot of attention, which increases the rate of pregnancy and reduces the chance of miscarriage (8-10).

Various studies have been performed on the type of progesterone for LPS (1-9). Different types of progesterone are used for this purpose, including

1) Oral micronized progesterone (300-600 mg per day): One of this method's advantages is easy administration, but it is metabolized quickly by the liver. Its absorption rate is different in different people. Dydrogesterone, as the optical isomer of progesterone, is similar in formulation and structure to natural progesterone, and no specific side effects have been reported (10,11).

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2) Subcutaneous progesterone injection (25 mg twice daily): Requires daily injection and may cause a local reaction at the injection site. Its advantages are comfortable, easy to use, and efficient (12).

3) Vaginal progesterone: in the form of a gel (Crinone 8% of an applicator 90 mg per day) of the advantages of the vaginal method is the easy administration, but the relatively expensive price and the rare cases of vaginal irritation, inflammation, itching, leakage, and bleeding are its disadvantages (20).

Today, vaginal progesterone is more widely used in medical centers due to the high concentration of progesterone and its direct effect on the endometrium (20). The preferred regimen of LPS in FET cycles is unclear. Therefore, this study investigates the effect of three treatment regimens for LPS in FET cycles: vaginal, subcutaneous, and oral progesterone.

MATERIAL AND METHOD

This retrospective cohort study was approved by the Ethics Board of Erciyes University (number 2020/341, date 24.06.2020). The study was performed on 434 patients referred to the Kayseri Memorial In Vitro Fertilisation (IVF) Center from December 2018 to May 2020. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients provided their informed consent before enrolling.

All woman undergoing FET cycle at this time period were given vaginal, subcutaneous or oral progesterone for LPS according to patient preference or physician preference.

Patients with unknown infertility problems and women using birth control methods were excluded from the study. The mean age of participants was 33.5, with a minimum age of 20 and a maximum age of 39. Older subjects were excluded from the study.

Participants were divided into three groups: receiving progesterone vaginal, oral, and subcutaneous. There were 200 people in the vaginal group, 124 in the subcutaneous group, and 110 in the oral group. Participants in the first group received 90 mg of vaginal progesterone twice daily (Crinone gel, Merck, United Kingdom). The second group received 25 mg of subcutaneous progestogen twice a day (Prolutex, IBSA, Switzerland). The third group received 10 mg of oral dydrogesterone three times a day (Duphaston, Abbott Healthcare Products, Netherlands).

The routine IVF protocol utilized at our center was started with an initial examination performed on the first three days of menstruation. Briefly, patients' endometrial development was serially monitored with ultrasonography by evaluating endometrial thickness, pattern, and the presence/absence of active or residual follicles. Also, estradiol and progesterone's blood levels were determined and recorded, again with routine serial measurements. In preparation for IVF, patients were started on estradiol valerate 2 mg tablet (Estrofem tablet, three times daily), roxithromycin 300 mg tablet (Rulid tablet, once daily), acetylsalicylic acid 100 mg tablet (Coraspin tablet, once daily), and folic acid 5 mg tablet (Folbiol tablet, once daily) on the second day of menstruation. Patients continued to receive these treatments until the 12th day of menstruation. Progesterone initiation was begun after detecting 8-mm endometrium thickness and observing the characteristic "triple line" pattern. Progesterone was given through oral, vaginal, and subcutaneous applications to induce endometrial secretion to transfer the thawed embryo. As a rule of thumb, normal transfers were planned on the 18th or 19th days of the menstrual cycle, corresponding to 5 days of embryo development. Embryo transfers were performed under ultrasound guidance using a soft tip catheter (Cook Medical, USA) on the sixth day of progesterone administration. One or two blastocyst embryo transfer was performed at the stage of top quality or good quality embryos according to Gardner and Schoolcraft blastocyst grading system. Estradiol and progesterone supplementation were continued until the day of the pregnancy test, carried out 10 days after the embryo transfer. If the test was positive, estradiol and progesterone was maintained due to 10th gestational week.

Main outcome was to detect clinical pregnancy rate, ongoing pregnancy rate and live birth rate according to different progesterone regimens. Clinical pregnancy was defined as the confirmation of an intrauterine gestational sac at 6-7 weeks of pregnancy. Ongoing pregnancy was defined as a pregnancy proceeding beyond 20th gestational week and live birth was defined as the delivery of a living newborn after 24th gestational week. Miscarriage was defined as pregnancy loss after detection of a fetal heartbeat during TvUSG exam and before 20th gestational week.

Statistics

Chi-square test was used to examine the significant difference between each of the qualitative variables in the three groups. If the number of data in at least one cell was less than the other cells in the agreement table, Fisher's exact test was used and * denotes these variables. For quantitative variables, after examining their abnormality using the Kolmogorov-Smirnov test, the non-parametric equivalent of a one-way ANOVA test, the Kruskal-Wallis test was used. Statistical Package for Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA) was used to perform analysis.

RESULTS

There were 443 patients, and the patients were divided into three groups according to the progesterone administration route as vaginal, subcutaneous, and oral. The descriptive statistics of the participants are given in **Table 1**. The frequency of descriptive statics of patients within groups is also shown in **Table 2**. There was no significant difference between the descriptive statistics of the participants in the three different groups.

Table 1. The descriptive statics of patients

Variable	Mean (Sd)	Min-Max
Age	33.5 (4.7)	20-39
Cycles day in transfer	16.9 (1.4)	16-19
Embryo day	5 (0)	5-5
Endometrium thickness	10.45 (1.33)	8-16

Table 2. The frequency of descriptive statics of patients within groups

Variable	Frequency (%) within groups		
	Vaginal	Subcutaneous	Oral
Age	34.3 (4.9)	35.6 (3.9)	29.5 (2.5)
Cycles day in transfer	17.4 (0.9)	15.29 (1.07)	17.9 (1.2)
Embryo day	5 (0)	5 (0)	5 (0)
Endometrium thickness	10.37 (1.29)	10.48 (1.28)	10.57 (1.4)

As shown in **Table 1**, the participants' minimum age was 20, and the maximum age was 39 (mean±SD: 33.5±4.7). Also, the cycle days spent during the experiment for participants were a minimum of 16 days and a maximum of 19 days (mean±SD: 16.9±1.4). The day of receiving the embryo was the fifth day for all participants (Mean±SD: 5±0). The endometrium thickness was a minimum of 8 mm and a maximum of 16 mm in the participants (mean±SD: 10.45±1.33).

The descriptive statistics of the pregnancy variables are presented in **Table 3**. As shown in **Table 3**, in the participants' pregnancy beta test, 166 (38.2%) subjects were negative, and 268 (61.8%) were positive. The next examination was to test for the gestational sac formation. The examination result was negative for 167 (38.5%) subjects and positive for 267 (61.5%). The fetal heart rate was then examined, and results were negative for 173 (39.9%) subjects and positive for 261 (60.1%). The next field is related to miscarriage, which is divided according to the days after embryo transfer. The results show that 355 (81.8%) subjects did not experience a miscarriage. Also, 1 (0.2%) subject on the sixth day, 31 (7.1%) on the seventh day, 31 (7.1%) on the eighth day, and 16 (3.7%) on the ninth day experienced a miscarriage. The next field in the table shows that the child was born at the right time. This result was negative for 282 (65%) participants and positive for 152 (35%). The frequency and p-value of pregnancy variables within groups are shown in **Table 4**.

Table 3. The descriptive statistics pregnancy variables

Variable	Value	Frequency	Percent (%)
Beta pregnancy test	0	166	38.2
	1	268	61.8
Gestational sac	0	167	38.5
	1	267	61.5
Heartbeat	0	173	39.9
	1	261	60.1
Miscarriage	0	355	81.8
	6	1	0.2
	7	31	7.1
	8	31	7.1
	9	16	3.7
Live birth rate	0	282	65
	1	152	35
Ongoing pregnancy	0	246	56.7
	1	188	43.3

Table 4. The frequency and p-value of variables within groups Variable

Variable	Value	Frequency (%) within groups			p value
		Vaginal	Subcutaneous	Oral	
Beta pregnancy test	0	56 (28)	45 (36.3)	65 (59.1)	0.000
	1	144 (72)	79 (63.7)	45 (40.9)	
Gestational sac	0	56 (28)	46 (37.1)	65 (59.1)	0.000
	1	144 (72)	78 (62.9)	45 (40.9)	
Heartbeat	0	60 (30)	48 (38.7)	65 (59.1)	0.000
	1	140 (70)	76 (61.3)	45 (40.9)	
Miscarriage*	0	148 (74)	113 (91.1)	94 (85.5)	0.001
	6	0	1 (0.8)	0	
	7	21 (10.5)	5 (4)	5 (4.5)	
	8	18 (9)	3 (2.4)	10 (9.1)	
	9	13 (6.5)	2 (1.6)	1 (0.9)	
Total		52 (26)	11 (8.8)	16 (14.5)	
Live birth rate*	0	130 (65)	65 (52.4)	87 (79.1)	0.000
	1	70 (35)	59 (47.6)	23 (20.9)	
Ongoing pregnancy	0	108 (54)	57 (46)	81 (73.6)	0.000
	1	92 (46)	67 (54)	29 (26.4)	

*Fisher exact test

The last field indicated Ongoing pregnancy, which means a successful pregnancy. The participants' results showed that this variable was negative for 246 (56.7%) subjects and positive for 188 (43.3%).

As **Table 4** shows, Beta pregnancy test results differ significantly between the three groups (p-value=0.000). These results showed that the highest positive Beta pregnancy test was related to the vaginal group, with 144 (72%) cases. The subcutaneous group with 79 (63.7%) positive cases and the oral group, with 45 (40.09%) positive cases were also observed. The results of the gestational sac test also showed similar results.

The fetal heartbeat test showed that the highest positive rates were observed in the vaginal group with 140 (70%), followed by the subcutaneous group with 76 (61.3%) and the oral group with 45 (40.9%). These results were significantly different in the three groups (p-value=0.000).

Miscarriage was calculated according to the number of days after embryo transfer, and it was shown that the subcutaneous group with 11 (8.8%) had the lowest rate of miscarriage, followed by the oral group with 16 (14.5%) and the vaginal group with 52 (26%). Also, the seventh and eighth days with 31 cases recorded the highest miscarriage in a day.

Comparing the live birth rate showed a significant difference between the three groups (p-value=0.000). The highest rates of live birth were associated with the subcutaneous group with 59 (47.6%), followed by the vaginal group with 70 (35%) and the oral group with 29 (26.4%).

Finally, the results of successful pregnancies in participants also showed a significant difference between the three groups (p<0.001): the highest successful pregnancy rate was associated with the subcutaneous group with 67 (54%), followed by the vaginal group with 92 (46%) and the oral group with 29 (26.4%) cases. These results showed that the oral group was significantly less successful than the subcutaneous and vaginal groups.

DISCUSSION

There has been increasing trend in the use of frozen embryo transfer cycles in recent years. Although various protols have been used to prepare the endometrium during FET cycles, the ideal protocol for endometrial preparation and LPS in FET cycles is controversial yet. Endometrial and embryo coherence in frozen-thawed embryo transfer (FET) cycles is influenced by various factors, including the duration and type of progesterone treatment before embryo transfer (5).

Progesterone preparations can be administered by vaginal, subcutaneous, oral or intramuscular, routes. Vaginal progesterone preparations are considered as more patient-friendly; however, they may cause vaginal irritation, discomfort, and may not be preferred by some women owing to cultural reasons or medical conditions like vaginismus (13). Subcutanoeous progesterone preparations are the water-soluble forms of progesterone which is most recently developed. It was shown that absorbtion is faster and reaches peak serum concentration quicker than intramuscular progesterone (14).

Except dydrogesterone, other oral progesterone preparations are not suitable for LPS bacause of low bioavailabilities after hepatic first passage effect (15).

Our study examined three different progesterone preparations for LPS undergoing FET cycles. We found that the pregnancy outcomes including CPR, OPR and LBR were all significantly lower in the oral progesterone group than the other groups, while the vaginal and subcutaneous groups had comparable pregnancy outcomes in this regard. These results suggest that vaginal and subcutaneous progesterone are acceptable supplementations for LPS, but oral supplementation may not provide adequate LPS. This result of our study is consistent with a randomised controlled trial in the literature which compared oral dydrogesterone, vaginal progesterone, human corionic gonadotropin plus oral dydrogesterone and gonadotropin releasing hormone analogue plus oral dydrogesterone in FET cycles (16). Only oral dydrogesterone group was found to have lower pregnancy rates than the others. But there is also conflicting results in the literature concerning the efficacy of oral dydrogesterone for LPS in FET cycles. Guo et al. (17) investigated subcutaneous dydrogesterone and oral progesterone for LPS in FET cycles and concluded that oral dydrogesterone produces better results for LPS the FET cycles. Rashidi et al. (18) compared pregnancy outcomes of oral dydrogesterone, intramuscular progesterone and vaginal progesterone suppository use in FET cycles and found CPR 36.6%, 38.3% and 28.3% respectively and LBR 30%, 28.3% and 26.6% respectively which were similar between the groups. This result might be associated with a relatively high dosage of oral progesterone that 40 mg daily oral dydrogesterone was used in this trial.

In a recent randomised trial, LPS for FET cycles was performed by vaginal route, intramuscular route and their combination (19). Miscarriage rate was higher in the vaginal progesterone only group that resulted in significantly lower ongoing pregnancy rates than intramusculer route and the combination. This result might be considered as compatible with our study's miscarriage results that we also found higher miscarriage results in the vaginal and oral progesterone groups than the subcutaneous group.

One of the limitations of this study and studies in this area is the retrospective nature of the study. More detailed randomized clinical trials are needed to examine the efficacy of progesterone forms/combinations more accurately. In the same way with our study, pregnancy outcomes with different progesterone forms for LPS were measured only in quality embryos in the literature, while the results may be different in lower quality embryos. Real-world outcomes may be different from the selected study groups.

According to current literature, the available evidence on the optimal progesterone preparation in FET cycles is far from conclusive, and randomised controlled trials and further real-world studies on different progesterone forms and their combinations are needed.

CONCLUSION

The results of our study showed that in FET cycles, vaginal and subcutaneous progesterone provides better pregnancy outcomes than oral progesterone for LPS. Therefore, it may be recommended to use subcutaneous and vaginal forms for LPS in FET cycles.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Research Ethics Committee of Erciyes University (number 2020/341, date 24.06.2020).

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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The impact of laboratory features and comorbidities on the prognosis of patients with COVID-19

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ABSTRACT

Objective: Demographic and laboratory values predicting clinical severity in coronavirus disease 2019 (COVID-19) patients have been a matter of curiosity since the beginning of the disease. We aimed to show the relationship between the severity of COVID-19 disease and comorbidities, clinical and laboratory features of the patients.

Material and Method: The data of COVID-19 patients diagnosed with polymerase chain reaction (PCR), were analyzed retrospectively. The patients were divided into 3 groups according to their clinical severity as mild, moderate and severe. Comorbidities and the Charlson comorbidity index (CCI) at the time of diagnosis were calculated for each patient from the patients' records. Demographic data, laboratory values, comorbidity and CCI scores were compared between the patient groups. The effect of CCI on survival and length of hospital stay was examined.

Results: One hundred and four patients were included in the trial. The most common comorbid disease in the patients included in the trial was hypertension. The moderate-severe stage patients were statistically significantly older ($p<0.001$). The CCI was found to be statistically significantly different between mild, moderate and severe groups ($p<0.001$). When CCI increases by one unit, the risk of death increases by 1.193 times ($p=0.017$). The neutrophil-to-lymphocyte ratio (NLR) was statistically significantly different between the mild, moderate and severe patient groups. It was observed that as the severity of the disease increased, the NLR increased. Older age, WBC, neutrophil count, NLR, BUN, creatinine, AST, potassium level, C-reactive protein (CRP), procalcitonin, aPTT, fibrinogen, d-dimer, and ferritin levels were found to be higher in the clinically severe patient group. Lymphocyte and eosinophil counts, total protein, albumin and sodium levels were found to be lower in the clinically severe patient group.

Conclusion: This trial showed that calculating the CCI score in COVID-19 patients can be useful in predicting the severity of the disease. Examination of CCI, age, WBC, neutrophil, lymphocyte, eosinophil counts, BUN, creatinine, AST, total protein, albumin, sodium, potassium level, CRP, procalcitonin, aPTT, fibrinogen, d-dimer and ferritin levels at the time of diagnosis can be suggested.

Keywords: COVID-19, Charlson comorbidity index, mortality, neutrophil-to-lymphocyte ratio, laboratory features

INTRODUCTION

At the end of 2019, a new coronavirus was detected in Wuhan, China. It spread rapidly and caused an epidemic across China, followed by an increasing number of cases followed in other countries around the world, causing the pandemic.

Pneumonia appears to be the most common manifestation of infection, characterized by fever, cough, shortness of breath, and bilateral infiltrates on lung imaging (1). Other features such as upper respiratory symptoms, myalgias, diarrhea, and smell or taste disturbances have also been widely reported (2).

While coronavirus disease 2019 (COVID-19) is mild in some patients, it can be moderate or severe in some patients. It has been reported age, presence of comorbidity, lymphopenia, thrombocytopenia, acute renal failure, increased LDH, elevated transaminases, d-dimer, CRP, ferritin and IL-6 as factors determining the severity of the disease in COVID-19 patients (3).

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The clinical course and hospital stay are directly related to the comorbidities and ages of COVID-19 patients. People with chronic obstructive pulmonary disease (COPD) or any lung disease are candidates for more severe COVID-19 disease (4). It has been reported that patients diagnosed with diabetes mellitus (DM) have higher morbidity and mortality rates and longer hospital stay (5). Cardiovascular diseases and hypertension have also been reported as risk factors for mortality in COVID-19 patients (6,7).

We aimed to investigate the effect of age, laboratory features and comorbidities of patients followed up with a diagnosis of COVID-19 disease on the severity of the disease and survival. Charlson comorbidity index (CCI) was calculated as the comorbidity index and its effect on survival and hospital stay was examined.

MATERIAL AND METHOD

The trial was approved by İnonu University Research Ethics Committee (date/reference number: 10-11-2020/1255). The trial was conducted in accordance with the Helsinki Declaration principles.

Study Design

The data of COVID-19 patients diagnosed with polymerase chain reaction (PCR), between August 01, 2020 and August 30, 2020 were analyzed retrospectively. Laboratory results were analyzed retrospectively from patients' files. Comorbidities and the CCI at the time of diagnosis were calculated for each patient from the patients' records (8).

The patients were divided into 3 groups as mild, moderate and severe. Stage 1 (mild): Patients with mild and nonspecific symptoms and no findings other than lymphopenia and neutrophilia in the complete blood count. Stage 2 (moderate): There is viral involvement and localized inflammation in the lung. Patients have fever, cough, and hypoxia (PaO_2/FiO_2<300 mm Hg). There are bilateral infiltrates or grass opacids on chest radiography or computed tomography. Stage 3 (severe): There is an extrapulmonary systemic hyperinflammation syndrome. Inflammatory markers (IL-2, IL-6, tumor necrosis factor-alpha, CRP, ferritin, and d-dimer) were increased. Shock, respiratory failure and cardiopulmonary collapse may occur. Systemic organ involvement, even myocarditis, can occur at this stage (9).

Real-time reverse transcriptase-PCR tests for SARS-CoV-2 RNA were performed using nasopharyngeal swabs. Total nucleic acid extraction of nasopharyngeal swabs of viral isolates was performed using a biospeedy and coyote extraction system (Bioeksen ltd and Coyote Bioscience ltd). Real-time PCR (RT-PCR) assays for SARS-CoV-2 RNA detection were performed using Biospeedy COVID-19 RT-qPCR Detection Kit (Bioeksen, İstanbul, Turkey). The ARCHITECT ci16200 automatic biochemistry analyzer was used to measure the biochemical parameters and Sysmex Corporation was used to measure the hematologic parameters.

One hundred and four COVID-19 patients were included in this trial. The patients were divided into 3 groups according to their clinical severity as mild, moderate and severe. Among these 3 groups age, gender, leukocytes, neutrophils, lymphocytes, eosinophils, monocytes, hemoglobin, hematocrit, platelets, BUN, creatinine, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), sodium, potassium, CRP, procalcitonin, International Normalized Ratio (INR), activated Partial Thromboplastin Time (aPTT), fibrinogen, d-dimer, ferritin, hospitalization time, CCI and mortality rates data were compared.

Statistical Analysis

Data analysis was performed using IBM SPSS v22 software. Descriptive statistics were used to summarize data. Variables assessed for normal distribution with the Kolmogorov Smirnov test. Categorical data were presented as number-percentages, and numerical data were presented as median, minimum, and maximum. Differences between categorical variables were analyzed with the Chi-Square test, and numeric variables were compared with the Mann-Whitney U test. Statistical significance level was accepted as $p<0.05$.

RESULTS

A total of 104 patients were included in the trial (66 males, 38 females). The demographic and characteristic characteristics of the patients are summarized in **Table 1**.

Table 1. Patient characteristics

Total patients, n	104
Age, years (median, min.-max.)	58 (12-92)
Gender, n (M/F),	66 (63.5)/38 (36.5)
Stage, n (%)	
Stage I (Mild)	31 (29.8)
Stage II (Moderate)	60 (57.7)
Stage III (Severe)	13 (12.5)
Hospitalization (day)	6 (1-32)
Mortality, n (%)	11 (10.6)

The most common comorbid disease in the patients included in the trial was hypertension, which occurred in 17 patients (13.6%). 64 patients (51.2%) had no comorbid disease. Comorbid diseases seen in patients and their frequency are in **Table 2**.

The clinical and laboratory values of the patients who were divided into 3 groups as mild, moderate and severe patients according to their clinical severity are shown in **Table 3**.

Table 2. Comorbidities

Disease	n (%)
Hypertension (HT)	17 (13.6)
Coronary arter disease	15 (12)
DM	9 (7.2)
Asthma/COPD	9 (7.2)
Chronic renal failure	4 (3.2)
Arrhythmia	2 (1.6)
Cerebrovascular disease (CVD)	2 (1.6)
Benign prostat hyperplasia	1 (0.8)
Hypothroidism	1 (0.8)
Psychiatric disease	1 (0.8)
None	64 (51.2)
Total number of comorbidities	125 (100)

Among the patients whose ages at the time of diagnosis were between mild stage and moderate-sever stages, the moderate-severe stage patients were statistically significantly older ($p<0.001$).

In accordance with the literature, age, WBC, neutrophil count, neutrophil-to-lymphocyte ratio (NLR), BUN, creatinine, AST, potassium level, CRP, procalcitonin, aPTT, fibrinogen, d-dimer, and ferritin levels were found to be higher in the clinically severe patient group. Lymphocyte and eosinophil counts, total protein, albumin and sodium levels were found to be lower in the clinically severe patient group.

The NLR was statistically significantly different between the mild, moderate and severe patient groups. It was observed that as the severity of the disease increased, the NLR increased.

Table 3. Comparison of mild, moderate and severe patients

		Stage 1 (mild) (n=31)	Stage 2[a] (moderate) (n=60)	Stage 3 (severe) (n=13)	p
Age at diagnosis		29 (12-73)[a]	59.5 (30-92)[b]	68 (48-85)[b]	<0.001
Sex n (%)	Female	14 (45.2)	20 (33.3)	4 (30.8)	0.485
	Male	17 (54.8)	40 (66.7)	9 (69.2)	
CCI		0 (0-1)[a]	0 (0-5)[b]	1 (0-2)[c]	<0.001
WBC at diagnosis ($\times10^9$/L)		6.09 (2.93-11.75)[a]	6.31 (2.7-20.57)[a]	13.1 (4.89-23.7)[b]	0.001
Neutrophil (2-6$\times10^3$/μL)		3.63 (1.20-6.84)[a]	4.95 (0.03-18.81)[b]	11.99 (4.51-22.02)[c]	<0.001
Hemoglobin at diagnosis (g/dL)		13.9 (10.5-16.4)	13.45 (8.5-16.5)	13.2 (11.1-15.4)	0.372
Hematocrit (39-50%)		39.7 (34.7-52.2)	40.75 (25.6-49)	40.6 (34.7-48.1)	0.943
Platelet at diagnosis ($\times10^9$/L)		248 (170-389)[a]	209 (100-506)[b]	219 (89-467)[a,b]	0.040
Lymphocyte (1.3-3.5$\times10^3$/μL)		1.28 (0.79-4.56)[a]	1 (0.3-15.3)[b]	0.7 (0.31-3.89)[c]	0.001
Monocyte (0.3-0.9$\times10^3$/μL)		0.49 (0.25-1.14)	0.47 (0.16-10.4)	0.44 (0.07-0.80)	0.207
Eosinophils (0-0.5$\times10^3$/μL)		0.05 (0.00-0.35)[a]	0.01 (0.00-0.15)[b]	0 (0-0.1)[b]	<0.001
BUN (5.1-16.8 mg/dL)		28 (15-47)[a]	36 (19-270)[b]	76 (29-187)[c]	<0.001
Creatinine (0.57-1.25 mg/dL)		0.71 (0.20-1.14)[a]	0.9 (0.4-3.23)[b]	1.69 (0.7-2.87)[c]	<0.001
Neutrophil/Lymphocyte		2.13 (0.75-6.58)[a]	5.01 (0.01-29.74)[b]	15 (3.52-31.46)[c]	<0.001
Total protein (6.4-8.3 g/dL)		7.5 (6.4-8.7)[a]	6.8 (4.6-8.6)[b]	6.5 (5.6-7.6)[c]	<0.001
Albumin (3.5-5 gr/dL)		4.4 (3.9-5.04)[a]	3.4 (1.8-4.6)[b]	2.9 (2.2-3.6)[c]	<0.001
AST (5-34 U/L)		25 (11-73)[a]	35.5 (15-575)[b]	49 (29-1654)[c]	<0.001
ALT (0-55 U/L)		21 (9-86)[a]	29.5 (10-532)[b]	28 (15-882)[a,b]	0.033
ALP (40-150U/L)		80 (11-284)[a,b]	74 (41-584)[b]	97 (53-199)[a]	0.047
GGT (9-64 U/L)		17 (8-189)[a]	32 (10-819)[b]	36 (15-106)[b]	<0.001
Sodium (136-145 mmol/L)		138 (134-144)[a]	136 (127-151)[b]	135 (124-151)[b]	0.013
Potassium (3.5-5.1 mmol/L)		4.08 (3.25-5.08)[a]	4.3 (2.9-8.4)[b]	5 (4.3-6.2)[c]	<0.001
C-reactive protein (0-0.35 mg/dL)		0.26 (0.02-1.98)[a]	5.09 (0.03-35)[b]	18.36 (0.14-35.2)[c]	<0.001
Procalcitonin (0-0.5 ng/mL)		0.04 (0.02-0.27)[a]	0.1 (0.02-10.32)[b]	1.22 (0.06-33.2)[c]	<0.001
INR (0.8-1.2)		1.08 (0.83-1.97)[a]	1.16 (0.92-2.6)[b]	1.2 (1-1.48)[a,b]	0.048
APTT (23-35 sn)		22.2 (17.9-26.5)[a]	24.9 (15-63.3)[b]	25.3 (21.2-30.1)[b]	0.001
Fibrinogen (150-350 mg/dL)		244.5 (177-413)[a]	470 (76.7-1187)[b]	609 (49-1218)[b]	<0.001
d-dimer (0-0.55 mg/L)		0.10 (0.00-1.08)[a]	0.52 (0.01-9.16)[b]	2.4 (0.5-32.5)[c]	<0.001
Ferritin level (22-322 ng/mL)		63 (7.69-268)[a]	354.75 (21-2300)[b]	834 (363-2002)[c]	<0.001
Hospitalization (day)		6 (1-17)[a,b]	7 (1-32)[a]	5 (2-12)[b]	0.047
Mortality, n (%)		0 (0)[a]	4 (6.7)[a]	7 (53.8)[b]	<0.001

Abbreviations: It shows a statistically significant difference among the a, b, c markers.

The CCI was found to be statistically significantly different between mild, moderate and severe groups (p<0.001). Univariate logistic regression analysis was performed to determine the effect of CCI on survival. When CCI increases by one unit, the risk of death increases by 1.193 (95% CI=1.120-3.268) times (p=0.017). However, no relationship was determined between CCI and length of stay.

DISCUSSION

Since the beginning of the pandemic, it has been a matter of wonder in which patients the clinical course of COVID-19 disease will be mild or severe. The presence of comorbidity has been associated with the severity of COVID-19 disease. It has been reported that older age, presence of lymphopenia and/or thrombocytopenia, increased LDH, d-dimer, CRP, fibrinogen, IL-6, transaminases are associated with severe disease (10). In our trial, age, WBC, neutrophil count, NLR, AST, CRP, procalcitonin, fibrinogen, d-dimer, and ferritin levels were found to be higher in the clinically severe patient group.

Christensen et al. (11) investigated the effect on survival by calculating the CCI score in 4480 COVID-19 patients. The median age of the patients included in the study was 55 years. The patients were classified according to the CCI score as 0, 1–2, 3–4, and >4. The likelihood of severe COVID-19 increased significantly in CCI score 1–2 (odds ratio [OR], 1.76), CCI 3-4 (OR, 2.36) and CCI >4 (OR, 2.67) compared to those in CCI 0. Mortality rates for CCI score 1–2 (OR, 2.13), CCI 3–4 (OR, 3.00) and CCI >4 (OR, 3.85) were significantly increased compared to those for CCI 0. In our trial, we also found a statistically significant difference in CCI score between mild, moderate and severe patient groups (p<0.001). When CCI increases by one unit, the risk of death increases by 1.193 (95% CI=1.120-3.268) times (p=0.017).

Sun et al. (12) evaluated 63 COVID-19 patients. The median age of the patients was 47 years. The patients were divided into 4 groups as mild, moderate, severe and critically ill. Nineteen of the patients (30.2%) were in the severe and critically ill group. Patients with comorbidity were in the severe patient group. Twenty nine of 63 patients had 1 or more comorbid diseases. Comorbid diseases are hypertension in 12 patients, DM in 5 patients, thyroid disease in 3 patients, cerebral infarction in 2 patients, cardiac arrhythmia in 2 patients, bronchial asthma in 2 patients, respectively. Leukocyte, neutrophil, lymphocyte, eosinophil counts and hemoglobin levels differed statistically significantly among the four patient groups (p=0.007, p=0.001, p=0.001, p=0.000, p=0.021, respectively). In our trial, the most common comorbid disease in the patients included in the trial was hypertension, which occurred in 17 patients (13.6%).

Garcia et al. (13) included 639 serious COVID-19 patients, with a median age of 63 [53–71] years. In a multivariate Cox proportional hazard regression model, patients' creatinine, d-dimer, lactate, and potassium levels at presentation were independently associated with intensive care unit (ICU) mortality (p<0.01). In our trial, creatinine, potassium level, and d-dimer levels were found to be higher in the clinically severe patient group.

Bastug et al. (14) reported 191 hospitalized COVID-19 patients that patients who needed an intensive care unit were elderly and had more comorbidity. Lower lymphocyte count, hemoglobin, total protein and albumin levels were reported in patients in the ICU compared with non-critical patients. However, higher WBC, neutrophil count, urea, creatinine, AST, LDH, d-dimer levels were reported (p<0.001). Hypertension, DM and cardiovascular diseases were reported to be the most common comorbidities in the patients included in the study (30.9%, 14.1% and 10.5%, respectively. Comorbidity was found more frequently in patients who needed ICU [35 (76.1%) versus 48 (33.1%); p<0.001].

Liu et al. (15) reported that the rate of NLR was an independent measure of mortality in hospitalized patients in a study in which they studied 245 COVID-19 patients. Lagunas-Rangel et al. (16) reported that in a meta-analysis involving 6 severe COVID-19 patients, NLR values increased significantly in COVID-19 patients with severe disease. Liu et al. (17) identified NLR as an independent risk factor for critical disease in their study of 61 patients with COVID-19 infections. In our trial, the NLR was statistically significantly different between the mild, moderate and severe patient groups. It was observed that as the severity of the disease increased, the NLR increased.

In our trial, in accordance with the literature, age, WBC, neutrophil count, BUN, creatinine, AST, potassium level, CRP, procalcitonin, aPTT, fibrinogen, d-dimer, and ferritin levels were found to be higher in the clinically severe patient group. In addition, lymphocyte and eosinophil counts, total protein, albumin and sodium levels were found to be lower in the clinically severe patient group.

This trial showed that calculating the CCI score in COVID-19 patients can be useful in predicting the severity of the disease. Examination of CCI, age, WBC, neutrophil, lymphocyte, NLR, eosinophil counts, BUN, creatinine, AST, total protein, albumin, sodium, potassium level, CRP, procalcitonin, aPTT, fibrinogen, d-dimer and ferritin levels at the time of diagnosis can be suggested.

ETHICAL DECLARATIONS

Ethics Committee Approval: The trial was approved by İnonu University Research Ethics Committee (date/ reference number: 10-11-2020/1255).

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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The evaluation of sleep quality, anxiety disorder and depression in older adults with Parkinson disease

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ABSTRACT

Aim: The aim of this study was to examine the prevalence ratios of sleep disorder, anxiety and depression in older adults with Parkinson disease in addition to the relationship between these complaints and the stage and duration of the disease.

Material and Method: Epworth sleepiness scale, Pittsburgh sleep quality index, Insomnia severity index, Beck depression inventory, Hamilton anxiety scale were applied on a total of 585 older adults followed up with Parkinson disease diagnosis. The acquired results were interpreted by comparing with the data of the control group comprised of 585 healthy individuals.

Results: A total of 585 older adult with Parkinson disease and 585 healthy controls were included in the study. Each group was comprised of 255 female (43.59%) and 330 male (56.41%) participants. Mean age of the Parkinson group was 68.14±7.01 years, whereas mean age of the control group was 65.08±6.43 years. Mean Hoehn Yahr scale was determined in the Parkinson group as 1.8; while mean UPDRS was 29.8. Daytime functions and subjective sleep quality were worse at a statistically significant level in the patient group according to Pittsburgh sleep quality index (p=0.014, p=0.019, respectively). The most significant difference was observed in ESS when the scale scores of the patient and control groups were compared (p<0.001). A positive and statistically significant correlation was determined between the Beck depression inventory score and unified Parkinson's disease rating scale and Hoehn Yahr scale (p=0.023, p=0.034, respectively). While depression ratio increases with increasing stages of the disease, anxiety ratios increased with increasing disease duration. A positive and statistically significant correlation was determined between the weight of the disease and the score obtained from the somatic section of HAM-A (p=0.022).

Conclusion: The presence of depression and anxiety should be questioned especially in older adult with Parkinson disease who are clinically more severe and who have been undergoing treatment for a longer period of time; patients with treatment related complications should be evaluated with regard to sleep disorders. It should be kept in mind that the Parkinson disease is not related only with motor symptoms but that an effective treatment of the non-motor symptoms will also improve the quality of life of the patients.

Keywords: Anxiety, depression, older adults, Parkinson disease, sleep disorders

INTRODUCTION

Sleep disturbances associated with Parkinson disease are common and have many negative effects on the quality of life of patients with Parkinson disease (1). Daytime sleepiness, insomnia, REM-sleep behaviour disorder, and restless-legs syndrome are the most of observed sleep disorders in people with Parkinson disease (2). In order to identify sleep disturbances, regular screening using validated questionnaires such as the Pittsburgh sleep quality index (PSQI) or the medical outcomes study sleep scale are recommended (3). In order to assess daytime sleepiness, the usage of Epworth sleepiness scale (ESS) (4), the inappropriate sleep composite score or the Stanford sleepiness scale are recommended (5).

Parkinson disease which is the second most frequently observed neurodegenerative disease in the world affects 1% of individuals above the age of 60 (6). Whereas it has a prevalence ratio of 111/100.000 in our country (7). Known as a movement disorder and characterized primarily by motor symptoms such as tremor, rigidity, bradykinesia; the non-motor symptoms of the disease which significantly reduce the quality of life of patients such as depression, anxiety disorder, daytime sleepiness and insomnia are generally ignored (8).

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As is the case in all other chronic diseases, various psychiatric problems can also be observed in Parkinson disease. Depression is the most frequently observed psychiatric problem in patients with Parkinson disease with a prevalence ranging between 17-50% (9,10). Anxiety disorders can be seen in patients with Parkinson disease at ratios of 40-82% which is higher when compared with the normal population or other chronic neurological diseases (11-15). It has been reported in previous studies that the number of depression and anxiety cases increase with increasing stage and duration of the disease (16). Various problems such as insomnia, daytime sleepiness can be observed in patients with Parkinson disease. The prevalence of insomnia is around 60-76% in patients with Parkinson disease (17,18). The correlation between depression and sleep disorder in patients with Parkinson disease has been put forth in previous studies (19). Sleep disorders can be observed due to the disease itself as well as the medications used. Daytime sleepiness is observed most frequently in this patient group from among all sleep disorders. It may be followed by insomnia, sleep quality disorder at varying ratios (17,18,20).

This study specifically focused on older adult with Parkinson disease. In the study, the ratios of depression, anxiety and sleep disorders in older patients with Parkinson disease were examined along with the relationship between these complaints and the stage and duration of the disease. So our study tries to put forth the necessity for clinicians to question these generally overlooked symptoms in older adult with Parkinson disease.

MATERIAL AND METHOD

Study Design and Participants

The study was performed by the local ethical committee of Bezmialem Vakif University, including the number of the approval document and the date of the approval (Registration number: 2011- KAEK-25 2019/01-03). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

We recruited 585 adults aged 65 and over with Parkinson disease consecutively from the Department of Neurology, Training and Research Hospital in İstanbul, Metropolitan Municipality Hospital in Izmir, from September 2019 to January 2020. The control group was comprised of patient relatives and healthy hospital staff members without any neurological and systemic disease.

The patients diagnosed with Parkinson disease by a neurology specialist according to the United Kingdom Brain Bank Parkinson Disease Diagnostic Criteria were included in the study (21). Staging of the disease was carried out via Hoehn Yahr scale (HYS), whereas the clinical weight assessment was performed via unified Parkinson's disease rating scale (UPDRS). Unified Parkinson's disease rating scale is comprised of four main sections evaluating the mental states, behaviors and psychological moods of the patients along with their daily life activities, motor functions and treatment complications. The 42 items of the test were scored between 0-4. High scores indicated bad clinical development (22). There is a Turkish inter-rater reliability study of UPDRS (23).

Hoehn-Yahr scale was used for evaluating the severity and progression of the disease (24). Brain tomography or cranial magnetic resonances were examined for all patients. Factors such as medications, poisonings, vascular reasons, hypoxia, trauma, infections, normal pressure hydrocephaly and brain tumors which may lead to secondary Parkinsonism along with Parkinson plus syndromes were excluded from the study. All participants were subject to detailed physical and neurological examination after which their histories and demographic characteristics were recorded.

Beck depression inventory developed by Beck et al. (25) was used for evaluating depression. The purpose of this test is to measure the level of depressive symptoms and higher total scores indicate the severity of the level of depression. The inventory is comprised of 21 self-report questions with a maximum score of 63. In the present study, scores of 0-9 represented=minimal depression, 10-16=mild depression, 17-29=moderate depression, 30-63=severe depression (25). It was shown by Hisli to be valid and reliable in a Turkish sample (26).

The Hamilton anxiety scale (HAM-A) developed by Hamilton is comprised of two sections that question the physical and mental effects of anxiety. Including a total of 14 questions, the test has a maximum score of 56 with scores ranging between 6-14 indicating minor anxiety; whereas scores of 15 and above indicated major anxiety. Those with scores of 6 and above from the HAM-A scale were evaluated as having anxiety (27). The Turkish validity and reliability study of HAM was conducted by Yazıcı et al. (28).

Three different tests were used for evaluating the sleep quality in patients with Parkinson disease. These were ESS, PSQI and Insomnia severity index (ISI).

Epworth sleepiness scale developed by Johns MJ (4) in 1991 was used for evaluating daytime sleepiness. The maximum score that can be obtained from this test is 24 with scores of and above indicating issues of daytime sleepiness (4). The Turkish validity and reliability study of ESS was conducted by İzci et al. (29).

The Pittsburgh sleep quality index developed by Buysse et al. (3) is a test that evaluates sleep quality and disorder during the past one month. The scale is comprised of seven sub-titles of Subjective sleep quality (K1), sleep latency (K2), sleep duration (K3), sleep efficiency (K4), sleep disturbances (K5), use of sleeping medication (K6) and daytime dysfunction (K7) with a maximum score of 21. Total PUKİ scores of above 5 indicate bad sleep quality (3). The Turkish validity and reliability of the Pittsburgh Sleep Quality Index was conducted by Ağargün et al. (30)

Insomnia severity index was developed in 2001 by Bastien et al. (31) for evaluating insomnia severity. Comprised of a total of 7 questions, the maximum score that can be obtained from the test is 28. Scores of 0-7 indicate clinically insignificant insomnia, scores of 8-14 indicate insomnia lower threshold, scores of 15-21 indicate clinical insomnia (moderate level) whereas scores of 22-28 indicate clinical insomnia (severe) (31). The Turkish validity and reliability of the Pittsburgh Sleep Quality Index was conducted by Boysan et. al. (32).

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS Inc. Chicago, IL, USA). Shapiro-Wilk test was used for evaluating whether the variables comply with the normal distribution or not. The variables that comply with the normal distribution were presented as mean±standard deviation with the t-test used for comparisons between two independent groups. The variables that do not comply with the normal distribution were presented as median (minimum-maximum) values with the Mann-Whitney U test used for the comparisons between two independent groups. Categorical variables were presented with frequency and percentage values [n (%)] with the Pearson chi-square test, Fisher's exact chi-square test and Fisher-Freeman-Halton tests used for comparisons. Spearman correlation coefficient was used for examining the relationships between the variables. The level of significance was taken as $p<0.05$.

RESULTS

A total of 585 older adult with Parkinson disease and 585 healthy controls were included in the study. Each group was comprised of 255 female and 330 male participants. Mean age of the Parkinson group was 68.14±7.01 years, whereas mean age of the control group was 65.08±6.43 years. Mean HYS was determined in the Parkinson group as 1.8; while mean UPDRS was 29.8. There were no statistically significant differences between the two groups with regard to age, gender, BMI, hypertension, diabetes and restless leg syndrome (RLS) prevalence. **Table 1** presents the demographic characteristics of the patient and control groups.

Table 1. Comparison of demographic characteristics of patients and control groups.

Variables	Patient, (n,%)	Control (n,%)	p
Gender			1.000
Male	330 (56.41)	330 (56.41)	-
Female	255 (43.59)	255 (43.59)	-
Age*	68.14±7.01	65.08±6.43	0.079
BMI*	29.01±5.41	27.31±3.11	0.161
Marital status			0.621
Married	30 (5.13)	15 (2.56)	-
Single	465 (79.49)	435 (74.36)	-
Widow	90 (15.38)	135 (23.08)	-
Hypertension	255 (43.59)	210 (35.89)	0.711
Diabetes mellitus	180 (30.77)	60 (10.25)	0.389
Restless leg syndrome	120 (20.51)	105 (17.95)	1.087

* Variables are given as mean±standard deviation; BMI, Body mass index

Daytime sleepiness was observed to be high at a statistically significant level in the Parkinson group according to ESS ($p<0.001$). While the daytime sleepiness was 53.85% in the Parkinson group; this ratio was determined as 5.12% in the control group. The ratio of those with bad sleep quality according to PSQI was 82.06% in the Parkinson group, whereas the ratio was 61.54% in the control group; however, the difference was not statistically significant ($p=0.219$). A statistically significant difference could not be determined between the two groups when BDI, HAM-A, ISI and PUKI were compared. **Table 2** presents the correlations for the patient and control groups.

Table 2. Comparison of patient and control groups

Variables	Patient (n,%)	Control (n,%)	p
HAM-A			**0.211**
None	315 (53.85)	195 (33.33)	
Minor	240 (41.03)	270 (46.15)	
Major	30 (5.12)	120 (20.52)	
BDI			**0.692**
Minimal	435 (74.36)	360 (61.53)	
Mild	105 (17.95)	165 (28.21)	
Moderate	45 (7.69)	60 (10.26)	
ISI			**0.889**
Insignificant	510 (87.17)	525 (89.74)	
Lower threshold	30 (5.13)	45 (3.33)	
Moderate	30 (5.13)	15 (2.56)	
Severe	15 (2.57)	0 (0.00)	
ESS			**<0.001**
Negative	270 (46.15)	555 (94.88)	
Positive	315 (53.85)	30 (5.12)	
PSQI			**0.219**
Good	105 (17.94)	225 (38.46)	
Poor	480 (82.06)	360 (61.54)	

HAM-A: Hamilton anxiety scale, BDI: Beck depression inventory, ISI: Insomnia severity Index, ESS: Epworth sleepiness scale, PSQI: Pittsburg sleep quality index

The most significant difference was observed in ESS when the scale scores of the patient and control groups were compared. While the mean ESS score for the patient group was 9; the mean score was 3 for the control group (p<0.001). The scores obtained from the 1st component of PSQI that reflects subjective sleep quality and the 7th component that reflects the daytime functions were higher at a statistically significant level in the Parkinson group compared to the control group (p=0.014, p=0.019, respectively). Interestingly, ISI score was higher in the control group (p=0.045). There were no statistically significant differences between the two groups with regard to the other variables. **Table 3** presents the comparison of the test scores for the patient and control groups.

