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The Full Outline of UnResponsiveness score is more efficient than the Glasgow Coma Scale in the prognosis of stroke

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ABSTRACT

Objectives: After being a solid method of measuring consciousness for decades,the Glasgow Coma Scale (GCS) was offered to be replaced with a better option: The Full Outline of Unresponsiveness (FOUR) score, which several studies later confirmed to be superior due to its independence from verbal response and ability to consider brain stem reflexes. In this study, we decided to compare the two scales in the prognosis of stroke, which has never been conducted before.

Patients and Methods: We conducted both methods on 150 stroke patients during admission to the emergency service and assessed them during a 3-month follow-up after discharge.

Results: The type of stroke was 80% ischemic and 20% hemorrhagic. 55.3% survived after a 3-month follow-up. The FOUR score had a significant converse relation with hospitalization, a relation with the GCS lacked. The FOUR score also had a stronger correlation with the outcome of stroke than the GCS. Additionally, the receiver operating characteristic (ROC) curve showed greater sensitivity and specificity in the FOUR score in the prognoses of stroke patients.

Conclusion: We recommend using the FOUR score as the first priority.

Keywords: Glasgow Coma Scale, Full Outline of Unresponsiveness score, Stroke, Consciousness, Emergency medicine

1. INTRODUCTION

Stroke is the third most common cause of disability and the second most common cause of death worldwide. This condition can cause a wide variety of symptoms, one of which is loss of consciousness. Any neural damage due to hypoxia of a cerebrovascular accident can reduce the level of consciousness. There exist variable tools for measurement and assessment of consciousness [1].The Glasgow Coma Scale (GCS) has still remained as a common and simple method that is used in several health care centers, even the small ones, and can be provided easily by all clinicians, even general practitioners. It is scored by three items: eye opening, motor response and verbal response [2]. There is also another scale for evaluating the level of consciousness. The Full Outline of UnResponsiveness (FOUR) score has recently gained attention, which is composed of four clinically distinct categories of evaluation: eye reaction, motor function, brainstem reflexes and the respiratory pattern [3]. This scale has demonstrated superiorities to the GCS, leading clinicians to utilize it for more confidence and accuracy [4].

Discovering the prognosis of stroke in different patients at initial moments can help the doctors to make a reliable decision and perform an appropriate management [5, 6]. Different factors influence the prognosis of stroke and finding scales that show outcomes of patients with the most simple and fast way and highest possible accuracy is a challenge these days. Several studies have been performed to evaluate the prognosis of stroke and outcomes due to initial onsets and several scales used for this purpose.

In this study, we investigate using the GCS and FOUR score versus the other tools and also compare each other in order to evaluate the loss of consciousness due to stroke, and predict the outcomes of stroke by these scales, seeking to further assess the superiority of the FOUR score relative to the GCS.

2. PATIENTS and METHODS

Study Design

In this cross-sectional study, after receiving approval (approval number: 5/4/3858), from the Ethics Committee in Research, Tabriz University of Medical Sciences, Tabriz, Iran, the selection

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criteria were established as follows: Inclusion criteria: all patients with signs of stroke attending the emergency department between April 2013 and March 2014. All of these patients underwent biomedical imaging after being included in the study and the diagnosis was established in all cases.

Exclusion criteria: patients with serum electrolyte disorders, patients with any abnormalities in blood glucose level, patients with diagnoses apart from stroke, patients who received anesthesia, neuromuscular blockers, any sedative agents such as : midazolam, morphine, fentanyl, sufentanil, pancuronium bromide, atracurium besylate, propofol, within the past 48 hours, patients with hearing impairment and/or muteness were excluded.

Implementation

The sample size was calculated by the Morgan's Table, requiring 384 patients in a citywide population of 1.5 million with a 5% Margin of Error. All the patients were randomly selected. The patients' next of kin obtained written assurances of information confidentiality and provided consent with the study. The patients were assessed during the first hours after admission to the emergency room. Emergency specialists were the investigators who performed the assessment during admission and before examining the patients. All emergency specialists were re-educated of the procedures of calculation and the criteria of inclusion and exclusion. The physicians graded the patients, according to both the GCS and FOUR score at the time of arrival, and evaluated the patients' levels of consciousness.

The demographics and other information recorded for each patient were as follows: age, sex, the FOUR score and the GCS score at the time of arrival and date of discharge, prognosis of each patient according to the modified Rankin Scale (MRS), which was used to measure the degree of disability in patients who have had a stroke and included morbidity, duration of hospitalization and mortality during discharge from the emergency department. All the patients were followed up according to the mRS questionnaire, which continued for three months. Forty-four patients were missed during the follow-up. Additionally, adjusted regression models were calculated for possible confounders; and the mRS and/or the incidence of death were noted as well.

Statistical analysis

Eventually, the relation between the GCS and FOUR scores at the time of arrival was reviewed and compared with the prognosis and outcome during discharge according to the mRS. The data was collected and analysed using the SPSS software version 21.0 (IBM Corporation, Armonk, NY, USA). A discriptive study was run for demographic data (mean, mode, median) and the Pearson correlation coefficient (PCC) was used in order to find associations between the prognoses, mean hospital durations, mRS and each rating scale. The p-value below 0.05 was considered meaningful.

3. RESULTS

A total of 150 patients participated in the study: 66 males (44%