A positive and statistically significant correlation was determined between the BDI score and UPDRS and HYS. A positive and statistically significant correlation was determined between the weight of the disease and the score obtained from the somatic section of HAM-A. This is an indication that the prevalence of anxiety increased with increasing duration of the disease. A positive and statistically significant correlation was determined between the 4th section of UPDRS including treatment complications and the 5th component of HAM-A and PSQI that includes sleep disorders. The prevalence of anxiety and sleep disorders increased with increasing complications of the disease. A negative and statistically significant correlation was determined between the 3rd component of PSQI defining sleep duration and the 1st section of UPDRS that reflects the mental state, behavior and mental state. **Table 4** presents the correlations in the patient group.

Table 3. Testing between patient and control groups comparison of scores

Variables	Patient (n)	Control (n)	p
Beck depression inventory (BDI)	90	120	0.633
Hamilton anxiety scale-psychic HAM-A(P)	30	15	0.201
Hamilton anxiety scale-somatic HAM-A(S)	45	65	0.614
Hamilton anxiety scale (HAM-A)	75	105	0.236
Insomnia Severity Index (ISI)	15	60	0.045
Epworth sleepiness scale (ESS)	135	45	<0.001
Pittsburg sleep quality index (PSQI) K1	15	0	0.013
Pittsburg sleep quality index (PSQI) K2	15	15	0.558
Pittsburg sleep quality index (PSQI) K3	35	15	0.075
Pittsburg sleep quality index (PSQI) K4	0	0	0.153
Pittsburg sleep quality index (PSQI) K5	15	15	0.880
Pittsburg sleep quality index (PSQI) K6	0	15	0.374
Pittsburg sleep quality index (PSQI) K7	15	0	0.020
Pittsburg sleep quality index Total	105	105	0.343

PSQI, Subjective sleep quality; K1, Subjective sleep quality; K2, Sleep latency; K3, Sleep duration; K4, Sleep efficiency; K5, Sleep disturbances; K6, Use of sleeping medication; K7, Daytime dysfunction

Table 4. Correlations in the patient group

Variables	HYS	Initial	UPDRS1	UPDRS2	UPDRS3	UPDRS4	UPDRS-T	p
BDI	0.004	0.720	0.470	0.181	0.599	0.209	0.051	0.120
HAM-A (P)	0.035	0.359	0.911	0.259	0.839	0.129	0.519	0.481
HAM-A (S)	0.669	0.709	0.512	0.851	0.461	0.141	0.931	0.010
HAM-A (T)	0.107	0.581	0.321	0.391	0.810	0.007	0.710	0.121
ISI	0.510	0.371	0.931	0.549	0.869	0.389	0.571	0.959
ESS	0.418	0.089	0.279	0.841	0.949	0.391	0.841	0.609
PSQI K1	0.311	0.669	0.701	0.829	0.351	0.869	0.349	0.949
PSQI K2	0.719	0.389	0.359	0.709	0.951	0.709	0.851	0.689
PSQI K3	0.279	0.349	0.029	0.741	0.571	0.249	0.899	0.851
PSQI K4	0.467	0.329	0.468	0.679	0.749	0.281	0.443	0.877
PSQI K5	0.426	0.439	0.581	0.062	0.281	0.039	0.339	0.069
PSQI K6	0.249	0.610	0.359	0.179	0.571	0.321	0.211	0.161
PSQI K7	0.631	0.589	0.131	0.331	0.161	0.731	0.599	0.861
PSQI Total	0.919	0.331	0.219	0.191	0.531	0.589	0.459	0.259

BDI, Beck depression inventory; HYS, Hoehn Yahr scale; UPDRS, Unified Parkinson's disease rating scale; HAM-A, Hamilton anxiety scale; (P), psychic; (S), Somatic; (T), Total; ISI, Insomnia severity index; ESS, Epworth sleepiness scale; PSQI, Subjective sleep quality; K1, Subjective sleep quality; K2, Sleep latency; K3, Sleep duration; K4, Sleep efficiency; K5, Sleep disturbances; K6, Use of sleeping medication; K7, Daytime dysfunction

DISCUSSION

In our study, minimal depressive symptoms were observed in 7% of the patients with Parkinson disease, mild depression in 17% and moderate depression in 3%. Severe depression was not observed in any of our patients. A positive correlation was determined in our study between the BDI score and UPDRS and HYS. These findings supported the opinion that the prevalence of depression increases with increasing severity of the diseases and were in accordance with literature. In this study, daytime sleepiness was higher at a statistically significant level in the Parkinson group. Another interesting result of our study is; while depression ratio increases with increasing stages of the disease, anxiety ratios increased with increasing disease duration.

When we make an evaluation with the results of similar studies in the literature; it was determined as a result of a study carried out on patients with Parkinson disease with 80 participants that the ratios are 41.25%, 32.5% and 87% for depression, anxiety and the coupling of depression and anxiety, respectively. The number of depression and anxiety cases increased with increasing of UPDRS severity score (16,17).

It was determined in another study that the ratio of anxiety was higher at a statistically significant level among patients with Parkinson disease compared to the control group (17). The number of anxiety cases increased with increasing severity and duration of the disease. The anxiety ratio in our study in the Parkinson group was determined as 45.73%, however no statistically significant difference could be determined with the control group. High anxiety ratio in the control group may be due to the fact that majority of the control group is comprised of healthcare employees. While a statistically significant relationship could not be determined in our study between the severity of the disease and anxiety; a statistically significant relationship was determined between the somatic part of HAM-A and disease duration. This is an indication that the somatic complaints of the patients increased with increasing disease duration. anxiety prevalence also increased in our study with the increasing of treatment complications. This can be evaluated as a non-motor symptom that develops during the course of the disease while it should also be kept in mind that it can be related with a side effect of the medications used. These results put forth the necessity of questioning anxiety during polyclinic assessment of complicated patients with Parkinson disease who have been undergoing treatment for long periods of time.

It was determined in another study carried out with 636 patients with Parkinson disease that the ratio of sleep disorder among patients with Parkinson disease was higher at a statistically significant level compared with the control group. While it was observed according to PSQI that K1, K4, K5 and K7 have been disrupted in patients with Parkinson disease; K2 has not been affected. This is an indication that sleeplessness observed among patients with Parkinson disease affects the unity and totality of sleep but does not have any impact on the start of sleep (33). It was observed in our study that the K1 and K7 scores were high at a statistically significant level in the patient group. K3 score was also determined to be high in the patient group but not at a statistically significant level. A statistically significant difference could not be determined between the K2, K4, K5, K6 scores. In conclusion, we observed in our study that the subjective sleep quality and daytime functions have been affected adversely at a statistically significant level in the Parkinson group. Even though sleep duration was disrupted in the Parkinson group, the difference was not statistically significant. A statistically significant difference could not be observed between the Parkinson and control groups with regard to sleep latency, sleep effectiveness, sleep disorder and use of medication. The acquired results were in accordance with the findings in literature. While it was observed in a study during which ESS and multiple sleep latency test (MSLT) were used for evaluating daytime sleepiness in patients with Parkinson disease that daytime sleepiness is observed in 46.3% of the patients according to ESS which is a subjective test; it was observed that the ratio decreased to 13.4% when MSLT is used which is an objective test (34). We also determined a high ratio of 53.85% in our study for daytime sleepiness according to ESS. Even though this is in accordance with literature, this may be due to the fact that an objective test was not used in our study for determining daytime sleepiness.

All components of PSQI were observed to be distinctively disrupted in patients with Parkinson disease in another study on sleep disorders. Highest score was observed in the daytime functions according to PSQI (K7=1.6). This was followed respectively by sleep disorders (K5=1.3), sleep latency (K2=1.2) and sleep quality (K1=1.2) (37). It was also determined in our study in accordance with literature findings that daytime functions (K7=1) and sleep quality (K1=1) scores were higher in the Parkinson group compared with the controls.

It was determined in our study that the ratio of insomnia is 27.5% among young patients with Parkinson disease, while this ratio was 55.2% among older adults with Parkinson disease. Insomnia, nightmares and RLS are observed less frequently in young-onset patients with Parkinson disease and they had better sleep quality (36). The mean age of the patients with Parkinson disease in our study was 68.14±7.01 years. We are of the opinion that the advanced mean age of our study group is related with the negative results related with sleep disorders and daytime sleepiness.

Similar to the findings in literature, daytime sleepiness was observed to be higher in patients with Parkinson disease in our study. Daytime sleepiness may have developed secondary to disrupted nighttime sleep. Pain, nocturia, RLS, obstructive sleep apnea syndrome, depression can be indicated as reasons for nighttime sleep disorder. These have to be examined and treated if possible in order to prevent daytime sleepiness. Daytime sleepiness may have also developed secondary to dopaminergic treatment. Finally, daytime sleepiness may develop due to the disease itself by affecting the sleep-wake cycle (37). Medications such as L-dopa and dopamine agonist that may lead to daytime sleepiness were not classified separately in our study. Even though this can be considered as a limitation, almost all of our patients were using L-dopa, dopamine agonist or both. Taking this into consideration, we thought that such a classification will not be very meaningful.

It was reported in a study carried out on 128 patients with Parkinson disease that K2 is correlated with the first section of UPDRS that reflects the mental state, behavior and mood. Similarly, a statistically significant relationship was determined between the first part of ISI and measured insomnia. A statistically significant relationship could not be determined between any part of UPDRS and daytime sleepiness measured via ESS (38). We also determined statistically significant relationships in our study between K3 and behavior and mood, between K5 and treatment complications. A statistically significant relationship could not be determined in our study between any section of UPDRS and insomnia measured via ISI and daytime sleepiness measured via ESS. In conclusion, sleep duration decreased in our patients with worsening mental state, behavior and mood. The ratio of sleep disorders increased with increasing treatment complications. The acquired findings in our study indicate that it is necessary to consider treatment complications when evaluating the patients with Parkinson disease with regard to sleep disorders.

Disease duration was determined to be correlated with K2 and K4 in another study (39). A statistically significant relationship could not be determined in our study between the disease duration and any component of PSQI. The impact of disease duration on any of the components of sleep could not be put forth in our study.

Nocturia was among the primary reasons for sleep disorders in older patients. ISI scores were determined to be correlated with urinary problems and constipation (38). The ISI scores of the control group in our study were higher at a statistically significant level. This may be due to the fact that majority of our patients were early stage patients with Parkinson disease and also to the fact that insomnia related with motor symptoms has not yet emerged. In addition, the fact that the control group was comprised of patient relatives and hospital employees may have resulted in the high ISI scores in this group.

The fact that medication use has not been reported in our study is a limitation. Another limitation of the study is that objective tests such as polysomnography have not been carried out to put forth sleep disorders. All of our patients were either early or medium stage patients with Parkinson disease according to HYS. This may have also been effective in the fact that insomnia and daytime sleepiness related with motor symptoms have not yet developed.

CONCLUSION

The presence of depression and anxiety should be questioned especially in more severe Parkinson patients who have been undergoing treatment for longer periods of time and the patients with treatment related complications should be evaluated with regard to sleep disorders. It should be kept in mind that the Parkinson disease is not comprised only of motor symptoms and that an effective treatment of non-motor symptoms will improve the quality of life of the patients significantly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was performed by the local ethical committee of Bezmialem Vakıf University, including the number of the approval document and the date of the approval. (The registration number: 2011- KAEK-25 2019/01-03).

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

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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The place of transvaginal ultrasonography saline infusion sonohysterography and hysteroscopy in the diagnosis of abnormal uterin bleedings

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ABSTRACT

Aim: To establish the accuracy of transvaginal ultrasonography, saline infusion sonohysterography and hysteroscopy in diagnosing uterine pathology in patients with abnormal uterine bleeding.

Material and Method: In our study, Transvaginal ultrasonography (TVUSG), saline infusion sonohysterography (SIS) and hysteroscopy were applied to 60 patients in the reproductive period, who did not use any contraception method other than condom, who did not have chronic and systemic diseases, who applied to our clinic with abnormal uterine bleeding. Dilatation & Curettage (D&C) was applied to all patients after the procedure. Histopathology results obtained with D&C were compared with those obtained with TVUSG, SIS and hysteroscopy.

Results: Histopathologically, 28 patients (46.7%) endometrial polyp, 4 patients (6.7%) submucous myoma, 10 patients (16.7%) endometrial hyperplasia was in the form of endometrial changes due to cycle irregularities in 18 patients (30%). The sensitivity, specificity, positive and negative predictive values for detection of intracavitary pathology by transvaginal sonography were 71.42%, 77.78%, 88.23%, 83.85%. The values determined by saline infusion sonohysterography were respectively 88.89%, 90.48%, 80.00%, 95.0% and by hysteroscopy were respectively 100%, 90.00%, 95,24%, 100%.

Conclusion: TVUSG and SIS is a cheaper, easier and highly diagnostic value procedure for detecting endometrial pathologies such as endometrial polyps and submucous myomas. However, due to the high diagnostic values, low complication rates, direct biopsy and simultaneous treatment, we believe that hysteroscopy will maintain it's 'gold standard' feature in the diagnosis and treatment of endometrial pathologies for many years.

Keywords: Abnormal uterine bleeding, hysteroscopy, endometrial polyp, saline infusion sonohystygraphy

INTRODUCTION

Most women experience menstrual cycle irregularities at some part of their lives. Abnormal uterine bleeding (AUB), which is one of those kind irregularities, is up to one third of reason for admission to gynecology outpatient clinic throughout life and postmenopausal period. The main causes of abnormal uterine bleeding are classified as; polyp, adenomyosis, leiomyoma, malignancy and hyperplasia, coagulopathy, ovulatory dysfunction, endometrial causes,iatrogenic and those not yet classified (1). The diagnosis of space-occupying lesions in the endometrial cavity with transvaginal ultrasonography (USG) and saline infusion hydrosonography (SIS) has been used safely for many years. However, endometrial sampling is an important diagnostic method in the diagnosis of endometrial pathologies (2). Dilatation/curettage (D&C) procedure, was first applied by Recaimer in 1843, has been used in the detection of endometrial pathologies and accepted as a gold standard (3). First the cervix is dilated, then all parts of the endometrium from fundus to internal os are curetted, it is an invasive method with a high risk of complication (4). Hysteroscopy (H/S) can be performed in an outpatient examination environment because of hysteroscopy is a low cost, effective and reliable method that does not require anesthesia (5). SIS,

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is another diagnostic and treatment method which can be used in abnormal uterine bleeding and infertility. SIS, can be used as providing distension of the endometrial cavity with sterile saline and visualized the uterine cavity with ultrasonography (6,7).

In our study, the patients who had complaint of abnormal uterine bleeding in their reproductive and perimenopausal period were detected with transvaginal ultrasonography (TVUSG), saline infusion sonohysterography (SIS), Hysteroscopy and Dilatation & Curettage (D&C) as a diagnostic methods and comparing our visual diagnosis with the histopathological diagnosis of tissue obtained by D&C, according to literature information to reveal the accuracy and reliability of these diagnostic methods.

MATERIAL AND METHOD

Patient in this study was selected between June 2008 and November 2009, in İstanbul Bakırköy Dr. Sadi Konuk Education and Research Hospital Gynecology and Obstetrics Clinic, a total of 74 patients of childbearing age who presented with the disease of abnormal uterine bleeding complaint were included. The study was approved by İstanbul Bakırköy Dr. Sadi Konuk Education and Research Hospital Ethics Committee (date: June 01, 2008; Approval number: 303). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of principles.

Patients who has chronic and systemic disease, continuous use of medication, take hormone replacement therapy, use intrauterine device or using contraceptive medication, pregnant or genital tract infection were not included in the study. The study was carried out in the gynecology service of the hospital, following the approval of the hospital training planning and ethics committee. All patients included the study were informed about the procedure which performed and their complications, and bill of consent was signed. Detailed gynecological anamnesis of all patients were taken, their systemic and pelvic examinations done. TVUSG, SIS, H/S and D&C respectively performed to all patients, obtaining material was send to histopathological evaluation and results were recorded. 14 patient was excluded from the study because they did not accept the intervention in different part of the stages. Study completed with 60 patients.

Transvaginal ultrasonography was performed by single observer using General Electric Logıc 200 brand 3.5 mHz vaginal probe. Cervix, cervical canal, myometrium and ovaries were examined in both sagittal and coronal planes. After evaluating the endometrial morphological pattern and endometrial junction, endometrial thickness measured from the outside to the outside in the thickest place (in the longitudinal plane). SIS process performed early or mid proliferative phase. While the patients were in dorsolithotomy position, a speculum was inserted into their vagina. After the cervix was made visible, wipe it with an antiseptic solution (Povidone iodine) cleaned. The cervix is fixed with a teneculum if necessary and 8 fr catheter was placed in the uterine cavity. After fixing the catheter with forceps, the speculum was removed and a transvaginal ultrasound probe was placed in to the vagina. A 20-50 cc syringes was attached to the tip of the catheter, saline is given slowly into the cavity until sufficient distension was achieved and the findings were recorded. Office hysteroscopy was performed by 3 mm hysteroscope (Karl Storz, Tutlingen, Germany). Office hysteroscopy was performed in the dorsal lithotomy position while the bladder was empty. The procedure was performed after cleaning the External genitals, vagina and cervix with antiseptic solution povidone-iodine. Uterine distention was carried out using saline. After hysteroscopy D&C performed for all patients. The material taken was kept in alcohol and sent to the pathology laboratory. Cases in which sufficient material cannot be obtained, was evaluated as cases with no pathology detected. No complications occurred due to the procedures performed. By comparing our visual diagnosis with the histopathological diagnosis of tissue obtained by dilatation curettage, we tried to reveal the accuracy and reliability of these diagnostic methods in the light of the literature.

Statistical Analysis

While evaluating the findings obtained in the study, NCSS for statistical analysis 2007 & PASS 2008 Statistical Software (Utah, USA) program was used. While evaluating the study data after H/S in addition to descriptive statistical methods (Mean, Standard deviation) it was handled as biopsy results are the gold standard in comparison of qualitative data. According to TVUSG, SIS and H/S results, diagnostic screening tests for each test separate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and kappa scores were calculated. Statistical significance was evaluated at the $p<0.05$ level.

RESULTS

Sixty patients with abnormal uterine bleeding were included in the study. All patients consisted of gave birth and fertile women. According to SIS results; 33.3% of patient had normal histology, 16.7% hyperplasia, 46.7% polyps and 3.3% myoma. H/S results; normal histology in 30%, hyperplasia in 13.3%, polyp in 50.0% and myoma in 6.7% of the patient seen. The clinical and demographic characteristics of the patients are given in **Table 1**.

The histopathological distribution of patients who underwent TVUSG, SIS, and H/S are given in **Table 2**.

Table 1. Clinical and demographic characteristics of the patients

	Min-Max		Ort±SD (Median)	
Age	32-45		39.70±3.04	
BMI	17.36±29.42		23.43±4.72	
Gravida	2-7		4.0±1.5	
Parity	1-6		2.95±1.05	
Abortus	1-8		4.02±2.86	
Histopathological diagnosis	**TVUSG n (%)**	**SIS n (%)**	**H/S n (%)**	**Toplam n:60**
Normal	16 (26.7%)	20 (33.3%)	18 (30%)	18 (30.0%)
Hyperplasia	18 (30.0%)	10 (16.7%)	8 (13.3%)	10 (16.7%)
Polyp	22 (36.7%)	28 (46.7%)	30 (50%)	28 (46.7%)
Myoma	4 (6.7%)	2 (3.3%)	4 (6.7%)	4 (6.7%)

TVUSG: Transvaginal ultrasonography, SIS: Saline infusion sonohysterography, H/S: Hysteroscopy

Table 2. Histopathological distribution of patients who underwent TVUSG, SIS, and H/S

		Pathology Results				
		Normal n (%)	**Hyperplasia n (%)**	**Polyp n (%)**	**Myoma n (%)**	**Total n (%)**
TVUSG	Normal	14 (23.3%)	2 (3.3%)	0 (0%)	0 (0%)	16 (26.7%)
	Hperplasia	4 (6.7%)	8 (13.3%)	6 (10%)	0 (0%)	18 (30%)
	Polyp	0 (0%)	0 (0%)	20 (33.3%)	2 (3.3%)	22 (36.7%)
	Myoma	0 (0%)	0 (0%)	2 (3.3%)	2 (3.3%)	4 (6.7%)
	Total	18 (30%)	10 (16.7%)	28 (46.7%)	4 (6.7%)	60 (100%)
SIS	Normal	16 (26.7%)	4 (6.7%)	0 (0%)	0 (0%)	20 (33.3%)
	Hyperplasia	2 (3.3%)	4 (6.7%)	4 (6.7%)	0 (0%)	10 (16.7%)
	Polyp	0 (0%)	2 (3.3%)	24 (40%)	2 (3.3%)	28 (46.7%)
	Myoma	0 (0%)	0 (0%)	0 (0%)	2 (3.3%)	2 (3.3%)
	Total	18 (30%)	10 (516.7%)	28 (46.7%)	4 (6.7%)	60 (100%)
H/S	Normal	18 (30%)	0 (0%)	0 (0%)	0 (%)	18 (30%)
	Hyperplasia	0 (0%)	8 (13.3%)	0 (0%)	0 (0%)	8 (13.3%)
	Polyp	0 (0%)	2 (3.3%)	28 (46.7%)	0 (0%)	30 (50%)
	Myoma	0 (0%)	0 (0%)	0 (0%)	4 (6.7%)	4 (6.7%)
	Total	18 (30%)	10 (16.7%)	28 (546.7%)	4 (6.7%)	60 (100%)

McNemar-Bowker Test p=0.083 p=0.343 p=0.157 p>0,05 TVUSG: Transvaginal ultrasonography, SİS: Saline infusion sonohysterography, H/S:Hysteroscopy

When looking at the efficiency of TVUSG in the diagnosis of endometrial pathologies, sensitivity is 71.42%; specificity is 77.78%, positive predictive value is 88.23%, and negative predictive value is 53.85%. In endometrial hyperplasia, sensitivity is 80%, specificity is 80%, in polyps sensitivity is 71.43% and specificity was evaluated as 93.75%. Sensitivity is 50%, specificity was determined as 96.4% in endometrial myomas. When looking at the effectiveness of SIS in the diagnosis of endometrial pathologies, the sensitivity is 88.89%, specificity 90.48%, positive predictive value 80.00% and the negative predictive value is 95.0%. In endometrial hyperplasia; It was evaluated as sensitivity 40% and specificity 88%. In polyps; sensitivity 85.71%, specificity 87.50%. In endometrial myomas; sensitivity was 50% and specificity was 100%. Considering the effectiveness of hysteroscopy in the diagnosis of endometrial pathologies, sensitivity 100%; specificity is 90%, positive predictive value is 95.24%, and negative predictive value is 100%. In endometrial hyperplasia, sensitivity is 80%, specificity is 100%, and in polyps sensitivity is 100% and specificity was evaluated as 93.75%. Sensitivity and specificity was determined as 100% in endometrial myomas. Diagnostic accuracy values of TVUSG, SIS, H/S is given in **Table 3**.

Table 3. Diagnostic accuracy values of TVUSG, SIS, H/S

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
TVUSG				
Normal	77.78	95.24	87.50	90.91
Hyperplasia	80.00	80.00	44.44	95.24
Polyp	71.43	93.75	90.91	78.95
Myoma	50.00	96.43	50.00	96.43
SİS				
Normal	88.89	90.48	80.00	95.00
Hyperplasia	40.00	88.00	40.00	88.00
Polyp	85.71	87.50	85.71	87.50
Myoma	50.00	100.00	100.00	96.55
H/S				
Normal	100.00	100.00	100.00	100.00
Hyperplasia	80.00	100.00	100.00	96.15
Polyp	100.00	93.75	93.33	100.00
Myoma	100.00	100.00	100.00	100.00

TVUSG: Transvaginal ultrasonography, SİS: Saline infusion sonohisterography, H/S:Hysteroscopy

DISCUSSION

Irregularities in gonadotropin hormones in women and hyperandrogenemia caused by hyperinsulinemia ia main reason in anovulation. In addition, androgens are converted to estrogen in adipose tissue and increased estrogens have a proliferative and hyperplasic effect on the endometrium. All of these factors together can cause abnormal uterine bleeding in women (8). The estrogen hormone has a stimulating effect on the endometrium. Continuous estrogen stimulation in the endometrium causes many gynecological problems. Estrogen plays a role in the etiopathogenesis of endometrial polyp, which is one of the causes of abnormal uterine bleeding. Increasing estrogens trigger proliferative, hyperplasic and eventually endometrial cancer pathways for the endometrium. Estrogen is one of the most important factors involved in the formation of endometrial cancer precursors such as endometrial cancer and hyperplasia. (9-12) Abnormal uterine bleeding (AUB) is one of the most common reasons for referrals to gynecology outpatient clinics. This clinical situation, which is often an indicator of a pathology in the reproductive system, sometimes it can be a symptom of disorders of other systems (13).

In the evaluation of the uterine cavity, TVUSG can directly detect abnormal uterine structures or it can be used for imaging or detecting anomalies. In general for determining endometrial pathologies, TVUSG sensitivity is reported between 46-100% (14). TVUSG can detect submucous myomas with a sensitivity of 21-100%, specificity range is between 33-100%. Sensitivity for endometrial hyperplasia and cancer is between 33% 100, specificity is between 79-99% (14). Endometrial pathologies of TVUSG, has low diagnostic value particularly in evaluating focal abnormalities, but it has been reported that, this could be the first step method which will reduce the need for invasive procedures (15). In the study of Dijkhuizen et al. (16) in 50 premenopausal women's hysterectomy specimens compared the TVUSG and SIS findings with the pathological examination findings, they detected 13 myomas and 10 polyps, detection of polyps with TVUSG has sensitivity 40%, specificity 100%, positive predictive value (PPD) 100%, negative predictive value (NPD) reported as 87%. Direct detection of endometrial polyps has a low sensitivity with TVUS. In our study, the diagnosis of endometrial pathologies with TVUSG has a sensitivity of 71.42%, and a specificity of 77.78%. Sensitivity and specificity of TVUSG in the diagnosis of submucous myoma 96.43%; sensitivity for endometrial polyp 71.43%, specificity 93.75%. in endometrial hyperplasia, the sensitivity is 80.0% and the specificity is 80.0%, similar rates to the literature has been found.

In determining focal intrauterine pathologies SIS has a prominent advantage over TVUSG. It gives a higher diagnostic accuracy rate in detecting intracavitary lesions. Sensitivity and specificity of TVUSG in the diagnosis of intracavitary lesions reported by Williams et al. (17) as 67% and 93%, it was emphasized by De Vries et al (14) that the same rates were 60% and 93% with TVUSG respectively. As a result, SIS is superior to TVUSG in recognizing intracavitary pathologies, however It is found that SIS is insufficient to recognize endometrial pathologies such as hyperplasia other than polyp and myoma (18). In our study, the efficiancy of SIS in determining intracavitary lesions has asensitivity of 88.89% and specificity of 90.48%. Determination of endometrial polyps sensitivity 85.71% specificity 87.50%, in submucous myomas sensitivity 50%, specificity 100%. In endometrial hyperplasia, sensitivity 40% specificity 88%. These results were also found similar to the literature.

As a sensitive method hysteroscopy is and highly used in the detection of endometrial pathologies in the current literature. In 419 postmenopausal women Garutti et al. (19) performed hysteroscopy for determining endometrial pathologies, in their study sensitivity 96.5%, specificity 93.6%, positive predictive value 92.9% reported as. The purpose of invasive techniques used in patients with abnormal uterine bleeding is to detect benign intrauterine pathologies and especially to determine the diagnosis of endometrium cancer correctly. In recognition of normal and abnormal endometrial structure; sensitivity, specificity, negative predictive value and positive predictive values of H/S is respectively was finded as 94.2%, 88.8%, 96.3% and 83.1% (20). Although H/S can detect polyps with a high accuracy rate, it is not sufficient in detecting hyperplasia. In a study Lo et al. (21) showed that the sensitivity and positive predictive value of hysteroscopy without biopsy in diagnosing endometrial carcinoma were only 58.8% and 20.8%, respectively. In another study, the superiority of H/S over D&C in recognizing endometrial hyperplasia and cancer was investigated. It is stated that, in the diagnosis of cancer and hyperplasia the gold standard is D&C but H/S is more sensitive in detecting polyps and fibroids (16).

According to the study of Fukuda et al. (22), as a method hysteroscopy in determining endometrial pathologies has a sensitivity of 90%, specificity of 82% was find. The sensitivity is 95% and specificity is 92% in determination of submucous fibroids, also in determing endometrial hyperplasia it was mentioned that the sensitivity is 97% and specificity 100% (22). Since the sensitivity of hysteroscopy in endometrial hyperplasia is very low, taking biopsy from endometrium is required. Vanderley

et al. in the study where they compared ultrasonography and hysteroscopy in the evaluation of endometrial lesions; 191 patients were included in the study and although the accuracy rate of hysteroscopy in the evaluation of endometrial pathologies was above 90%, TVUSG was 65.9% in endometrial polyps, 71.9% in myomas and in endometrial hyperplasia, it has an accuracy rate of 63.2% (23). According to our study, the effectiveness of hysteroscopy in evaluating endometrial pathologies; sensitivity 100%, specificity 90%, PPD 95.24%, NPD 100% was determined as. In endometrial polyps; sensitivity 100%, specificity 93.75%, in submucous myomas; sensitivity 100%, specificity 100% was found as. In endometrial hyperplasia,it is determined that sensitivity is 80%, specificity is 100%. The results were found to be similar to the literature.

CONCLUSION

Abnormal uterine bleeding is one of the most common causes of gynecological complaints. In terms of presenting treatment options to the patient, careful diagnosis of uterine cavity pathologies are necessary. According to the results of our study, for diagnosis; firstly an inexpensive and simple method should be chosen, later complicated and expensive methods must be tried. Hysteroscopy is the most reliable method in diagnosis of endometrial pathologies, is also the gold standard in diagnosis-treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by İstanbul Bakırköy Dr. Sadi Konuk Education and Research Hospital Ethics Committee (date: June 01, 2008; Approval number: 303).

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

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The effect of phosphorylcoline-coated cardiopulmonary by-pass circuits on morbidity and mortality in patients with congenital open cardiac surgery

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ABSTRACT

Objective: Our aim in this study is to investigate the relationship between mortality and morbidity of phosphorylcholine coated oxygenator circuit used in heart-lung machine in congenital open-heart surgery operations.

Material and Method: The study was conducted in Dr. Sami Ulus Child Diseases Training and Research Hospital Cardiovascular Surgery Clinic between 2008-2009. 30 congenital heart patients were included. The patients were divided into 2 groups of 15 people. In one of the groups, a phosphorylcholine coated oxygenator circuit was used in the heart lung machine (Group P). In the other group, a standard oxygenator circuit was used (Group C).Congenital heart surgery was performed for 19 ventricular septal defects (VSD), 5 secundum atrial septal defects (ASD), 3 primum ASD, 2 mitral insufficiency and 1 discret subaortic membrane. Extubation times, intensive care and discharge times, 24-hour drainage follow-up, inotropic drug use, blood and fresh frozen plasma (FFP) transfusion amount, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase-MB, urea, blood urea nitrogen, creatinine, white cell number of platelets, lactate dehydrogenase, albumin, total protein, C-reactive protein, prothrombin time, partial thromboplastin time, fibrinogen, D-dimer, C5a and elastase levels were compared perioperatively.

Results: In the study, it was determined that the discharge time was shorter in Group P. It was found that the increase in d-dimer values with fibrinogen was less in Group P. These were found to be statistically significant ($p<0.05$). There was no significant difference between groups in other parameters ($p>0.05$). There was no mortality in either group.

Conclusion: In this study, phosphorylcholine-coated oxygenator did not significantly reduce the inflammatory response during cardiopulmonary by-pass (CPB). There was no difference between the two groups in terms of morbitidity and mortality. However, the fact that fibrinogen values, which are the acute phase reactants, are lower than the control group and the increase in d-dimer values remain limited may be important in terms of hemocompatibility of the phosphorylcholine coated circuit.

Keywords: Phosphorylcholine coated oxygenator, congenital heart surgery, cardiopulmonary bypass, inflammation

INTRODUCTION

Cardiac surgery is a procedure widely used in congenital and adult cardiac patients all over the world (1). Open-heart surgery was first performed by Gibbon (2). A cardiopulmonary bypass (CPB) machine is used to perform heart surgery in a still and bloodless environment (3). In surgery, myocardial damage and contact of patient blood with large artificial surfaces during CPB results in a strong systemic inflammatory response. The oxygenator, one of the main parts of the heart-lung machine, consists of a membrane with a large foreign surface area where oxygen and carbon dioxide are exchanged (4). Children may experience a much more severe inflammatory response during CPB than adults. The reason for this is that their immunological development has not yet been completed and the blood touches a larger foreign surface than the body area (5). Due to the release of

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many inflammatory cytokines by CPB, activation of complement and coagulofibrinolytic systems is the main reason of this systemic inflammatory response (6,7).The complement system consists of more than 30 plasma proteins. They are potent vasoactive anaphylotoxins interacting with each other (8). C5a, formed in the early phase of CPB, is a potent chemotactic protein that promotes neutrophil chemotaxis, degranulation, and superoxide formation (9). Neutrophils have a strong proteolytic and cytotoxic substance stores. Azurophilic granules contain lysozyme, myeloperoxidase, cationic proteins, elastase, collagenase, proteinase-3, acid hydrolase, defensins and phospholipase. These substances, which enter the circulation from neutrophils activated by C5a, mediate the symptoms of "systemic inflammatory response syndrome" (SIRS) seen in CPB and cardiac surgery (10,11). Neutrophil-endothelial interaction, which is formed by increasing adhesion molecules during systemic inflammatory response, has been shown as the cause of organ failure that develops after CPB (12).

This inflammatory response may cause complications such as myocardial dysfunction, respiratory failure, renal and neurological disorders, bleeding diathesis, liver dysfunction and multi-organ failure that may occur in the postoperative period (13). Foreign surfaces in the circuits used in cardiopulmonary bypass are the main cause of SIRS. Therefore, in practice, many mechanical and pharmacological methods are used to prevent this negative inflammation caused by CPB. Different surface coatings are used for the control and reduction of SIRS occurring in these methods. These are Polymethoxyethylacrylate, Albumin, heparin and heparin-polymer combinations (14).

The hemo and biocompatibility of surface coatings is very important. The hemocompatibility and biocompatibility criteria of the CPB circuit are as follows. It is because the circuit does not cause adverse reactions due to contact with blood. It does not cause changes in blood elements and thrombogenic phenomenon. It does not cause hemolysis or complement system activation, inflammatory response, direct or indirect toxicity, and particle separation from the circuit surface. It is chemically inert (15).

Phosphorylcholine, a biocompatible surface coating, forms a layer between the blood and the surface. In vitro and in vivo studies have shown that it reduces the inflammatory response and microbial adhesion. Thrombogenic, toxic and allergic reactions are minimal and have no immunological and carcinogenic effects (16,17).

The aim of this study is to investigate the effects of phosphorylcholine-coated oxygenator circuits on morbidity and mortality by evaluating systemic inflammation in congenital heart surgery operations performed with CPB.

MATERIAL AND METHOD

This study was approved by Dr. Sami Ulus Child Health and Diseases Training and Research Hospital Ethics Committee (date: 10.01.2008, No: 2008/05). It was performed on 30 patients who underwent congenital open heart surgery between February 2008 and July 2008 in the cardiovascular surgery clinic. Informed consent form was obtained from all patients and it was made in accordance with the Helsinki Declaration of 1964 and subsequent amendments or comparable ethical standards.

In the study, 30 congenital heart patients were divided into two groups as 15-person control and 15-person phosphorylcholine group. In the control group (Group C, n=15), the standard CPB oxygenator circuit (Dideco Mirandola, Italy) and in the phosphorylcholine group (Group P, n=15) phosphorylcholine coated CPB circuit (Dideco Phisio Mirandola, Italy) was used. Anesthesia was induced with 15 mcg/kg fentanyl, 0.2-0.3 mg/kg midazolam and 0.1 mg/kg vecuronium and connected to the ventilator. For maintenance of anesthesia, 2 µcg/kg fentanyl, 0.1 mg/kg midazolam (dormicum) and 0.05 mg/kg vecuronium chloride (norcuron) were added. Sevoflurane (sevorane) at approximately 1 MAC (Minimum alveolar concentration) was added if necessary. Operations were performed with a median sternotomy. Arterial cannulation was performed from the ascending aorta. Two stage venous cannulation was applied and CPB was entered. It was cooled down to 28-32°C according to cardiac pathologies. Cardiac arrest was achieved with antegrade hypothermic crystalloid cardioplegia. Crystalloid cardioplegia was administered at 20-minute intervals. The amount to be administered was calculated as 20 ml/kg for the first dose and 10 ml/kg for maintenance doses. The prime solution was prepared using isolyte with Hct 30% and 50 mg/kg cefazolin was added. Age, gender, congenital heart diseases, total CPB times, crosclamping times, extubation times, intensive care and discharge times, 24-hour drainage follow-up, inotropic drug use, blood and FFP transfusion were recorded.

Blood samples were taken for aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase MB (CKMB isoenzyme level), urea, blood urea nitrogen (BUN), creatinine, white cell count (WBC), platelet (PLT), lactate dehydrogenase (LDH),

albumin, total protein, C-reactive protein (CRP), prothrombin time (PT), partial thromboplastin time (aPTT), fibrinogen and D-dimer levels on preoperatively (T1), postoperatively (T2) hour and postoperative 24th hour (T3). Blood samples for C5a and elastase were taken preoperatively (T1), before CBP (T2), before protamine administration (T3), postoperatively (T4) and at the postoperative 24th hour (T5).

Statistical package for the social sciences (SPSS) for Windows 16.0 statistics package program was used to evaluate the data. Measuring data were expressed as mean and standard deviation. Fisher's exact test was used in quantitative data, the Mann-Whitney U test was used to compare the means of the two groups, and the Wilcoxon test was used for repeated measurements within the group. P<0.05 was considered statistically significant.

RESULTS

Thirty patients diagnosed with congenital heart disease were divided into two equal groups; Group P and Group C. The ages of the patients participating in the study are between 1-12; mean age was calculated as 7.03±4.41 in group P and 6.86±5.00 in group C. The demographic data of the groups are shown in **Table 1**. There was no statistically significant difference between the groups in terms of demographic data (p>0.05).

Table 1. Demographic data

	Group C	Group P
Gender (F/M)	5/10	6/9
Age (year)	6.86±5.00	7.03±4.41
Height (cm)	115.27±33.37	111.93±26.87
Weight (kg)	27.92±24.33	23.44±17.36

Congenital heart disease diagnoses in Group C and P are given in **Table 2**. All patients consist of non-cyanotic congenital heart diseases.

Table 2. Patient diagnoses

	Group C	Group P
VSD	10	9
Secundum ASD	2	3
Primum ASD	1	2
Mitral insufficiency	1	1
Discret aortic membrane	1	-

VSD: Ventricular septal defect, ASD: Atrial septal defect,

Intraoperative and postoperative data of the groups are given in **Table 3**. The discharge time in Group P was determined to be shorter than in Group C. This is statistically significant (p<0.05).

Table 3. Preoperative and postoperative data

	Group C	Group P
CPB (dk)	43.93±5.05	46.96±23.59
Cross clemp (min)	26.86±15.56	29.10±18.03
Extubation (h)	8.30±8.38	6.70±6.74
Intensive care (h)	37.00±14.19	29.93±8.72
Discharge (days)	9.86±5.05	7.13±2.82*
Inotrope (μg kg dk^{-1})	3.33±4.49	6.00±4.70
Inotrope time (h)	8.80±11.53	13.06±11.48
Drainage amount (ml)	227.40±119.96	192.33±120.68
Given erythrocyte suspension	50.00±154.39	55.33±95.90
Given TDP (ml)	207.66±155.83	213.66±168.44

CPB: Cardiopulmonary by-pass, * p<0.05

Fibrinogen and D-dimer values at the 24th hour increased less in group P than in group C. This is statistically significant (p<0.05).

There was no statistically significant difference between the groups in all measurements of C5a and elastase (p>0.05).

In the within-group evaluation, a significant difference was found between postoperative and preoperative values in both groups (p<0.05).

DISCUSSION

Cardiac surgery mortality is higher than other surgeons. It is a surgery with high postoperative morbidity. Using CPB during the operation increases the inflammatory response. The main reason for this is the contact of blood with foreign surfaces. Complications such as rhythm disturbance, ventricular dysfunction requiring inotropic support, infection, gastrointestinal dysfunction, acute lung injury, and renal failure may develop. Increased systemic inflammatory response to surgical trauma may cause many postoperative complications (18).

In the pediatric age group, age-related differences in inflammatory response with immature organ systems may lead to increased damage due to CPB. In addition, larger extracorporeal circulation volume compared to body surface area in this patient group may result in more complications related to CBP (19,20).

The most severe consequence of the inflammatory response is multiple organ dysfunction and death. Mild inflammatory responses do not cause severe organ dysfunction requiring intensive care, but increase hospital stay and cost. Systemic inflammatory response is a multifactorial event and has secondary effects on damaged and intact tissue. Proinflammatory mediators can have beneficial effects on many organ systems as well as harmful effects. Tissue damage, endotoxemia and contact of blood with a foreign surface during CPB are the main causes that lead to systemic inflammatory response (21). For this reason, different surface coatings are used to control and reduce the resulting SIRS.

Zwaal at al. (22) showed that the outer surfaces of the erythrocyte membranes are antithrombogenic and that the phospholipids on the cell membrane are in asymmetric distribution. Lipids containing phosphorylcholine cell is found to be concentrated in a bilayer membrane outer layer. Chapman et al. (23) succeeded in binding methacrylolphosphorylcholine/lauryl-methacrylate co-polymers to metal and synthetic surfaces and the term "biomembrane mimicry" first appeared for phosphorylcholine-coated surfaces. In vitro and animal experiments have shown that phosphorylcholine coated artificial polymers, which are one of the surface coating technologies used to improve the hemo and biocompatibility of artificial devices, have thrombogenic resistance. These polymers exhibit minimal plasma protein and platelet adhesion and are used in contact lenses and CPB circuits (24-26).

In another experimental study conducted by De Somer et al. (27) they stated that C5a levels slightly increased in phosphorylcholine-coated circuits. In our study, an increase was observed in the post-bypass period, and results similar to preoperative values were obtained in later values. In a study conducted by Draaisma et al. (28) in 28 neonatal and infants, they compared phosphorylcholine-coated circuits with circuits without surface coating. When they evaluated the groups in terms of complement factor C3b/c, elastase, CRP values, duration of intensive care, ventilation duration, body temperature and inotropic medication, they stated that there was no difference between the groups. In our study, there was no difference in C5a and elastase results between groups ($p>0.05$). However, when the groups were compared within themselves, we found that it was higher in the postoperative period. This indicates that there is inflammation during the surgery. However, we can say that there is no difference between the groups in terms of inflammatory severity, which is consistent with the study of Draaisma et al. (28).

In their study on 39 high-risk patients, Pappalardo et al. (29) stated that the platelet count, soluble CD40 ligand, fibrinogen, antithrombin, D-dimer, prothrombin fragments and free hemoglobin values and postoperative bleeding, the amount of blood administered and clinical results were similar. Nevertheless, they stated that when phosphorylcholine coated circuits are used together with tracamide acid, intraoperative thrombin formation can be improved and platelet, fibrinogen and antithrombin consumption may be reduced. In our study, we found that the increase in fibrinogen and D-dimer values among the parameters. We used to evaluate the bio and hemocompatibility of phosphorylcholine-coated circuits increased significantly less in group P. This is important for hemocompatibility, but there was no difference in the amount of drainage between the groups. There was also no difference in the use of blood and FFP ($p>0.05$). On the other hand, De Somer et al. (30) found no difference in the formation of hemolysis and thrombin formation between the phosphorylcholine coated group and the non-surface coated group, however, they stated that the amount of blood loss was 30% less in the phosphorylcholine coated group. Again, they reported that the most beneficial effect of phosphorylcholine-coated circuits was on thrombocytes and therefore reduced blood loss. Kirshbom et al. (31) showed that phosphorylcholine and heparin coated circuits were not different from circuits without surface coating in terms of platelet count, beta-thromboglubulin values, thromboelastographic measurements of platelet function, and postoperative bleeding amounts. They stated that clinical results did not change in pediatric cardiac surgery. Thrombocytopenia is due to dilution, platelet adhesion to lines, aggregation, activation and removal of damaged platelets by the reticuloendothelial system. After CPB, the platelet count is 30-50% below the preoperative level. (32). In our study, significant decreases in platelet values were observed, but no difference was found between the groups. Again, in the comparisons between groups, there was a significant prolongation in postoperative values according to preoperative PT and aPTT values, but no difference was found between groups.

Harig et al. (33) stated that phospholipid-coated CPB circuits have beneficial effects on clinical parameters such as ventilator and ICU stay in pediatric patients. Nevertheless, they reported that it should be confirmed by studies involving many patients. In our study, there was no difference between the groups in terms of length of stay in the intensive care unit.

Urea, BUN and creatinine parameters were measured to evaluate renal functions. Serum creatinine and creatinine clearance is the most commonly used and practical method in clinical use (34). In our study, although the postoperative creatinine value was significantly higher than the preoperative value in the control group, it was within normal limits. Acute or chronic renal failure was not observed.

Liver enzymes may increase slightly in the postoperative period, and mild icter may be observed in 10-20% of the cases. In the majority of cases with icterus, the etiology is hemolysis rather than liver (35). In the study, Although there was no difference between the groups in terms of AST, ALT, LDH, total protein, albümin and CKMB values, there was a significant difference in these parameters compared to the preop values in the measurements within the group. This is a result of the surgery and CBP's inflammatory response in the liver.

There was no statistically significant difference between the groups in terms of X-clamp and CBP times. All of the patients in the study consisted of isolated cogenital heart disease. This resulted in short X-clamp and CBP times. This caused a limitation of the study. Inclusion of complex congenital heart patients in the study may increase the mean CBP duration and inflammatory response (36). We think that better data can be reached to measure inflammation in terms of evaluating biocompatibility.

Limitation of the Study

All patients in the study consisted of non-cyanotic cogenital heart disease. This resulted in shorter cross-clamp and CBP times. Including cyanotic groups in the study and conducting more studies on more patients can be more accurate in terms of inflammation.

CONCLUSION

Phosphorylcholine coated circuits do not significantly reduce complement and neutrophil activation during CPB. Because the artificial surface does not trigger the inflammation that occurs after CPB. It depends on many factors, as noted in other studies. We believe that additional applications are required in order to control inflammation in CPB. On the other hand, we think that the lower fibrinogen values, which are the acute phase reactants, and the limited rise in D-dimer values, the short hospital discharge period, the shorter extubation and intensive care stay, although not statistically significant, may be important in terms of biocompatibility and hemocompatibility.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Dr. Sami Ulus Child Health and Diseases Training and Research Hospital Ethics Committee (date: 10.01.2008, No: 2008/05).

Informed Consent: All patıents participating in the study have signed the informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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Comparison of hydroxyethylstarch 130/0.4 and ringer's lactate on fibrinogen level in cesarean operations: a randomized clinical trial

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ABSTRACT

Aim: Colloids are effective in volume resuscitation but they have been shown to have negative impacts on fibrin formation. The aim of this study is to evaluate the effect of using low dose HES solutions on fibrinogen level in cesarean operations.

Material and Method: 100 women, scheduled for cesarean surgery with spinal anesthesia were enrolled in the study. Patients were assigned to receive hydroxyethyl starch (HES) 130/0.4 in addition to a basic infusion of Ringer's lactate (RL) solution (GROUP HES) or exclusively RL solution throughout the intraoperative study period (GROUP RL). Patients in the Group HES received HES 130/0.4 8 ml.kg^{-1}h^{-1} with RL solution 5 mL.kg^{-1}.h^{-1} and Group RL received RL solution 20 mL kg^{-1}.h^{-1}. Fibrinogen, haemoglobin, platelet values were recorded preoperatively and at postoperative first hour.

Results: HES 130/0.4 was used only in Group HES, amount consumed was 418±90 mL. Ringer's lactate consumption was 276±58 mL for Group HES and 1197±197 mL for Group RL. There was a significant change in haemoglobin and fibrinogen values before and after surgery between groups. The mean decrease in fibrinogen was 92.18±60.12 mg/dL in Group HES and 65.70±83.61 mg/dL in Group RL.

Conclusion: HES 130/0.4 solution can be used in elective cesarean operations without predicted or active hemorrhage. When there is a hemorrhage of any reason in HES 130/0.4 used cases, the decrease in fibrinogen level must be anticipated and rapid replacement of fibrinogen must be kept in mind.

Keywords: Fibrinogen, hydroxyethyl starch, cesarean operations

INTRODUCTION

Colloids are more effective in volume resuscitation than the cristalloids based on their longer duration in intravascular area and higher volume effect, Recent studies showed that, colloids have negative impacts on fibrin formation. Especially when administered in high doses (>50 ml.kg.d^{-1} or >1.5 liters) or in patients who has prior coagulation problems; they had been shown to cause more haemodilution and have serious adverse effects (1,2).

Low doses of hydroxyethyl starch (HES) 130/0.4 solutions are frequently used at perioperative period to prevent hypotension and related complications. In case of unexpected hemorrhage, the coagulation defect related to the use of HES 130/0.4 solutions may enhance blood loss. The aim of this study is to evaluate the effect of using low dose HES solutions on fibrinogen level in non-complicated cesarean operations and guide intraoperative fluid management in accordance with this information.

MATERIAL AND METHOD

The study was carried out with the permission of Zekai Tahir Burak Women's Health Training and Research Hospital's institutional review board (2011-KAEK-19, Approval code: 41/2018).

After Ethics Committee approval, one hundred pregnant women, ASA I or II, scheduled for cesarean surgery with spinal anesthesia were enrolled in the study. An informed written consent was taken from the patients before participation. Exclusion criteria were; refusal or contraindication for regional anaesthesia; age under 18 or over 40 years; body weight over 100 kg and body height below 150 cm; pregnancy with gestational age under 36 weeks and multiple gestations, fetal anomaly, placental invasion anomalies; history of preeclampsia/eclampsia, abnormal values of fibrinogen, coagulation tests and haemoglobin, haematocrit and platelet.

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Using a computer-generated randomization list, patients were assigned to receive medium molecular-weight medium-substituted hydroxyethyl starch (6% Voluven® 130/0.4, Fresenius, Pharma Austria GmbH, Graz, Austria) in addition to a basic infusion of Ringer's lactate solution (Ringer Laktat®, Osel İlaç Sanayi, İstanbul, Turkey) (GROUP HES) or exclusively Ringer's lactate solution throughout the intraoperative study period (GROUP RL). Patients in the Group HES received hydroxyethyl starch 130/0.4 8 $mL.kg^{-1}h^{-1}$ with Ringer lactate solution 5 $mL.kg^{-1}.h^{-1}$ and Group RL received only Ringer lactate solution 20 mL $kg^{-1}.h^{-1}$. The local pharmacy prepared the study solutions that were supplied in identical 500 ml bottles. An IV line was established and flushed with the randomized fluid placed in an opaque bag to blind the outcome assessor, who recorded the volume of lost blood by suction and weight of swabs.

The volume regimen was based on the presumption that hydroxyethyl starch and Ringer lactate solution show different volume effects. Fluids were actively warmed with fluid warmer to 41°C. The safety of usage and stability of HES that had been warmed has been shown (3,4).

Preoperative fibrinogen, haemoglobin, and platelet values were recorded.

With patients in the sitting position, spinal anaesthesia was performed with 26 gauge atraucan (atrau-com®, egemen INTERNATIONAL, İzmir) needle. A standart solution of 10 mg hyperbaric bupivacaine was injected in 30 seconds to cerebrospinal fluid. After the procedure, the patients lied supine with 20° tilt to left to prevent hypotension caused by aorta-caval pressure.

Hypotension was defined as a decrease of 20% or more below baseline mean arterial pressure value and treatment was made with ephedrine bolus of 10 mg until the mean arterial pressure returned to normal values. Bradycardia was defined as heart rate <50 beats. min^{-1} and treatment was made with atropine.

After taking the patients to recovery room, infusion of HES 130/0.4 was terminated and fluid infusion continued with Ringer's lactate in both groups. Patients who were given more than 1500 mL HES 130/0.4 were excluded from the analysis. Patients who had bleeding during/after operation; given any blood product, tranexamic acid or fibrinogen or given oxytocin more than routine practice were also excluded from the analysis.

Blood loss during the operation was recorded. Patients were excluded from the analysis if blood loss was more than 1000 mL. Fibrinogen, haemoglobin, platelet values were recorded at postoperative first hour.

Statistical analysies were performed using SPSS Software (Version 21.0, SPSS Inc., IL, USA). The sample size of study was calculated based on the sample size of previous studies, using a significance level of 5% (p=0.05) and power of 97.5% Categorical data are expresses as counts and percentages (%) and continuous data as mean±SD (range). After determining normal distrubution using Kolmogorov-Smirnov test for quantitative data, analysis were performed using Student's t-test or Mann–Whitney U-test. $\chi 2$ test was used for qualitative data. $p<0.05$ was considered significant.

RESULTS

The data of one hundred patients, consisting of fifty patients from each group were analysed. Hydroxyethyl starch 130/0.4 was used only in Group HES, amount of voluven consumed was 41±90 mL. Mean Ringer's lactate consumption was 276±58 mL for Group HES and 1197±197 mL for Group RL.

Patients' characteristics, duration of surgery and blood loss in surgery are shown in **Table 1**. Body weight, body height, duration of surgery and blood loss did not differ among groups, with the only exception that mean age was higher in Group RL as compared with group HES. This difference was statistically significant (p=0.02) but without clinical relevance.

Table 1. Demographic characteristics of patients and duration and blood loss in surgery

	Group HES mean±SD	Group RL mean±SD	p
Weight (kg)	73.3±8.1	75.6±9.6	0.320
Height (cm)	159.9±5.6	160.4±5.4	0.495
Age (years)	27.7±5.4	30.1±4.8	0.02*
Blood loss (mL)	596.8±203.4	618±198.6	0.275
Duration of surgery (min)	43.9±7.3	45.3±6.8	0.162

*p<0.05

As shown in **Table 2**, the distribution of operation indications did not differ among groups (p=0.568).

Table 2. cesarean indication of the study participants

	Group HES N (%)	Group RL N (%)
Fetal distress	4 (8%)	5 (10%)
Cephalopelvic disproportion	7 (14%)	3 (6%)
Previous uterin surgery	32 (64%)	36 (72%)
Abnormal presentation	7 (14%)	6 (12%)
Total	50 (100%)	50 (100%)

The change in haemoglobin values before and after surgery was statistically significantly different among groups (p=0.05). Preoperative and postoperative change in platelet values did not differ among groups (p=0.08) (**Table 3**).

Table 3. Change in laboratory parameters

	Group HES Mean (±SD)	Group RL Mean(±SD)	p
Change in haemoglobin value (g/dl)	1.24 (±0.71) ↓↓	0.69 (±0.66) ↓↓	0.00*
Change in platelet value (n)	28380 (±26949) ↓↓	24195 (±38742) ↓↓	0.08
Change in fibrinogen value (mg/dl)	92.18 (±60.12) ↓↓	65.70 (±83.61) ↓↓	0.004*

*p<0.05

There was a statistically significant change in fibrinogen value before and after surgery between Group HES and Group RL. As shown in **Table 3**, the mean decrease in fibrinogen value was 92.18±60.12 mg/dL in Group HES and 65.70±83.61 mg/dL in Group RL.

Number of patients with a fibrinogen level below 200 mg/dL did not differ among groups (p=0.24) (**Table 4**).

Table 4. The distribution of cases with fibrinogen value below 200 mg/dL

	Fibrinogen >200 mg/dl N (%)	Fibrinogen <200 mg/dl N (%)	Total
Group HES	50 (100%)	0 (0%)	50 (100%)
Group RL	47 (94%)	3 (6%)	50 (100%)

DISCUSSION

Hydroxyethyl starch 130/0.4 solution is shown to be more effective than cristalloids in maintaining systolic and diastolic blood pressure, regulating changes in heart rate, ephedrine use and total amount of infused solution (5). Their use especially in cesarean section with spinal anaesthesia has many benefits in controlling the blood pressure of the patient and helps preventing the effects of hypotension on mother and fetus.

Fibrinogen level is found to be the main determinant of coagulopathy in postpartum hemorrhage (6) and postoperative bleeding in cardiac surgery (7). Although HES solutions are more effective than Ringer's lactate solution in volume resuscitation, the most remarkable effect of HES is reported to be a decrease in fibrinogen level and impairment in fibrin polymerisation (8,9). The impairment in total cloth strength when colloids were used is reported to be with a decrease in the fibrinogen component of the cloth; furthermore with both HES 200/0.5 and 4% modified gelatin a more decrease in fibrinogen concentration is observed (10).

Maximum clot firmness (MCF) is known to be associated with function and level of platelets and fibrinogen level (11). Fenger and colleagues demonstrated that hemodulition with HES solutions leads to a decrement in MCF and a coagulopathy with unchanged clotting time, maximum velocity and time to maximum velocity (9). It is reported in a study that colloids affect the speed and quality of clot formation by interacting with functional measured polimerisation of fibrinogen/fibrin and fibrinogen concentration; as a result although coagulation factors and coagulation time remain unchanged, results of functional measured fibrinogen/fibrin polymerisation tests become impaired (12).

Based on the long half-life of HES solutions; ending the infusion of HES solution does not resolve coagulopathy in a short duration of time (13). The insufficiency of fibrinogen is reported to occur when colloids and red blood cell components are used for compansation of blood loss during major urologic or abdominal surgery In this study, fibrinogen concentrations reported to decrease in the colloid groups by average 100 mg/dl throughout the observation period (14). This shows that severely decreased fibrinogen concentrations might be reached earlier than expected time.

In our study, there was a significant reduction with HES 130/0.4 when compared with Ringer's lactate solution in fibrinogen values although average amount of used HES 130/0.4 was 418 ml-considering the fact that the clinical coagulopathic effects of HES 130/0.4 are mostly seen when large volume (>1.5 L) of colloids have been infused.

Intraoperative and postoperative major bleeding did not occur in any of the patients. The patients with a blood loss of more than 1000 mL would be excluded from the study but none of the patients exceeded this limit. The change in haemoglobin values before and after surgery was statistically significantly different among groups. The reduction in haemoglobin value in group HES is greater than the reduction in haemoglobin value in group RL. The reduction in haemoglobin value can be explained either by the negative impact of HES 130/0.4 on blood coagulation or by the more profound haemodiluting effect; but in any case it leads to a quicker reach to red blood cell transfusion tresholds.

When a cesarean section without expectable bleeding is anticipated in preoperative evaluation, HES 130/0.4 solution can be used to prevent hypotension related to spinal anaesthesia. Considering that fibrinogen concentrations below 200 mg/dL is found to be predictive for serious postopartum hemorrhage (6), it would be useful to determine preoperative fibrinogen level in regulating intraoperative fluid regimen.

One limitation of our study seems to be the lack of viscoelastic tests as standard laboratory tests are not able to evaluate the platelet interactions with the endothelium, erythrocytes and fibrinogen but as the main purpose of our study is focus on the final fibrinogen level to evaluate the effect of using low dose HES solutions, performing viscoelastic tests is not considered necessary.

CONCLUSION

Hydroxyethyl starch 130/0.4 solution can be used in elective cesarean operations without predicted or active hemorrhage. According to the results of our study, although it causes a significant reduction in fibrinogen level when compared with Ringer's Lactat solution, this situation does not lead to an consequential clinical outcome in patients. In consideration of above-mentioned studies, when there is a hemorrhage of any reason in HES 130/0.4 used cases, the decrease in fibrinogen level must be anticipated and rapid replacement of fibrinogen must be kept in mind.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was received from the institutional review board (Zekai Tahir Burak Women's Health Training and Research Hospital Institutional Review Board (2011-KAEK-2019, approval code: 41/2018).

Informed Consent: Written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Original Article

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Factors associated with the development of screw cut-out after the fixation of intertrochanteric femoral fractures with a proximal femoral nail

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ABSTRACT

Objective: To reveal the factors associated with screw cut-out in the fixation of proximal femur intertrochanteric fractures with a proximal femoral nail (PFN).

Material and Method: Patients who were diagnosed with proximal femoral intertrochanteric fractures and were being treated and followed up in our hospital between January 2014 and January 2019 were retrospectively analyzed. The hip fracture types of the patients were determined according to the American Foundation/American Orthopedic Trauma Association (AO/OTA) classification. AO/OTA 31-A1, A2 and A3 type fractures were included in the study. Twenty-seven patients with PFN fixation failure and screw cut-out (cut-out group, 11.4%) were compared with 208 patients who had successful osteosynthesis without cut-out (non-cut-out group, 88.6%). Age, gender, affected side, follow-up time, PFN design, tip-apex distance (TAD), calcar-referenced TAD, fracture type, reduction quality, posteromedial support loss, lag screw position, Singh index, and collo-diaphyseal angle (CDA) were compared between the two groups.

Results: The two groups significantly differed in terms of TAD and calcar-referenced TAD (p=0.002 and 0.001, respectively). In the evaluation of reduction quality according to the Baumgaertner scale and the Garden alignment index, a significant difference was found between the two groups (p=0.021 and 0.002, respectively). A significant difference was also observed between the two groups in terms of screw position and posteromedial cortex continuity (p=0.009 and 0.037, respectively). However, there was no significant difference in relation to age, gender, affected side, CDA, PFN design, and osteoporosis severity.

Conclusion: Fracture type, poor reduction quality, loss of posteromedial support, TAD, calcar-referenced TAD, and lag screw position were found to be associated factors in the development of screw cut-out. Apart from the type of fracture, these factors that are under the control of the surgeon generally show the importance of anatomical reduction and accurate screw placement. According to the results obtained, the risk of screw cut-out can be reduced by preoperative planning and intraoperative evaluation.

Keywords: Intertrochanteric femoral fractures, proximal femoral nail, associated factors, implant failure, cut-out

INTRODUCTION

Hip fractures are a major cause of increased mortality and morbidity, especially in the elderly. Trochanteric hip fractures constitute about half of all hip fractures (1). According to the American Foundation/American Orthopedic Trauma Association (AO/OTA) classification system, fractures involving the trochanteric region of the proximal femur are classified as AO/OTA 31-A. These fractures are further divided into the groups of A1, A2 and A3. A1 is defined as simple, two-part fractures; A2 fractures are those with multiple fragments; and A3 refers to more complex fractures, including reverse oblique and transverse fracture patterns (2).

Current treatment options for intertrochanteric fractures are cephalomedullary nails and dynamic hip screws, with the former being considered to be superior to the latter (3). Cephalomedullary nails are the preferred implants, especially since they allow for acceptable closed reduction. In addition, intramedullary fixation is associated with reduced soft tissue trauma, decreased blood loss, and lower infection rates and wound complications (4). On the other hand, the use of the proximal femoral nail (PFN) is associated with certain complications, including thigh pain, displacement of interlocking head screws (Z-effect and reverse Z-effect), varus collapse, screw cut-out, peri-

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implant fracture, non-union, delayed union, femoral neck shortening, and infection development. The most frequently reported complication in PFN application in hip fractures is screw cut-out, which is defined as the extrusion of the screw from the femoral head with the varus collapse of the femoral neck (5).

The aim of this study was to discuss the factors associated with screw cut-out, which is the most common complication in fixation with PFN, which remains controversial in the literature. We hypothesized that the development of screw cut-out was related to factors under the surgeon's control and therefore they could be altered.

MATERIAL AND METHOD

The study was approved by Gaziosmanpaşa Training and Research Hospital Clinical Researchs Ethics Committee (approval date and number: 02.12.2020/192). The study was conducted in accordance with the principles of the Declaration of Helsinki. Patients who were diagnosed with proximal femoral intertrochanteric fractures and were being treated and followed up in our hospital between January 2014 and January 2019 were retrospectively analyzed. Patients under 65 years of age (n=42), those with pathological fractures (n=13 patients) or ipsilateral knee or ankle fractures (n=2), those that had undergone open reduction (n=24), those without a follow-up for at least one year (n=51), and cases in which a fixation material other than PFN was used (n=21) were excluded from the study. The hip fracture types of the patients included in the study were determined according to the AO/OTA classification system (4), and only AO/OTA 31- A1, A2 and A3 type fractures were included in the sample. Trigen InterTan (Smith & Nephew, Memphis, TN, USA) nails were used in 61 patients and Profin (TST SAN, İstanbul, Turkey) nails in 26 patients.

Twenty-seven patients with PFN fixation failure and screw cut-out were evaluated as the cut-out group and 208 patients with successful osteosynthesis constituted the non-cut-out group. Age, gender, affected side, tip-apex distance (TAD), fracture type, reduction quality, loss of posteromedial support, lag screw position, angle between the lag screw and femoral neck axis, Singh index, neck-shaft angle, and the Garden alignment index (GAI) were compared.

Preoperative and postoperative anteroposterior (AP) and lateral radiographs were used to classify the fractures and determine the quality of fracture reduction, collo-diaphyseal angle (CDA), lag screw position, TAD (5), and calcar-referenced TAD (CalTAD) (6). Fracture reduction was evaluated in the first postoperative radiograph using GAI (7). GAI was used to define the angle between neck and shaft in AP and lateral view. The results were classified as very good (AP 160°), good (AP 180-160°), acceptable (AP 160-150°) and poor (AP<150°). The postoperative reduction quality of the patients was determined as good, acceptable, or poor according to the reduction criteria defined by Baumgaertner et al. (5). The state of posteromedial support was defined as present or absent according to the amount of the displacement of the posteromedial segment. A displacement of less than the cortical thickness means that there is contact between proximal and distal fragments, and it is interpreted as the presence of posteromedial support (8).

The osteoporosis severity of the patients was evaluated according to the Singh index (9). The lag screw position was determined according to the method described by Cleveland (**Figure 1**) (10). Radiographic measurements were performed at two separate times in a month by two different orthopedic surgeons. High intra-observer [intraclass correlation coefficient (ICC) 0.91 (95% CI 0.82–0.96)] and inter-observer (ICC 0.83 [95% CI 0.81–0.93] agreement was observed in the measurements.

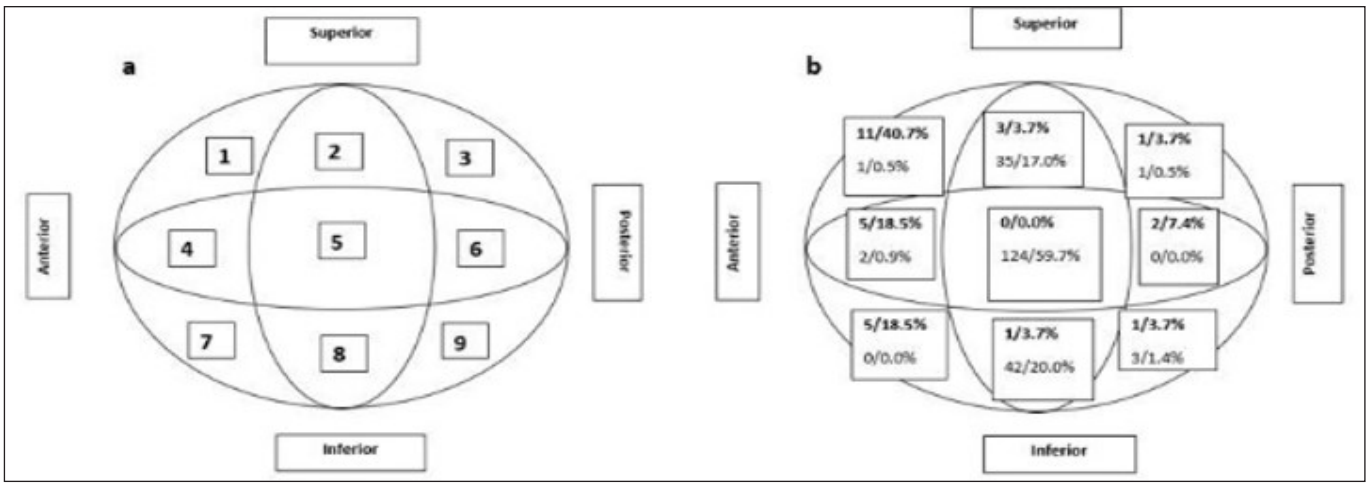

Figure 1. Lag screw positioning within the femoral head. a: Cleveland's definition of the location of lag screw in the femoral head. The nine-zone template of the head, b: The number of implant failures and failure rate in each zone is represented. Bold-italics indicates the cut-out group values.

Surgical Protocol

All the patients underwent surgery under general or regional anesthesia on the traction table in the supine position. Closed reduction and minimally invasive nailing were performed under fluoroscopic imaging. The patients then underwent routine surgical procedures for PFN implantation according to the manufacturer's protocol. Both PFN types had trochanter major tips. Postoperatively, the patients received standard prophylaxis for deep vein thrombosis. Partial weight bearing was initiated after fracture healing was seen on radiographs, and total weight bearing was allowed once clinical fracture healing was observed. Follow-up evaluations were undertaken at postoperative months 1, 3, 6 and 12, and annually thereafter.

Statistical Analysis

Statistical analyses were performed using SPSS v. 22.0 (SPSS Inc., IBM, NY, USA). The distribution of variables was analyzed using the Kolmogorov-Smirnov test. The independent-samples t-test was used for the comparison of continuous quantitative data with a normal distribution, the Mann-Whitney U test was used to compare continuous quantitative data without a normal distribution, and the chi-square or Fischer's exact test was conducted for the comparison of categorical data. The level of significance was set at $p<0.05$ for all statistical tests. A post-hoc power analysis was performed to determine whether the number of patients in each group was adequate to achieve statistical significance and to avoid type II error. More than 80% power was detected for the comparison of the measured parameters between the groups. ICC with a 95% confidence interval was used to quantify intra- and inter-observer agreement regarding the measured parameters. Based on the study of Landis and Koch (11), we defined an ICC value of 0-0.2 as indicating slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.8 substantial agreement, and >0.80 perfect agreement.

RESULTS

Between January 2014 and January 2019, 386 consecutive trochanteric fractures patients underwent surgery in our institution. The mean age of the patients was 78.91±10.71 (range, 65-95) years, and the mean follow-up time was 30.1±6.9 (12-70) months. Screw cut-out was seen in 27 (11.4%) of 235 patients. Four (14.8%) patients underwent plate-screw fixation, twelve (44.2%) patients underwent partial hip replacement and 11 (41%) required revision surgery with total hip replacement (**Figure 2**). The demographic characteristics and clinical data of the patients are summarized in **Table 1**. There was no significant difference between the two groups in terms of age, gender, and affected side (**Table 1**). There was also no significant difference between the two groups in relation to CDA, PFN design, and osteoporosis severity (p=0.156, p=0.218, and p=0.291, respectively) (**Table 2** and **Table 3**).

Table 1. Demographic characteristics of the patientsv

	Cut-out Group (n=27)	Non-cut-out Group (n=208)	p
Age	76.85±9.11 (65-91)	80.08±11.21 (65-95)	0.475
Affected side			
Right	16 (59.2%)	102 (48.3%)	0.487
Left	11 (40.8%)	106 (51.7%)	0.262
Gender			
Male	10 (37.0%)	87 (41.6%)	0.814
Female	17 (63.0%)	121 (58.4%)	0.434
Follow-up time (months)	28.1±5.6 (12-66)	31.3±7.7 (13-70)	0.706
AO/OTA classification			0.011
31-A1	2 (7.4%)	142 (68.2%)	
31-A2	18 (66.6%)	34 (16.3%)	
31-A3	7 (26.0%)	32 (14.5%)	

p<0.05

Table 2. Comparison of the groups in terms of the Singh index and radiological measurements

	Cut-out Group (n=27)	Non-cut-out Group (n=208)	p
Mean TAD (mm)	33.19± 7.64	22.18± 4.58	0.002
Mean CaIDAT (mm)	29.74± 7.33	23.32± 7.33	0.001
Mean CDA (°)	132.67± 10.79	135.82± 8.56	0.156
Singh index	3.37± 0.92	3.63± 0.75	0.291

Bold values indicate statistical significance. P < 0.05
(TAD: Tip-apex distance, CDA: Collo-diaphyseal angle, CaIDAT: Calcarreferenced tip-apex distance)

Table 3. Comparison of the groups in terms of nail design and reduction quality

	Cut-out Group (n=27)	Non-cut-out Group (n=208)	p
Proximal Femoral Nail Design			0.218
InterTan (intertrochanteric antegrade nail)	16 (59.2%)	145 (70.0%)	
Profin (proximal femoral nail)	11 (40.8%)	63 (30.0%)	
Posteromedial cortex continuity			0.037
Existence	8 (29.7%)	116 (56.7%)	
Loss	19 (70.3%)	92 (43.3%)	
Garden alignment index			0.002
Very good	3 (11.1%)	131 (62.9%)	
Good	6 (22.2%)	57 (27.4%)	
Acceptable	7 (26.7%)	16 (7.8%)	
Poor	11 (40.0%)	4 (1.9%)	
Baumgaertner Reduction Quality			0.021
Good	5 (18.5%)	176 (84.6%)	
Acceptable	8 (29.6%)	29 (13.9%)	
Poor	14 (51.9%)	3 (1.5%)	

P < 0.05

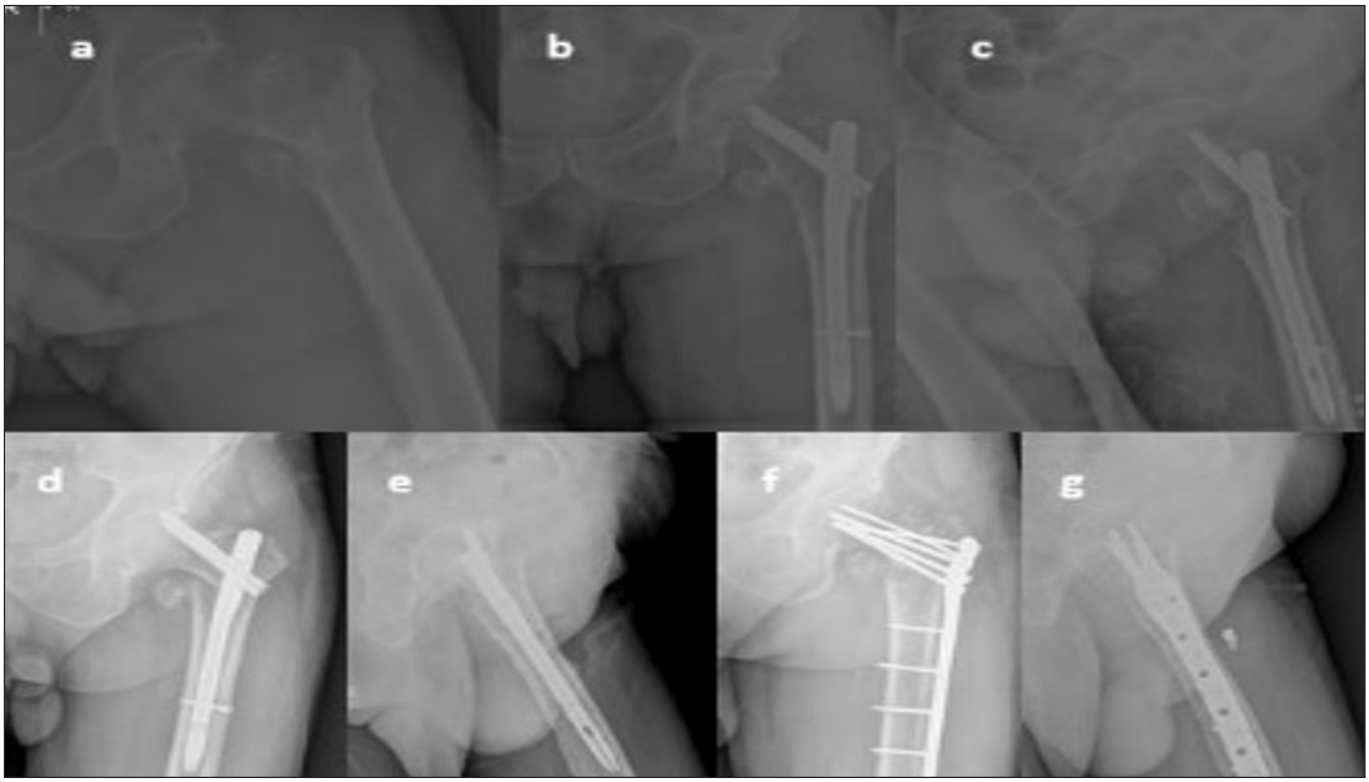

Figure 2. 68-year-old male patient who has AO type 31-A2 femur fracture. a: a preoperative anteroposterior hip radiography, b-c: early postoperative anteroposterior and lateral hip radiographs show fracture reduction and fxation using PFN, d-e: postoperative anteroposterior and lateral hip radiographs show screw cut-out, f-g: Image of loss of reduction in the patient's first month follow-up radiography. Revision surgery with plate-screw was applied as a salvage option.

The groups significantly differed in respect of fracture types according to the AO/OTA classification (p=0.011) (**Table 1**). There were also significant differences in terms of TAD and CaITAD values (p=0.002 and 0.001, respectively) (**Table 2**). When reduction quality was evaluated according to the Baumgaertner scale, a significant difference was observed between the two groups (p=0.021). Similarly, according to GAI, there was a significant difference in the reduction quality of the two groups (p=0.002) (**Table 3**). A further significant difference was found in relation to posteromedial cortex continuity (p=0.037) (**Table 3**).

According to the locations defined by Cleveland, the lag screw position was zone 1 in 11 (40.7%) patients, zone 4 in five (18.5%) patients, and zone 7 in five (18.5%) patients in the cut-out group. In the non-cut-out group, the lag screw position was zone 2 for 35 (17%) patients, zone 5 for 124 (59.7%), and zone 8 for 42 (20%). There was a significant difference between the two groups in terms of lag screw position (p=0.009) (**Figure 2**).

DISCUSSION

The most important finding of this study was the association of postoperative cut-out development with fracture type, reduction quality, TAD, CaITAD, loss of posteromedial support, and lag screw position in the fixation of intertrochanteric hip fractures with PFN. In addition, the screw cut-out complication rate was determined as 11.4% in PFN applications among the 235 consecutive patients with proximal femoral intertrochanteric fractures.

TAD and CaITAD

The literature reports that there is a correlation between implant cut-out and TAD and CaITAD in hip fractures. Baumgaertner et al. (5) determined that the TAD value was the best predictive parameter in determining the cut-out rates during a three-month follow-up period (6,12).

CalTAD differs from TAD only in AP view; the apex of the femoral head is determined using a line parallel to the femoral neck that runs adjacent to the calcar rather than the center of the femoral neck (13). The maximum values of both TAD and CalTAD have been defined as 25 mm to prevent implant cut-out complications (6,13). Many studies have shown the importance of an appropriate TAD and CaITAD to avoid implant failure (6,7,12-15). In the current study, TAD and CalTAD were found to significantly differ between the two groups, with excellent inter-observer reliability. Both values were found to be higher in the cut-out group.

Screw Location

The central location of the screw is well known. The literature reports that there is a significant relationship between incorrect lag screw positioning and cut-out complication (16-18). Valentini stated that the highest

cut-out rate was seen in the posterior section (19). De Bruijn et al. (20) reported no cut-outs in the anterior-inferior lag screws. It has also been suggested that the highest rate of cut-outs are observed in the central zone. In the current study, 27 cut-out cases were determined and when their graphs were examined, it was found that they were either superior in the AP plane or anterior in the lateral plane.

Collodiaphyseal Angle

The relationship between CDA in AP view and cut-out remains controversial (13,21). In the current study, there was no significant relationship between CDA and screw cut-out.

Implant Design

The relationship between the screw cut-out complication and implant design has been evaluated in many studies, but the results are inconsistent (22-24). In the current study, two types of PFN designs were used: InterTan (intertrochanteric antegrade nail) and Profin (proximal femoral intramedullary nail). InterTan PFN is made of titanium alloy and has a 4° proximal valgus offset. Intertan PFN has two types with 125° or 130° CDA and includes two screws (11 mm lag screw and 7 mm compression screw). Profin PFN is made of titanium alloy and has a 6° proximal valgus offset. It is applied with 135° CDA and two 8.5 mm lag screws. The results of the current study revealed no significant relationship between the screw cut-out complication and the design of the implant used.

Bone Quality and Age

In the literature, studies report conflicting results on the relationship between bone quality and age and implant cut-out (21,25,26). In the current study, no significant relationship was found between these two parameters and the cut-out complication ($p=0.291$ and $p=0.475$, respectively).

Fracture Type

De Brujin et al. (20) explained that AO/OTA 31-A3 fractures posed a 14 times higher cut-out risk due to their instability and difficulty of reduction. In a study evaluating 295 patients, Domingo et al. (27) reported that all the patients requiring revision procedure (3.3%) had AO/OTA 31 A2 and A3 fractures. In contrast, Büyükdoğan et al. (28) suggested that fracture type was not a risk factor for the development of screw cut-out in PFN application. In the current study, 66% of the patients in the cut-out group had AO/OTA 31-A2 type fractures and 26% had 31-A3 type fractures. There was a significant difference in fracture types between the cut-out and non-cut-out groups.

Fracture Reduction

Many studies in the literature show that there is a statistically significant relationship between non-anatomical reduction and screw cut-out (12,20,29). In the current study, we observed that significantly higher rates of poor reduction in the cut-out group according to the evaluation performed using both GAI and Baumgaertner's reduction criteria.

Posteromedial Cortex Continuity

It is known that posteromedial support is an important factor for the stability of intertrochanteric fractures (30). Hao et al. (8) reported that the loss of posteromedial support was a risk factor for implant failure in AO/OTA 31-A3 fractures. In our study, we also determined that loss of posteromedial support was significantly higher in the cut-out group.

Study Limitations

This study has certain limitations. First, it was designed retrospectively. Second, the number of patients was relatively small. Third, there was no control group that included an alternative treatment method to PFN.

CONCLUSION

Fracture type, poor reduction quality, loss of posteromedial support, TAD, calcar-referenced TAD, and lag screw position were found to be associated factors in the development of screw cut-out. Apart from the type of fracture, these factors that are under the control of the surgeon generally show the importance of anatomical reduction and accurate screw placement. According to the results obtained, the risk of screw cut-out can be reduced by preoperative planning and intraoperative evaluation.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Gaziosmanpaşa Training and Research Hospital Clinical Researchs Ethics Committee (Approval date and number: 02.12.2020/192).

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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Effect of number of uses on the cyclic fatigue resistance of single-file rotary instruments

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ABSTRACT

Aim: The purpose of this study was to evaluate and compare the cyclic fatigue resistance of two different single file nickel-titanium instruments -Reciproc blue (RB) and One Curve (OC)- used for single or in a certain number of times.

Material and Method: One Curve (n=40) and Reciproc Blue (n=40) files were used in this study. The instruments were divided into two subgroups (n=20); those to be used for the first time (N) and those that were previously used (U) only once in the clinic for the root canal treatment of one vital mandibular molar with three root canals. Cyclic fatigue testing of these instruments was performed in an artificial stainless-steel canal with 60° curvature, 5 mm radius 1.5 mm width, and 3.0 mm depth. All the tested files were rotated/reciprocated under the continuous irrigation with distilled water at 37°C until fracture occurred. The time to failure (TTF) in seconds and the number of cycles to failure (NCF) were recorded. Data were statistically analyzed and the significance level was set at $p<0.05$.

Results: A significant difference was observed among the four groups and between RB-N/RB-U, OC-N/OC-U, RB-N/OC-N, and RB-U/OC-U, regarding TTF and NCF values ($p<0.05$). Predicted time for 99% survival were RB-N>RB-U>OC-N>OC-U. The TTF value of RB is greater than OC in both the new instrument groups and the used instrument groups.

Conclusions: RB showed higher cyclic fatigue resistance than OC files in both new and used instrument groups. Reuse of RB and OC instruments significantly reduced the cyclic fatigue resistance of the instrument.

Keywords: Cyclic fatigue, nickel-titanium, one curve, reciproc blue, single file

INTRODUCTION

Despite all technological advances in endodontics, file separation is still a vexing problem for dentists. Torsional failure and cyclic fatigue (flexural fatigue) are the two main causes of file separation. If the tip of the instrument stuck in the root canals but the coronal segment continues to rotate, torsional failure occurs (1). Besides, repeated cycles of tension and compression of the metal cause metal fatigue and thereby give rise to cyclic fatigue (2). The features of rotary instruments (such as kinematic, alloy type or manufacturing process) (3,4) and factors depending on usage pattern (such as autoclave process, irrigation solutions) (1,5) can cause cyclic fatigue. The single-file systems introduced to the market in 2008 (6) broke new ground in the world of endodontics and minimized the risk of instrument breakage as well as saving time, preventing cross-contamination and being cost-effective (7).

Recirpoc blue (RB) (VDW, GmbH, Munich, Germany) is a thermally treated single file nickel-titanium (NiTi) instrument. It works in the principles of reciprocation with unequal forward and reverse rotation which was reported as a unique feature that can reduce the incidence of file separation (5,8). It can be used in both instrumentation and retreatment of the root canals. The file has a non-cutting tip with a S-shaped cross section and variable taper. According to the manufacturer's instructions, it can be used without any prior instrumentation or glide path creation (9).

One Curve (Micro Mega, Besançon, France) (OC) was introduced as the evolution of One Shape and was released in the market as a single file rotary instrument in 2018 by MicroMega. OC has improved mechanical properties thanks to its novel proprietary C-Wire heat-treatment controlled memory of NiTi (10). It has variable cross-sections with a triangular-shaped at the tip and S-shaped near the shaft which enhances its cutting efficiency and centering ability (10).

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Although there are several in vitro studies in the literature that assess the influence of heat-treatment procedure, autoclave process, irrigation solutions and design of the single file rotary systems on the cyclic fatigue resistance, there is not any study that evaluates the cyclic fatigue resistance of the single-file systems that were either never used or used in a certain number of times in clinic, yet. For this reason, the purpose of this study was to evaluate and compare the cyclic fatigue resistance of new or were previously used (U) only one time in the clinic for the root canal treatment of one vital mandibular molar with three root canals. The null hypothesis was that the cyclic fatigue resistance of OC and RB used for the first time is higher than the previously used ones in the clinic.

MATERIAL AND METHOD

The authors declare that this study does not carry the qualification of experiments on humans and animals, biological materials (biological fluids such as blood, urine, extracted human teeth and tissue samples, etc.) and the present study was conducted in accordance with the principles of the Declaration of Helsinki.

Forty One Curve (25/.06) and 40 Reciproc Blue (25/.08) which were either new or used previously in certain times were used for the study. The summary of the group contents was as follows; New One Curve instruments (OC-N), One Curve used in the clinic (OC-U), New Reciproc blue instruments (RB-N), and Reciproc blue used in the clinic (RB-U).

Backstage of New Instruments

All the new files (20 OC-N/20 RB-N) were inspected at 40× magnification under a surgical microscope (Leica M320, Leica microsystems, Wetzlar, Germany). If any defect or deformity was observed, replaced it with a new one. No imperfect instrument was detected.

Backstage of Used Instruments

For multiple use groups (20 OC-U/20 RB-U), the files were selected from the pool of the used files which were classified and stored according to the cases in the clinic. These instruments were inspected at 40× magnification under a surgical microscope before use. Those selected instruments where the ones which were used only once in the treatment of a vital mandibular molar with three root canals in the clinic. The instruments which were used in the treatment of four root canals, calcified root canals, open apices, the teeth with a strongly curved root, maxillary molars or teeth with necrotic pulp were excluded from the study. All the root canal instrumentation was performed by a single operator.

In OC-N group, the apical patency of the teeth was checked with #10 K file and the working length (WL) was determined with an apex locator. It was also checked with a periapical radiograph. The glide path was created with One G (300 rpm and 1.2 N/cm torque) according to the manufacturer's instructions. OC was used in the root canals with back-and-forth motion without any apical pressure. If any resistance was experienced, the file was removed. The flutes were cleaned and the root canal was irrigated with 2 mL 5.25% sodium hypochlorite. This procedure was repeated until reaching the WL.

In RB-N group, the apical patency was checked with #10 K file. WL was determined with an apex locator and checked with a periapical radiograph. The glide path was created by R-Pilot with original VDW Reciproc settings. The irrigant was placed to access the cavity and then RB was gently used in the canal with in-and-out pecking motion. After 3 pecking motions or any resistance was encountered, the instrument was removed from the canal and the debris on the flutes was cleaned with a sponge. The root canal was irrigated and this procedure repeated until reaching the WL.

The root canals were irrigated with 2 mL sodium hypochlorite (NaOCl) after every withdrawal of instruments and after the completion of root canal instrumentation. All the teeth were obturated with AH Plus and gutta-percha using cold lateral condensation. The temporary crown restoration was done with glass ionomer cement. If there was a visible deformation of the instrument after use in a clinical case, it was discarded from the study.

Before sterilization, the debris on the used instruments was cleaned with an alcohol sponge and then files were rinsed with distilled water. The instruments were packaged singularly and subjected to 1 cycle of autoclave sterilization at the temperature of 134°C for 17 minutes (11). Before performing the cyclic fatigue test, the autoclaved files were allowed to cool at room temperature (5).

Cyclic Fatigue Test

The artificial stainless-steel canal with 60° curvature, 5 mm radius, 1.5 mm width and 3.0 mm depth was used for the study. All the files were operated with a torque-controlled endodontic motor (X-smart Plus, Dentsply Maillefer, Switzerland) following the recommendations of the manufacturers; Reciproc Blue in "Reciproc ALL" mode (Note: The manufacturers claim that the Reciproc mode has 300 rpm) and One Curve with 350 rpm, 1.5 N/cm torque. After the sterilization of the instruments in RB-U group, the expanded ring on the file was removed as explained in the previous study (12). To both mimic the clinical conditions and minimize the friction between the files and the steel, 37°C distilled water with continuous irrigation was used. The files were freely rotated/reciprocated in the

static mode without any pecking motion until the fracture occurred. All the cyclic fatigue test steps were done by a second operator. The time to failure (TTF) was recorded in seconds by a chronometer both visually and audibly. The number of cycles to failure (NCF) for each instrument was calculated as follows; Time to fracture (in seconds) X The number of rotations or cycles/60 seconds.

Statistical Analysis

Data were analyzed with IBM SPSS Version 23. Conformity to normal distribution was examined using the Shapiro-Wilk test. One-way analysis of variance and Tamhane test for multiple comparisons were used to compare normally distributed NCF and TTF values according to the groups. Independent two sample t-test was used to compare normally distributed NCF and TTF values according to OC and RB instruments. Paired two sample t-test was used to compare NCF and TTF values according to time within groups. Evaluation of the variability of NCF and TTF between samples was performed by Weibull reliability analysis using Minitab 17 program. The significance level was set as $p<0.05$.

RESULTS

Comparison of NCF and TTF values between and within the groups are presented in **Table 1**. A significant difference was observed among the four groups and between RB-N/RB-U, OC-N/OC-U, RB-N/OC-N, and RB-U/OC-U, regarding TTF and NCF values ($p<0.05$). However, no significant difference was observed between OC-N and RB-U ($P>0.05$). While RB-N had the highest mean values of NCF and TTF, OC-U had the worst results in these two parameters ($p<0.05$).

Weibull reliability plots with the probability of survival values for NCF and TTF are shown in Figure 1 and Figure 2, and the related Weibull calculations (Weibull modulus, R2, predicted cycles, and time for 99% survival), mean values, and standard deviations are given in **Table 2**. According to Weibull calculations, around 1500 to 1750 rotations for RB-N, 1000 to 1250 for OC-N and RB-U, 750 to 1000 for OC-U were predicted at given speeds and 80% reliability. Predicted time for 99% survival were RB-N>RB-U>OC-N>OC-U. The TTF value of RB is greater than OC in both the new instrument groups and the used instrument groups.

DISCUSSION

While NiTi rotary instruments caused a revolution in endodontic therapy, it brought some concerns such as file separation. Considering the Pubmed literatures in the last decade, the finding of nearly 500 studies about instrument fractures and cyclic fatigue is the greatest proof of this situation. In a comprehensive literature review, there was no study evaluating cyclic fatigue resistance of RB and OC instruments that were either never used or used in certain times. Therefore, the aim of this was study was to evaluate the usage of these two single file instrument systems on CFR of the files. In the light of the findings, new instruments showed higher CFR than the used ones and so, the null hypothesis was accepted.

The main idea of single-use NiTi instruments is generally related to safety, workflow, and cross-contamination (13). Another drawback that creates this approach could be the autoclaving process and corrosion effect of NaOCl which is associated with file separation due to cyclic fatigue. However, there are studies in the

Table 1. Comparison of NCF and TTF values between and within the groups

		RB		OC		p
		Mean± Sd	Median (min. – max.)	Mean± Sd	Median (min. – max.)	
TTF	New	350.3±35.2	342.3 (301.7 - 408.7)	239.3±39.2	227.3 (189.4 - 308.6)	<0.001
	Used	248.5±24.7	245.5 (215.3 - 292.5)	165.3±23.3	165.5 (123.3 - 205.5)	<0.001
	p	<0.001		<0.001		
NCF	New	1751.7±175.8	1711.3 (1508.4 - 2043.4)	1395.7±228.9	1326.2 (1104.9 - 1800.2)	<0.001
	Used	1242.7±123.7	1227.7 (1076.7 - 1462.6)	964.3±135.7	965.7 (719.4 - 1198.6)	<0.001
	p	<0.001		<0.001		

Table 2. Weibull calculations and One-way analysis of variance results for NCF and TTF

	n	Speed (rpm)	NCF's Mean± Sd	TTF's Mean± Sd	Weibull modulus	R2	Predicted cycles for 99% survival	Predicted time (s) for 99% survival
RB-N	20	300	1751.7±175.8[a]	350.3±35.2[a]	12.6	0.88	1240	247.392
OC-N	20	350	1395.7±228.9[b]	239.3±39.2[b]	7.9	0.85	760	132.905
RB-U	20	300	1242.7±123.7[b]	248.5±24.7[b]	12.4	0.9	840	181.971
OC-U	20	350	964.3±135.7[c]	165.3±23.3[c]	8.3	0.98	600	100.195

Sd: Standard deviation, TTF: time to fracture, NCF: number of cycle to failure

literature showing that extra heat treatment occurred during autoclave sterilization has a positive effect on the cyclic fatigue resistance of the rotary files (14,15). In the present study, the used instruments were exposed to both extra heat due to autoclaving process and sodium hypochlorite during the shaping procedures. The positive effect of the former and the negative effect of the latter on the cyclic fatigue resistance may neutralize each other's effect and may have caused lower NCF and TTF values in the RB-U and OC-U groups.

According to the results of the present study: 1-OC file showed lower cyclic fatigue resistance than RB in both used and new instruments groups; 2- New RB and OC files had higher CFR than the used ones; 3- Used RB instruments had statistically better CFR than the new OC files. Gündoğar and Ozyurek (14) reported a parallel result with the first results of the present study that the CFR of RB was statistically higher than OC instruments. The second results of this study were also comparable with Pirani et al. (16) who showed the deformation on the tip and a certain degree of surface wear on the multi used single-file reciprocating systems. On the other hand, the third results are not comparable because there is no study on this topic, yet. In the authors' opinion, some properties such as M-Wire heat-treated alloy, the thin blue titanium oxide layer (17), and the small austenite grains on the surface (18) of RB may improve its flexibility and cyclic fatigue resistance and caused the third result. Above all, the main difference between the two instruments that gave rise to this finding is the motion kinematic. RB works on the principle of clockwise and counterclockwise motion which makes continuously progress the file towards the apex and reduce the CFR caused by taper lock, tension, and compression (12,16,19).

Today we know that separation of root canal instruments depends on several factors such as challenging root canal anatomy, size of the rotary file, tooth type, operator's experience, location of the canal, incorrect or excessive use of the files (20-22). These situations are tried to be imitated in all ex vivo studies. In our study, the tooth type in the used instrument groups was standardized as vital mandibular molars with three root canals. All the treatments were performed by a single experienced endodontist. The instruments had the same tip size but different tapers.

Although NiTi instruments started a new era in endodontics in the early 1990s, they have increased the cost for dentists. Only 6% of endodontists identified rotary instrument systems economically (23). A current study conducted in the USA showed that 74% of the endodontists reuse the NiTi rotary instruments (24). Another study showed that 25.6% of general dentists and 36.1% of endodontists used rotary tools up to 6-10 times and none of them mentioned single-use of these instruments (25). Patturaja et al. (23) indicated that 41.3% of endodontists used the files until a visible distortion was observed. When taking into consideration that most of the company launching the rotary instruments as disposable instruments, these results are quite dramatic. Since it is a disputable fact that clinicians want to provide the best healthcare to their patients, only economic reasons can explain this high reuse rate.

The reuse of engine-driven instruments has been the subject of studies in the literature (12,20,26) (Wolcott, Vieira, Bueno). Wolcott et al. (26) conducted a clinical study about the separation incidence of Protaper (Dentsply Maillefer, Ballaigues, Switzerland) rotary instruments and announced that the fracture resistance of the Protaper instrument was the same for the first 4 uses. Vieira et al (20) reported that the Protaper rotary instrument can be safely used by an experienced endodontist up to eight molars without a file separation. Bueno et al. (12) used the WaveOne and Reciproc files three times each on 358 posterior teeth in the clinic and reported only three instrument fractures. Although such studies encourage clinicians to reuse engine-driven instruments, it should be kept in mind that the instrument damage is cumulative (20,27). The mechanical properties and cutting efficiency of the instrument may also change due to multiple uses and sterilization procedures. Therefore, CFR is not the only factor that determines the number of instruments used.

CONCLUSION

Within the limitations of this in vitro study, the reuse of RB and OC instruments significantly reduced the cyclic fatigue resistance of the instrument. RB showed higher cyclic fatigue resistance than OC files in both new and used instrument groups. According to the results of this study, although RB seems safer to use a second time than OC, caution should be exercised in the second use of both devices in the clinic. The finding that the predicted cycles for the survival of RB-U to be similar to OC-N may pave the way to further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The authors declare that this study does not carry the qualification of experiments on humans and animals, biological materials (biological fluids such as blood, urine, extracted human teeth and tissue samples, etc.), observational and descriptive research without intervention (questionnaire, scale file scans, system model development, audio and video recordings) and does not require ethics committee approval.

Informed Consent: For this type of study, formal consent is not required.

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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The effect of normotensive arterial pressure on cerebral saturation during carotid endarterectomy

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ABSTRACT

Aim: Monitoring of cerebral perfusion during carotid endarterectomy helps to institute early intervention to prevent risk of ischemia during the operation. We aimed to evaluate if the disadvantages of high systemic pressure can be avoided by keeping arterial blood pressure below 150 mmHg during cross-clamping of the internal carotid artery by correlating our findings of NIRS values with clinical results.

Material and Method: This was designed as a prospective study that included elective patients scheduled for an operation of carotid artery stenosis under general anesthesia. A total of 60 patients operated on between August and October 2019 were included in the study.

Results: Patients' NIRS findings on the right and left hemispheres were analyzed separately to be able to differentiate any possible changes of NIRS of the cerebral hemisphere ipsilateral to the clamped carotid artery. The analysis of the right and left NIRS findings in these 60 patients revealed a significant decrease between intubation and incision NIRS values (p=0.008 and p=0.02 respectively).

Conclusion: Considering the NIRS findings and clinical observations of the patients in the postoperative period, we think that ICA endarterectomy operations may be safe to perform in a suitable patient group while the systolic arterial blood pressure is maintained below 150mmHg during cross-clamping.

Keywords: Carotid endarterectomy, near-infrared spectroscopy, stroke

INTRODUCTION

The technique of carotid endarterectomy is known to bear a relatively low perioperative stroke risk of around 0.7-1.5%. However, it remains to be a social health problem in terms of outcomes and long duration of treatment after stroke despite this relatively low risk (1). Monitoring of cerebral perfusion during carotid endarterectomy (CEA) helps to institute early intervention to prevent risk of ischemia during the operation.

The awake test under regional anesthesia is the most reliable method among all techniques of cerebral perfusion monitoring (2). However, different methods are used to monitor cerebral perfusion such as Transcranial doppler (TCD) and Near-Infrared Spectroscopy (NIRS) which have come into prominence in recent years (3). NIRS is a test that can measure changes in cerebral oxygenation continuously and non-invasively. NIRS measures regional oxygenation by interpreting oxyhemoglobin and deoxyhemoglobin signals. Normal cerebral NIRS values are around 60% (4). A decrease in regional cerebral oxygen saturation ($rScO_2$) below 40% or 25% decline from baseline may be indicative of cerebral ischemia (5).

The development of cerebral ischemia during carotid endarterectomy usually requires insertion of a shunt to prevent stroke. Another time honoured approach is to increase blood pressure during carotid clamping although there are no studies showing the effects of blood pressure changes on NIRS values at the time of clamping. There are also no studies related to the clinical results of blood pressure management strategies during carotid surgery. In our study, we examined patients with carotid artery stenosis operated on under general anesthesia. We aimed to evaluate if the disadvantages of high systemic pressure can be avoided by keeping arterial blood pressure below 150 mmHg during cross-clamping of the internal carotid artery by correlating our findings of NIRS values and clinical results.

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MATERIAL AND METHOD

This was designed as a prospective study that included elective patients scheduled for an operation of carotid artery stenosis under general anesthesia. Ethical approval and informed consent of the patients were obtained. The study was carried out with the permission of Ethics Committee of Okmeydanı Training and Research Hospital (ethic number: 48670771-514.10). A total of 60 patients operated on between August and October 2019 were included in the study. All patients underwent carotid CT angiography before the operation. Patients with unilateral or bilateral carotid artery stenosis above 70% were operated (6). All patients underwent a detailed preoperative cardiac evaluation.

Invasive radial arterial pressure monitoring was performed along with standard monitorization. The frontal region was cleansed before the patients were anesthetized and NIRS probes were placed on the left and right sides of the frontal region over the eyebrows. Preanaesthetic NIRS values of the right and left sides, systolic and diastolic arterial pressures, mean arterial pressure (MAP), peripheral oxygen saturation (SpO_2) were recorded. These values were continuously monitored and recorded at intubation, at incision, and every 5 minutes starting from the first minute of ICA cross clamping. The values after removal of carotid clamp, at the end of operation and after the extubation of patients were also recorded.

A standard longitudinal endarterectomy was performed in all patients with primary or patch closure of arteriotomy with ven graft, depending on the arterial diameter and surgeon preference. Our strategy to use shunting is when NIRS values drop 20-25% from baseline or when the clamp time is anticipated to be long due to the anatomy of the patient and the carotid lesion. 5000 units of systemic intravenous (IV) heparin were administered before the carotid arterial cross-clamp was placed with the aim of an ACT value above 200. The blood pressure was controlled in order to prevent its increase and systolic pressure was kept below 150 mmHg. Cross-clamp durations were recorded.

Patients were taken to the Cardiovascular Surgery Intensive Care Unit after the operation. Patients were followed up closely for hemodynamic and neurological sequelae. Major cerebral event was defined as cerebral infarction or cerebral hemorrhage within postoperative 30 days, while minor events were defined as transient hemiplegia or hemiparesis with no pathology detected on MR/CT.

Statistical Analysis

The data obtained from the study were analyzed using the SPSS (Statistical Package for Social Sciences) version 21 package program. In the descriptive statistics, categorical variables were expressed as numbers and percentages, while numerical variables were expressed as mean, standard deviation, median, minimum and maximum values, and interquartile range (IQR). The Shapiro Wilk test was used to evaluate the normality of variable distribution. In the comparison of two independent groups, the Student T-test was used for numerical variables with normal distribution while the non-parametric Mann Whitney U test was used when the assumptions for a parametric test were not met. The repeated measures ANOVA was used to evaluate repeated NIRS values during and after the operation. The Mauchly's test was used to test for sphericity and the Greenhouse-Geisser correction was used when the assumption of sphericity was violated. The correlational analysis was performed by Pearson's tests for parametric conditions while the Spearman's tests were used for non-parametric conditions. The type-1 error rate below 5% ($p<0.05$) was considered statistically significant.

RESULTS

Between August 2019 and October 2019, carotid endarterectomy was performed in a total of 60 patients (16 women (26.7%), 44 men (73.3%) with a mean age of 68.27±6.62 years. All patient data such as demographics, smoking status, concomitant diseases, neurological symptoms (previous stroke, transient ischemic attack (TIA), ejection fraction (EF), and the presence of bilateral carotid artery lesions were recorded. Bilateral lesion was defined as carotid stenosis >70% in both carotid arteries.

Hypertension was the most common concomitant disease among patients (46 patients (76.7%). Of the patients, 32 (53.3%) were asymptomatic. All our symptomatic patients were recently referred with new onset of symptoms which started within a few months. The mean ejection fraction (EF) was 55.17±5.49%. Patients' clinical characteristics and comorbidities are given in **Table 1**.

A total of 14 patients (23.3%) had bilateral carotid artery disease. 22 patients (36.7%) underwent right carotid endarterectomy and 38 (63.3%) underwent left carotid endarterectomy. Saphenous patch plasty was performed in 10 patients (16.6%). No postoperative mortality, myocardial infarction or permanent stroke was recorded, but one patient (1.6%) had a transient ischemic attack. Patient's MRI showed no lesions and it was thought to result from hypoperfusion although intraoperative NIRS values did not show a significant decrease. Patients' operative and postoperative characteristics are given in **Table 2**.

Table 1. Preoperative data of patients		
Age (years)	68.27±6.6	54-82
Ejection fraction	55.17±5.49	45-65
Variable	**n**	**% of total**
Sex		
Male	44	73.3
Female	16	26.7
Hypertension	46	76.7
Smoking	34	56.7
Diabetes mellitus	32	53.3
Coronary artery disease	24	40
Concomitant CABG	12	20
COPD	14	23.3
Symptom		
Stroke	16	26.7
TIA	12	20
Asymptomatic	32	53.3
Degree of right carotid stenosis (%)		
<50	16	26.7
50-70	15	23.3
70-99	30	50
Degree of left carotid stenosis (%)		
<50	14	23.3
50-70	2	3.4
70-99	44	73.3
Bilateral lesion	14	23.3

COPD: Chronic obstructive pulmonary disease, TIA: Transient ischemic attack

Table 2. Operative and postoperative data of patients		
Variable	**n**	**% of total**
Side of endarterectomy		
Right	22	36.7
Left	38	63.3
Arteriotomy closure		
Primary	50	83.3
Patch	10	16.7
Shunt use	0	0
Postoperative complications		
Transient hemiparesis	1	1.6
Local hematoma	4	6.6
Exploration for bleeding/hematoma	0	0
Myocardial infarction	0	0
Mortality	0	0
Variable	**Mean±SD (range)**	
Clamp duration (minutes)	20.97±7.52 (7-33)	
ICU stay (days)	1.3±0.65 (1-3)	
Hospital stay (days)	3.67±1.3 (3-8)	

ICU: Intensive care unit

The present study showed a correlation of right and left sided NIRS values with each other regardless of the side of the lesion. This was true for all time points of measurements including those after clamping. Therefore to simplify the comparison of results we gave only right and left sided results. The analysis of the right and left NIRS findings in these 60 patients revealed a significant decrease between intubation and incision NIRS values (p=0.008 and p=0.02 respectively). No significant change was detected in other duration times . Further analysis showed no difference between NIRS findings of right and left hemispheres at all points of recordings (**Table 3**).

Table 3. NIRS findings of right and left hemispheres at all points			
Measurement times	**Right sided NIRS**	**Left sided NIRS**	**p**
Awake	62.57±7.48	61.80±6.77	0.679
Intubation	63.20±9.96	63.50±9.73	0.859
Incision	59.87±8.92	61.00±8.85	0.623
Clamp on 1 min.	58.33±8.86	58.87±8.88	0.817
Clamp on 5 min.	59.03±8.42	58.30±8.52	0.739
Clamp on 10 min.	59.25±8.79	58.64±8.27	0.791
Clamp on 15 min.	60.13±7.97	60.33±7.29	0.925
Clamp on 20 min.	61.31±7.69	60.69±7.70	0.820
Clamp off 1 min	60.93±8.06	60.57±7.85	0.859
Clamp off 5 min	60.37±8.33	60.60±8.13	0.913
End of the operation	60.87±8.01	61.63±7.57	0.705
After extubation	62.97±8.28	63.37±8.18	0.851

NIRS: Near-infrared spectroscopy

Moreover, patients with bilateral lesions showed similar pattern of NIRS changes to the patients with unilateral lesions (e.g. right and left sided NIRS in those with bilateral lesions; at incision 59.6±8.5 and 59.8±8.7, after CC of carotid artery 58.1±8.6 and 58.5±8.7).

There was no statistically significant decrease of the NIRS values of either the right or the left hemisphere after cross-clamping of the ICA and no correlation was found between blood pressure indices and the NIRS values at that time (**Table 4**). In three patients NIRS values dropped more than 20% from baseline values just before declamping of carotid artery. The clamp was removed from carotid artery in a very short time and a shunt was not necessary in any. No neurological deficit was observed in any of these patients.

Table 4. Correlations of NIRS values and blood pressure indices after carotid clamping			
	Systolic blood pressure	**Diastolic blood pressure**	**Mean blood pressure**
Right sided NIRS	r=0.048	r=0.352	r=0.234
	p=0.800	p=0.057	p=0.214
Left sided NIRS	r=-0.089	r=0.258	r=0.098
	p=0.638	p=0.169	p=0.606

NIRS: Near-infrared spectroscopy

Almost half of our patients (53.3%) were asymptomatic. Awake NIRS values of symptomatic and asymptomatic patients were compared to see if there was any association between symptoms and global oxygenation of cerebral hemispheres. Mean awake NIRS value on the right hemisphere was 62.71±9.7 in symptomatic patients and was 62.4±5.1 in asymptomatic patients (p=0.9) where as on the left side these values were 60.7±8.4 in symptomatic and 62.6±4.9 in asymptomatic cases (p=0.4). Therefore no association was found between NIRS values and the presence of symptoms in patients with carotid disease.

DISCUSSION

Stroke is ranked as the third leading cause of mortality and morbidity worldwide. One-fifth of all strokes are caused by extracranial internal carotid artery stenosis (7). Carotid endarterectomy (CEA) is considered the gold standard treatment for stenosis of the internal carotid artery above 70% (6). However, despite the relatively low perioperative stroke risk of this surgical technique (0.7-1.5%), there is a high number of patients suffering from complications as 100,000 patients are operated per year in the United States only (1).

It is highly important to monitor cerebral perfusion during CEA in order to prevent neurological complications and to avoid unnecessary shunting in patients operated on under general anesthesia. Several cerebral monitoring techniques such as electroencephalogram (EEG), somatosensory evoked potentials (SSEP), Transcranial Doppler (TCD) and near-infrared spectroscopy (NIRS) are used during CEA operations (8-11).

Yu Wang et al. (12) found that the sensitivity and specificity of NIRS monitoring for intraoperative hypoperfusion were 64.3% and 90.0%, resulting in a strong consistency with TCD monitoring results. JM Findlay et al. (13) compared stump pressures and NIRS measurements in CEA operations in terms of shunt placement and reported that NIRS was more accurate than stump pressure measurement. We routinely use NIRS monitorization during CEA operations. It is both non-invasive and offers continuous assessment of cerebral perfusion in target brain tissue (14,15). We believe that one of the major advantages of NIRS monitorization is the spontaneous observation of changes in cerebral oxygenation in accordance to changes in cerebral circulation. This helps us to assess the factors changing cerebral oxygenation immediately and to react to solve any problems in a short time.

A common surgical practice is to maintain mean arterial pressure above baseline or arterial pressure above 150 mmHg by using vasoactive drugs during cross-clamping of the internal carotid artery (16). This is an unpublished rule of thumb and mean arterial pressure is often targeted to be approximately 20% above baseline. Although generally acceptable, this approach may not be suitable for all patients due to the undesirable outcomes such as cerebral hemorrhage and fatal cardiac events under systemic heparinization. This maneuver essentially aims to increase total cerebral blood flow as well as flow to the cerebral hemisphere ipsilateral to the clamped carotid artery by increasing collateral flow. However, our review of the literature revealed that this approach has not been justified by any study showing the clinical effects of management strategies for blood pressure during cross clamping of carotid artery. Moreover, there are no studies which evaluate the impact of keeping blood pressure close to normal on cerebral oxygenation during CEA. The analysis of the right and left NIRS findings in our patients revealed a significant decrease between intubation and incision NIRS values ($p=0.008$ and $p=0.02$ respectively). The NIRS value at the time of incision was essentially picked to represent the duration while patient was asleep but carotid artery was not manipulated yet. Therefore, we interpreted the difference in NIRS values between awake state (and at the time of intubation) and at the time of incision as the impact of general anaesthesia and its effects on cerebral perfusion.

The analysis of NIRS values at other time points showed no significant difference while patient was asleep. It is important to emphasize that there was no statistically significant decrease in the NIRS values of either the right or the left hemisphere after cross-clamping of the ICA. Besides, no correlation was found between blood pressure indices and the NIRS values at that time (**Table 4**). The latter may be the result of blood pressure control below 150 mmHg and therefore relatively stable blood pressure during cross clamping and after the clamp was removed. Nevertheless, the absence of reduction in NIRS values after carotid clamping suggests that CEA may safely be performed without increasing blood pressures during clamping. This is further supported by the lack of any neurological complications in this patient group. These findings are especially important for a select patient group for whom the control of blood pressure is particularly important which include those with aortic and peripheral aneurysms at risk of rupture and those at risk of cardiac events. This study is a first study to show changes in NIRS values during various stages of carotid endarterectomy and more importantly effect of blood pressure during these stages. These results may help to develop a blood pressure strategy in different patient groups.

We did not use carotid shunting in any patients in this study group. We prefer to use shunting when NIRS values drop 20-25% from baseline and when the clamp time is anticipated to be long due to the anatomy of the patient and the carotid lesion. Although this is not our primary end point, the absence of any neurological complications and any serious decline in NIRS values suggests that carotid endarterectomy can be performed safely without shunting while keeping normal blood pressures.

We performed a subanalysis to see if there was any association between NIRS values and the presence of symptoms in patients. We couldn't find any difference in awake NIRS values of symptomatic and asymptomatic patients. This finding may imply that the symptoms are probably not related to the global cerebral oxygenation in resting state but to either instant changes in cerebral flow or embolic events.

CONCLUSION

Considering the NIRS findings and clinical observations of the patients in the postoperative period, we think that ICA endarterectomy operations may be safe to perform in a suitable patient group while the systolic arterial blood pressure is maintained below 150 mmHg during cross-clamping. It may thus be possible to prevent complications caused by high arterial blood pressure under systemic heparinization. Further studies with larger patient groups are needed for more accurate results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ethics Committee of Okmeydanı Training and Research Hospital (Ethic number: 48670771-514.10).

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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Our results of cardiac surgery performed with a right infra-axillary mini thoracotomy

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ABSTRACT

Aim: The aim of the study is to share the technique and results of heart surgery performed with right infra-axillary mini thoracotomy.

Materyal and Method: Between March 2013 and July 2014, 16 heart operations were performed with right infra-axillary mini thoracotomy incision. Patient data were analyzed retrospectively. These are 6 mitral valve replacement (MVR), 3 aortic valve replacement (AVR), 2 AVR+MVR, 2 atrial septal defect (ASD) repair, 1 myxoma excision. One of them is AVR+tricuspid ring annuloplasty operation and MVR performed due to reoperation. Radiofrequency (RF) ablation was also applied to 1 patient who underwent MVR due to atrial fibrillation. In these operations, thoracotomy was performed through the right anterior infra-axillary line. A parabolic incision of 6-8 cm was made at the 3rd intercostal space (ICA) for aortic valve interventions and at the 4th ICA level for other valve interventions. No special surgical instruments were used in the operations except standard surgical instruments. All cannulation procedures were carried out through the existing thoracotomy incision.

Results: No mortality or morbidity was observed as a result of the operations. The mean extracorporeal circulation (ECC) time was 95±11 minutes, and the mean cross clamp (CC) time was 61±9 minutes. There was an average of 380±35 cc drainage. Extubation time was 7±2.3 hours on average. All patients were taken to the service after 1±0.5 day of intensive care treatment. They were discharged from the hospital in an average of 6±1.5 days. Incision site infection was not observed in any patient. No patients were revised due to bleeding. One patient who was ablated for atrial fibrillation (AF) developed postoperative AF again. He was discharged with medical treatment and speed control.

Conclusion: In this study, we think that heart surgeries with right infra-axillary mini thoracotomy incision may be a good alternative to standard sternotomy and other minimally invasive methods in heart surgery. With standard surgical instruments and cannulation through a single incision, not only the mitral valve but also the aortic valve, tricuspid valve and other cardiac pathologies can be easily intervened. However, very good results are obtained in patients cosmetically.

Keywords: Open heart surgery, right infra-axillary thoracotomy, heart valve replacement

INTRODUCTION

Today, median sternotomy is used as the gold standard incision for heart surgeries (1). For the first time, Lillehei et al. (2) performed a mitral valve replacement under cardiopulmonary bypass (CPB) with a right thoracotomy incision. In recent years, this incision is preferred especially in reoperations. Minimally invasive methods have long attracted the attention of cardiac surgeons due to reasons such as less need for blood transfusion, low morbidity, good cosmetic results, short intensive care and hospital stay (3).

Recently, the development of thoracoscopic and robotic technologies in the health sector has also been reflected in minimally invasive cardiac surgery. However, their financial costs, long operation times and difficult learning process are still a major problem. Among the increasingly minimally invasive approaches, mini sternotomy, anterolateral thoracotomy, and posterolateral thoracotomy stand out. (4-6). Also, right infra-axillary mini thoracotomy is another method preferred in recent years due to its cosmetic results and surgical vision (7).

In this study, we aimed to share the results of heart surgeries performed using the right infra-axillary mini thoracotomy method, which is one of the minimally invasive methods.

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MATERIAL AND METHOD

The results of 16 open heart surgeries performed using right infra-axillary mini thoracotomy method between March 2013 and July 2014 were retrospectively analyzed. Patients' data were obtained from hospital digital data and patient files. The study was conducted in accordance with the Helsinki Declaration of 1964 and later amendments or comparable ethical standards, and permission was obtained from the institution. All patients have a consent form for surgery. The study was approved by the EMSEY Hospital Ethics Committee (date: 10.8.2014, no: 2014/4).

By examining the data of the patients; diagnoses, demographic data, comorbidity, types of surgery, CC and ECC times, postoperative bleeding, amount of blood products used, reoperation due to bleeding, mechanical ventilation durations, intensive care and hospital stay, incision infection, hospital and postoperative 3-month mortality rates were evaluated.

The operations were 6 MVR, 3 AVR, 2 AVR and MVR, 2 ASD repair, 1 myxoma excision. Two patients who had previous valve replacement were operated due to paravalvular leakage. AVR and TA was applied to one of them, and MVR was applied to the other one.

This method was not applied to patients who had previously undergone thoracic surgery, received radiotherapy and whose body mass index was over 30 kg/m^2. Operations carried out by the same team.

Electrocardiography, invasive artery monitoring, central venous pressure monitoring and pulse oximetry monitoring were performed for all patients who were taken into the operating room. A foley urinary catheter and nasal temperature probe were routinely used before the operation. In addition, external defibrillation pads were also attached to each patient. For anesthesia, 30 minutes before the operation, 0.05 mg/kg morphine was administered intramuscularly for premedication. Intravenous 0.4 mg/kg etomidate was given for induction. Then, the neuromuscular blocker 0.8 mg/kg rocuronium was administered. Sevoflurane anesthetic was started at 2-4% MAC value. All patients were intubated endotracheally with a double lumen intubation tube. The patient was placed in a 60° anterior oblique position with the right thorax above, and a support pad was placed under the thorax to raise the thorax. The right arm was abducted approximately 120° and was suspended over the head by breaking 90° at the elbow. A 6-8 cm parabolic skin incision was made from the right anterior axillary line, targeting the 3rd intercostal space (ICS) for aortic valve interventions and the 4th ICS for other interventions. The incision started at the 2nd ICA level and was extended to the 5th ICS level. The size of this incision may vary according to the height and weight of the patient. Except for the intercostal muscles, no muscles or ribs were cut. When the skin and subcutaneous tissues reached the target costal space, right lung ventilation was stopped and the thorax was entered. Conversion to median sternotomy was not required in any patient. The lung was excluded and the heart was reached. The pericardium was opened 2 cm above the phrenic nerve. The pericardium was hung from various points on the thorax wall with the help of multiple 1/0 silk sutures (**Figure 1**). In this way, the most appropriate exposure for the surgical procedure was achieved by ensuring that the heart came close to the right thorax. Cannulation sutures were placed. After heparinization, when the appropriate activated coagulation time (ACT) reached the value (ACT≥400 sec), the standard cannulation procedure was started. Arterial cannulation was performed from the aorta in all cases. A single two-stage venous cannula was placed through the right atrium auricle for aortic valve surgery (**Figure 2**). For others, bicaval venous cannulation was performed. Vena cava superior and vena cava inferior turned. Routinely, cardiac arrest was achieved with standard isothermal blood cardioplegia and CPB was initiated. For mitral valve interventions, the mitral valve was reached through the right atrium using the transseptal route. Mechanical prosthetic valves were used for valve replacements. Standard surgical instruments were used in all of these operations. There was no need to use an extra surgical instrument. Cannulation was done through the thoracotomy incision. The cross clamp was placed through the middle thoracotomy incision. Temporary pacemaker wire was placed in all patients. Temporary pacemaker wire was placed in the heart before the cross clamp was lifted and the heart was working. At the end of the operation, a thoracic drain was placed in the thorax from the 7th ICA. The ribs were approximated with three absorbable sutures. Tissue layers were sutured in accordance with anatomical folds. A skin suture line of approximately 6-8 cm was formed (**Figure 3**). After the operation, the patients were taken to intensive care by changing the double lumen intubation tube with a single lumen tube.

SPSS for Windows version 15.0 pocket program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Arithmetic mean and standard deviation as measures of central tendency in numerical data with normal distribution; Median, minimum and maximum values were used in numerical data that did not show normal distribution. Categorical variables are shown as percentages.

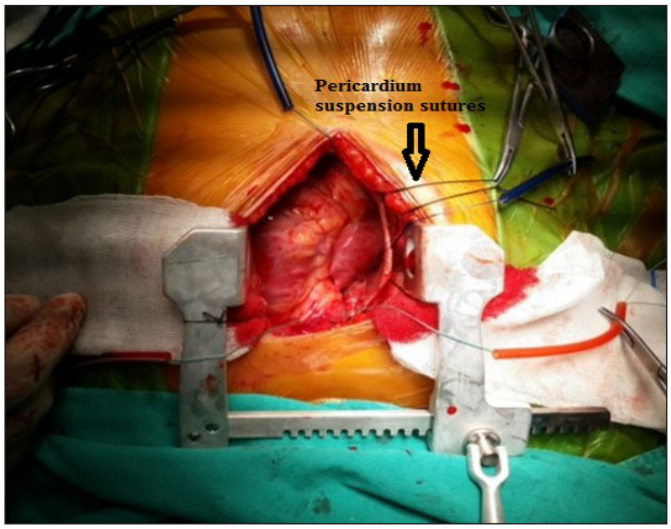

Figure 1. Right infra-axillary thoracotomy

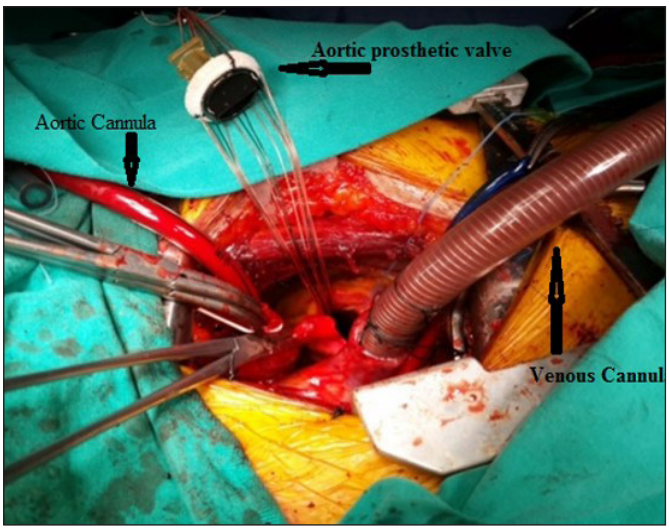

Figure 2. Cannulation and aortic valve replacement

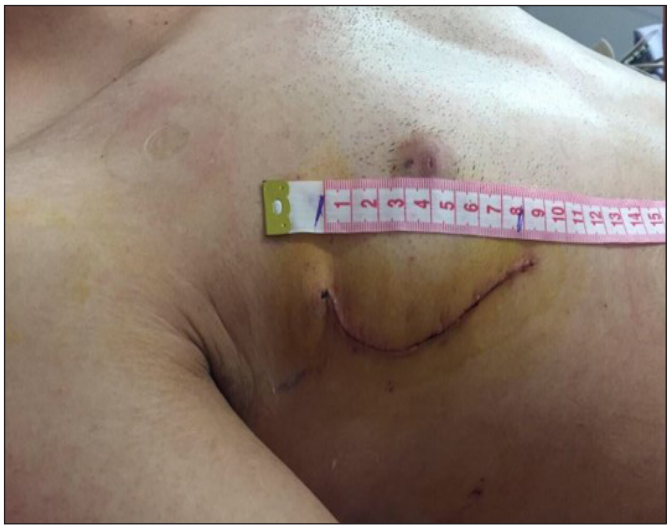

Figure 3. Thoracotomy skin incision

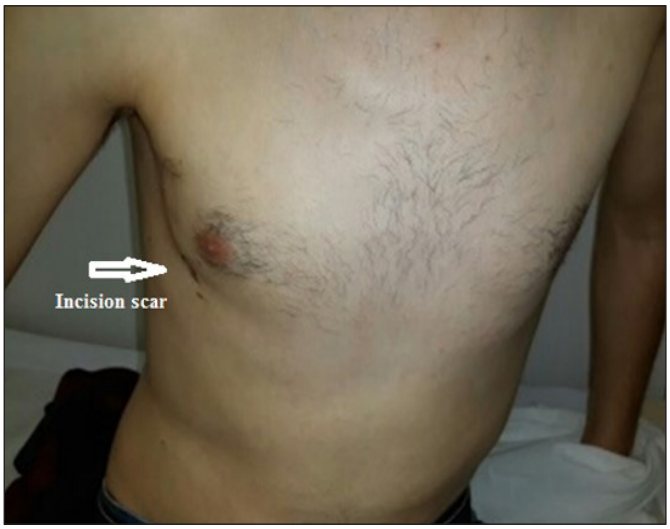

Figure 4. The cosmetic result of the postoperative incision

RESULTS

A total of 16 patients underwent cardiac surgery with a right infra-axillary mini thoracotomy. 9 of them were men and 7 of them were women. The average age was 47.5±11.2 years, the mean weight was 73.4±9.2 kg, and the average body mass index was 25.3±3.2 kg/m². Diabetes mellutus, renal insufficiency and hypertension were not present in any of the patients. Demographic and comorbidity data are shown in **Table 1.**

Table 1. Preoperative and comorbitide data

	MVR	AVR	AVR+MVR	AVR+TRA Reoperation	MVR Reoperation	ASD	MYXOMA
Age (mean)	54±11.4	45±8.5	61±12.1	65±14.3	75	22	31
Weight (mean)	63.16	87	77	75	80	67.5	71
Height (mean)	1.68	1.8	1.61	1.72	1,78	1.68	1.74
Male	3	1	1	1	1	1	1
Female	3	2	1	-	-	1	-
BMI (mean)	22.4	26.9	29.7	25.4	25.24	23.9	23.5
BSA (mean)	1.72	2.07	1.81	1.88	1,95	1.77	1.85
COPD	2	1	1	-	-	-	-
HT	-	-	-	-	-	-	-
DM	-	-	-	-	-	-	-
CRF	-	-	-	-	-	-	-

AVR: Aortic valve replacement, MVR: Mitral valve replacement, ASD: Atrial septal defect,
TRA: Tricuspid annuloplasty, BMI: Body mass index, BSA: Body surface area, COPD: Chronic obstructive pulmonary disease, HT: Hypertension, DM: Diabetes mellitus, CRF: Chronic renal failure.

Mitral valve replacement, AVR, AVR and MVR, ASD repair, myxoma excision, reoperation AVR+TRA, reoperation MVR operations were performed on the patients. The data are shown in **Table 2.**

Table 2. Cardiac pathologies and surgeries performed

			n
Valve diseases	MS		4
	MF		2
	AF		1
	AS		2
	AF+MF		1
Reoperation	Paravalvular aortic valve leak+TF		1
	Paravalvular mitral valve leak		1
Other heart diseases	ASD		2
	Miksoma		1
Operations	Single valve surgery	MVR	6
		AVR	3
	Double valve surgery	AVR+MVR	2
	Others	ASD repair	2
		Myxoma excision	1
		Reoperation AVR+TRA	1
		Reoperation MVR	1

MS: Mitral stenosis, MF: Mitral failure, AS: Aortic stenosis, aortic gailure, TF: Tricuspid failure, AVR: Aortıc valve replacement, MVR: Mitral valve replacement, ASD: Atrial septal defect.

The mean cannulation time was 50±9 minutes, the mean ECC time was 95±11 minutes, and the mean CC time was 61±9 minutes. The mean extubation time was 7±2.3 hours. Incision site infection did not develop in patients. The intensive care stay of the patients was 1±0.5 days and the hospital stay was 6±1.5 days. No mortality was observed in the 3-month follow-up of the patients. Operative and postoperative data are shown in **Table 3.**

Table 3. Intraoperative and postoperative data

	mean±std
Average cannulation time (min)	50±9
ECC time (min)	95±11
Cross clamp time (min)	61±90
Intensive care stay (day)	1±0.5
Hospital stay (day)	6±1.5
Average drainage (cc/day)	380±35
Blood transfusion (ES/unit)	1.57±1.13
Extubation time (hrs)	7±2.3
Postoperative incision infection	0
Postoperative atrial fibrillation	1
Low cardiac output syndrome	0
Exploration by bleeding	0
Stroke	0
Sepsis	0
Pulmonary complication	0
Postoperative pacemaker	0
Renal insufficiency	0
Hospital mortality	0

ECC: Extra corpareal circulation

DISCUSSION

The most important advantage of right infra-axillary mini thoracotomy is the minimum injury that occurs during surgery. In addition to the small incision made, reaching the thorax through the muscle tissue without cutting any muscle or bone tissue during thoracotomy causes the surgical injury to be minimal (8). Another advantage is that a very good cosmetic result is obtained with a short skin incision under the armpit. There is no visible incision like in the median sternotomy and anterolateral thoracotomy incision (**Figure 4**). Kaneda et al. (9) conducted a study with right infra-axillary thoracotomy on 20 patients. They stated that this technique could minimize skin incision and improve both cosmetic and functional results compared to the methods previously described. In addition, incisions in the foreskin tend to result in hypertrophic scarring, such as in the shoulder or groin areas. Anterior chest surgical incisions can be easily seen and attract attention. This may be a cause of social maladjustment in patients (10).

Heart surgeries with thoracotomy first started as simple ASD repair operations (11). Despite the advantages of minimally invasive cardiac surgery, its development in the world is slow. Only 5 to 10% of isolated valve operations in the USA and Europe have been performed using a minimally invasive approach (12). We have shown that procedures such as ASD, myxoma excision, reoperations, AVR, MVR or double valve interventions, as well as RF ablation for atrial fibrillation can be easily performed with this method.

Today, femoral artery-vein cannulation is frequently performed in open heart surgery performed with minimally invasive methods) (13,14). In this method, cannulation is performed from a single incision without the need for femoral cannulation and the surgery can be carried out more practically. Thus, incision infection that may originate from the inguinal region and injuries to the artery (dissection in the femoral artery, thromboembolism in the artery or vein, aneurysm, bleeding, leg ischemia, incision infection) will be avoided (15). Although surgeons have prejudices about the approach to the aorta, an aortic exposure similar to the median sternotomy is obtained. The aorta can be safely intervened. Another advantage is that it does not require special surgical instruments during the procedure and there are no additional costs caused by these. It is an easy method to learn and practice compared to other minimally invasive methods such as thoracocospopic and robotic surgeries.

Although it seems like a disadvantage that the pre-cannulation preparation period takes longer than the standard sternotomy method during the learning period, this period is shortened with the practical application in the studies. Masuda et al. (16) performed 126 isolated

aortic valve surgeries using right infra-axillary mini thoracotomy. In this study, they stated that this learning process could occur with approximately 40 patients. We think this process will be completed in much less cases. In our study, the mean cannulation time was determined as 50±9 minutes. Since an exposure similar to median sternotomy can be obtained, total ECD and CC times are not very different from each other (17). In the study, the mean duration of ECD was 95±11 minutes, and the mean duration of CC was 61±9 minutes.

Re-exploration due to bleeding and tamponade leads to morbidity such as increased blood transfusion, development of infection, prolonged ventilation, neurological problems and renal failure. Technological advances in cardiac surgery and the development of CPB techniques and equipment have reduced these rates. In recent studies, these rates have decreased to around 3% (18). In median sternotomy, bone incision and related increase in the amount of bleeding are found less in the postoperative infra-axillary method (19). While the mean amount of bleeding in cardiac surgery performed with sternotomy was 589±136 cc/day in studies performed, they were found to be 351±63 cc/day in cases performed with thoracotomy (20). In our study, the average postoperative drainage amount was found to be 380±35 cc/day, and there were no patients undergoing revision due to bleeding and tamponade.

In cardiac surgery cases requiring CPB, coagulation factors and platelets are consumed depending on the size of the surgical area, overactivation of the hemostatic system, hemodilution, inflammation and the mechanical effects of the perfusion system. Therefore, it is inevitable that the need for transfusion will increase in the perioperative and postoperative period (21). Transfusions performed in cardiac surgery, where blood use is needed so much, also brings complications. The amount of bleeding brings along the negative effects of blood transfusion (22). Postoperative bleeding may be excessive as a result of the instability of the periosteum and bone in sternotomy. Infra-axillary thoracotomy is a less traumatic method that can be reached to the thorax and heart without cutting muscle and bone tissue. (23). Kalender et al. (24), in their study on 137 patients, showed that blood transfusion of 2 units or more in cardiac surgery negatively affected mortality and morbidity. In our study, it was found that an average of 1.57±1.13 units of erythrocyte suspension was used. In heart surgeries performed with sternotomy, this rate is above 2 units on average (25).

Respiratory support with a prolonged ventilator may disrupt the stability of the sternum, as well as cause post-extubation pain and increased secretion, atalectasis and fever as a result of respiratory muscle fatigue. Long-term physiotherapy is required to prevent these complications and to strengthen the respiratory muscles. As a result, the intensive care treatment process may be prolonged. Respiratory physiology changes and related complications due to thoracotomy, stay in the ventilator and intensive care periods are shorter (26). In this study, the mean duration of stay in the ventilator was 7±2.3 hours, the duration of stay in the intensive care unit was 1±0.5 days and the hospital stay was 6±1.5 days. These results are consistent with the literature. One of the most feared complications in open heart surgery is sternum infection. Sternal detachment, superficial surgical site infection and deep surgical site infections were evaluated in patients who underwent standard sternotomy and were found to be 1%, 3.4%, and 2.5%, respectively (27). The risk of sternal dehiscence, sternal infection and mediastinitis is protected with right infra-axillary thoracotomy.

Studies have shown that the right infra-axillary thoracotomy incision is mostly made vertically (28,29). We performed the thoracotomy skin incision parabolically to reduce skin necrosis due to tension caused by the thorax retractor. This also gave us a larger exposure area. In addition, since it is an incision suitable for the anatomy of the breast tissue in women, it allows penetration into the thorax without touching the breast tissue. This provides an advantage in wound healing. While sternotomy surgery requires a lying position, sternal support brace and movement restriction in order to prevent sternal dehiscence during the healing process, infra-axillary thoracotomy does not require any movement restriction after 24 hours. This provides convenience in respiratory rehabilitation (coughing, secretion removal). As a result, it enables the patient to adapt more easily to daily life (30). In a meta-analysis performed MVR operation with right mini thoracotomy, the mortality rates were similar to those performed with MVR with median sternotomy (31). In the 3-month follow-up of the patients, echocardiography findings were normal and no mortality was observed.

Limitations: The first limitation of the study is that it is a single center. The second limitation is that it was done retrospectively. The third limitation is the small number of patients in the study group.

RESULT

We think that the right infra-axillary mini torochatomy method can be used safely in many heart surgeries including multiple valve procedures. In addition to a good cosmetic result, we recommend the right infra-axillary mini thoracotomy method in suitable patients to reduce intensive care and hospital stays, protect from sternum infections, return to normal daily activities without the need for movement restriction, and a quality life physically and psychologically.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission EMSEY Hospital of Research Ethics Committee (permission date: 10.08.2014, decision no: 4).

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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Evaluation of effect of frailty on warfarin compliance among older patients

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ABSTRACT

Aim: Warfarin has a narrow therapeutic index. We aimed to evaluate the effect of frailty on compliance to warfarin pharmacotherapy and the attainment of international normalized ratio (INR) goals in patients aged over 65 years receiving warfarin.

Material and Method: We recruited 473 elderly subjects aged 65 years and over. Indications for the administration of warfarin and INR values were recorded. All patients were assessed according to the Clinical Frailty Scale of the Canadian Health and Aging Study. Whether or not the target of INR values and the degree of frailty were compared.

Results: Of the 473 patients, 401 patients were assigned to the non-frail group and 72 patients were assigned to the frail group. When patients were compared according to the attainment of target INR values, a negative correlation between frailty and numerical INR values was observed.

Conclusion: It can be said that the presence of frailty negatively affects reaching the target INR values in patients over 65 years of age using warfarin. If patients in this population are indicated for warfarin use, it is necessary to be sure that INR monitoring will be done well.

Keywords: Frailty, geriatric population, warfarin

INTRODUCTION

The American Medical Association refers those with 'frailty' as 'the patient group posing the most complicated and coercive problems for physicians and all health professionals' (1-3). Increased understanding among healthcare professionals regarding frailty in elderly patients may improve the follow-up and management of frail individuals.

A limited number of previous clinical studies have demonstrated that frailty may affect drug compliance in elderly patients (4,5). Warfarin is the most widely used oral anticoagulant in the world. Warfarin, which acts by inhibiting vitamin K epoxide reductase, is used in primary and secondary prevention of thromboembolic diseases. However, studies regarding compliance to treatments such as warfarin requiring a close follow-up are yet to be reported (4,5). Scoring systems commonly used at the initiation of warfarin therapy do not take into account frailty, other than patient age.

In the present study, we aimed to determine the severity of frailty in elderly patients receiving warfarin and to evaluate the relationship between frailty and compliance to pharmacotherapy.

MATERIAL AND METHOD

The study protocol was explained to all participants. Informed consent was obtained from all patients. The study was conducted in accordance with the Declaration of Helsinki and Ethical approval was received from the Health Science University, Ümraniye Training and Research Hospital Ethics Committee, who also approved the study protocol (approval no. 18467, 23.11.12).

In total, 473 patients aged over 65 years who were receiving warfarin treatment for any reason were included in the present study. Exclusion criteria for the study included all patients who did not want to participate in the study, had congenital disability, and had any active diagnosis of malignancy. The medical

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history of all patients was recorded and international normalized ratio (INR) levels were analyzed during three months. The therapeutic INR range was defined as 2.5-3.5. Turkish version of The Criteria of Canada Health and Age Study (CTHAS) were applied to all patients to assess frailty (2). All criteria components were assessed by the same person using a proper and clear tone of voice such that patients were able to fully understand. The degree of frailty was determined according to the ability to walk without assistance, perform activities of daily living without assistance, the presence of urinary incontinence (UI), the presence of stool incontinence (FI), impairment of cognitive function without dementia, the presence of dementia and complete dependence during mobilisation. Patients with only urinary incontinence were considered to be mildly fragile. The need for help in mobility and activities of daily living, cognitive impairment without dementia, and inability to incontinence urine and feces were considered to be moderately to severely fragile.

Statistical Analysis

All statistical analyses were performed using the SPSS 20.0 (Statistical Package for the Social Sciences) IBM Software program. In addition to definitive statistical measures (mean, standard deviation), the Mann–Whitney U test, Kruskal–Wallis test and Spearman's correlation were used to compare non-normally distributed data. The chi-square test was used to compare qualitative data. Statistical significance was assessed at $p<0.05$.

RESULTS

The mean age patients were 71.68±0.23 years (non-frail group), 77.32±0.12 years (mild frail group) and 79.68±0.43 years (moderate/severe frail group). Of all 473 included patients, 263 (55.6%) and 210 (44.4%) were females and males, respectively. Patient characteristics and diagnoses are summarized in **Table 1**.

The severity of frailty was found to be related to walking condition and age. The mean age of patients able to walk unaided was lower than for patients unable to walk unaided. Ability to walk unaided, urinary continence, stool continence, cognitive state, presence of dementia, Daily Life Activity (DLA) and complete dependency in performing DLA were all positively related to patient age **(Table 2).**

A significant relation between the severity of frailty and the attainment of therapeutic INR target was observed in patients with mild frailty or moderate/severe frailty and INR targets <2.5 or 2.5–3.5. A greater relation was observed in patients with target INR >3.5. Whereas 7.3% and 15.3% of patients with moderate/severe frailty failed to attain target INR values of <2.5 or >3.5, respectively, and 2.9% of patients with this degree of frailty did achieve the target INR value. A significant relation was observed between DLA and the attainment of INR targets. The proportion of respondents that reported the ability to perform DLA was higher among patients who achieved INR target values compared to patients with INR values <2.5 or 2.5–3.5. A significant relation was observed between urinary incontinence and the attainment of INR target values. The proportion of respondents with urine incontinence was higher among patients with INR values >3.5 compared to patients with INR values <2.5 or 2.5–3.5. A significant relation was observed between stool incontinence and the attainment of INR target values. The proportion of respondents with stool incontinence was higher among patients with INR values >3.5 compared to patients with INR values <2.5 or 2.5–3.5. A significant relation was observed between dementia and the attainment of INR target values. The proportion of respondents with dementia was higher among patients with INR values >3.5 compared to patients with INR values <2.5 or 2.5–3.5 **(Table 3).**

A significant relationship was observed between the severity of frailty in patients and the achievement of the therapeutic INR target. In patients with increased frailty, the rate of reaching the therapeutic INR value was low. In patients with moderate to severe frailty, only 6 of 31 (19.35%) patients achieved the therapeutic INR. In 41 patients with mild frail, the rate of achieving therapeutic INR was higher with 20 (48.71%) patients than moderate to severe frail patients.

Table 1. Overall patient characteristics and diagnoses

		N	%
Gender	Female	263	55.6%
	Male	210	44.4%
Diagnosis	Atrial fibrillation	299	63.2%
	Valve replacement	112	23.7%
	Deep vein thrombosis	17	3.6%
	Pulmonary embolism	34	7.2%
	Atrial thrombus	6	1.3%
	Cerebrovascular disease	2	0.4%
	Valve + neonatal valve repair	2	0.4%
	Af + atrial thrombus	1	0.2%
Frailty	Normal	401	84.8%
	Frail	72	15.2%
Dla*	No	37	7.8%
	Yes	436	92.2%
Urine	No	453	95.8%
	Yes	20	4.2%
Stool	No	458	96.8%
	Yes	15	3.2%
Cognitive	No	469	99.2%
	Yes	4	0.8%
Dlacomdep**	No	442	93.4%
	Yes	31	6.6%
Dementia	No	459	97.0%
	Yes	14	3.0%
Inr target	On-target	296	62.6%
	Off-target	177	37.4%

*: Daily life activity, **: Complete dependency in performing DLA

Table 2. Comparison of variables according to frailty degree

		Frailty degree						
		Non-frail		Mild		Moderate/severe		P-value†*
		N	%	N	%	N	%	
Walking	No	3	0.7%	41	100%	31	100%	
	Yes	398	99.3%	0	0%	0	0%	<0.001**
Dla§	No	1	0.2%	6	14.6%	30	96.8%	
	Yes	400	99.8%	35	85.4%	1	3.2%	<0.001**
Urine	No	401	100%	37	90.2%	15	48.4%	
	Yes	0	0%	4	9.8%	16	51.6%	<0.001**
Stool	No	400	99.8%	41	100%	17	54.8%	
	Yes	1	0.2%	0	0%	14	45.2%	<0.001**
Cognitive	No	400	99.8%	41	100%	28	90.3%	
	Yes	1	0.2%	0	0%	3	9.7%	<0.001**
Dla-comdep¶	No	400	99.8%	37	90.2%	5	16.1%	
	Yes	1	0.2%	4	9.8%	26	83.9%	<0.001**
Dementia	No	401	100%	41	100%	17	54.8%	
	Yes	0	0%	0	0%	14	45.2%	<0.001**
Sex	Female	224	55.9%	21	51.2%	18	58.1%	
	Male	177	44.1%	20	48.8%	13	41.9%	0.816
Age	Mean±sd	71.68±0.23		77.32±0.12		79.68±0.43		<0.001‡**

*: p< 0.05; **: p< 0.01,
§: daily life activity, ¶: complete dependency in performing dla
†: chi-square test; ‡: kruskal–wallis test

Table 3. Comparison of the variables according to inr groups

		Inr value						
		<2.5		2.5-3.5		>3.5		P-value
		N	%	N	%	N	%	
Frailty	No	164	85.4%	183	87.6%	54	75%	
Degree	Mild	14	7.3%	20	9.5%	7	9.7%	
	Mod/severe	14	7.3%	6	2.9%	11	15.3%	0.0006†**
Walking	No	28	14.6%	28	13.4%	19	26.4%	
	Yes	164	85.4%	181	86.6%	53	73.6%	0.078†
Dla§	No	15	7.8%	11	5.3%	11	15.3%	
	Yes	177	92.2%	198	94.7%	61	84.7%	0.024†*
Urine	No	184	95.8%	209	100%	60	83.3%	
	Yes	8	4.2%	0	0%	12	16.7%	<0.001†**
Stool	No	185	96.4%	209	100%	64	88.9%	
	Yes	7	3.6%	0	0%	8	11.1%	<0.001†**
Cognitive	No	189	98.4%	209	100%	71	98.6%	
	Yes	3	1.6%	0	0%	1	1.4%	0.201
Dlacomdep¶	No	180	93.8%	199	95.2%	63	87.5%	
	Yes	12	6.2%	10	4.8%	9	12.5%	0.072
Dementia	No	189	98.4%	206	98.6%	64	88.9%	
	Yes	3	1.6%	3	1.4%	8	11.1%	<0.001†**
Sex	Female	108	56.2%	113	54.1%	42	58.3%	
	Male	84	43.8%	96	45.9%	30	41.7%	0.79
Age	Mean±sd	72.91±0.45		72.43±0.32		72.9±0.40		0.691##

*: p< 0.05; **: p< 0.01; †: chi-square test; ‡: kruskal–wallis test; §: daily life activity, ¶: complete dependency in performing dla

DISCUSSION

The most important finding of the present study was the observation that the proportion of patients attaining INR target values was lower in frail patients aged over 65 years using warfarin than non-frail patients aged over 65 years using warfarin. Frailty was seen to negatively affect the attainment of INR target values in patients receiving warfarin.

The increasing elderly population has increased the prevalence of age-related diseases, and this makes the concept of frailty an increasingly important clinical issue. A total of 9008 patients aged over 65 years were evaluated in the (CHAS) with the measurements of clinical parameters such as walking without assistance, performance of daily life activities without assistance, urinary and stool incontinence, impairment of cognitive function and dementia used to assess frailty (6). In the present study, the prevalence of frailty was lower than reported in the Cardiovascular Health Study; with the prevalence of frailty determined as 0.7%, 2% and 4% in the 65–74 years age group, 75–84 years age group and the

group aged 85 and over, respectively (6). In the present study, in which the same parameters were used to assess frailty, we found the 15.3% and 9.7% of patients aged over 65 years receiving warfarin had moderate/severe frailty or mild frailty, respectively.

Several previous studies have examined pharmacotherapy compliance in patients aged over 65 years for a number of diseases. Krousel-Wood et al. (7) should that very few elderly individuals were found to comply with pharmacotherapy, with mild and moderate levels of non-compliance observed in a considerable number of patients. Cooney et al. (8) found that drug interactions and antihypertensive drug compliance in elderly patients, the presence of multiple chronic diseases, polypharmacy and decreases in cognitive and functional capacity were all related to decreased compliance.

Numerous factors have been previously reported to negatively affect the attainment of INR target values. Nutrition status, hepatic and renal function, intestinal absorption rate, genetic factors affecting warfarin pharmacokinetics, patient compliance to pharmacotherapy and drug interactions have the greater reported contributions (9). Decreases in K vitamin-dependent factors and increases in warfarin sensitivity necessitate closer follow-up of elderly patients compared to normal patients to attain target INR values. Low doses of warfarin should be used with caution in elderly populations (10).

The relationship between frailty and the attainment of target INR values and factors preventing the attainment of target INR values in geriatric populations receiving warfarin have yet to be fully elucidated. In the present study, we found that frailty negatively affected the attainment of target INR vales in patients aged over 65 years in addition to the negative effects of other clinical parameters such as DLA, dementia, impairment of cognitive activity, urinary and fecal incontinence (FI).

Corroborating the study by Ertas et al. (11) we found age and sex had no effect on the attainment of target INR values.

Compared to patients without dementia or impaired cognitive function, a lower proportion of patients with dementia and/or impaired cognitive function have been shown to attain target INR values (12,13). In the present study, 45.2% of frail patients had dementia. On the other hand, none of the non-frail patients had dementia. In the present study, dementia negatively affected both frailty and the attainment of target INR values.

On the other hand, the proportion of patients able to performed DLA independently was 99.8% in the non-frail group of the present study. However, the proportion of patients able to perform DLA independently decreased to 85.4% and 3.2% in the mild and moderate/severe frailty groups, respectively, with a significant negative correlation observed between DLA independence and frailty. Dependency on others for DLA has a negative effect on the attainment of target INR values.

Previous studies have reported urinary incontinence (UI) as a leading cause of permanent admission to nursing homes (14). Urinary incontinence may preclude individuals from social environments and physical activities and lead to disruptions in DLA, including regular drug use. In the present study, urine incontinence was not observed in any patients in the non-frail group. The proportion of patients with urinary incontinence was found to be 9.8% and 51.6% in the mild and moderate/severe frailty groups, respectively, with a significant relationship between frailty and urinary incontinence observed. In the present study, in which both urinary incontinence and INR targets were evaluated, no patient who achieved target INR had urinary incontinence, whereas the proportions of patients with urinary incontinence patients with INR values below 2.5 and above 3.5 were determined to be 4.2% and 16.7%, respectively. Thus, urine incontinence was found to have a negative effect on the attainment of target INR values.

Although fecal incontinence is not life threatening, it is regarded as an important health problem due to its social, financial, hygienic and emotional pressures on the elder individual in addition to causing significant reductions in the health and quality of life of elderly individuals. No direct studies of the relationship between UI, FI and warfarin compliance have previously been reported. Other studies have demonstrated that UI and FI typically decrease patient quality of life and lead to disruptions in the performance of daily activities. Patients with FI and UI therefore tend to self-administer drugs irregularly leading to decreased pharmacotherapy compliance that further decreases their confidence in treatments and physicians. In the present study, the proportion of patients with FI was 0.2%, 14.6% and 96.8% in non-frail, mild frailty and moderate/severe frailty groups, respectively. A strong relationship was not only observed between frailty and FI, but also between the attainment of target INR and FI. Although no patients who attained INR target values had FI, the proportions of patients with FI in off-target groups with INR values below 2.5 and above 3.5 were 3.6% and 11.1%, respectively. As this result was statistically significant, FI apparently has a negative effect on the attainment of target INR values.

Warfarin and acetylsalicylic acid (ASA) have proven efficacy as first-line treatments for the prevention of stroke and thromboembolic complications. In previous

studies comparing the administration of warfarin with placebo, warfarin was shown to significantly reduce stroke risk compared to placebo. The use of warfarin was unquestionably superior to ASA in preventing chronic atrial fibrillation dependent stroke and thromboembolism (15). Oral anticoagulant treatment with warfarin reduces ischemic stroke risk by 68%, however, it also increases the risk of major concurrent hemorrhagic complications. The proportion of AF patients who do not use warfarin that suffer ischemic stroke in any given year is approximately 12% (16). Hylek et al. (17) reported that 59% of patients have substantial functional disability following AF-related ischaemic stroke. The proportion of patients who do not receive warfarin treatment that develop thromboembolism annually was reported as 2.5 per 100 individuals in the ATRIA cohort, a proportion higher than in other cohorts. Singer et al. (18) reported the high risk of thromboembolism can be reduced with warfarin treatment by 50%. This benefit exceeds the additional risk of warfarin-related intracranial hemorrhage (0.47 per 100 individuals per year with warfarin treatment compared to 0.29 per 100 individuals per year without treatment) (19). Accordingly, the benefits of warfarin use are considered to outweigh the related risks in AF patients (16).

All of these studies indicate warfarin absolutely requires close follow-up. Many studies have shown the failure to attain target INR values can lead to life-threatening complications (9,10). INR values below the target value increase the risk of thromboembolism and values above the target may cause major hemorrhagic complications. In the present study, we assessed frailty in addition to numerous parameters known to affect the attainment of target INR values in elderly patients. As frailty has been shown to have a negative effect on the attainment of target INR values in patients aged over 65 years using warfarin, new generation oral anticoagulants may be considered in such patients.

CONCLUSION

We demonstrated a negative relationship between frailty and the attainment of target INR values, with fewer patients with frailty achieving INR targets. Accordingly, frailty in individuals aged over 65 years apparently has a negative effect on the attainment of target INR values. Therefore, as complications such as embolism and hemorrhage may develop in elderly patients receiving warfarin who are frail or fail to attain target INR values, the close follow-up of such patients should be ensured during warfarin use. Further, we believe that frailty should be included in scoring systems used to assess patients at the initiation of warfarin therapy.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethical approval was received from the Health Science University, Ümraniye Training and Research Hospital Ethics Committee, who also approved the study protocol (approval no. 18467, 23.11.12).

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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Evaluation of the hematologic indices in patients with thyrotoxicosis with distinct etiologies: a case-control study

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ABSTRACT

Aim: Thyrotoxicosis is a clinical state of inappropriately high levels of free T4 (thyroxine) and/or free T3 (tri-iodothyronine) in the body caused by distinct etiologies including Graves' disease (GD), subacute thyroiditis (SAT), toxic adenoma and toxic multinodular goiter (TMNG). Simple hematologic indices such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and mean platelet volume (MPV) have increasingly been mentioned as measures of presence and severity of thyrotoxicosis. In this study, we aimed to analyze whether there is a link between these peripheral blood parameters and the presence of thyrotoxicosis.

Material and Method: A total of 46 GD, 46 TMNG, 39 TA and 45 SAT patients and 45 healthy controls were included. Laboratory parameters and NLR, PLR, and MPV values were recorded from peripheral blood complete blood cell counts for each patient.

Results: This study showed that NLR and PLR levels are elevated in patients with SAT in comparison with other thyrotoxic patient groups and controls. The post hoc analysis of comparison of NLR and PLR in each study groups revealed that NLR and PLR were statistically different in the SAT group in comparison to the GD, TMNG, TA, and healthy controls. A significant decrease in the level of MPV was demonstrated in thyrotoxicosis patients ($p<0.001$).

Conclusion: NLR, PLR and MPV that is routinely and automatically calculated from complete blood count plays an important diagnostic role in thyrotoxicosis.

Keywords: Thyrotoxicosis, mean platelet volume, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

INTRODUCTION

Thyrotoxicosis is usually characterized by the clinical manifestations of unsuitably elevated thyroid hormone (TH) action in the body. Hyperthyroidism is a kind of thyrotoxicosis owing to elevated TH synthesis and secretion in the thyroid gland. Although Graves' disease (GD) is one of the most common reason of hyperthyroidism in iodine-sufficient regions all over the world that affects <1.0% of the entire population, the other three most frequent causes of hyperthyroidism are toxic multinodular goiter (TMNG), toxic adenoma (TA), and subacute thyroiditis (SAT) (1-3). Patient characteristics and laboratory work-up are crucial in order to differentiate the underlying disorder. Unfortunately due to some confounding factors, the exact diagnosis cannot be performed in some cases. Moreover, differential identification of exact cause of thyrotoxicosis is also important for medical therapy and follow-up visits for subsequent observations, because GD and TA require further therapy with antithyroid medications and likely requires thyroid operation and SAT is a self-limited inflammation related disorder in which the usual therapy is anti-inflammatory drugs (4,5).

Thyroid hormones are crucial for the normal development, differentiation, proliferation, metabolic balance and physiological function of virtually all tissues (6). The functions of THs are also exceedingly pleiotropic, influencing a number of body tissues at diverse developmental stages. By affecting and regulating haematopoiesis in the bone marrow, it has been reported that thyroid dysfunction can cause alterations in haematological parameters, such as haematocrit (Hct), haemoglobin (Hgb), mean platelet volume (MPV), white

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blood cell (WBC) and its differentials (7,8). Enlarging literature data suggests that hyperthyroidism is related with increased risks for leucopenia, neutropenia, thrombocytopaenia, and WBC differentials including altered platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) (1,9).

Mean platelet volume (MPV), NLR, and PLR are simple and quick to compute hematologic markers that have been demonstrated to suggest inflammatory response and disease activity in several disease conditions comprising ulcerative colitis, acute pancreatitis, acute appendicitis, gestational diabetes mellitus, cirrhosis, coronary artery disease, neoplastic disorders (10-14). Moreover, there are several reports depicting the importance of NLR, PLR and MPV in differentiating the central causes of thyrotoxicosis (7-9). Unfortunately, these findings were inconsistent across studies, many of which were restricted by relatively undersized sample range and by the lack of replication. Thus, there are still no clinically defined diagnostic characteristics with respect to complete blood cell counts (CBC) which can discriminate the etiology of thyrotoxicosis. In this manner, the present research aimed to analyze the diagnostic significance of NLR, PLR and MPV in thyrotoxicosis patients with distinct etiologies.

MATERIAL AND METHOD

The study was approved by the institutional ethics committee of COMU (decision no:14-22, dated 09.12.2020). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

A total of 46 patients with GD, 39 patients with TA, 46 patients with TMNG and 45 patients with SAT admitted to the endocrinology clinic of Çanakkale Onsekiz Mart University (COMU) Training and Research Hospital between April 2016 and November 2020 included in the present study. The healthy controls were recruited from healthy persons without any history of acute or chronic inflammatory diseases admitted to internal medicine department for routine check-up.

Graves disease diagnosis was established on the existence of regular symptoms of hyperthyroidism and laboratory criteria, including elevated serum TH levels, decreased thyroid-stimulating hormone (TSH) values, positive TSH receptor antibody (TRAb), and/or high radioactive iodine (RAI) uptake tests. Toxic adenoma/multinodular goiter was diagnosed by the use of RAI uptake with/without thyroid scan and fine-needle aspiration. Diagnosis of SAT was made on the basis of conventional clinical indicators, elevated erythrocyte sedimentation rate (ESR), and pronounced decrease of thyroid RAI uptake.

Exclusion criterias included patients under 18 years old, pregnant women, patients having hematologic or neoplastic disease conditions, serious renal or hepatic disease, chronic inflammatory or autoimmune disorders, use of antithyroid drugs at the time of disease diagnosis. Those having chronic rheumatologic conditions, active or chronic infectious disease, diabetes mellitus, active thyroid orbitopathy and smokers were also excluded.

After database access was granted by the management of the COMU medical center, demographic data of the patients and biochemical and hormonal parameters including CBC parameters including Hgb, Htc, WBC, neutrophil count, lymphocyte count and MPV at diagnosis were extracted from COMU Hospital Information and Management System (HIMS). NLR and PLR were calculated for each study participant from the differential count by dividing the absolute neutrophil count by the absolute lymphocyte count and by dividing the platelet count by the lymphocyte count respectively. All blood samples were collected without the use of any anticoagulant from the antecubital vein after an overnight fast and all CBC analyses were carried out in the hematology laboratory of COMU medical center.

Statistical Analysis

Statistical analysis was done by using SPSS 22 (SPSS Inc., Chicago, IL, USA). Data are presented as mean±standard deviation (SD) for normally distributed variables, median (minimum–maximum) for non-normally distributed variables, and as the number of cases (%) for nominal variables. To assess differences between groups, a chi-square test was performed for categorical variables. The comparisons between groups were performed by ANOVA and Kruskal–Wallis for parametric and nonparametric variables respectively. Post hoc comparisons were evaluated with Tukey's test for parametric variables and Bonferroni correction Mann–Whitney U test for nonparametric variables. $P<0.05$ were considered as significant.

RESULTS

This study included 176 patients (46 GD; 46 TMNG; 45 SAT; and 39 TA patients) with a diagnosis of thyrotoxicosis and a control group with 45 healthy individuals. NLR, PLR were 176.07±59.59 and 112.04±46.49 respectively. Mean MPV levels of GD, SAT, TMNG, TA and controls were 9.04±1.35, 8.34±0.96, 8.88±1.25, 8.88±0.92 and 9.99±1.39 respectively. A statistically significant decrease in MPV levels was observed in patients with thyrotoxicosis ($p<0.001$). **Table 1** summarizes demographic characteristics and biochemical and hormonal data of study participants and controls. Mean NLR levels of SAT patients and controls were 2.99±1.47 and 1.73±0.66 respectively. Mean MPV and PLR levels of SAT patients and controls were significantly different between groups.

Table 1. Demographic and laboratory characteristics of study participants

	TA patients (n =39)	TMNG patients (n =46)	GD patients (n =46)	SAT patients (n =45)	Control group (n =45)	P value*
Age, years	64.97±10.14	69.23±8.33	51.00±16.37	44.62±9.86	49.22±14.49	0.000
Gender (F/M)	24/15	34/12	31/15	42/3	27/18	0.003
Hgb (g/dl)	13.64±1.58	13.62±1.56	12.91±1.44	12.01±1.25	13.46±1.79	0.000
Plt (x1000/ml)	241.30±71.34	242.93±56.43	247.15±65.37	341.86±80.89	260.33±80.25	0.000
WBC (x1000/ml)	7.38±2.17	7.73±1.98	6.75±1.75	8.46±1.69	7.39±1.94	0.001
TSH (mIU/L)	0.09±0.24	0.12±0.24	0.03±0.05	0.07±0.11	2.20±0.98	0.000
Free T4 (ng/dL)	1.43±0.81	1.23±0.33	2.31±1.69	2.33±0.98	-	0.000
Free T3 (ng/dL)	5.24±2.93	4.73±2.35	7.87±6.94	5.20±1.54	-	0.001
Anti-TPO (+/-)	3/36	6/40	8/38	2/43	-	0.204
Anti-Tg (+/-)	2/37	6/40	8/38	4/41	-	0.312
CRP (mg/dl)	0.64±0.63	0.62±0.48	0.60±0.71	5.04±4.13	0.49±0.55	0.000
ESR (mm/h)	19.51±12.88	20.97±10.10	18.00±10.90	61.95±27.72	15.60±10.16	0.000
NLR	2.21±1.43	2.18±1.10	2.17±2.17	2.99±1.47	1.73±0.66	0.002
PLR	118.78±56.93	114.81±41.57	131.76±82.63	176.07±59.59	112.04±46.49	0.000
MPV (fl)	8.88±0.92	8.88±1.25	9.04±1.35	8.34±0.96	9.99±1.39	0.000

Values are presented as median (range) or mean±SD as appropriate. WBC=White blood cells, TSH=thyroid-stimulating hormone, Anti-TPO=thyroid peroxidase antibody, Anti-Tg=antithyroglobulin, CRP= C-Reactive protein, ESR= Erythrocyte sedimentation rate,NLR=neutrophil-to-lymphocyte ratio, PLR=platelet-to-lymphocyte ratio, MPV=mean platelet volume,
*P-value, calculated by chi-square (for categorical variables), ANOVA (for parametric variables), and Kruskal–Wallis (for nonparametric variables). P<0.05 is significant.

In post hoc analysis, NLR and PLR were significantly higher in the SAT group when compared to the GD, TA, TMNG and control groups (p<0.005 for each group). MPV levels were different between groups (p=0.000) in post hoc analysis. **Figure 1** shows the levels of hematologic parameters within each study groups and controls.

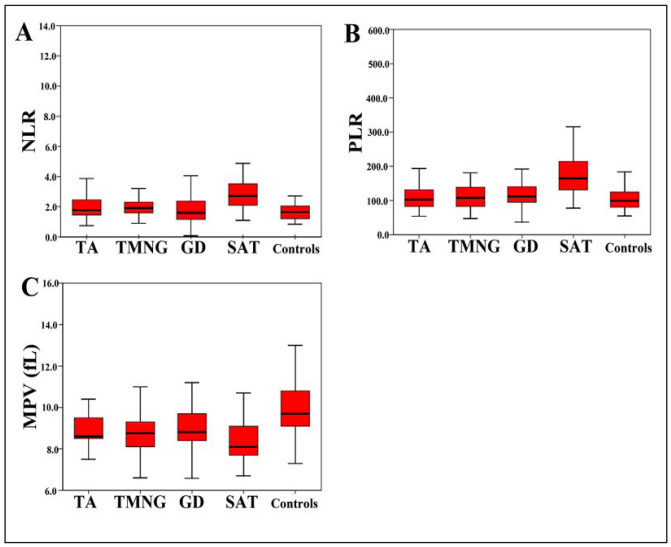

Figure 1. Comparison of NLR (A), PLR (B) and MPV (C) levels within each study group (NLR: Neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, MPV: Mean platelet volume, TA: Toxic adenoma, TMNG: Toxic multinodular goiter, GD: Graves' disease, SAT: Subacute thyroiditis).

DISCUSSION

In this study, we revealed that NLR and PLR is increased in SAT patients and MPV is decreased in all causes of thyrotoxicosis. These findings clearly suggests the role of MPV and WBC differentials in the pathophysiology of thyrotoxicosis particularly in SAT.

Thyroid hormones take part a significant physiological role in the metabolism, growth and development of the human body. The relation between thyroid disorders and abnormalities in haematological parameters by affecting bone marrow is a well-known issue (8). In this context, MPV is one of the determinants of the platelet function which is situated in the routine CBC analysis and was shown to be affected in distinct disease conditions including acute pancreatitis, inflammatory bowel diseases, cardiac disorders, peritonitis, autoimmune disorders and tumoral diseases (10-13,15,16). The diagnostic role of MPV in thyroid diseases is a novel topic of research with conflicting results. In a study by Simsek et al. (17) the role of MPV in hyperthyroid toxic adenoma patients undergoing radioactive iodine (RAI) ablation treatment was evaluated. It was demonstrated that MPV levels are decreased after radioactive iodine therapy in hyperthyroid patients. In a recent study by Bagir et al. (18) higher initial MPV levels in GD patients and significant drop following the restoration of hyperthyroidism was reported. Lippi et al. (19) reported a considerable relation between MPV and TSH valevels

in both univariable (p<0.001; r=0.12) and multivariable logistic regression analysis (beta coefficient, 0.07; p<0.001) after adjustment for multiple confounders including patient age, gender, free T4 values and thrombocyte counts. After homogenization of the study population in context to quartiles of serum TSH levels, a graded increase of MPV values was observed from the first to the fourth quartile of TSH. Contrary to these findings a recent study from China reported no significant relationship between MPV or platelet distribution width (PDW) and THs in a Chinese patient cohort (20). This study demonstrated low MPV levels in all 4 groups of patients with thyrotoxicosis. Low grade inflammation that can be seen in the clinical course of the disease may be responsible for the relation between MPV and thyrotoxicosis. MPV is an initial parameter of platelet activation and big platelets are further reactive than their smaller counterparts in releasing different kinds of pro-inflammatory cytokines that is more likely to aggregate. Therefore, aggregation that is triggered by inflammation causes severe infiltration of large platelets into vascular and intestinal wall, and a final decrease in platelet size (21-23).

In addition to the change of total leukocytic count, alterations of WBC and its differentials, such as NLR and PLR have been proved to be associated with distinct causes of thyrotoxicosis. Moreover, the final outcome of anti-thyroid medical treatment on the granulocytic series are notable and the data on the direct effect of thyroid disorders on granulocytes are partially restricted. Furthermore, literature data on lymphocyte subpopulation distributions in thyroid patients with or without neutropenia are also insufficient (24-25). In this context, SAT which is distinguishable by painful swelling of the thyroid, systemic inflammatory symptoms, and transient thyrotoxicosis is reported to be associated with higher NLR and PLR levels in several studies (4,26). An elegant study by Taşkaldıran et al. (4) the relationship between WBC differentials and the most common causes of thyrotoxicosis including GD, toxic multinodular goiter (TMNG), TA and SAT were investigated. Authors demonstrated higher NLR and PLR levels in the SAT group when compared to other etiologies of thyrotoxicosis. The present study demonstrated elevated NLR and PLR levels in SAT patients compared to the GD, TA, TMNG and healthy controls. Among these, the difference in NLR and PLR was statistically significant. We think that this association might be related to the activation of monocytes and platelets early in the disease process seen in SAT as well as an increase in the number of platelets.

This study didn't observe any significant association between NLR and PLR with other causes of thyrotoxicosis including GD which is the most common reason of hyperthyroidism. GD is an autoimmune disorder, in which the human body produces specific antibodies to the receptor for TSH. Based on this inflammatory background, it is not surprising to find out studies evaluating the role of NLR and PLR in GD. In this context, Taskaldiran et al. (4) investigated the role of these peripheral blood differentials in distinct causes of thyrotoxicosis including GD. Similar to our results authors' demonstrated insignificant association between these parameters and GD. Contrary to our findings Turan et al. (27) revealed higher lymphocyte, monocyte, platelet levels and lower NLR levels in GD patients compared to the healthy controls. Although not compared to healthy population, a study by Alay et al. (28) revealed a statistically significant decrease in NLR in GD compared to TMNG group after treatment.

The main limitation of the present study is the retrospective nature of analysis and relatively low numbers of cases. Moreover, due to the different age distribution of thyrotoxicosis causes, mean ages were different between groups. Another important limitation of our study was the lack of simultaneous measurement of inflammatory markers such as tumor necrosis factor alfa, interleukin 1 beta, interleukin 6 and interleukin 8 with CBC. We accept that it would be noteworthy to demonstrate a possible association between these inflammatory markers and CBC indices.

CONCLUSION

This study evaluated the role of NLR, PLR and MPV in newly diagnosed thyrotoxic patients. We strongly believe that NLR and PLR may be helpful to differentiate SAT from other causes of thyrotoxicosis before applying more expensive and difficult diagnostic studies such as RAI uptake.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Research Ethics COMU (decision no:14-22, dated: 09.12.2020).

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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Efficacy of reduced dose melphalan conditioning for multiple myeloma patients undergoing autologous stem cell transplantation: in the era of combined induction with novel agents

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ABSTRACT

Aim: Melphalan 200 mg/m² (MEL 200) is known as the standard conditioning regimen for Multiple Myeloma (MM) patients in autologous stem cell transplantation (ASCT). Most of the studies showing the superiority of MEL 200 versus melphalan 140 mg/m² (MEL 140) were performed in the era of conventional chemotherapies. However, today, several novel agents such as proteasome inhibitors, immunomodulatory agents have been introduced in MM treatment algorithms. There is limited data on the impact of this dose reduction on progression-free survival (PFS) or overall survival (OS). The present study compares MEL 140 and MEL 200 conditioning for ASCT in patients treated with combination therapy of bortezomib-containing induction.

Material and Method: Results of 84 MM patients who underwent ASCT at our center between 2010 and 2018 were analyzed retrospectively.

Results: In the MEL 140 group, PFS was 9 months (95% CI 2.2-15.8) and OS was 30 months (95% CI 9.5-50.4), while PFS was 13 months (95% CI 10.5-15.5) and OS was 34 months (95% CI 6.9-61) in the MEL 200 group. There was no statistically significant difference in PFS and OS between the two groups (p:0.6, p:0.7).

Conclusion: Consequently, MEL 140 and MEL 200 were found similar in terms of engraftment duration, transplant-related mortality rate, and survival rates. The idea that similar outcomes in both MEL 140 and MEL 200 group in patients who received combined induction treatment with novel agent suggested that MEL 140 may be used more commonly than the standard approach of MEL 200.

Keywords: Melphalan, autologous stem cell transplantation, multiple myeloma

INTRODUCTION

For more than 20 years, autologous hematopoietic stem cell transplantation (ASCT) after high-dose chemotherapy has been the standard consolidation therapy for newly diagnosed, fit multiple myeloma (MM) patients. High-dose chemotherapy following ASCT is superior to conventional chemotherapy in MM patients (1,2). After high-dose chemotherapy following induction with novel treatment approaches such as thalidomide analogs and proteasome inhibitors, the benefit of ASCT has been confirmed (3-5). Compared to conventional chemotherapies, ASCT provides the patient advantages in progression-free survival (PFS) and overall survival (OS) (2,4). ASCT is also widely administered in elderly, fit MM patients (6,7). Furthermore, some studies also show the superiority of ASCT versus conventional chemotherapy in elderly patients (7,8). In these studies, patients were administered high-dose chemotherapy with the dose of 200 mg/m² melphalan (MEL 200) (1-4). MEL 200 was found to be less toxic than other high-dose combination regimens (9,10). For this reason, MEL 200 has been accepted as the standard conditioning regimen for ASCT and remains widely used in current practice (11,12). Otherwise, some studies with

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MEL 200 related increased toxicity in elderly patients and those with renal failure (13-15). Organ dysfunction and decreased drug metabolism have been considered causes of increased melphalan toxicity in elderly MM patients. As a result, the dose of 140 mg/m² Melphalan (MEL 140) is widely preferred in elderly patients and patients with renal failure in clinical practice (15-19). However, in studies comparing MEL 140 and MEL 200, MEL 140 was associated with lower response rates and shorter survival than MEL 200 (17-20). There is limited data on the impact of reduced dose reduction on progression-free survival (PFS) or overall survival (OS) in the era of induction with novel agents. This study aims to compare the efficacy of the dose of conditioning regimen as melphalan 140 mg/m² or 200 mg/m² in terms of the effect on survival and transplantation response in MM patients who received a bortezomib-containing combination as induction therapy at our center.

MATERIAL AND METHOD

This study was approved by the local human research ethics committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was carried out with the permission of Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital Ethics Committee (date: 06.11.2019, decision no: 2019-11/421).

The results of 84 multiple myeloma patients who underwent ASCT following induction therapy between 2010 and 2018 at Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital Bone Marrow Transplantation Center were analyzed retrospectively. Patients' age, gender, myeloma subgroup, disease stage, number of treatments they received before transplantation, history of radiotherapy, presence of renal failure, the melphalan dose they received, the quantity of CD 34+ infused, and disease status before transplantation were recorded. The International Staging System (ISS) calculated from serum β_2-microglobulin and albumin levels at the time of diagnosis was used for risk classification (21). Patients treated with bortezomib-containing combination treatments in induction therapy were enrolled in the study, while patients who received non-bortezomib induction treatment were excluded. Patients with tandem transplants, defined as second transplantation performed within six months without progression or recurrence after the first ASCT, were not included in the study. OS was defined as the time from transplantation to death or the last follow-up date for those who survived. PFS was defined as the time from transplantation to the date of disease progression or death (whichever occurs earlier) or the last follow-up date for those without death or disease progression. Transplant-related mortality (TRM) was defined as death within the first 100 days after ASCT. Assessment of treatment response was performed according to the guidelines from the International Multiple Myeloma Working Group (IMWG) guidelines (22). Patients were stratified into two groups according to the dose of conditioning regimen they received, i.e., 140 mg/m² melphalan (MEL140) or 200 mg/m² melphalan (MEL200). Patients receiving doses other than these were not included in the study. MEL140 was administered for those over 70 years old and/or with serum creatinine levels equal to or higher than 2 mg/dL. In contrast, MEL 200 was administered as the conditioning regimen for the other patients. Glomerular filtration rates (GFRs) of patients were calculated with chronic kidney disease epidemiology collaboration (CKD-EPI) equation using plasma creatinine, age, gender, and race variables (23). The renal function evaluation was performed by stratifying glomerular filtration rate (GFR) into two groups as >50 mL/min and ≤50 mL/min. Engraftment definition; for neutrophils, it was defined as the first day of absolute neutrophil count (ANC) >500/mm³ or 1000/mm³ for three consecutive days. It was defined as the first day of thrombocytes >20000/mm³ for three consecutive days without transfusion for thrombocytes.

Statistical Analysis

All statistical analyses were performed with the SPSS V21.0 (SPSS Inc., Chicago, IL) program. Descriptive statistics were used to summarize the data. Categorical data were presented as ratios and numerical data as median and mean ± standard deviation. The chi-square test was used for categorical data and the Kruskal Wallis test for numerical data in comparing the groups. Kaplan-Meier was used for PFS and OS, and log-rank tests were used for confounding factors. P values of ≤0.05 were considered statistically significant.

RESULTS

Of the 84 patients, 21 patients (25%) were enrolled in the MEL 140 group, and the median age was 61 years (41-72) in patients. 63 (75%) patients were in the MEL 200 group, and the median age was 57 years (36-66) in this group. The clinical characteristics of patients are given in **Table 1**. MEL 200 and MEL 140 groups were similar in the myeloma subgroup and ISS staging (p=0.1, p=0.35, respectively). GFR was ≤50 mL/min in (14%) patients in the MEL 140 group and ≤50 mL/min in two (3.4%) patients in the MEL 200 group. In both MEL 140 and MEL 200 groups, neutrophil engraftment occurred on a median Day 11 (9-21 days in the MEL 140 group, 9-14 days in the MEL 200 group), and thrombocyte engraftment occurred on a median Day 12 (10-23 days in the MEL 140 group, 7-24 days in the MEL 200 group).

Table 1. Clinical characteristics of patients

	Patient Population (n), Median (range)
Age (years)	58 (36-72)
Gender (number)	Female/Male: 33/51
Multiple myeloma subgroup (n)	Heavy chain: 70 Light chain: 13 Non-secretory: 1
International Staging System (ISS) stage (n)	ISS I: 19 ISS II: 26 ISS III: 24 Not Evaluated: 15
Durie Salmon stage (n)	DS1: 6 DS2: 8 DS3: 66 Not Evaluated: 4
Disease status before transplantation (n)	CR: 27 VGPR: 22 PR: 22 Stable: 8 Refractory: 2 Not evaluated: 3
Melphalan dose (n)	140 mg/m²: 21 200 mg/m²: 63
Renal failure (GFR) * (n)	GFR >50 mL/min: 79 GFR ≤50 mL/min: 5
Number of chemotherapy courses (n)	1: 23 2: 44 3: 13 4: 2 5: 1
History of radiotherapy (n)	Yes/No: 10/74
Quantity of CD34+ infused (median)	4.54×10^6/ kg (2.3-9.1)

Abbreviations: International Staging System (ISS), GFR: Glomerular filtration rate, *GFR was calculated with the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation.

Patients were stratified into subgroups according to disease status at the time of transplantation (complete response (CR), very good partial response (VGPR), partial response (PR), stable disease, progressive disease). There was no statistically significant difference between MEL 140 and MEL 200 in PFS and OS according to disease status before transplantation (p=0.3; p=0.7, respectively) (**Table 2**).

When the patients were grouped as those under 60 years of age and 60 years and above, there was no statistically significant difference between MEL 140 and MEL 200 conditioning regimens in PFS and OS (p=0.9; p=0.5, respectively). When GFR was stratified into two groups as >50 mL/min and ≤50 mL/min, there was no statistically significant difference between MEL 140 and MEL 200 conditioning regimes in terms of PFS (p=0.7) (**Table 3**).

In the MEL 140 group, PFS was nine months (95% CI 2.2-15.8) and OS was 30 months (95% CI 9.5-50.4), while PFS was 13 months (95% CI 10.5-15.5) and OS was 34 months (95% CI 6.9-61) in the MEL 200 group. There was no statistically significant difference in PFS and OS between the two groups (p=0.6, p=0.7, respectively) (**Figures 1** and **2**). None TRM was observed in both groups.

Table 3. PFS and OS in Melphalan 140 and Melphalan 200 arms according to age group and renal failure stage

Age group	MEL 140 (n)	PFS (months) 95% CI	OS (months) 95% CI	MEL 200 (n)	PFS (months) 95% CI	OS (months) 95% CI	p value
<60 years	9	7 (1.9-12.1)	23 (7,6-38,4)	38	14 (0.6-27.4)	42 (13.8-70.2)	p=0.5 (OS)
≥60 years	12	41 (22.6-59.4)	47	25	12 (6.2-17.8)	13 (0-41.8)	p=0.9 (PFS)
GFR (ml/min)	**MEL 140 (n)**	**PFS (months) 95% CI**	**OS (months) 95% CI**	**MEL 200 (n)**	**PFS (months) 95% CI**	**OS (months) 95% CI**	**p value**
≥50 ml/min	18	9 (6.1-11.9)	17 (0-35)	61	13 (10.1-15.8)	34 (8.4-59.5)	N/A (OS) p=0.7 (PFS)
<50 ml/min	3	7	30	2	12	-	

Mel 140: Melphalan 140 mg/m²; Mel 200: Melphalan 200 mg/m², PFS: Progression-free survival, OS: Overall survival
GFR: Glomerular filtration rate, GFR was calculated with the Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation.

Table 2. PFS and OS in Melphalan 140 and Melphalan 200 arms according to the disease status before transplant

Disease status before transplant	MEL140 (n)	PFS (months) 95% CI	OS (months) 95% CI	MEL200 (n)	PFS (months) 95% CI	OS (months) 95% CI	p-value
Complete remission	6	12 (7.2-16.8)	23	16	14 (0-40.3)	50 (0-110)	p=0.7 (OS)
Very good partial response	6	20 (0-48.8)	31	16	12 (7.8-16.2)	12 (0.2-23.8)	
Partial response	7	8 (0-41.3)	30 (0-60.4)	15	12 (6.3-17.7)	34	p=0.3 (PFS)
Stable disease	2	7	3	6	13	13	

Mel 140: Melphalan 140 mg/m²; Mel 200: Melphalan 200 mg/m², PFS: Progression-free survival, OS: Overall survival
(Those with no response assessment and those with progressive disease were not included in the above OS and PFS subgroup analysis)

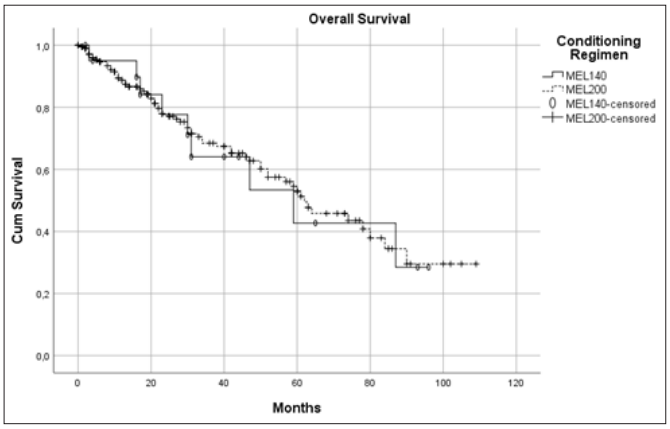

Figure 1. Overall Survival Curve
Abbreviations: Mel 140: Melphalan 140 mg/m²; Mel 200: Melphalan 200 mg/m²

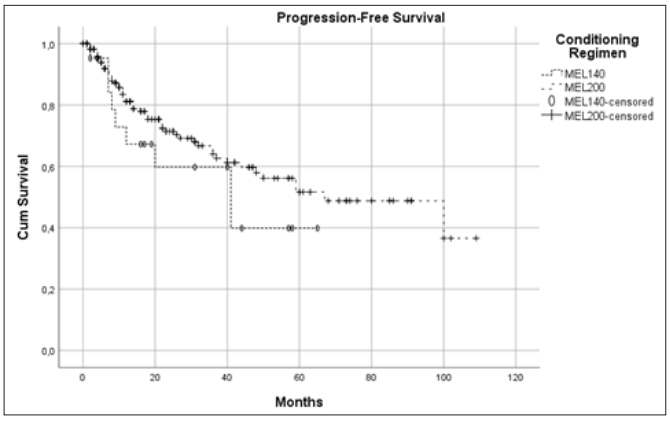

Figure 2. Progression Free Survival Curve
Abbreviations: Mel 140: Melphalan 140 mg/m²; Mel 200: Melphalan 200 mg/m²

DISCUSSION

The data about the studies showing the superiority of MEL 200 over MEL 140 for the conditioning regimen of ASCT in the multiple myeloma patients were performed in the era of conventional chemotherapies. Currently, several novel agents such as proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies have been included in the treatment of MM. The lack of significant difference in OS and PFS between MEL 140 and MEL 200 in patients who received combination therapies containing novel agents for induction therapy has led to the idea that MEL 140 may be used more widely than the standard approach, MEL 200.

In the CALM study conducted by Auner et al. (19) MEL 200 was superior to MEL 140 in terms of OS, PFS, and relapse risk in patients with a response rate less than the partial response at transplantation time. This result has been explained by the higher dose dependence of melphalan-induced anti-myeloma effects in cells with limited chemosensitivity. However, the study's conclusions also support that MEL 140 may be more administered versus MEL 200 due to the disease response of VGPR/CR before the ASCT and raise the question of whether more patients should receive MEL 140 (19). This question becomes essential considering that novel combined induction regimens provide higher rates of VGPR/CR. Therefore, the remission status of MM before the first ASCT should be taken into account when deciding the melphalan dose (19). In our study, when patients were stratified into subgroups according to their disease status at the time of transplantation, there was no statistically significant difference between MEL 140 and MEL 200 in terms of PFS and OS according to the disease response before transplantation suggesting that MEL 140 may be preferred since it is associated with a lower risk of toxicity in ASCT.

In the same study by Auner et al. (19) the superiority of MEL 200 over MEL 140 could not be demonstrated in patients with high-risk genetic features or higher ISS stages. Similarly, in our study, the superiority of MEL 200 over MEL 140 could not be demonstrated in patients with higher ISS, and Durie Salmon stages at the time of diagnosis. Melphalan 200 mg/m² is insufficient to overcome poor cytogenetic characteristics and high tumor burden, so that new strategies should be developed for this high-risk group.

In the studies of myeloma patients treated with high-dose melphalan following ASCT, higher dose melphalan exposure has been associated with higher toxicity and also better disease responses (11,12,24). In a recent study, exposure to high-dose melphalan was found to increase overall survival in myeloma patients; however, despite the net survival benefit, there was no association between melphalan exposure and progression-free survival (24). PFS was nine months in our study, and OS was 30 months in the MEL 140 group, while PFS was 13 months, and OS was 34 months in the MEL 200 group. There was no statistically significant difference in PFS and OS between both groups, and survivors were very similar. Similarly, in the study by Katragad et al. (16) there was no difference between MEL 140 and MEL 200 arms in PFS and OS. In the study conducted by Badros et al. (13) there was an improvement in event-free survival with MEL 200 compared to MEL 140; however, they did not report any OS progress.

In a recent The Center For International Blood and Marrow Transplant Research (CIBMTR) analysis of elderly patients undergoing ASCT for MM, examined the effect of the MEL conditioning dose (25). One thousand two hundred twenty-three patients received reduced dose (MEL140) conditioning, whereas 868 patients received a standard dose in patients ≥70 years. The analysis focused on the patients aged ≥70 years, at a dose of MEL 200 was associated with superior PFS and OS and a lower rate of non-relapse mortality rate compared with MEL 140 (25). The authors discussed that sicker patients were expected to have more complications and TRM. They underlined

that without understanding the reason for choosing Mel 140 vs. Mel 200 beyond performance status and comorbidity score, it was not possible to recommend Mel 200 in the elderly group. The results of CIBMTR analysis provided MEL 200 safely in some older adults aged ≥70 years (25). We did not observe the superiority of standard-dose melphalan in our cohort; the lower patients number included is a significant limitation of our study. Rather than age, evaluating the patients' comorbidities and performance regarding geriatric assessments are more critical points when deciding on conditioning.

While some studies suggest that renal failure may be associated with the excess toxicity of MEL 200, others have not reported such association (15-17,20,26). In our study, GFR was stratified into two groups as >50 mL/min and ≤50 mL/min; there was no statistically significant difference between melphalan doses. A study conducted by Badros et al. (14) demonstrated better tolerability with MEL 140 than MEL 200, with similar survival in patients older than 70 years of age. Similarly, we could not demonstrate any difference between MEL 140 and MEL 200 in terms of OS and PFS according to the age (as those under 60 years of age, 60 years and above) current study. In the study by Katragad et al. (16) an increased frequency of prolonged neutropenia and neutropenic fever was observed in patients receiving MEL 140, however, in our study, both in MEL 140 and MEL 200 groups, neutrophil engraftment was observed on a median Day 11, and thrombocyte engraftment was observed on a median Day 12, both with similar frequency.

If Mel 140 becomes more widely used, it will be a more cost-effective treatment option, predicting that the lower doses of melphalan will reduce ASCT costs by 30%. Considering the increased costs associated with novel myeloma treatment modalities, that is predictable to be offset costs by a reduced conditioning regimen like MEL 140.

Unlike other studies, the present study has analyzed data from patients with renal failure or the elderly and data from all patients who received MEL 140 regardless of the cause of dose reduction. Patients who had only one ASCT were analyzed, thus minimizing the confounding effect of a second ASCT or tandem transplantation. However, due to MEL 200 as the standard dose in our clinic and dose reduction is performed just when required clinically for limited number of patients in the MEL 140 group.

CONCLUSION

Melphalan 140 and MEL 200 were found similar in terms of engraftment duration, transplant-related mortality rate, and survival rates in patients who received combination treatment with new agents in induction therapy. Reduced dose melphalan (MEL140) may replace the standard dose melphalan in the group of patients who do not meet high-risk criteria during the era of novel induction agents and those who undergo transplantation with complete response or very good partial response. On the other hand, more extensive studies are warranted for MEL 140 to replace MEL 200 in patients with normal renal function and young patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital Ethics Committee (date: 06.11.2019, decision no: 2019-11/421).

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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Cytotoxicity of two self-adhesive flowable composites on bovine dental pulp-derived cells

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ABSTRACT

Aim: The use of self-adhesive composites in dental treatment relatively a new concept. The aim of this study was to in vitro evaluation cytotoxicity of Vertise Flow and Nova Compo SF Flow self-adhesive flowable composites on bovine dental pulp-derived cells.

Material and Method: Experimental test samples (2×5mm) of Vertise Flow and Nova Compo SF Flow were prepared. Bovine dental pulp-derived cells were incubated in MEM-Alpha (Gibco-Invitrogen). The material samples to be tested were stored in the culture medium for 24 hours and therefore obtained extracts were applied onto the cells. The cell viability was determined by MTT assay. One-way ANOVA and Tukey-HSD post hoc tests used for statistical analysis.

Results: The percentage of cell viability of Vertise Flow found as 81.55%, Nova Compo SF Flow found as 71.40%. There is statistically significant difference between the control group and the test groups in term of the percentages of cell viability ($p<0.05$).

Conclusion: Self-adhesive flowable composites affect cell viability but they not have cytotoxic effects.

Keywords: Self-adhesive flowable composite, cytotoxicity, cell culture, MTT, bDPCs

INTRODUCTION

Adhesive dentistry is developing rapidly every day. The first factor accelerating this development is the increasing demand of patients to aesthetic restorative materials. The other factor is that the physicians want to perform restorative procedures with minimum intervention in less time (1,2). The most commonly used restorative system in today's dentistry practice is the combination of adhesive resin/composite resin (1). Adhesive systems are mainly divided into two groups; 'etch & rinse' and 'self-etch' adhesives. Before placing the resin composite, etch & rinse adhesive system require acid etching, rinsing and drying as a first step then requires applying a priming agent and adhesive. This greatly prolongs the clinical application time (3). However, physicians must finish their restorations as soon as possible. Self-etch adhesives eliminate the acid etching and rinsing steps and contain weakly acidic monomers in the primer. It is therefore very popular now. In particular, single-step self-etch adhesive system that combines the etching, priming and adhesion in one stage. Therefore, this system is very successful in shortening the clinical application time. However, even single-step self-etch adhesives have a considerable clinical application time and some technical sensitivity (4).

There are exciting advances in the development of restorative materials that can be directly adhered to the dental hard tissues without requiring any adhesive system. The first material produced for this purpose was Vertise Flow, Kerr which is a flowable composite. The self-adhesive flowable composite is a restorative material that is formed by adding a single-stage self-etch adhesive resin and applied directly to the cavity. It is based on the use of acidic monomers. HEMA monomer is another functional monomer which can be used in self-adhesive flowable composites (5). It is aimed to simplify restorative processes by eliminating the additional adhesive resin application phase by the using of self-adhesive flowable composites (6).

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There are many materials used in the restorative treatment of decayed teeth. however, these materials help to restore the health of the tooth, they also may have the potential to produce undesirable effects on body tissues (7). In our days, advances in biomaterials focus on simplifying techniques improving material performance and improving biocompatibility and to achieve better results in less time (8,9). Since this material will be in close relationship with the pulp-dentin complex for a long time, its effects on pulp tissue is very important and should be investigated.

The aim of this in-vitro study was to evaluate the cytotoxicity of Vertise Flow and Nova Compo SF Flow self-adhesive flowable composites on bovine dental pulp-derived cells. Our null hypothesis is that self-adhesive flowable composites have no effect on cell viability.

MATERIAL AND METHOD

The study was carried out with the permission of Selçuk University Faculty of Dentistry Ethics Committee (permission granted: 2020/60, decision no: 07). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of principles.

Standardized cylindrical test samples with 2 mm height and 5 mm diameter of Vertise Flow, Kerr and Nova Compo SF Flow, Imicryl were prepared according to their manufacturers' instructions (n=15). The contents, lot numbers and manufacturers of the materials used in the study are shown in **Table**. The test samples stored in the culture medium for 24 hours and subsequently extracts obtained.

Table. The contents, Lot numbers and manufacturers of the self-adhesive flowable composites used in the study

Materials	Content	Lot Number	Manufacturer and Country
Vertise Flow	GPDM, HEMA, 4-Methoxy phenol, Nano-ytterbium fluoride, barium glass, nano-size colloidal silica, zinc oxide, activator, stabilizer and colorants	3488779	Kerr, Germany
Nova Compo SF Flow	10-MDP, Bis-GPDM P, Bis-HEMA P, HEMA P, 4-META, 10-MDP + 4-META	2027A	Imicryl Turkey

bDPCs (bovine dental pulp-derived cells) were cultured in a 96-well plate which have MEM Alpha containing 20% FBS (fetal bovine serum), 1% geneticin and 5% penicillin/streptomycin at 37°C with humid air containing 5% CO_2. Obtained test extracts applied to experiment group cells and original culture medium was used for control group. bDPCs viability was analyzed by measuring the mitochondrial activity with the methyl tetrazolium assay (MTT) after 24 hours of exposure. The absorbance spectrophotometrically measured at 540 nm. The mean values of experiment groups proportioned to mean of control group. Therefore, viability value obtained as a percentage. One-way ANOVA and Tukey-HSD post hoc tests used for statistical analysis.

RESULTS

The results of the cytotoxicity test are summarized in **Figure**. The percentage of cell viability of self-adhesive flowable composites were found as 81.55% (Vertise Flow), 71.40% (Nova Compo SF Flow) and 100% (Control) respectively. There is statistically significant difference between the control group and the test groups in term of the percentages of cell viability ($p<0.05$). Both Vertise Flow and Nova Compo SF Flow showed lower percentages of cell viability than the control group and there was no statistically significant difference among the percentages of cell viability of them ($p>0.05$).

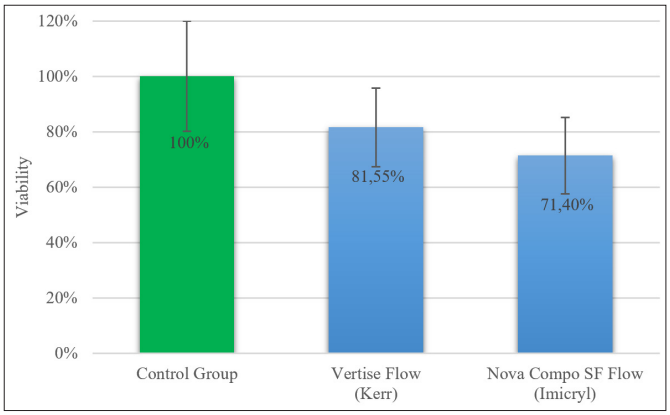

Figure. Percentages of cell viability of bovine dental pulp-derived cells exposed to self-adhesive flowable composites.

DISCUSSION

In minimally invasive dentistry composite resins, which are applied together with adhesive systems, stand out as the most preferred material. Due to the widespread use of composites over the years, the focus has been on shortening the application procedures of these materials and in this direction, self-bonding fluid composites have been introduced to the market (10) .

All biomaterials used in dentistry must be evaluated for biocompatibility using screening assays to protect patient health and safety. Biocompatibility implies that a material does not cause systemic and local toxic, allergic, mutagenic and carcinogenic effects when in contact with vital tissues (9). Non-biocompatible or cytotoxic restorative materials can cause reactions ranging from short or long term post-

operative hypersensitivity to irreversible pulp damage (11). Many information on the harmful effects of the components of resin-based materials has been obtained from in vitro studies (12,13). Each material should be tested for biocompatibility before application on patients. Materials approved by independent researchers with biocompatibility are more reliable. In the investigation of biocompatibility, it should be preferred to use a standard, simple and quick test method. Cell culture assays, which are reliable, reproducible are frequently used to investigate biocompatibility (14,15). In our study, direct contact test method in which material extracts come into direct contact with cells was used and supportive results were obtained. Biocompatibility of dental materials can be measured with three types of biologic tests: in vitro, animal and usage tests (14). The in vitro MTT test has been shown to be a suitable in vitro method for assessing the cytotoxicity of dental materials. It has therefore become a standard test commonly used to assess the cytotoxicity of new biomaterials (16). Recently bovine dental pulp-derived cell line was developed for better mimicking of primary pulp cells by transfection with large T-antigen of SV40 (Simian Virus 40) (17). For these reasons, MTT assay and bovine dental pulp-derived cells were chosen as methods in our study.

One of the important ingredients self-adhesive flowable composite is glycerophosphate dimethacrylate (GPDM), which is a phosphate-based self-etch acidic functional monomer, and its task is to etch a rough surface required for preservation of enamel and dentin and to increase the wettability of this rough surface. Vajrabhaya et al. (18) evaluated the cytotoxicity of the Optibond Solo Plus SE (Kerr, USA), a dental adhesive containing GPDM as a functional monomer by dentine barrier test, and reported that the Optibond Solo Plus SE did not show cytotoxic effect. The other important functional monomer is hydroxy-ethyl methacrylate (HEMA). HEMA is a monomer which is added to many dental adhesive contents to increase wettability and to improve penetration of the resin into dentin. It has been shown that HEMA can be released from methacrylate-based resin composites and even physiological concentrations can affect pulp cells (19,20). On the other hand, HEMA, a small hydrophilic monomer, has been reported to diffuse even from sclerotic dentin (21). Pawlowska et al. (22) reported that HEMA could induce harmful biological effects such as DNA damage, apoptosis and delay in cell cycle. According to the results of a study conducted by Ülker et al. (23) residual monomers or other harmful components in Vertise Flow are difficult to diffuse from dentin and affect pulp cells. In this study, our null hypothesis is rejected. Although Vertise Flow and Nova Compo SF Flow decreased cell viability compared to the control group, it cannot be said that they have cytotoxic effects since the cell viability is over 70% according to ISO 10993-5 standard (24). Also, the cell viability of Nova Compo SF Flow was less, no statistically significant difference was found between Vertise Flow and this group.

The clinical relevance of the in vitro data presented has to be interpreted with caution. Oral cavity condition differs from in vitro status and contains many factors such as saliva, mucus, creatine levels, food and drink intake, and normal flora. Only one method was used in this study to evaluate the cytotoxicity of self-adhesive composites, further studies may help to better understanding.

CONCLUSION

In this study, it was determined that self-adhesive flowable composites affect cell viability but they not have cytotoxic effects. These materials are in close relationship with the pulp-dentin complex for a long time, so their effects on pulp tissue are very important and should be investigated by further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Selçuk University Faculty of Dentistry Ethics Committee (permission granted: 2020/60, decision no: 07).

Informed Consent: For this type of study, formal consent is not required.

Referee Evaluation Process: Externally peer-reviewed.

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

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The effectiveness of electroneuromyography in the early diagnosis of diabetic foot development

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ABSTRACT

Objective: Diabetic foot is one of the basic causes of lower extremity amputation. The aim of this study is to determine which examination method of nerve conduction disorders may be used predominantly for early diagnosis of diabetic foot development in the follow-up of diabetic patients.

Material and Method: The study consists of 3 different groups (n=150) of patients diagnosed with type 2 diabetes (DM). Group 1; 50 patients with diabetic foot complications (DFC+), Group 2; 50 patients without diabetic foot complications and with polyneuropathy (DFC-/PNP+), Group 3; 50 patients without diabetic foot complications and without polyneuropathy (DFC-/PNP-). Diabetic foot wounds were grouped by PEDIS classification. A total of 150 DM patients were included. The age, sex, diabetes duration, blood glucose levels, HbA1c measurements, and standard electroneuromyography (ENMG) findings were compared.

Findings: Age, sex, diabetes duration, blood glucose, HbA1c values and electroneuromyography (ENMG) for nerve amplitude, velocity and latency results were compared among the groups. A significant statistical difference was found between three groups when age, sex, HgbA1c, fasting blood glucose, diabetes duration was evaluated ($p<0.05$). All DFC+ patients had PNP+. In the DFC+ group, unlike DFC-/PNP+ group, the motor nerves of the lower extremities were also involved. Tibial nerve velocity was lower than normal in DFC+ patients and normal in other groups ($p<0.05$). A statistically significant difference was found in peroneal nerve conduction velocity between the DFC+ group and the DFC- groups ($p<0.05$). Peroneal nerve conduction velocity was not statistically significant between DFC-/PNP+ and DFC-/PNP- groups ($p>0.05$). Peroneal nerve conduction velocity was lowest in the DFC+ group. This factor was considered as a risk factor for DFC development.

Conclusions: The slowdown in peroneal nerve conduction velocity and the increase in diabetes duration were the primary risk factors for diabetic foot development, and the decrease in tibial nerve velocity was also considered as significant. This study showed that the involvement of motor nerve conduction in the lower extremity was considered as a signal for diabetic foot development.

Keywords: Diabetic foot, polyneuropathy, Electroneuromyography

INTRODUCTION

The prevalence of diabetes and diabetes complications has become an increasing global health problem in the world (1). Peripheral neuropathy may cause several complications, including chronic pain, foot ulcers, foot infections and amputations. The prevalence of diabetic foot ulcers in the world is 6%. Approximately 25% of diabetic people experience foot ulcers at least once in their lifetime (2). Diabetic foot ulcers are one of the main causes of lower extremity amputation. Diabetic peripheral neuropathy, history of foot ulcers, structural foot deformity, peripheral artery disease, visual impairment, diabetic nephropathy, poor glycemic control and smoking history are considered as high risk for the development of foot ulcers. It is estimated that approximately 14–24% of people with foot ulcers will require amputation. Patient training has been shown to decrease the incidence of foot ulcers and amputations by up to 50%. Therefore, early diagnosis and treatment is fundamental (3).

The role of neuropathy as a diabetes complication in the development of diabetic foot ulcers is important. Diabetic peripheral neuropathy is the most common form of neuropathy worldwide. Neuropathy is associated with pain, sensory impairment, impairment in quality of life, restrictions in daily activities and depression (4). There are several forms of diabetic peripheral neuropathy. The

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most common type is distal symmetrical polyneuropathy. It constitutes approximately 75% of all diabetic neuropathies (5). Distal symmetrical polyneuropathy causes neuropathic pain symptoms in approximately 10-30% of affected patients (6). It may be characterized as pain, burning, drowsiness, hyperesthesia. It usually affects the lower legs and feet (7). Other forms of diabetic peripheral neuropathy include mononeuropathies and radiculopathy. Mononeuropathies may affect the median, ulnar, radial, tibial, sural, and peroneal nerves (8).

The effect of nerve conduction impairment in diabetic foot development is known, however, a few studies have examined the severity of the impairment on the nerve. The aim of this study is to compare electroneuromyography (ENMG) findings in diabetic patients, to determine the condition of the most affected nerve due to diabetic foot development and to follow up the electrophysiological changes indicating a risk and to take the required measures.

MATERIAL AND METHOD

Ethics committee approval for the study was obtained from Atatürk University Faculty of Medicine Clinical Research Ethics Committee (permission granted: 28.05.2020, decision no: B.30.2.ATA.0.01.00/278). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

The study consisting of 3 different patient groups diagnosed with type 2 diabetes (DM) was designed prospectively. Group 1; 50 patients followed up in the Infectious Diseases clinic, diagnosed with type 2 diabetes and diabetic foot complication (DFC+), Group 2; 50 patients admitted to neurology outpatient clinic with polyneuropathy and without diabetic foot complications (DFC-/PNP+), Group 3; 50 patients admitted to neurology outpatient clinic without polyneuropathy and without diabetic foot complication (DFC-/PNP-). A total of 150 DM patients were included. The age, sex, diabetes duration, blood glucose levels, HbA1c measurements, and standard electroneuromyography (ENMG) findings were compared. Motor and sensory action potentials latency (peak delay time), velocity, amplitude data were noted in the median, ulnar, tibial, peroneal and sural nerves by ENMG procedure. Foot wounds of DFC + patients were divided into four groups by PEDIS classification. Demographic characteristics, blood glucose levels, and HbA1c measurements of the patients were compared between the groups. Several rating systems by the ulcer condition were used for the classification of diabetic foot ulcers. In this study, PEDIS classification was used by the "Diabetic Foot International Study Group" to evaluate ulcer perfusion, width, depth, infection and sensory loss (9).

Nihon Kohden Neuropack M1 ENMG measurement unit was used in our study. The patients were prepared by resting for 15 minutes at 22–24°C room temperature before the examination. In all cases, skin resistance was minimized by cleaning the skin using alcohol before the examination. In the electrodiagnostic study; sensory and motor nerve action potentials of the median and ulnar nerves in the upper extremities, peroneal, posterior tibial motor nerve action potentials and sural nerve action potential in the lower extremities were measured. The nerve conduction velocity, amplitude and latency values were considered as normal by the cut-off values of our local hospital and the literature (10). All nerve conduction studies were performed by the same investigator.

Statistical analysis was performed by SPSS 22.0 package program. Kolmogrof Smirnow test was used to evaluate the compliance of the data to normal distribution. When the numerical data were compared, Anova analysis was used if the number of groups was more than three in normal distributions and Kruskal Wallis analysis was used in non-normal distribution. Post-hoc Tukey analysis was used in the groups distributed homogeneously after Anova test if the data differed significantly between the groups. Mann-WhitneyU test was used for binary comparisons in the groups when data were not normally distributed. Pearason correlation test was used for correlation analysis. Chi-square test was used to evaluate categorical data. $p < 0.05$ was considered statistically significant in all tests.

RESULTS

The study results showed that all patients with DFC development had PNP+. Given the mean age of the patients, a statistically significant difference was found between three groups. This difference was found between two DFC-/PNP + and DFC-/PNP- groups. The mean age of the DFC + patients was (62.06±10.48). The mean age of the patients with DFC-/PNP+ (62.84±11.7) was significantly higher than the patients with DFC-/ NP- (57±11.08). A significant statistical difference was found between three groups in terms of sex ($p < 0.05$). The male ratio was higher in DFC patients than the other two groups. HgbA1c levels were statistically significantly different between three groups. HgbA1c level was the highest in the DFC+ group, and the lowest in the DFC-/PNP- group ($p < 0.05$). The blood glucose level was the highest in the DFC+ group, the lowest in the DM +/PNP- group. DM duration was significantly different between three groups ($p < 0.05$). This difference was found between DFC+ group and other two DFC- groups. Disease duration was determined as the highest causative parameter for DFC development (**Table 1**).

Table 1. Comparison of the demographic characteristics, HbA1c and blood glucose levels of DFC + and DFC- patients*

	DFC+ n=50	DFC-/PNP+ n=50	DFC-/PNP- n=50	p
Age (years)	62.06 ±10.48[ab]	62.84±11.7[a]	57±11.08[b]	0.019*
Sex n (%)				0.000***
Female	13(26%)	21(42%)	40(80%)	
Male	37(74%)	29(58%)	10(20%)	
HbA1c	9.63±2.27	8.32±1.56	7.56±2.11	0.000**
Blood glucose (mg/dL)	245.08±95.29	169.04±53.08	151.80±81.22	0.000**
DM duration (years)	11.36±5.96	7.34±6.34[a]	4.80±4.49[a]	0.000*

* Anova Test, ** Kruskal - Wallis, *** Pearson chi-square, ab: No difference was found between composites with the same letter for each measurement value. DM: Diabetes Mellitus DFC: Diabetic foot, PNP: Polyneuropathy

Our study showed that the latency values were within normal limits in all nerve parameters of the upper and lower extremities examined by ENMG in three groups, except for the prolonged median motor latency in DFC group. Nerve conduction studies showed that the patients in DFC+ group had sensorial polyneuropathy in the upper extremities and sensor-motor axonal type polyneuropathy in the lower extremities. Sensory axonal type polyneuropathy was present in the upper and lower extremities in DFC-/PNP + group. In DFC+ group, unlike DFC-/PNP+ group, the motor nerves of the lower extremities were also involved (**Table 2**).

In our study, amplitudes and velocities of all motor and sensory nerve parameters examined in the upper and lower extremities were lowest in DFC+ group and the highest in DFC-/PNP- group. A statistically significant difference was found between three groups in amplitude and velocities of all nerves except median motor amplitudes ($p<0.05$).

Tibial nerve velocity was lower than normal in DFC+ patients and normal in other groups. Tibial nerve velocity was lowest in DFC+ group and was statistically significantly lower than the other two groups. A significant difference was also found between DFC-/PNP+ and DFC-/PNP- groups ($p<0.05$). A statistically significant difference was found in peroneal nerve conduction velocity between DFC+ group and DFC-/PNP+ and DFC-/PNP- groups ($p<0.05$). Peroneal nerve conduction velocity was lowest in the DFC+ group (**Table 2**). However, peroneal nerve conduction velocity was not statistically significant between DFC-/PNP+ and DFC-/PNP- groups ($p>0.05$). No statistically significant correlation was found between peroneal velocity and DM duration, HgbA1c level, blood glucose, and age in DFC+ patients (Pearson's correlation test $p<0.05$).

Table 2. Comparison of nerve conduction findings of patients with and without diabetic foot*

	DFC+ med±sd	DFC- PNP+ med±sd	DFC-PNP- med±sd	p
Median nerve motor latency (msec)	4.13±1.38	3.83±1.06	3.32±0.35	0.000**
Median nerve motor amplitude (mV)	7.63±3.57	7.57±1.84	8.39±1.55	0.106**
Median nerve motor velocity (m/sec)	47.64±10.11	54.08±5.07	60.50±8.26	0.000**
Median nerve sensory latency (msn)	2.61±4.28	2.95±1.14	3.12±0.43	0.433**
Median nerve sensory amplitude (μV)	5.22±6.27	11.02±8.53	26.86±7.85	0.000*
Median nerve sensory velocity (m/sec)	24.21±23.11	42.11±16.85	54.32±4.83	0.000**
Ulnar nerve motor latency (msec)	3.02±0.69	2.49±0.34	2.25±0.20	0.000**
Ulnar nerve motor amplitude (mV)	8.43±3.93	10.71±5.90	13.06±3.39	0.000*
Ulnar nerve motor velocity (m/sec)	46.62±8.93	54.08±8.57	59.88±9.13	0.000**
Ulnar nerve sensory latency (msn)	1.64±1.58	2.29±0.83	2.03±0.96	0.692**
Ulnar nerve sensory amplitude (μV)	4.40±6.25	17.02±18.02	25.60±8.86	0.000*
Ulnar nerve sensory velocity (m/sn)	25.27±24.95	46.45±18.55	58.36±7.70	0.000**
Peroneal nerve latency (msn)	3.72±1.81	3.63±0.83	3.27±0.70	0.000**
Peroneal nerve amplitude (mV)	3.11±2.34	5.30±6.89	5.32±2.26	0.017*
Peroneal nerve velocity (m/sec)	46.04±23.52	52.27±10.22[b]	56.64±15.26[b]	0.027**
Posterior tibial nerve latency (msn)	3.73±3.16[a]	3.84±1.32[a]	3.31±0.55	0.007**
Posterior tibial nerve amplitude (mV)	2.10±2.4	5.37±2.97	11.67±3.84	0.000**
Posterior tibial nerve velocity (m/sec)	24.59±21.18	43.04±11.65	47.98±8.31	0.000**
Sural nerve latency (msec)	0.67±1.98	1.67±1.29	2.39±0.39	0.000**
Sural nerve amplitude (μV)	1.80±4.92	3.95±3.64	20.58±7.69	0.000**
Sural nerve velocity (m/sn)	6.38±18.32	30.42±24.35	64.86±12.83	0.000*

* Anova Test (Tukey analysis in binary comparisons) ** Kruskal-Wallis (Man Whihtey U Test in binary comparisons) *** Pearson chi-square. In binary comparison A-B: No difference was found between composites with the same letter for each measurement value.

When diabetic foot wounds were grouped by PEDIS classification, the highest was PEDIS 3 (42%), the lowest was PEDIS 1 (10%). Wound classification showed that no significant difference was found in age, sex, DM duration and fasting blood glucose between four groups, however, a statistically significant difference was found in HgbA1c levels. HbA1c levels were the highest in PEDIS 4 group and this was significant as compared to PEDIS 2 (p=0.007) and PEDIS 3 (p=0.003) groups. Compared to PEDIS 1, HbA1c level was higher in PEDIS 4, but it was not statistically significant (p=0.061), this was attributed to the low number of patients in the groups (**Table 3**).

DISCUSSION

It is known that factors such as age, gender, and duration of diabetes also play a role in the development of diabetic foot ulcers and other complications. In our study, the mean age was 62.06±10.48 in DFC+ group. The male ratio was higher (74%). DM duration was significantly higher in DFC+ patients as compared to two groups with DFC-, no difference was found in DM duration between DFC- groups. We may suggest that the increase in diabetes duration is a risk factor for DFC development. HbA1c and blood glucose levels were the highest in the DFC+ group, and lowest in the DFC-/PNP- group. These results showed that a better glucose regulation may reduce the risk of PNP and DFC development in diabetic patients. The data of the previous studies showed that elderly age, male sex, impaired glycemic control, and increased diabetes duration are the risk factors for the diabetic foot development (11,12). In our study, patients with diabetic foot were in the elderly age group and male ratio was higher. Patients in the DFC+ group had longer diabetes duration and all patients had PNP. Our results were compatible with the literature. PNP incidence increases with the age and diabetes duration in DM patients. PNP may affect both large and small fibers, causing pain symptoms depending on its size. It may often be asymptomatic. As peripheral neuropathy progresses, the patient becomes insensitive due to loss of protective sensory in distal extremities. This problem may significantly increase the risk of extremity loss. Diabetic patients cannot take protective measures since they do not feel the trauma in foot. Therefore, the development of diabetic foot ulcers is easier (11). Early detection of PNP by evaluating early nerve conduction in DM patients will contribute to prevention of diabetic foot development and decrease the administration of expensive treatment applications.

In our study, there was motor nerve involvement in addition to sensory nerve involvement in the lower extremities of DFC+ patients, unlike the DFC-group. When neuropathic involvement especially affects motor nerves, muscle weakness occurs in diabetic patients, facilitating the diabetic foot development by pressure changes on feet (13).

The study conducted by Karsidag et al. (14) in 30 patients with type 1 diabetes showed that the percentage of abnormal electrophysiological parameters in different motor and sensory nerves was 86.7% in the sural nerve, 83.3% in the peroneal motor nerve, 73.3% in the posterior tibial motor nerve, and the percentage of nerve involvement in the lower extremity was 90% motor, 86.7% sensory and 76.7% sympathetic nerves. They noted that the significance of nerve dysfunction in the lower extremity is associated with the length of these nerves. Again, the neuropathy study conducted by Kakrani et al. (15) on 50 type 2 diabetic patients showed that posterior tibial and sural nerve involvement was more common in diabetic neuropathy. They concluded that the long nerves are often affected by these involvements, and the lower extremity is affected more because of long nerves, and upper extremity involvement requires a longer diabetes duration. In our study, a statistically significantly difference was found in the mean motor amplitude and velocity of posterior tibial nerve and peroneal nerve between the groups, and the values were lowest in DFC + group among 3 groups. However, the motor amplitude of the posterior tibial nerve was within the normal range, and the velocity of posterior tibial nerve was lower than normal. Similarly, the amplitude and velocity of the sural nerve were lower than normal in DFC group and the values were lowest in

Table 3. Comparison of demographic characteristics, DM duration, HbA1c and blood glucose levels of DFC+ patients by PEDIS classification*

	PEDIS 1	PEDIS 2	PEDIS 3	PEDIS 4	p
n (%)	5 (%10)	17(%34)	21(%42)	7(%14)	
Age (years)	63.60±12.17	60.06±9.27	64±10.32	60±13.52	0.646*
Sex n (%)					0.114***
Female	1 (%7.7)	3(%23.1)	5(%38.5)	4(%30.8)	
Male	4 (%82.3)	14(%76.9)	16(%61.5)	3(%69.2)	
DM duration (years)	11±6.51	10±5.5	12.9±6	10.29±6.8	0.481*
HbA1c (%)	9.20±2.8 [abc]	9.27±1.74 [ad]	9.07±1.91[bd]	12.51±2.30[c]	0.022**
Fasting Blood glucose: (mg/dl)	224.60±37.08	237.88±79.70	239.52±120.46	293.85±67.47	0.106**

* ANOVA, ** Kruskal Wallis *** Pearson chi square, Binary comparisons: Man Whitey U Test; p <0.05 statistically significant. AD: No difference was found between composites with the same letter for each measurement value.

DFC+ group among three groups. This finding showed that the long nerves in DFC+ group were affected more as compared to those without DFC development. All necessary proteins synthesized in the cell body are transmitted to the distal parts of the nerves by axoplasmic flow, protecting the anatomical and functional integrity of the nerve (14). Termination of axoplasmic flow in the long nerves is more apparent than in short nerves. Also, in our study, the involvement of long nerves such as peroneal, tibial and sural nerves in the lower extremities in DFC+ group is supported by this information.

In their study, Kızıltan et al. (16) measured only the peroneal nerve and sural nerve conduction levels in the patients with diabetic foot, and they could not find a correlation between these nerve conduction levels and diabetic foot development. In their electrophysiological evaluation on the newly diagnosed diabetic patients, Kulkarni et al. (17) reported that they detected an increase in peroneal nerve latencies and a decrease in motor conduction velocity and amplitudes. As a first proof of diabetic neuropathy, they suggest following up the slowdown of motor conduction velocity to detect the subclinical dysfunctions. Similarly, in our study, the mean peroneal nerve motor velocity (even though at normal level) was statistically significantly lower in DFC+ patients as compared to DFC-/PNP+ and DFC-/PNP- diabetic patients. No significant difference was found between DFC- cases. When the results were evaluated, the slowdown of peroneal nerve velocity was considered as a risk factor for diabetic foot development.

In their study, Taşkiran et al. (18) reported that the velocity of posterior tibial nerve impairs as the duration of diabetes increases. No relationship was found in our study between the slowdown of peroneal velocity, which is considered as a risk factor in DFC+ patients, and DM duration, HbA1c, blood glucose level, and age.

When DFC+ patients were grouped by PEDIS wound classification, the highest was PEDIS 3 (42%) and the lowest was PEDIS 1 (10%). Wound classification showed that no significant difference was found in age, sex, DM duration and fasting blood glucose between four groups, however, a statistically significant difference was found in HgbA1c levels. HbA1c levels were the highest in the PEDIS 4 group. According to PEDIS 1, HbA1c level was higher in PEDIS 4, but it was not statistically significant. This was associated with the low number of patients in the groups. Literature data showed that the incidence of ulcer development and ulcer recurrence increased and the healing times were prolonged in diabetic patients with high HbA1c levels (HbA1c >9%) (19, 20). The data suggested that better glucose regulation in the long term will be effective in the prevention of diabetic foot development and the wound progression.

CONCLUSIONS

The slowdown in peroneal nerve conduction velocity and the increase in diabetes duration were the risk factors for diabetic foot development, and the decrease in tibial nerve velocity was also considered as significant. The disease duration should be considered for early determination of the risk of diabetic foot development, and nerve conduction should be measured at certain intervals. The involvement of motor nerve conduction in the lower extremity, especially, the slowdown in peroneal nerve conduction velocity should be considered as a signal for diabetic foot development.

ETHICAL CONSIDERATIONS

Ethics Committee Approval: The study was carried out with the permission of Atatürk University Faculty of Medicine Clinical Research Ethics Committee (permission granted 28.05.2020, decision no. B.30.2.ATA.0.01.00/278).

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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Original Article

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Antibiotic resistance pattern in *Shigella* species isolated from children with acute diarrhea in Tabriz city, Iran

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ABSTRACT

Aim: *Shigella* is one of the leading causes of acute diarrhea in children worldwide. Antibiotic resistance in this bacterium has increased due to the indiscriminate use of common antibiotics. Therefore, the aim of this study was to investigate the prevalence and pattern of antibiotic resistance in *Shigella* species isolated from children with acute diarrhea in Tabriz hospitals.

Material and Method: In this descriptive cross-sectional study, 321 samples suspected of *Shigella* were collected from children's diarrhea stool samples and identified using standard microbiological and biochemical tests. Antibiotic resistance was also determined using disk diffusion by the Kirby-Bauer method.

Results: A total of 84 samples were positive for *Shigella*. Among them, *S. flexneri* species with 83.3% had the highest frequency and *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysanteriae* with 1.2% had the lowest frequency. The highest resistance was related to the antibiotics cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%) and the lowest resistance was related to imipenem antibiotics (2.38%) and ceftizoxime (9.52%).

Conclusion: The present study showed that *Shigella flexneri* is the predominant species isolated from children with Shigellosis in hospitals in Tabriz city. Our results also indicate an increase in resistance to common antibiotics. Therefore, it is recommended that antimicrobial susceptibility testing be performed in the study area prior to antibiotic administration.

Keywords: Prevalence, *Shigella*, diarrhea, drug resistance

INTRODUCTION

Diarrhea is one of the most serious intestinal infections in children, which is a major health problem in developing countries and one of the leading causes of illness and mortality (1,2). *Shigella* belongs to the family *Enterobacteriaceae*, gram negative coccobacilli, nonmotile, spore-forming rods, and facultative anaerobes. This bacterium causes shigellosis or bloody diarrhea in humans. *Shigella* species are among the most common Enteropathogenic bacteria that have the most diarrheal infections in developing countries. About 200 million *Shigella* infections and 3 to 5 million deaths occur annually in developing countries, most of which involve children under 5 years of age (3). Shigellosis is recognized as a global problem by the World Health Organization (4). *Shigella* contains four species of *S. flexneri*, *S. dysanteriae*, *S. boydii*, and *S. sonnei*, which can cause shigellosis by the presence of blood in the stool (5). Epidemiological data has shown that *S. sonnei* is the predominant species of *Shigella* in Europe, and the United States. But it is noteworthy that *S. flexneri* is more common in Asian and African countries, especially Iran (6). In terms of pathogenicity, the bacteria involved in diarrhea are divided into two groups: invasive bacteria and toxin-producing bacteria. Bacterial pathogens are involved in adhesion, invasion and toxin production. *Shigella* penetrates the mucosal epithelium, begins to grow and penetrates into adjacent cells, eventually causing cell damage. These bacteria multiply in the gut or on the surface of small intestinal epithelial cells to produce toxins that cause water and electrolytes to be secreted by gut cells (7). The bacterium can be transmitted directly from person to person, and indirectly through the consumption of contaminated food and water (8).

The most important symptoms of infection with this bacterium include: anorexia, fever, intestinal inflammation, bloody-purulent stools, abdominal pain, and feeling of incomplete emptying of the intestine with anal pain (9). One of the most important problems for the treatment of Shigellosis-positive people today is the development of antibiotic resistance by the plasmids and integrons.

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Therefore, susceptibility to resistant strains rarely occurs (7). *Shigella* antibiotic resistance is a growing problem in Asia, Africa and South America (10). Because shigellosis is highly contagious, information about the prevalence of the disease and the antimicrobial susceptibility of the strains is crucial to ensure appropriate clinical treatment, and patient management. Therefore, the aim of this study was to determine the pattern of antibiotic resistance in *Shigella* sppisolated from children with diarrhea in Tabriz city.

MATERIAL AND METHOD

In this descriptive cross-sectional study, 321 samples suspected of *Shigella* were collected from stool (diarrhea) samples during one year from November 2016 to November 2017 by random sampling method in special and disposable sterile containers. Stool samples from sick children admitted to different wards of the hospital with positive culture according to *Shigella*, are collected weekly, and then by obtaining the consent of each patient and filling out their personal and clinical profile form and observing the statute of the Commitment Committee Ethical regarding the secrecy of the name and details of each of the test subjects, we transferred the samples to the specialized microbiology laboratory of the Islamic Azad University, Ahar Branch, and proceeded to isolate and identify the bacteria causing the infection. Samples were examined for macroscopic characteristics (consistency, mucus, and blood) and microscopy (white, and red blood cells). Isolates on Salmonella-*Shigella* agar (SS), McConkey agar, xylose lysine deoxycholate agar (XLD) and selenite-F (SF) (manufactured by Merck Germany) for 1 to 4 days at 35 to 37°C was placed in the incubator. Suspected colonies then grown by biochemical and differential tests including oxidase, catalase, SIM, MR/VP, citrate consumption, TSI, urease, phenylalanine deaminase, decarboxylation of the amino acids ornithine and mannitol, the consumption of lysine decarboxylase, XLD and hydrogen sulfide production (all media were provided by Merck, Germany) were identified.

Antibiotic resistance pattern of all *Shigella* isolates by Kirby Baur standard method, and through the instructions of Clinical and Laboratory Standards Institute (CLSI) (11) and preparation of 0.5 McFarland concentration of bacteria and culture on Mueller hinton agar medium against cefotaxime, ceftazidime, ceftriaxone, ampicillin, ciprofloxacin, ceftizoxime, imipenem, nalidixic acid, tetracycline, cotrimoxazole and cefxime (Mast, UK) were performed at the plate level according to the standard, antibiogram. Results were reported according to CLSI guidelines. In order to statistically analyze the data, the twentieth version of SPSS software and Chi-square and Fisher tests were used. P<0.05 values were considered statistically significant.

RESULTS

Out of 321 stool (diarrhea) samples, 84 (26.17%) samples were positive for *Shigella*. Of these, 46 (54.76%) samples belonged to males and 38 (45.24%) samples belonged to females. The mean age of patients varied from 6.07±2.12 to at least one year to a maximum of 12 years. *S. flexneri* species with 83.3% had the highest frequency and *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysanteriae* with 1.2% had the lowest frequency. According to Fisher test, there was no statistically significant difference between *Shigella* species in sex and age (p>0.05). Antibiogram results show that the highest resistance is related to the antibiotics cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%) (**Figure 1**). The results of Fisher's exact test showed there was no significant relationship between *Shigella* and antibiotic resistance (p>0.05).

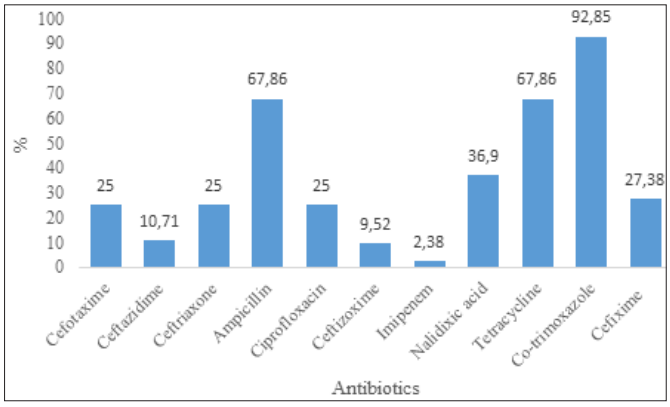

Figure 1. Percentage of antibiotic resistance in *Shigella* bacteria

DISCUSSION

Shigella is the causative agent of Shigellosis, a bacterial diarrhea that develops in adults and is very dangerous in infants and children, and can lead to death if left untreated. The disease is endemic worldwide, affecting 163 million people worldwide each year (12,13). The findings of the present study shows that out of 321 stool (diarrhea) samples, 84 (26.17%) samples are positive for *Shigella*. Of these species, *S. flexneri* with 83.3% had the highest frequency, *S. boydii* with 13.1%, *S. sonnei* with 2.4% and *S. dysanteriae* with 1.2% had the lowest frequency. In developing countries, the prevalence of *S. flexneri* is 60%, *S. sonnei* 15%, *S. boydii* 6% and *S. dysanteriae* 6%. In developed countries, this amount is 16%, 77%, 2, and 1, respectively (4).

Yavari et al. (14), Khorshidi et al. (15), Abbasi et al.(16), Hosseini Nave et al. (17), Kahsay et al. (18), and Tajaddini et al. (19), by examining *Shigella* on patients with diarrhea, the incidence of Shigellosis was reported to be 7.8%, 7.6%, 8.2%, 9%, 13.3% and 14.1%. Which are less than the findings of the present study. In the present study, the most common *Shigella* species are related to *S. flexneri* with 83.3%. Which is in line with the findings of the study of Moosavian et al. (20), in Ahvaz and Hosseini Nave et al. (17), in Kerman.

In 2014, Abbaspour and colleagues isolated 90 *Shigella* cases from a total of 9,131 stool samples in Tehran. Of these, 70% belonged to the S.sonnei, 28.9% belonged to the *S. flexneri* and 1.1% belonged to the *S. boydii* (21). In 2020, Karimi-Yazdi et al. (22), reported a prevalence of *S. sonnei* of 78.7%, *S. flexneri* of 19.9%, and *S. boydii* of 1.4%. Abbasi et al. (16), examined multidrug-resistant *Shigella* infection in children with diarrhea, out of 19 positive *Shigella* specimens, 21% belonged to flexneri species and 78.9% belonged to sonnei species. Differences in the frequency of *Shigella* and its genera may be related to age, geography, climate, as well as many other environmental conditions. Facilities such as a public water supply, and sewerage systems, close relationship with the level of health and personal hygiene can be another reason for these differences. *S. sonnei* is generally found in industrialized countries, while *S. flexneri* is highly prevalent in developing countries (23). Analysis of antibiotic resistance data in *Shigella* shows that the highest resistance is related to cotrimoxazole (92.85%), tetracycline and ampicillin (67.86%), and the lowest resistance is related to imipenem 2.38% and ceftizoxime 9.52%. During a study in Tehran, the *Shigella* resistance to common antibiotics was reported as follows: cotrimoxazole 92.2%, tetracycline 65.6%, ampicillin 65.6%, nalidixic acid 34.4%, cefixime 24.4%, Ceftriaxone 23.3%, cefotaxime 22.2%, ciprofloxacin 22.2%, ceftazidime 7.8%, ceftizoxime 7.8%, and imipenem 1.1%. Which is consistent with the findings of the present study (21).

Dolatshahi and Amini from a total of 300 stool samples tested, obtained 60 (20%) *S. sonnei* strains. All isolates (100%) were resistant to streptomycin and nalidixic acid. Also, the results of antimicrobial susceptibility test showed that percentages of resistance to tetracycline, chloramphenicol and ampicillin were 86.7%, 91.8% and 50%, respectively (24). Previous reports in Iran has reported resistance to cotrimoxazole from 92.2% to 94%. Which is close to the findings of the present study (25,26).

According to reports by Ashkenazi et al. (27), in 2003 and Akçali et al. (28), in 2008, ampicillin resistance ranged from 12-20% to 87%. Which is less and more of the findings of the present study, respectively. In a study by Zamanlou et al. (29), and Aggarwal et al. (30), in 2018 and 2016, ciprofloxacin antibiotic resistance reported 4.2% and 56.2%, respectively. Which is less and more from the present study, respectively. According to the findings of Zamanlou et al. (29), and Ranjbar et al. (31), in Iran, the resistance to nalidixic acid antibiotic has been reported as 17.4% and 31%, respectively. Which are less than the findings of the present study.

CONCLUSION

Due to the increasing in prevalence of *Shigella* species, and the increasing resistance of intestinal pathogenic strains to widely use and inexpensive antibiotics, it seems that effective antibiotic treatment is becoming more difficult. Our results therefore raise concerns about the spread of *Shigella* among children with diarrhea in the study area. Therefore, to prevent the spread of these resistant isolates, monitoring of the antimicrobial resistance of *Shigella* species should be considered on an ongoing basis and common antibiotics should be used properly.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study has been approved by the ethical committee of Ahar University of Sciences (permission granted: 12.06.2020, decision no: 2020-02).

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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The frequency and relationship of osteoporosis and vitamin D deficiency in the female geriatric population in Central Anatolia

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ABSTRACT

Aims: The frequency of osteoporosis increases with age and is an important cause of mortality and morbidity due to hip fractures. The aim of our study is to determine the frequency of osteoporosis and vitamin D deficiency in female geriatric population and to investigate the relationship of vertebral and femur bone mineral densities (BMD) with vitamin D level in this population.

Material and Method: The study included 457 women aged 65 years and older. Vertebra and femur bone mineral densities (BMD) and serum 25-hydroxy vitamin D3 (25 (OH) D3) levels were measured.

Results: The mean age was found to be 72.4±5.7 years. The mean L1-L4 and Femur neck BMD values in the participants were -2.74±0.77, -2.31±0.95 respectively, and the rate of osteoporosis was 72.2% (n=330). There was no statistically significant difference between age groups in respect to frequency of osteaporosis (p=0.58). Weak negative correlation was found between femur neck BMD value and age (r=-0.26, p<0.001). Vitamin D level was 13.00±8.80 ng/ml and vitamin D deficiency was 87.4% (n=387). There was no statistically significant difference between age groups in respect to frequency of vitamin D deficiency (p=0.16). Similarly, no statistically significant relationship was found between osteoporosis and vitamin D deficiency (p=0.65).

Conclusion: This study suggested that geographic, cultural, and genetic factors are more important determinants in the development of osteoporosis.

Keywords: Bone mineral density, Central Anatolia, female geriatric population, vitamin D

INTRODUCTION

Osteoporosis is a disease characterized by a decrease in bone density and deterioration in bone microarchitecture, and it is the most common metabolic disease of bone (1). Senile osteoporosis (Type II osteoporosis) is the type of osteoporosis seen in both women and men, especially after the age of 70. Although many factors play a role in the pathogenesis of senile osteoporosis, the most important and emphasized cause is vitamin D deficiency and secondary hyperparathyroidism resulting from it (2).

Vitamin D is a steroid hormone that is essential for normal calcium and bone metabolism. The source of vitamin D in the body is cholecalciferol synthesized on the skin with the effect of ultraviolet light and ergocalciferol taken with diet. The initial metabolism of vitamin D precursors occurs in the form of hydroxylation (25-hydroxy vitamin D3 [25 (OH)D3]) in the 25th position of the carbon atom in the structure of the precursors in the liver. It is then transformed into its active form (1,25-hydroxy vitamin D3 [1,25 (OH)D3]) by hydroxylation of the carbon atom in its first position in 25 (OH)D3 in the kidney. This active form provides bone mineralization by increasing absorption of calcium and phosphorus from the intestines (3).

The best indicator of vitamin D status in tissues is serum 25 (OH)D3 level. Vitamin D deficiency is defined as the level of vitamin D at which histological, laboratory and clinical findings occur. Different threshold values are used for vitamin D deficiency (4,5). Vitamin D deficiency is especially common in closed clothing societies, northern European countries, the elderly, and those living in nursing homes. The frequency of vitamin D deficiency increases in the elderly due to the decrease in ultraviolet light exposure, decreased skin's vitamin D synthesis capacity, inadequate intake of vitamin D with nutrients, increased renal dysfunction and malabsorption with aging (6).

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When there is a deficiency of Vitamin D, 1,25 (OH)D3 level decreases, in response, the level of parathormone (PTH) increases. Increasing PTH level increases at 1,25 (OH) D3 level and causes bone destruction. Thus, a sufficient level of 1,25 (OH)D3 is achieved despite increased bone destruction. This condition is called secondary hyperparathyroidism (7).

The aim of our study is to determine the frequency of vitamin D deficiency, which is one of the most important factors contributing to osteoporosis and senile osteoporosis in the female geriatric age group in Central Anatolia; to reveal the relationship between BMD values and osteoporosis and vitamin D deficiency in this population.

MATERIAL AND METHOD

Ethics committee approval was provided from the Yozgat Bozok University Medical Faculty Local Ethics Committee (approval no: 2017-KAEK-189_2019.02.28-21), and informed consent of each patient was obtained for the study. All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

This prospective, cross-sectional study was conducted between March 1, 2019, and March 1, 2020 at the outpatient clinics of the internal medicine department in a tertiary hospital. The study consecutively included 457 women aged 65 years and older, who were admitted to the outpatient unit of Internal Medicine Department in the tertiary hospital, living in the community and independently acting in her daily life activities. The exclusion criteria were as follows: those who had renal failure (glomerular filtration rate less than 90 ml/min), liver failure (liver transaminases levels more than two times of upper range of the limit), patients with secondary osteoporosis disease, patients with metabolic bone disease other than osteoporosis, bedridden patients, patient using drugs that may affect bone metabolism (thyroid hormones, estrogen, selective estrogen receptors, glucocorticoids, bisphosphonates, calcium, vitamin D+calcium). The individuals participating in the study were divided into three groups as per age: 65-74 years old; 75-84 years old; and 85 years and older.

BMD Measurement and Diagnosis of Osteoporosis

Bone mineral densities (BMD) values were measured by using DEXA device (Discovery DXA, Hologic, Marlborough, Massachusetts, USA). The diagnosis of osteoporosis was made according to the World Health Organization (WHO) criteria for osteoporosis. Patients were divided into three groups according to the T score: Those with T score >-1 were normal, those with -1≥T score >-2.5 were considered osteopenic and those with T score ≤-2.5 were considered osteoporosis.

Diagnosis of Vitamin D Deficiency

25 (OH) D3 level was measured by radioimmunassay method (Diasorid, 25-Hydroxyvitamin D 125I RIA Kit, Stillwater, Minnesota, USA). Vitamin D levels were divided into 4 groups: ≥30 ng/ml was considered as sufficient Vitamin D level; ≥20 ng/ml but <30 ng/ml as insufficient Vitamin D level; ≥10 ng/ml but <20 ng/ml as deficient Vitamin D level; <10 ng/ml level as osteomalasic. All individuals with values below 20 ng/ml were also considered as vitamin D deficiency.

Statistical Analysis

SPSS version 20.0 (Statistical Package for Social Sciences) program was used for statistical analysis of the findings. Descriptive statistics were used in the analysis of the data. Continuous variables are expressed as mean±standard deviation (SD) and nominal variables as percentage. Pearson's correlation test was used to evaluate the correlation between variables. Chi-square test was used to evaluate the significance of the differences between the groups. P value <0.05 was considered statistically significant.

RESULTS

The study included 457 women aged 65 years and older consecutively. The ages of the patients ranged from 65 to 95 years old, and the mean age of the study population was found to be 72.4±5.7 years.

The mean values of L1-L4 and femur neck BMD were -2.74±0.77 and -2.31±0.95, respectively. Osteoporosis rate was 72.2% (n=330) among the study population. In terms of osteoporosis, the distribution of individuals was given in **Table 1**.

As per age groups, it was found that there was no statistically significant difference between age groups in respect to osteoporosis (p=0.58). Distribution of osteoporosis by age groups was shown in **Table 2**.

The average vitamin D level was 13.00±8.80 ng/ml. Rate of vitamin D deficiency was found to be 87.4% (n=387). Frequency of vitamin D deficiency at the osteomalasic level was found to be 46.8%. The distribution of individuals in terms of vitamin D deficiency was given in **Table 3**.

Considering the distribution of vitamin D deficiency according to age groups, there was no statistically significant difference between age groups (p=0.16). Distribution of vitamin D deficiency by age groups was shown in **Table 4**.

When looking at the relationship between osteoporosis and vitamin D deficiency, no statistically significant relation was found (p=0.65). When looking at the relationship between osteoporosis and vitamin D deficiency at osteomalasic level, there was no statistically significant relation (p=0.18). The relation between osteoporosis and vitamin D deficiency was expressed in **Table 5**.

Table 1. Osteoporosis status of individuals according to L1-L4 BMD, Femur neck BMD and evaluated together

	Femur neck BMD values		
	Normal (-1<Femur neck BMD)	Osteopenic (-1≥Femur neck BMD>-2.5)	Osteoporosis (Femur neck BMD≤-2.5)
Osteoporosis status (n=457)	19.5% (n=89)	35.4% (n=162)	45.1% (n=206)
	L1 –L4 BMD values		
	Normal (-1<L1–L4 BMD)	Osteopenic (-1≥L1–L4 BMD>-2.5)	Osteoporosis (L1 –L4 BMD≤-2.5)
Osteoporosis status (n=457)	5.5% (n=25)	30.2% (n=138)	64.3% (n=294)
	L1-L4 and Femur neck BMD values are evaluated together		
	Normal (-1<L1–L4 and Femur neck BMD)	Osteopenic (-1≥L1 –L4 and/or Femur neck BMD>-2.5)	Osteoporosis (L1 –L4 and/or Femur neck BMD≤-2.5)
Osteoporosis status (n=457)	3.7% (n=17)	24.1% (n=110)	72.2% (n=330)

BMD: Bone mineral density

Table 2. Distribution of osteoporosis by age groups

		Age groups			
		65≤Age<75 (n=313)	75≤Age<85 (n=127)	Age≥85 (n=17)	p value
Osteoporosis	Yes (n=330)	71.2% (n=223)	73.2% (n=93)	82.4% (n=14)	p=0.58
	No (n=127)	28.8% (n=90)	26.8% (n=34)	17.6% (n=3)	

Table 3. Distribution of individuals according to 25 (OH) D3 levels

	25(OH)D3 levels (ng/ml)			
	Osteomalasic (25(OH)D3<10)	Deficient (10≤25(OH)D3<20)	Insufficient (20≤25(OH)D3<30)	Sufficient (25(OH)D3≥30)
Vitamin D status (n=457)	46.8% (n=214)	37.9% (n=173)	9% (n=41)	6.3% (n=29)

25 (OH) D3: 25-hydroxy vitamin D3

Table 4. Distribution of Vitamin D deficiency by age groups

		Age groups			
		65≤Age<75 (n=313)	75≤Age<85 (n=127)	Age≥85 (n=17)	p value
Vitamin D deficiencyi (<20 ng/ml)	Yes (n =387)	85% (n=266)	81.9% (n=104)	100% (n=17)	p=0.16
	No (n=127)	15% (n=47)	18.1% (n=23)	0% (n=0)	

Table 5. The relationship between osteoporosis and vitamin D deficiency

		Vitamin D deficiency at osteomalasic levals (25(OH)D3 levels <10 ng/ml)		
		Yes (n=214)	**No (n=243)**	**p value**
Osteoporosis (L1 –L4 and/or Femur neck BMD≤-2.5)	Yes (n=330)	48.8% (n=161)	51.2% (n=169)	p=0.18
	No (n=127)	41.7% (n=53)	58.3% (n=74)	
		Vitamin D deficiency (25(OH)D3 levels <20 ng/ml)		
		Yes (n=387)	**No (n=70)**	**p value**
Osteoporosis (L1 –L4 and/or Femur neck BMD≤-2.5)	Yes (n=330)	85.2% (n=281)	14.8% (n=49)	p=0.65
	No (n=127)	83.5% (n=106)	16.5% (n=21)	

In correlation analysis of vitamin D level and age, there was no statistically significant correlation between the two variables (r=-0.027, p=0.56). In correlation analysis of L1-L4 with femoral neck BMD values and with vitamin D, there was no statistically significant correlation in both analyses (r =0.064, p =0.17; r=0.042, p=0.37, respectively). Similarly, there was no statistically significant correlation between L1-L4 BMD value and age (r=-0.091, p=0.052). However, statistically significant but weak negative correlation was found between femoral neck BMD and age (r=-0.26, p<0.001).

DISCUSSION

In our study, we evaluated vitamin D deficiency and frequency of osteoporosis in women aged 65 and older who applied to the internal medicine outpatient clinic. We found that 87.4% of the study population was found to have vitamin D deficiency. In the multi-centered "Survey in Europe on Nutrition and the Elderly; A Concerted Action" (SENECA) study that included individuals between the ages of 71-76 in European countries, 47% of female participants and 36% of male participants were found to have Vitamin D deficiency. In this study, the level for vitamin D deficiency was accepted as <12 ng/

ml. Interestingly, in the results of the study, the prevalence of vitamin D deficiency was found to be 83% in Greece among Mediterranean countries, while this rate was found to be 18% in Norway among Northern European countries (8). In a study conducted in the United States with the participation of 13,432 people, the rate of vitamin D deficiency (<23.3 ng/ml) was found to be 32% in individuals over 50 (9). In a study conducted by Hirani et al. (10) in individuals aged 65 and older in the UK, the rate of vitamin D deficiency (10 ng/ml) was found to be 15% in males and 9.6% in females. The prevalence of total vitamin D deficiency (<15 ng/ml) was found to be 33.4% in the study, which included 195 elderly people living at home and 225 elderly people living in the elderly nursing home. This rate was found to be 24.4% in the elderly living in their own homes and 40.1% in the elderly living in the nursing home (11). The frequency of vitamin D deficiency is higher in the elderly living in nursing homes compared to the elderly living in the community (10,11). The prevalence of vitamin D deficiency varies depending on the geographic region, population, 25 (OH)D3 threshold used for definition of vitamin D deficiency and dietary habits, but it is common in the geriatric population. The prevalence of vitamin D deficiency in the female elderly in our study was found to be higher than the elderly population described in the litareture. The reason for our higher rates may be because of religious beliefs that women are over-covered with clothing and the skin is less exposed to sunlight. Additionally, considering the fact that vitamin D deficiency was 83% in Greece in the SENECA study was close to the rate in our study, it suggests that geographic or especially genetic factors may be more important determinants in vitamin D deficiency and osteoporosis.

Osteoporosis is one of the most important causes of mortality and morbidity in this age group due to its frequency of up to 50% in the geriatric age group and increased risk of hip fracture. Although the prevalence of osteoporosis in women is higher than men in the postmenopausal period, the rate of osteoporosis detected in male gender after 65 years old approaches to that of female gender (12). In our study, the rate of osteoporosis was found to be 72.2% in women over 65 years old. It may be due to the fact that over-covering of women with clothes due to religious beliefs reduces the skin's exposure to sunlight. As a result, it may be that in women, bone mineralization has not reached the optimal level in the premenopausal period and the loss is faster and higher in the postmenopausal period. It also suggests that geographic or especially genetic factors may be more important determining factors.

Vitamin D deficiency is the most important cause of type II osteoporosis in the geriatric age group. Increased PTH levels cause increased bone production and destruction and decreased BMD. Secondary hyperparathyroidism, which develops due to vitamin D deficiency in the elderly, leads to a decrease in BMD and increase in fractures, especially in the hip region where the cortical bone is dense (13). In the Supplements en Vitamines et Mineraux Antioxydants (SUVIMAX) study conducted in France, a significant correlation was found between serum PTH and 25 (OH)D3 levels. In this study, in patients with vitamin D deficiency but without secondary hyperparathyroidism, the decrease in BMD values was found to be less than in cases with secondary hyperparathyroidism. This situation has been associated with insufficient elevation of PTH level and inadequate bone resorption in some of the patients with vitamin D deficiency (14). In a study by Sahota et al. (15), a negative correlation was found between vitamin D and PTH values in 421 postmenopausal women aged 60 to 80 years old. Similar to the SUVIMAX study, insufficient PTH response was observed in some cases with vitamin D deficiency. The reason for the lack of a significant relationship between vitamin D deficiency and osteoporosis in our study may be due to that insufficient PTH response or serum 25 (OH) D3 levels throughout the year in our study population.

In many studies conducted in the literature, while a relation was found between vitamin D deficiency and hip BMD values, the same relationship could not be revealed clearly with vertebral BMD values. The probable reason for not correlating with vertebral BMD values may be related with the increasing frequency of degenerative vertebral changes with increasing age, osteophytes and facet joint osteoarthritis frequently seen in lumbar vertebrae, and these degenerative changes appear as increased BMD values in DEXA measurements. In addition, due to the higher PTH activity in the cortical bone, the decrease in BMD is less in the vertebral bones where trabecular bone density is higher (16). In our study, no correlation was found between serum 25 (OH)D3 levels and BMD values of the femoral neck and vertebrae. In the study conducted by Sahota et al. (15), total hip BMD values were lower in women with vitamin D deficiency and secondary hyperparathyroidism and a correlation was found between serum PTH and hip BMD values. However, the similar relationship was not determined for lumbal vertebra BMD value. Similarly, in another study, a negative correlation was found between serum D vitamin level and PTH levels in 119 patients aged 65 - 90 years. There was a significant decrease in total hip BMD values in the group with vitamin D deficiency. However, lumbal vertebra BMD values were not found to have the same relationship (17). Contrary to the literature, in our study, the relationship between femur BMD values and serum 25 (OH)D3 levels may be due to the fact that the vitamin D levels of the participants were measured throughout the year. It is likely that the values observed in spring

and summer were higher and lower in winter. If all the participants' vitamin D levels in our study were examined in the same month, we would probably have shown the relationship more accurately. Perhaps geographic or genetic factors may be more important determinants of osteoporosis than vitamin D level.

Our study has several limitations. The first is that the study is cross-sectional. Causality cannot be evaluated in cross-sectional studies. The second limitation was that serum 25 (OH)D3 levels was not measured at the same season of the year.

CONCLUSION

Serum vitamin D deficiency and osteoporosis are frequently seen in people aged 65 and over who apply to the outpatient clinic. Increasing exposure to sunlight and enriching frequently consumed foods with calcium and vitamin D can be simple but effective methods for increasing vitamin D levels in the elderly. However, this study suggests that cultural, geographical, and genetic factors may be more important determinants in the development of osteoporosis. Especially in the premenopausal period in which bone mineralization has reached the maximum level, in the societies where the exposure of the skin to the sunlight is insufficient - excessive covering with clothes due to religious beliefs - calcium and vitamin D replacement may be decreased osteoporosis and fracture development at the geriatric age in these women. In other words, premenopausal calcium and vitamin D replacement may be more important than postmenopausal calcium and vitamin D replacement in these women.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Yozgat Bozok University Medical Faculty Local Ethics Committee (approval no: 2017-KAEK-189_2019.02.28-21).

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

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: Tekin Yıldırım declare that they have no conflict of interest.

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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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Can mini-tracheostomy needle be safer for residency training in percutaneous dilatation tracheostomy applications in intensive care unit?

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ABSTRACT

Aim: The aim of this study is to evaluate the bedside percutaneous dilatation tracheostomy (PDT), performed with fiberoptic bronchoscopy guided Griggs technique using a mini-tracheostomy needle, by residents in intensive care unit (ICU) retrospectively.

Material and Method: Twenty PDT applications were performed in ICU using mini-tracheostomy needle in the present study. All PDT procedures were performed by a resident who was currently undergoing ICU residency training. Griggs technique was used in all procedures. All complications, the time from needle insertion to the insertion of the tracheostomy cannula was also noted.

Results: The average age of the patients was 69.8±16.14 years. The mean Acute Physiology and Chronic Health Assessment (APACHE) II score of the patients was 23.05±6.16, Glasgow Coma Scale (GCS) score was 10±3.43, and Sepsis-Related Organ Failure Assessment (SOFA) score was 7.2±2.11. The mean procedure time was 13±1.68 minutes, and the day of tracheostomy application was 6.35±4.59. Twelve (60%) patients were switched to home-type ventilators. Ten (50%) of the patients were transferred to the palliative ward with home-type ventilator. Mortality rate was 20% (4 patients) at 90 days. When patients were evaluated in terms of complications; none of the patients had pneumothorax, subcutaneous emphysema, posterior tracheal wall damage, or tracheoesophageal fistula. Minimal bleeding that required no intervention was observed in only one patient.

Conclusion: Using mini-tracheostomy needle in PDTs performed via fiberoptic bronchoscopy by less experienced residents may be safer to prevent complications.

Keywords: Mini-tracheostomy, PDT, residency training, mini-tracheostomy needle

INTRODUCTION

Recently, the percutaneous dilatational tracheostomy (PDT) technique, which is used in intensive care unit (ICU), in patients who require long-term mechanical ventilation (MV), has been used increasingly and with a higher rate compared to the surgical technique (1). PDT has some advantages such as easy application with a small skin incision at the bedside, less tissue damage, and limited complications (2,3). It is possible to protect the airway, prevent complications due to intubation, clear airway secretions easily, and to facilitate the patient's speech and oral nutrition with this process (4-6). There are various methods in PDT applications. In one of these, Griggs technique; the cannula is placed in the trachea by performing tracheal dilatation with specially designed forceps (7). The most common complications of the PDT include bleeding, hypoxia, pneumothorax, pneumomediastinum, subcutaneous emphysema, malposition, and posterior tracheal wall damage (8). Because of serious complications enough experience could be crucial to achieve successful PDT procedure. Therefore adequate residency training is a key factor to prevent PDT complications. Manikin-based and cadaver studies have been performed to provide better hand-skill (9). We think that during PDT procedure needle puncture is a stressful period due to the risk of damage to the posterior wall of the trachea and nearby vascular structures and short needle length could be safer in PDT intervention.

Fiberoptic bronchoscopy (FOB) intervention helps the safe and easy application of PDT by providing vision

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inside the trachea. There are studies indicating that the FOB reduces complications such as pneumothorax, paratracheal placement of the tracheostomy cannula, posterior tracheal wall damage (10,11). The mini-tracheostomy is an intervention that performed easily and frequently in emergency services in case of emergent airway management. It is also used to facilitate secretion cleaning and reduce FOB requirements (12). Prophylactic use of mini-tracheostomy is also recommended in patients with high risk for secretion retention (12,13).

The mini-tracheostomy is applied through the cricothyroid membrane in clinical practice. It is also performed through the subcricoid region. This application provides some advantages such as less development of stenosis, and if needed standard percutaneous tracheostomy can be easily performed through this tract (14). Mini-tracheostomy needles are short and the tip of needle is curved. These properties of needle provide some advantages particularly to prevent complications. Although we couldn't find any data about the use of mini-tracheostomy needle in PDT applications in the literature, using mini-tracheostomy needle during PDT training might be safer for residents with fewer experiences.

We hypothesized that, the use of a mini-tracheostomy needle during PDT may be appropriate, especially for ICU residents' training program in order to minimize serious complications such as the posterior tracheal wall damage that may develop during the procedure. In addition, we considered performing the procedure with the aid of FOB will be effective in reducing complications.

In this descriptive study, we aimed to evaluate the bedside PDTs, performed with FOB-guided Griggs technique using a mini-tracheostomy needle, by residents in ICU patients retrospectively.

MATERIAL AND METHOD

The study was carried out with the permission of the Ethics Committee of Ankara Atatürk Chest Diseases and Chest Surgery Training and Research Hospital Clinical Researchs Ethics Committee (permission date: 14/01/2021, decision number: 709). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of Principles.

The PDTs performed in 20 ICU patients using a mini-tracheostomy needle (Minitrach® II, Portex, UK) (**Figure 1**) were examined. Demographic data, duration and number of intubation, the Glasgow Coma Scale (GCS), Acute Physiology and Chronic Health Assessment (APACHE) II, Sepsis-Related Organ Failure Assessment (SOFA) scores of the patients who were clinically suitable for PDT, duration of the procedure, those who switched to home-type MV, and those transferred from the ICU to the palliative care unit, procedure-related complications, and 90-day mortality were recorded retrospectively.

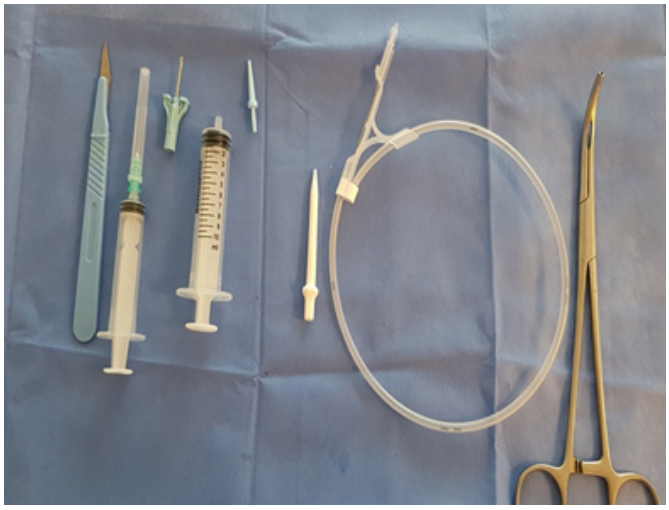

Figure 1. Mini-trach needle

Protocol

All patients were prepared for tracheostomy in accordance with the standard protocol used in our clinic. Patients were given intravenously 1 mcg/kg fentanyl, 0.5-1 mg/kg propofol, and 0.5 mg/kg rocuronium before the procedure. Five minutes before the procedure, the fraction of inspired oxygen (FiO2) of 100% and respiratory rate of 20/min were set in the controlled mechanical ventilation mode.

During the procedure, all patients were followed up with electrocardiogram, peripheral oxygen saturation and invasive arterial pressure monitoring.

A roller blanchett was placed under the shoulder of the patient with head in extension position to increase the view of the tracheostomy area. During the procedure, the eyelids were closed to protect the eyes. In sterile conditions, the application area was cleaned with povidin iodine and covered. Local anesthesia was applied with 3 ml of 2% lidocaine.

All PDT procedures were performed by a resident who was currently undergoing ICU residency training and had at least 10 PDT experience, in the presence of an experienced anesthesiologist.

During the procedure, an ICU nurse was also included in the team. Griggs technique was used in all procedures (7). A catheter mount was attached to the endotracheal tube to allow both passage of the bronchoscope and simultaneous ventilation.

The endotracheal tube was withdrawn from the trachea after determining the translumination effect of the light of the bronchoscope on the skin. It was entered with a 2 cm long, 16 Gauge and beveled Mini-trach® needle and advanced until air was aspirated into the injector (**Figure 2**). By seeing the tip of the needle on the screen of the bronchoscope, the guidewire was advanced through Mini-trach® needle (**Figure 3**). The skin and trachea were enlarged with forceps after dilated with an 8 French dilator (Portex®, Percutanous Dilation Tracheostomy Kit, UK), then an appropriate size of tracheostomy cannula was inserted over the guidewire. All patients' chest X-Ray were evaluated after procedure to rule out complications. Complications such as minor and major bleeding during and after the procedure, subcutaneous emphysema, pneumothorax, posterior tracheal wall injury, tracheal tube misplacement, and cuff puncture were recorded. Moreover, the time from needle insertion to the insertion of the tracheostomy cannula was also noted.

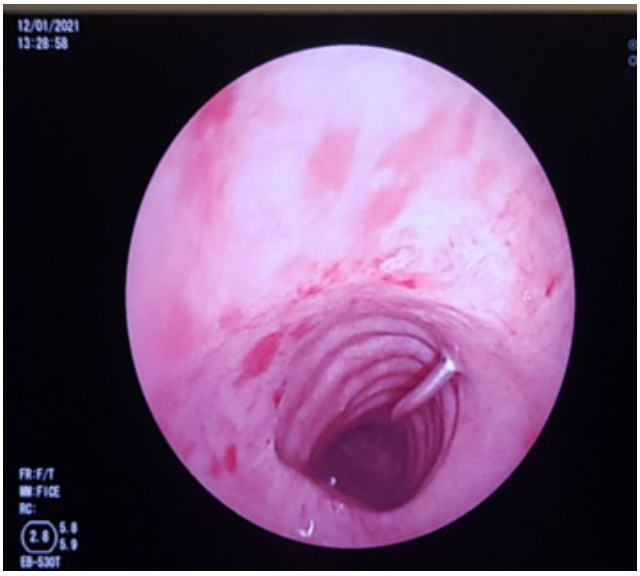

Figure 2. The view of entry with mini-trach needle

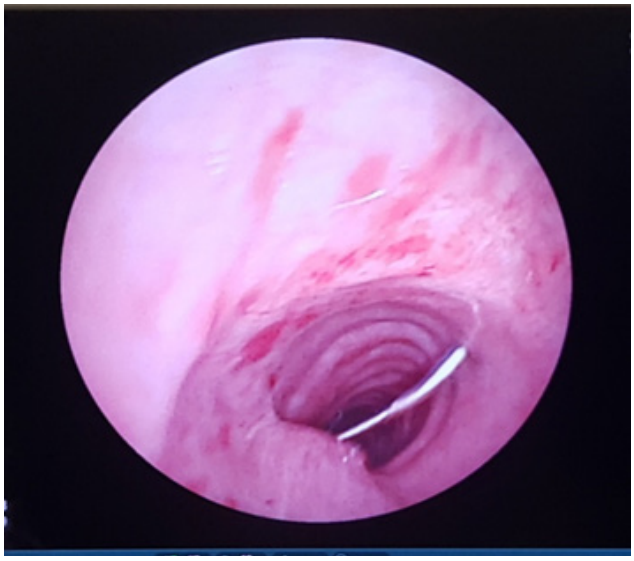

Figure 3. The view of guide wire passing through the needle

Statistical Analysis

Descriptive statistics of the data were presented as mean±standard deviation for continuous variables, and as number and (%) for categorical variables.

RESULTS

Data of 20 ICU patients who underwent PDT using Mini-trach® needle with FOB guidance were retrospectively evaluated. 8 of them were female (40%) and 12 (60%) were male. The average age of the patients was 69.8±16.14 years. Respiratory failure was present in 13 patients (65%) due to chronic obstructive pulmonary disease, 3 patients (15%) due to malignancy, and 4 (20%) patients due to neurological diseases. The mean APACHE II score of the patients was 23.05±6.16, GCS score was 10±3.43, and SOFA score was 7.2±2.11 (**Table 1**).

The mean procedure time was 13±1.68 minutes, and the day of tracheostomy application was 6.35±4.59. Twelve (60%) patients were switched to home-type ventilators. Ten (50%) of the patients were transferred to the palliative ward with home-type ventilator. Mortality rate was 20% (4 patients) at 90 days (**Table 2**).

Table 1. Demographic data and comorbidities of the patients

Variables	n (20)-%	Mean±sd
Gender (female/male)	8-40 / 12-60	-
Age (year)	-	69.8±16.14
BMI (kg/m^2)	-	25.05±2.19
APACHE II score	-	23.05±6.16
SOFA score	-	7.2±2.11
GCS score	-	10±3.43
COPD	13-65	-
Malignancy	3-15	-
Neurological diseases	4-20	-

Variables are given as number-percentage or mean±standard deviation. BMI: Body mass index, GCS: Glasgow coma scale, APACHE II: Acute physiology and chronic health evaluation II score, SOFA: Sepsis-related organ failure assessment score. COPD:Chronic obstructive pulmonary disease

Table 2. Percutaneous dilatational tracheostomy procedure duration, tracheostomy application day and intensive care outcomes of the patients

Variables	n (20)-%	Mean±sd
Procedure time (min)	-	13±1.68
Time until tracheostomy procedure (day)	-	6.6±4.59
Number of patients switching to home-type MV	12-60	-
90-day mortality	4-20	-

Variables are given as number-percentage or mean±standard deviation. MV: Mechanical ventilator.

In patients evaluation before the tracheostomy application, it was observed that, extubation was tried once in 3 patients and twice in 4 (20%) patients, while extubation could not be performed at all in 13 patients (65%). When patients were evaluated in terms of

complications; none of the patients had pneumothorax, subcutaneous emphysema, posterior tracheal wall damage, or tracheoesophageal fistula. Minimal bleeding that required no intervention was observed in only one patient.

DISCUSSION

The results of the present study showed that, there were no serious complication in PDTs performed using Mini-trach® needle by ICU residents with little experience in PDT procedure. We did not encounter any complications other than minimal bleeding in a patients who underwent PDT with this technique. Performing the PDTs with the FOB-guided Griggs technique may have contributed to procedure be faster and safer.

The PDT is one of the invasive methods commonly applied to critically ill patients in the ICU. Conditions such as the need for airway protection and avoiding long-term invasive mechanical ventilation are among its main indications (15).

There is no definitive protocol for its indications, timing, and methods. A comprehensive evaluation and teamwork are key to success in preventing procedural complications. The PDT in ICU is usually performed by two clinicians, at least one of which is experienced, and an ICU nurse.

The PDT application is one of the important intervention during ICU residency training. It is stated that, in PDT technique, familiarity with the process can be achieved after 5-10 procedures, but at least 20 PDT experiences are required to be familiar with possible complications and risks (9). All PDT training procedures are performed using a regular needle but in this study we performed this procedure by using mini-tracheostomy needle. The PDT procedures were applied by two intensive care physicians, at least one of which is experienced. The resident who performed the procedure, under the supervision of an experienced ICU doctor, had at least 10 PDT experiences. We think that mini-tracheostomy needle could provide safe procedure during residency training because of the short needle length.

Application of minitracheostomy from the subcricoid area has many advantages over the one performed through the cricothyroid membrane. Some of these advantages are; the procedure can be performed percutaneously in the intubated patient, it does not cause subglottic stenosis and voice change. In addition, application of PDT through this stoma could be easier and safer (14). Since the PDT needle is a plastic-sheathed needle, in our experience while plastic sheath is pushed towards the trachea after the needle is pulled-back, it may be bend in some interventions. However, the tip of the metal Mini-trach® needle is blunt, and curved with no tendency of bending or breaking. In addition, damage to the posterior wall of trachea could be technically rare, as its length is shorter than the standart PDT needle. In all cases we performed with the aid of FOB, the Mini-trach® needle remained in the trachea at a fairly safe distance from the posterior wall and the plastic wings of the needle prevented further advancement.

The most common complications of PDT include bleeding, subcutaneous emphysema, malposition, and posterior tracheal wall damage. Hazard et al. (16) evaluated bleeding and subcutaneous emphysema as minor complications of PDT, and reported that their incidence was low. In the same study, it was observed that the complications of minitracheostomy performed from subcricoid localization were less than PDT.

In cases of tracheostomy examined prospectively between 2005 and 2007, by Yeniaras et al. (8) it was found that, 4 (3.5%) cases had moderate bleeding, 1 case (0.8%) delayed bleeding, 3 cases (2.6%) stoma infection, and 1 case (0.8%) had subcutaneous emphysema due to malposition of the tracheostomy cannula. In present study, Mini-trach® needle was used in all patients, no complications were encountered during the procedure and at the early stage, except for minimal bleeding that developed in only one patient.

In a study comparing QuickTrach™ (for cricothyrotomy) with Melker™ kit (for percutaneous cricothyrotomy) in a patient simulation manikin; both cricothyrotomy and percutaneous cricothyrotomy have been evaluated as easy and rapid techniques with high success rates (17). According to the results of our study, PDTs performed by clinicians during the training process might have less complication rates with the Mini-trach® needle in combination with PDT set. However, we think that comparative studies are needed in larger study groups. Our study had some limitations. The study was planned retrospectively and there is no comparison group. Since the study was conducted with a specialized procedure, the number of cases was low, and this situation has significantly limited our capacity to identify the complications rate and deficiencies accurately.

CONCLUSION

There was no serious complication in PDTs performed using Mini-trach® needle in combination with the PDT set, by ICU residents with little experience other than minimal bleeding in a patient. Using Mini-trach® needle in PDTs performed via FOB by less experienced residents may be safer to prevent complications. On the other hand, more extensive and comparative studies are needed on this subject.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Ankara Atatürk Chest Diseases and Chest Surgery Training and Research Hospital Clinical Researchs Ethics Committee (permission date: 14/01/2021, decision number: 709).

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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Original Article

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Does red ginseng ameliorate liver damage caused by obstructive jaundice?: an experimental study

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ABSTRACT

Aim: This experimental study aimed to evaluate the hepatoprotective effect on obstructive jaundice (OJ) of oral Red Ginseng (RG) extract, which is known to have anti-inflammatory and antioxidant properties.

Material and Method: The rats were randomly separated into 3 groups of 10 rats: The sham group, the control group, and the treatment group. In Group 1 (sham), the common bile duct (CBD) was identified but no ligation or transection was performed. In Group 2 (control), the CBD was identified and ligation and transection were performed, but no treatment was given. In Group 3 (RG group), CBD ligation and transection were performed, then RG extract was administered via an orogastric tube at a dose of 100 mg/ kg/day for 10 days. After 10 days, blood samples were taken for biochemical analysis, and liver tissue samples for biochemical and histopathological analysis.

Results: Significantly higher serum albumin levels and lower serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined in the RG group than in the control group ($p=0.028$, $p=0.001$ and $p=0.034$, respectively). In the oxidative stress parameters, malondialdehyde (MDA) levels and catalase (CAT) levels were significantly different between the RG group and control group ($p\leq0.001$ for each). Total sulfhydryl (T-SH) was not at a statistically significant level, although it was high and approached the value of the sham group ($p=0.076$). In the histopathological evaluation, the RG group had statistically significantly lower scores in all parameters compared to the control group ($p<0.05$).

Conclusion: The results of this study showed that RG has a strong hepatoprotective effect as a result of its anti-inflammatory and antioxidant properties.

Keywords: Red ginseng, obstructive jaundice, anti-inflammatory properties, antioxidant properties, hepatoprotective effects

INTRODUCTION

The immune response is the most important defence system that protects the human body against attacks from harmful micro-organisms, and toxic, chemical, and carcinogenic substances. One of the mechanisms of the immune system is the balance between the pro-inflammatory cytokines and anti-inflammatory cytokines secreted in the body. Inflammation is the disruption of this homeostasis in favor of pro-inflammatory cytokines, and this forms the basis of many diseases ranging from various autoimmune diseases to different types of cancer (1-3). Not only is the liver a vital human organ in terms of metabolic, synthetic, and detoxification functions, but it is also the body's antioxidant and inflammatory balance store. Despite advances in technology and imaging techniques, liver diseases are still diagnosed at a late stage (4). The first sign of liver diseases is usually jaundice. Obstructive jaundice (OJ) may be due to intrahepatic diseases or generally due to diseases that cause extrahepatic biliary obstruction.

OJ may lead to liver-specific diseases that can progress to cholangitis, cirrhosis, and organ failure, and may result in many clinical sequelae if the treatment is not applied in a timely and adequate manner and bile flow

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is not provided. The clinical entities it may reveal are renal failure, encephalopathy, gastrointestinal system bleeding, coagulation disorders, malnutrition, and sepsis (5). Of these, sepsis is a very serious problem due to its consequences. An important consequence of obstructive liver disease in humans and experimental animals is hepatic inflammation, which causes tissue damage with the release of inflammatory cytokines and toxic free oxygen radicals (6). Oxidative stress and lipid peroxidation cause significant damage to the liver kupffer cells and other tissues, causing these cells to not work effectively in removing lipopolysaccharides and other toxins. In this context, antioxidant therapy is effective on an experimental basis. Although many studies have been conducted using various antioxidant and anti-inflammatory agents to prevent or reverse oxidative stress, which has an important role in the pathogenesis of OJ, there is no commonly used drug in clinical practice (6-8).

Panax ginseng is one of the most widely used herbal remedies in Asia and Western countries. Ginseng is a slow-growing succulent perennial plant, the root of which is made up of ginsenoids and the most important sub-component is red ginseng (RG). RG has been used in traditional medicine for many years in far eastern countries such as China, Korea, and Japan, and there are studies of the use of RG in many fields of medicine such as liver, cardiovascular diseases, kidney diseases, autoimmune systemic diseases, various malignancy diseases and in areas such as strengthening the immune system (9-11). Studies have shown that RG reveals anti-inflammatory activities in inflammatory responses by regulating the activities of inflammatory signalling pathways (12). Recent studies have shown the antioxidant and anti-inflammatory effects of RG in reducing the toxic effects of hepatoxic drugs and agents (11). According to the literature, this is the first study to examine the effect of RG on liver damage due to OJ.

The aim of this study was to evaluate the hepatoprotective effect of oral RG extract, which is known to have anti-inflammatory and antioxidant properties, on an experimental model of OJ.

MATERIAL AND METHOD

The experimental procedures and technique of this study met the requirements of the National Guidelines for the Use and Care of Laboratory Animals and The Animal Ethics Committee approved this study (approval date: 26.11.2020, number: 0063: 640). All procedures were performed adhered to the ethical rules and principles.

Animals and Experimental Surgical Procedure

The study sample consisted of 30 female adult Wistar albino rats, each weighing 230±22 g. The rats were kept in wire mesh cages at a permanent temperature of 21±2 ° C with a 12-hour light / dark cycle. The animals were fed a standard lab food diet with drinking water ad libitum. Access to food was stopped 12 hours before anesthesia and to water 2 hours before anesthesia. The same team carried out the anesthesia and surgical intervention stages in sterile conditions. Before the interventions, the rats were anaesthesized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Detroit, MI, USA) and 5 mg/kg Xylazine (Rompun; Bayer AG, Leverkusen, Germany). The rats were randomly separated into 3 groups of 10 rats: the sham group, the control group, and the treatment group. In Group 1 (sham), the common bile duct (CBD) was identified and no ligation or transection was performed. In Group 2 (control), after the CBD was identified, dissected, and skeletonized, it was double tied using 5/0 silk and cut between the sutures. No treatment was applied to this group. In Group 3 (RG group), following CBD ligation and transection, RG [Korean 6-Year Root Red Ginseng Extract (Samsung, Korea)] was administered via an orogastric tube at doses of 100 mg/kg/day for 10 days. After the specified procedures were completed, abdominal incisions were closed in two layers with 3/0 silk sutures in all three groups. The rats were allowed to be fed after surgery. All the animals were euthanized after 10 days using an overdose of ketamine. Following laparotomy and sternotomy, samples were taken from liver tissue and blood to be analyzed biochemically, then liver tissue samples

Biochemical Analysis

Evaluation of the liver functions in the serum was carried out in the Biochemistry Department of Ankara Training and Research Hospital. Serum samples were kept at -80°C until the day of analysis. Serum albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin, and direct bilirubin were measured by using a Roche Cobas 8000 biochemistry analyser.

The parameters of oxidative stress were evaluated in the Biochemistry Department of Ankara Training and Research Hospital. Liver tissues were kept at -80°C until the day of analysis. The levels of malondialdehyde (MDA), total sulfhydryl (T-SH), and catalase (CAT) were determined. Tissues were homogenized in phosphate buffer for the MDA, T-SH, and CAT measurements.

The levels of MDA were measured using the fluorometric method, as described by Wasowicz et al. (13) The levels of MDA were shown as nmol/g protein. CAT levels were determined using spectrophotometric measurement as defined by Hadwan (14). The value of CAT activity was determined using the first-order reaction rate constant as kU. CAT activity was stated as kU/g protein. The T-SH measurement was performed according to the principle described by Taylan et al, who applied this method by adapting the Ellman reaction to the microplate method (15). The T-SH levels were stated as μmol/g protein.

Histopathological Analysis

The liver tissue samples were stored in containers containing 10% formol until the day of macroscopic examination. In the macroscopic examination, tissue specimens were cut into strips at 3 mm intervals and transferred to tissue tracking cassettes and embedded in paraffin blocks. Sections 4-microns in thickness were cut from the blocks and stained with Hematoxylin-Eosin and Masson Trichrome.

The pathological evaluation was performed by a single pathologist blinded to the groups. The sections prepared were examined under a light microscope at ×40, ×100, ×200, and ×400 magnifications. Histopathological examinations were evaluated using a modified histological activity index scoring system (HAI). The modified HAI scoring system consists of a total of five parameters: focal necrosis, portal inflammation, interphase hepatitis, confluent necrosis, and fibrosis (16).

In addition to HAI scoring, bile duct proliferation was evaluated. For the grading of bile duct proliferation, 5 consecutive areas were counted at x 100 magnification and each proliferation was evaluated as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). These numbers were then averaged and scored as 0=None, 1=Mild, 2=Moderate, 3=Severe.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 25.0 software (IBM Corp., Armonk, N.Y., USA). The normality of the distribution of numerical variables was assessed using the Shapiro-Wilk test. Numerical variables were stated as median (minimum-maximum) or mean±standard deviation (SD) values and were compared using the Kruskal-Wallis test followed by the Tamhane T2 post-hoc test or the one-way ANOVA test followed by Tukey's HSD post hoc test, as appropriate. A value of $p<0.05$ was considered statistically significant.

RESULTS

Two rats in the control group died in the postoperative period and no other complication was observed during the study period.

Liver function tests

With the exception of total protein levels, all the other parameters differed significantly between the groups. Significantly lower serum albumin levels and higher levels of serum total protein, ALP, GGT, ALT, AST, total bilirubin, and direct bilirubin were determined in the control group compared to the sham group. With the exception of the ALT levels, these results were similar to the comparison of the RG group and the sham group.

Statistically significantly higher serum albumin levels and lower serum ALT and AST levels were determined in the RG group compared to the control group (p=0.028, p=0.001, and p=0.034 respectively). Although there was an improvement in ALP and GGT enzymes in the RG group compared to the control group, the difference was not statistically significant (p=0.139, p=0.188 respectively). The serum biochemical parameters of liver function are shown in **Table 1**.

Oxidative stress parameters

The MDA, CAT, and T-SH levels in liver tissue are presented in **Table 2**. The CAT and T-SH levels were significantly higher and MDA levels were significantly lower in the sham group compared to the control group and no significant difference was observed between the RG group and the sham group in these parameters. In the comparisons of the RG group and the control group, the MDA levels and CAT levels were significantly different (p=<0.001 for each). T-SH was higher in the RG group than in the control group and approached the value of the sham group, but the difference was not statistically significant (p=0.076).

Table 1. Results and comparisons of serum liver function parameters.

	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	p value	p value (1-2)	p value (1-3)	p value (2-3)
Albumin (g/L)	37.22±2.19	28.05± 3.41	31.68± 2.74	<0.001	<0.001	<0.001	0.028
Total protein(g/L)	57.69±3.90	57.80± 6.01	58.91± 2.48	0.782	0.998	0.796	0.845
ALP (IU/L)	95.5 (68-162)	435.5 (219-850)	275 (209-399)	<0.001	0.004	<0.001	0.139
GGT (IU/L)	0 (0-1)	33 (8-46)	19 (14-38)	<0.001	<0.001	<0.001	0.188
ALT (IU/L)	67 (51-100)	148.5 (101-189)	87.5 (41-113)	<0.001	<0.001	0.180	0.001
AST (IU/L)	237 (175-288)	477 (369-829)	336 (211-417)	<0.001	0.005	0.004	0.034
Total bilirubin (mg/dl)	0.05 (0.03-0.07)	10.18 (9.43-16.70)	10.03 (8.00-13.28)	<0.001	<0.001	<0.001	0.820
Direct bilirubin (mg/dl)	0.02 (0.02-0.04)	9.80 (8.54-16.00)	9.68 (6.88-13.00)	<0.001	<0.001	<0.001	0.751

RG: Red ginseng, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Values shown in bold are statistically significant ($p < 0.05$).

Table 2. Results and comparisons of malondialdehyde, catalase, and total sulfhydryl levels in liver tissue

	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	p value	P value (1-2)	P value (1-3)	P value (2-3)
MDA (nmol/g protein)	65.42 ± 17.45	102.13 ± 5.58	76.28 ± 6.40	<0.001	<0.001	0.109	<0.001
Catalase (kU/g protein)	44.64 ± 5.66	27.12 ± 6.41	42.91 ± 6.47	<0.001	<0.001	0.807	<0.001
Total SH µmol/g protein	167.51±23.84	129.66±16.16	151.23±18.09	<0.001	0.001	0.180	0.076

RG: Red ginseng, MDA: Malondialdehyde, SH: Sulfhydryl, Values shown in bold are statistically significant ($p < 0.05$)

Histopathological Results

The scores of the histopathological changes in the liver tissue are summarized in **Table 3**. The sham group had the lowest scores as expected and all parameters were significantly lower compared to the control group. The RG group had significantly lower scores for all parameters compared to the control group. In the comparison of the RG and sham groups, statistically different changes were determined in four of six parameters (**Figure 1**).

DISCUSSION

Benign and malignant diseases are among the causes of OJ. Biliary obstruction is known to cause hepatocellular damage, primarily, as a result of blocking the passage of bile into the intestine, high concentrations of bile acid accumulate in intrahepatic sinusoids, hepatocytes, and blood, and this triggers oxidative stress and inflammatory response (6). Inflammatory cell infiltration, accumulation of hydrophobic bile acids, endotoxemia, changes in mitochondrial permeability transition, and deleterious effects of oxygen free radicals are possible factors responsible for cholestatic liver damage (17, 18). These radicals damage the cell membrane as well as intracellular structures. Decreased absorption from the intestines causes the plasma levels of antioxidants to decrease and thus increases oxidative damage. Oxidative damage and lipid peroxidation exacerbate liver damage in OJ (19). Although the mechanism of liver damage due to OJ is complex and multifactorial, the two main factors affecting the degree of liver damage are oxidative stress and inflammation. In the literature, many experimental

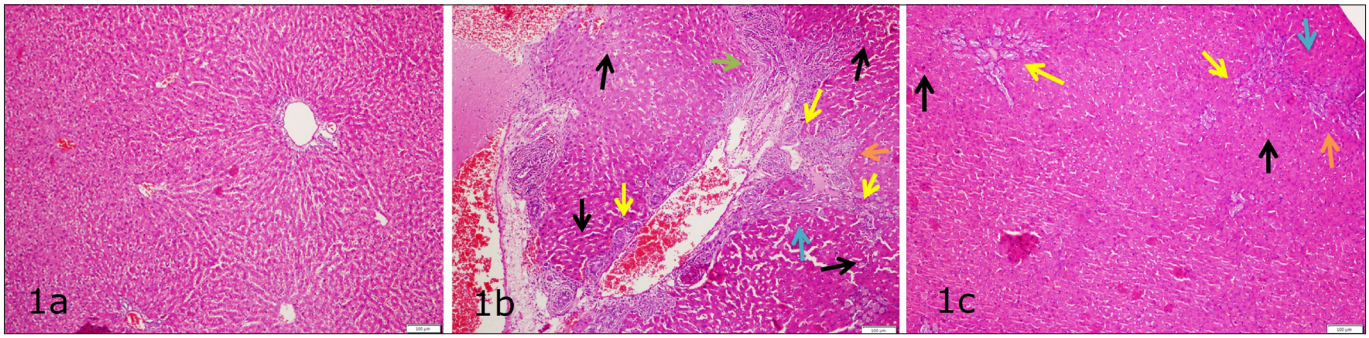

Figure 1. Histopathological findings: **1a:** Normal microanatomy of the liver was preserved. There is no portal inflammation, fibrosis and significant bile duct proliferation in the portal area. (H&E x100; Sham group). **1b:** Fibrous septa / bridging and nodules caused by severe biliary duct proliferation (yellow arrow). Nodules consist of fibrous (green arrow) and inflammatory bridges (blue arrow) in some areas. Focal necrosis foci and prominent necrotic keratinocytes (black arrow) are observed in hepatocytes. There are signs of severe interphase hepatitis (orange arrow) in the portal areas (H&E x100; Control group). **1c:** Sparse focal necrosis and necrotic keratinocyte foci (black arrow). Mild inflammation and fibrosis (blue arrow) and focal interphase hepatitis findings (orange arrow) are seen in one of the two Portal areas. Mild bile ductus proliferation (yellow arrow) is observed in both portal areas. (H&E x100; RG group).

Table 3. Results and comparisons of histopathological changes scores of liver tissue.

	Sham group (Group 1)	Control group (Group 2)	RG group (Group 3)	p value	p value (1-2)	p value (1-3)	p value (2-3)
Bile duct proliferation	0 (0-1)	3 (2-3)	2 (1-3)	<0.001	<0.001	<0.001	0.001
Focal necrosis	0 (0-1)	2 (1-3)	1 (0-2)	0.001	0.001	0.309	0.024
Portal inflammation	1 (0-1)	3 (2-4)	1 (1-2)	<0.001	<0.001	0.008	0.001
Interface hepatitis	0 (0-1)	3 (3-4)	1.5 (0-2)	<0.001	<0.001	0.025	<0.001
Confluent necrosis	0.5 (0-2)	3 (2-3)	1 (0-2)	<0.001	<0.001	0.252	<0.001
Fibrosis	1 (0-1)	3 (2-4)	2 (1-2)	<0.001	<0.001	0.002	<0.001

RG: Red ginseng. Values shown in bold are statistically significant ($p < 0.05$)

studies have been conducted with various drugs with antioxidant and anti-inflammatory properties to treat the damage caused by OJ. In an experimental study of OJ, Unal et al reported that Calcium Dobecylate significantly reduced the development of inflammation and fibrosis in the liver, and that serum and tissue enzyme activity decreased biochemically and oxidative stress significantly (20). Moran et al. demonstrated that nitric oxide (NO) plays a crucial role in inducing liver damage in rats with jaundice and that erythropoietin reduces oxidative damage both by decreasing NO levels and increasing or possibly preserving reduced glutathione levels (6). Padillo et al. reported that melatonin reduced liver damage in an experimental cholestasis study (17). In the literature, there are studies that have shown that OJ causes damage to distant organs as well as having a toxic effect on the liver. In one of these, it was stated that there is a relationship between high lipid peroxidation and damage to the heart, brain, and kidney in experimental OJ (21). In another experimental cholestasis study, Dilektasli et al. found brain damage to be irreversible if the duration of bile duct obstruction was > 7 days (22). A notable study from the experimental OJ studies was about the method of creating obstruction in the common bile duct. Yunfu et al. compared the methods of CBD ligation and transection with CBD ligation only and showed that the total bilirubin level returned to normal on day 21 only in the rats that were ligated, and therefore recommended the CBD ligation and transection method for experimental cholestasis studies (23). In the current study, OJ was created by the CBD ligation and transection method.

Ginseng, a plant belonging to the Panax genus, belongs to the Araliaceae family and has been used as a popular herbal medicine for thousands of years, especially in Far East Asia. There are 13 plants related to the Panax genus, the most commonly used of which is Panax ginseng (24). RG has traditionally been shown in studies to be more effective than white or fresh ginseng (25). Most of the biological activities of ginseng are due to its main constituent, ginsenosides. Also known as steroid-like saponins, ginsenosides are unique to the ginseng types. There are more than 100 ginsenosides expressed by Rx (26). Various studies have been conducted to understand the pharmacological mechanisms of ginseng and ginsenosides in diabetes mellitus, cardiovascular disease, stress, neurodegenerative diseases, various types of cancer, and immuno-stimulation (9,27, 28).

Inflammation is one of the natural immune mechanisms. While the dynamic balance between proinflammatory cytokines and anti-inflammatory cytokines modulates the state of inflammation, disruption of the balance in favour of pro-inflammatory cytokines causes inflammation-related diseases such as diabetes, cancer, cardiovascular disease, and neurological diseases. Considering the mechanism of action of ginsenosides, its anti-inflammatory effects have been proven by purified ginsenosides. Pro-inflammatory cytokine expressions (TNF-alpha, IL-1, and IL-6) have been shown to inhibit iNOS and COX-2 enzyme expressions while inducing M1 and M2 polarization of macrophages or microglia, which in turn has been found to be the anti-inflammatory mechanism of ginsenosides (12,29,30). An important consequence of obstructive liver disease in humans and experimental animals is hepatic inflammation, which causes tissue damage with the release of inflammatory cytokines and toxic free oxygen radicals. Oxidative stress and lipid peroxidation cause significant damage to the liver kupffer cells and other tissues, causing these cells to not work effectively in removing lipopolysaccharides and other toxins. In this context, antioxidant therapy is effective on an experimental basis (6). In current study, liver damage caused by obstructive jaundice in the control group and RG group caused an increase in liver function tests and oxidative stress parameters in both groups, but these values healed significantly in the RG group compared to the control group. We think that the anti-inflammatory and antioxidant effects of ginsenosides are very important in this healing process.

There are many studies in literature which have examined the hepatoprotective effect besides the anti-inflammatory effect of RG. Karakus et al. stated in an experimental study that serum biochemical liver function tests, which increased in liver damage due to carbon tetrachloride (CCl4) toxicity, improved significantly in rats receiving RG treatment (31). In another experimental study by Hong et al., 2 months of oral RG treatment in rats with non-alcoholic fatty liver was shown to inhibit steatohepatitis, improve high lipid profile values, and stimulate natural clay cell activity (32). RG administration has also been shown to provide a significant improvement in liver function tests in patients with chronic hepatitis C, lower viral titers, and lower the level of tumor marker alpha-fetoprotein (AFP) by showing potent therapeutic effects against liver cancer (33). In a recent experimental study, it was stated that serum liver function tests and oxidative parameters improved in the group treated with RG in rats given cyclophosphamide, which has hepatotoxic side effects, and its hepatoprotective effect was emphasized (11).

In this study, the histopathological evaluation of liver tissue samples and biochemically oxidative and antioxidant parameters of blood samples were examined. RG was found to significantly reduce inflammation, necrosis, and fibrosis in the histopathological evaluation. It was also observed that oxidative stress parameters and liver function tests improved significantly in the RG group compared to the control group.

CONCLUSION

In conclusion, the results of this study showed that RG had a strong hepatoprotective effect as a result of its anti-inflammatory and antioxidant properties. To the best of our knowledge, this is the first study in literature to have investigated the effect of RG on experimental hepatic OJ. Although promising beneficial hepatoprotective effects of RG have been demonstrated, there is a need for further clinical studies to apply these positive results in clinical settings.

ETHICAL DECLARATIONS

Ethics Committee Approval: The experimental procedures and technique of this study met the requirements of the National Guidelines for the Use and Care of Laboratory Animals and Ankara Training and Research Hospital The Animal Ethics Committee approved this study (approval date: 26.11.2020, number: 0063: 640). All procedures were performed adhered to the ethical rules and principles.

Referee Evaluation Process: Externally peer-reviewed.

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

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Case Report

HEALTH SCIENCES
MEDICINE

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Can persistent hiccups be a progression marker in COVID-19?

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ABSTRACT

Hiccup is a reflex action that may rarely be intractable. A 55-year-old man diagnosed with COVID-19 was hospitalized to our clinic with body temperature elevation, weakness, and headache persisting for the previous three days. Persistent hiccups were present during follow-up, and progression was observed at pulmonary tomography with an increase in the numbers and dimensions of focal ground glass areas. Lymphocyte count was 920/μL, platelet count 138×10^3/μL, sedimentation rate 6 mm/h, ferritin 1256 ng/mL, C-reactive protein 16.8 mg/L, aspartate aminotransferase 43 U/L, alanine aminotransferase 67 U/L, and lactate dehydrogenase 326 U/L. Other potential causes of persistent hiccups were excluded. COVID-19 immune plasma and remdesivir therapy were initiated. The hiccups resolved two days after treatment, and the patient was discharged on the 11th day of follow-up. Persistent hiccups should be remembered among the symptoms that may appear during the clinical progression of COVID-19.

Keywords: Persistent hiccups, progression, COVID-19

INTRODUCTION

Unidentified cases of viral pneumonia were detected in the city of Wuhan in the Chinese province of Hubei in December 2019. These spread rapidly, and growing numbers of cases also began being seen in other countries. The World Health Organization announced the existence of COVID-19 disease, meaning coronavirus disease 2019, in February 2020 (1). The virus uses angiotensin-converting enzyme type 2 (ACE-2) receptors to bind to cells (2). ACE-2 receptors are found in endothelial cells and in several organs, including nervous system tissue (3).

The most frequently seen symptoms include fever, cough, fatigue, phlegm, shortness of breath, sore throat, and headache. Gastrointestinal symptoms such as diarrhea and vomiting may also be seen, although less frequently (4). Anosmia has been widely reported as an early onset symptom (5). Neurological diseases such as cerebral hemorrhage and cerebral infarction have also been reported in addition to nonspecific symptoms such as headache, lethargy, and gait disturbance (6,7).

Hiccups are a widespread and transient entity that can affect almost all individuals during their lives. They results from involuntary contraction of the diaphragm and intercostal muscles. Hiccups exceeding 48 h in duration are rare and can derive from severe diseases (8). Etiological factors include cerebrovascular disease, coronary artery disease, diaphragmatic hernia, metabolic disease, duodenal ulcer, and psychogenic and other causes (9). Here, we describe a case report of a male patient with a rare association of COVID-19 and persistent hiccups.

CASE

A 55-year-old man receiving favipiravir therapy due to diagnosis of COVID-19 five days previously was hospitalized in our clinic due to fever, weakness, and headache persisting for three days despite treatment. The patient had no history of chronic disease, malignancy, or smoking. No nasal discharge, nasal obstruction, sore throat, or shortness of breath were present at system examination. The patient was a health worker, but had no history of unprotected high-risk contact or travel. Vital signs were body temperature 38.4°C, heart rate 78 beats/min, blood pressure 120/80 mmHg, respiratory rate 25/min, and oxygen saturation (SO_2) in room air 95. All systems were normal at physical examination. Pulmonary tomography findings were present in the form of ground glass opacities with irregular borders in the lower lobes

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of both lungs (**Figure 1**). White blood cell (WBC) count was 4.88×10^3/μL, lymphocyte count 1010/μL, platelet count 133×10^3/μL, sedimentation rate 5 mm/h, D-dimer 243 ng/mL, ferritin 529 ng/mL, procalcitonin 0.09 ng/ml, c-reactive protein (CRP) 3.17 mg/L, aspartate amino transferase (AST) 19 U/L, alanine aminotransferase (ALT) 34 U/L, and lactate dehydrogenase (LDH) 180 U/L. Metilprednizolon 60 mg/day, levofloxacin 750 mg/day, and enoxaparin sodium 0.6 ml/day were added to the existing favipavir therapy. Persistent hiccups occurred together with persisting and refractory fever on the third day of hospitalization. The patient's SO_2 in room air was 89, and increases in the numbers and dimensions of focal ground glass areas were observed at control pulmonary tomography (**Figure 2**). Laboratory parameters were WBC 7.78×10^3/μL, lymphocyte count 920/μL, platelet count 138×10^3/μL, sedimentation rate 6 mm/h, ferritin 1256 ng/mL, CRP 16.8 mg/L, AST 43 U/L, ALT 67 U/L, and LDH 326 U/L. The patient's fever persisted and no improvement was observed in symptoms, and convalescent plasma therapy was administered. Favipavir was stopped, and remdesivir was initiated at 1×100 mg/day (five days) after 1×200 mg 1×1. On the fourth day of follow-up, the patient's body temperature was 38.3°C. Oxygen was given at 4 lt/min through a nasal cannula, and the patient's SO_2 was 92. Our patient was evaluated by the neurology clinic. Neurological examination and cranial magnetic resonance imaging (MRI) were normal. The hiccups persisted, and a singular intramuscular 25 mg dose of chlorpromazine was administered. Other system evaluations potentially related to persistent hiccups were normal. The patient's body temperature returned to normal on the sixth day of follow-up, the hiccups resolved, and he was discharged in a healthy condition on the 11th day.

DISCUSSION

Spike proteins of severe acute respiratory syndrome virus-2 (SARS-CoV-2), which causes coronavirus disease, use ACE-2 receptors to bind to cells. ACE-2 receptors are also expressed in the central nervous system (2,3). ACE-2 receptors are also expressed in the central nervous system (2,3). In the literature, headache, dizziness, impaired consciousness, epilepsy, ataxia, acute disseminated encephalomyelitis and viral encephalitis are among the neurological symptoms of COVID-19 (10,11). Headache is the most common central nervous system symptom with a prevalence between 6.5 and 23% (12). In our case, headache was among the complaints at the time of admission to hospital.

New information regarding the clinical characteristics of COVID-19 is constantly emerging. The present case, diagnosed with COVID-19 and with persistent hiccups, will add to the abnormal clinical characteristics of COVID-19. This is the first report of this clinical feature from Turkey, and the second in the literature. The previous, first case report concerned a patient presenting to the emergency department with persistent hiccups and diagnosed with COVID-19 at subsequent follow-up. Apart from persistent hiccups, the patient who does not have fever, nasal congestion, sore throat, chest pain or shortness of breath is reported to have no travel or patient contact (13). In our case, fever, headache and malaise were the initial symptoms and persistent hiccups developed during follow-up.

In the case report in which COVID-19 was reported as an atypical admission complaint, it was reported that the case had leukopenia, thrombocytopenia, hyponatremia

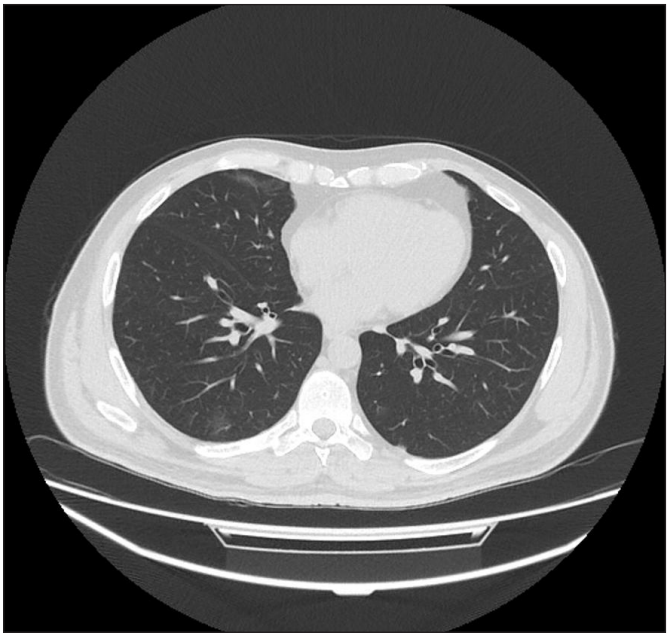

Figure 1. Ground glass opacities with indefinite borders located peripherally in the lower lobes of both lungs

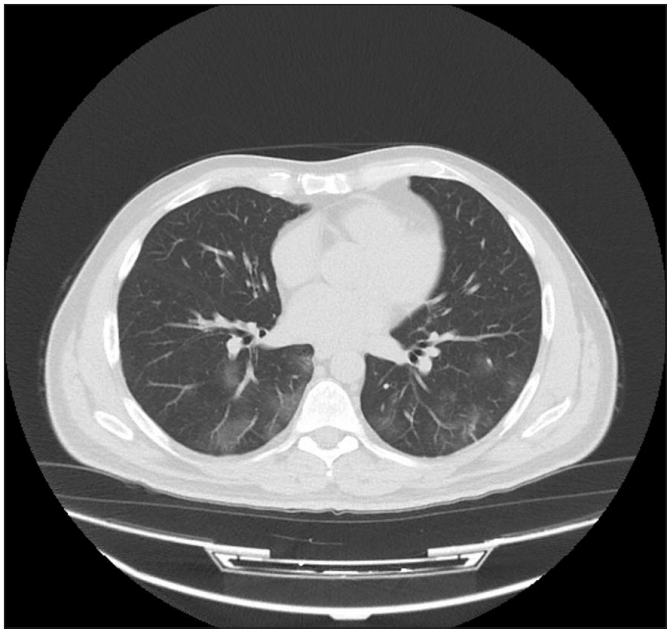

Figure 2. Increased numbers and dimensions of focal ground glass opacities in both lungs

and hypochloremia, on the other hand, troponin, metabolic panel, lactate and urine tests were normal (13). Metabolic conditions that could cause persistent hiccups were normal in our patient.

Vagus or phrenic nerve irritations can cause persistent hiccups (14,15). In the case with persistent hiccups and weight loss presented in the literature, contrast-enhanced chest CT performed to exclude a mediastinal mass and demonstrated small focal ground glass opacities scattered in the lungs and regional peripheral ground glass opacities of the upper lobes (13). In our case, which we followed up with the diagnosis of COVID-19, we saw that there was an increase in the numbers and dimensions of focal ground glass areas on contrast chest CT, which was taken with suspicion of a mass that could compress the vagus or phrenic nerve. No mediastinal pathology or diaphragmatic hernia was observed.

Vascular or infectious conditions of the central nervous system are included in the etiology of persistent hiccups (16,17). Neurological examination and cranial MRI of our patient were normal. Even if no specific cause is found, detailed history must be taken and physical examination and diagnostic tests must be performed to identify the underlying cause and provide appropriate treatment. Apart from COVID-19, no other disease that could cause persistent hiccups was detected in our patient.

It is important to treat the underlying cause in patients with persistent hiccups. The present case report emphasized that clinical persistent hiccups may emerge in the progression stage of COVID-19. Atypical symptoms such as persistent hiccups in patients with no clinical and laboratory improvement should suggest the presence of progression, and treatment should be revised accordingly.

ETHICAL DECLARATIONS

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

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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Case Report

HEALTH SCIENCES
MEDICINE

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A rare presentation of brucellosis: acute cholestatic hepatitis

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ABSTRACT

Brucellosis can present with clinical hepatitis and rarely as acute cholestatic hepatitis. The aim of the study is to demonstrate acute cholestatic hepatitis as a rare presentation caused by *Brucella melitensis*. A thirty-nine-years-old male patient was admitted to our department with the complaints of fever, malaise, fatigue and sweating. At hospitalization, the temperature was 39°C. Laboratory tests; white blood count (WBC) 4800 cells/mm^3, platelet (PLT) 173000/mm^3, erythrocyte sedimentation rate (ESR) 38 mm/h, C-reactive protein (CRP) 257 mg/L, alanine transferase (ALT) 284 U/L, aspartate transferase (AST) 300U/L, γ-glutamyl transpeptidase (GGT) 1152 U/L, alkaline phosphatase (ALP) 1762 U/L, total bilirubin (T-Bil) 2.8 mg/dL, direct bilirubin (D-Bil) 1.3 mg/dL, lactate dehydrogenase (LDH) 705 U/L. Gruber Widal test was negative. Wright serum agglutination test was found to be positive at 1/2560 titer. Brucellosis should be kept in mind, especially in the differential diagnosis of the patients with acute hepatitis and cholestasis accompanied by fever.

Keywords: Acute cholestatic hepatitis, brucellosis, fever

INTRODUCTION

Brucellosis is an important zoonotic disease and a major cause of morbidity worldwide. Brucellosis may present with different clinical manifestations. The hepatic involvement in brucellosis includes a wide spectrum that ranging from mild elevation of aminotransferases to the manifestation of hepatitis. Brucellosis can present with clinical hepatitis in 3% of the cases. In addition acute cholestatic hepatitis is a very rare complication of brucella (1). Cholestatic liver disease is characterised by a typical elevation of serum alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT) and bilirubin (2). The aim of the present study is to demonstrate acute cholestatic hepatitis as a rare presentation caused by *Brucella melitensis*.

CASE

A thirty-nine-years-old male patient was admitted to our department with the complaints of fever, malaise, fatigue and sweating for approximately 1 month. The patient had the history of animal husbandry, the consumption of raw milk and dairy products. Physical examination revealed yellow sclera and hepatosplenomegaly of 3 cm pathologically. At hospitalization, the temperature was 39°C, the pulse was 76/min, the respiratory rate was 18/min and blood pressure (BP) was 110/80 mm/Hg. Laboratory tests showed the following values: Hemoglobin (HgB) 12.7 g/d, hematocrit (HCT) 34, White blood count (WBC) 4800 cells/mm^3 (neutrophils 62%, lymphocytes 26%, monocytes 10% and eosinophils 3%), platelet (PLT) 173000/mm^3, erythrocyte sedimentation rate (ESR) 38 mm/h, C-reactive protein (CRP) 257 mg/L, alanine transferase (ALT) 284 U/L, aspartate transferase (AST) 300 U/L, GGT 1152 U/L, ALP 1762 U/L, total bilirubin 2.8 mg/dL, direct bilirubin 1.3 mg/dL, albumin 3.3 gr/dL and lactate dehydrogenase (LDH) 705 U/L. As the coagulation tests, the international normalized ratio (INR) and the prothrombin time (PTT) were measured as 1.1 and 16s, respectively. Among the viral serelogy findings, the hepatitis B surface antigen (HBs-Ag), the IgM antibody against hepatitis B core antigen (anti-HBc IgM), the anti-hepatitis A virus IgM (anti-HAV IgM), the anti-hepatitis C virus (anti-HCV), the anti-cytomegalovirus IgM (anti-CMV IgM), the Epstein-Barr virus anti-virus capsid antigen IgM (anti-EBV VCA IgM) and the Gruber-Widal tests were found to be negative. Autoimmune hepatitis markers (anti–smooth muscle antibody, antinuclear antibody, anti–liver/kidney microsomal antibody) were also negative. Turkey is an endemic region for brucellosis so we performed the Wright serum agglutination test, the titer was found to be positive at 1/2560. Abdominal ultrasonography

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(USG) showed moderate hepatosplenomegaly, Based on these findings, acute cholestatic hepatitis due to brucella was considered. Blood cultures had been performed after the admission to hospital. Therefore, the treatment of doxycycline (100 mg every 12 h) and streptomycin (1 g every 24 h) was started. *Brucella melitensis* was isolated from blood culture on the second day of the admission to hospital. The time to defervescence was on 12th day of the treatment. With the second week of the treatment, we observe the decrease of all elevated liver enzymes (ALT 52 U/L, AST 44 U/L, ALP 134 U/L, GGT 134 U/L) and so the patient was discharged. Then, the patient was evaluated every two weeks. During the follow-ups, the hepatic enzymes, leukocyte count, CRP and ESR were observed to return to normal limits in five weeks. The combination therapy was completed in six weeks with oral doxycycline (100 mg every 12 h)+rifampicin (600 mg every 24 h)+21 days of streptomycin (100 mg every 12 h). The laboratory features and treatment modalities are demonstrated in **Table.** No relapse was observed in the one-year follow-up after the treatment.

DISCUSSION

Brucella melitensis is the most pathogenic and invasive species of Brucella and more common, compared with other known species. Acute disease may include liver tenderness and mild elevation of transaminases and ALP (3).

Hepatic involvement in brucellosis includes a wide spectrum, ranging from mild elevation of aminotransferases to the manifestation of hepatitis. The hepatic involvement in brucellosis can present with clinical hepatitis in 3% of the cases. Brucella hepatitis may lead to liver decompensation and cirrhosis, if left untreated (1). Although cholestasis may occur due to the effects of proinflammatory cytokines and bacterial toxins on the bilirubin transport during bacterial infections, the exact mechanism of cholestasis in brucellosis still remains unknown (4). In a study evaluating 1028 brucellosis cases, hepatic involvement was found to be 24.8% (5). In another study involving 325 brucellosis patients with significant hepatobiliary efficacy, the clinical hepatitis was found in 284 (87.3%) patients, and cholestasis was found in 215 (66.1%) patients (1). In our report, we present an acute cholestatic hepatitis. The emergence of clinical hepatitis during the course of the disease was found to be associated with the presence of large amounts of bacteria in the liver (6). Our patient also had a significant elevation of transaminases during the hospitalization and brucella growth in blood cultures. Oztürket al reported that the greatest symptoms were; weakness in 91%, fever in 86%, sweating in 83% and joint pain in 79% (1). As similar with Öztürk et al. (7) the symptoms on admission were found as fever, weakness, malaise, fatigue and sweating in our case. In our study, the time to defervescence developing on 12th day of the treatment was noteworthy. In two studies evaluating the average time to defervescence achieved by doxycycline plus rifampicin and doxycycline plus streptomycin groups was 4.2 and 3.2 days and 3.5 and 3.5 days, respectively (8).

In addition, the use of doxycycline and aminoglycoside was reported to show more rapid normalization of liver aminotransferases in clinical brucellar hepatitis cases (1). In our study, we also used the combination of doxycycline and streptomycin, and the hepatic enzymes had returned to normal limits in five weeks. No relapse was observed in the one-year follow-up after the treatment.

CONCLUSION

Brucellosis should be kept in mind, especially in the differential diagnosis of the in-patients with acute hepatitis and cholestasis accompanied by fever. An attentive anamnesis for occupational and travel history is important for early diagnosis and effective treatment resulting with lower mortality and morbidity rates.

ETHICAL DECLARATIONS

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

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.

Table. The laboratory features of the patient

	AST/ALT U/L	ALP/GGT U/L	LDH U/L	T.Bil/D.Bil mg/dL	INR/ PTT(s)	WBC/NEU	HGB g/dL	PLT / mm^3	CRP mg/L
Admission	300/284	1762/1152	705	2.8/1.3	1.1/16	4800/2640	12.7	173000	257
Second week	52/44	52/44	522	0.8/0.2	0.7/14	7200/4200	12.2	230000	24
End of treatment	18/32	86/54	240	0.6/0.2	0.9/13.8	6400/4800	13.1	280000	3
Treatment regimen :(Six weeks of Doxycycline and Rifampicin+21 days of streptomycin)									

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Letter to the Editor

HEALTH SCIENCES
MEDICINE

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Spontaneous hemothorax under rivaroxaban treatment

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Dear Editor,

Rivaroxaban is a highly selective direct thrombin inhibitor indicated in stroke prevention, non-valvular atrial fibrilation, treatment of pulmonary embolism and deep vein thrombosis, prevention of recurrent venous thromboembolism and VTE after elective hip/knee replacement surgery and secondary prevention after acute coronary syndrome. Hemorrhage is the major concern of the drug use. Subdural hematoma, cerebral hemorrhage, GIS hemorrhage, and also intra-articular bleeding has been published in postmarketing reports. Here, we report a case of spontaneous hemothorax under rivaroxaban treatment.

A 73-year-old of man was consulted due to acute dyspnea and palpitation. His x-ray pulmonary images and thorax computed tomography were compatible with hemothorax, consequently, we performed thoracentesis, and macroscopic appearance, cell count revealed hemothorax. There was no sign of any disease, such as low pleural fluid pH and glucose, leucocytic dominance, dysplastic cell, which would indicate an another cause of the bleeding. He was taking rivaroxaban (15 mg daily) due to chronic atrial fibrillation. Hemorrhage was drained and the drug was cessated. The patient had a history of a 1-year anticoagulant treatment under warfarin therapy with very labile INR levels before rivaroxaban. We followed-up the patient with low molecular heparin therapy for one month after discharge and then switched to low dose (2.5 mg daily) apixaban. Hemothorax did not relapse in the three month-follow-up period (**Figure 1**).

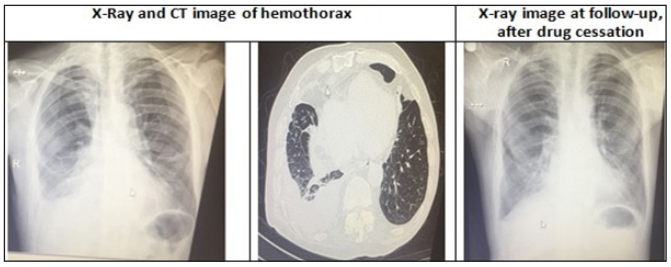

Figure. Right lung settled pleural fluid accumulation and X-ray image of the lung after recovery

Rivaroxaban (Xarelto) is a member of new anticoagulant drug class and has become favorable in the patient carrying systemic embolism risk with nonvalvular atrial fibrillation. It has predictable pharmacokinetics features, fewer food interactions, bleeding risk, and contrast to warfarin it does not need frequent INR monitoring. Despite promising features, serious hemorrhagic complications have been reported and bleeding risk is still its major challenge in clinical use. Cases of subdural hematoma, cerebral hemorrhage, GIS hemorrhage, and also intra-articular bleeding has been published (1). Spontaneous hemothorax was assumed as a rare complication until recently published case reports (2,3). Here, we announce our case to keep in mind that rivaroxaban is a cause of spontaneous hemothorax, as not uncommonly.

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ETHICAL DECLARATIONS

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

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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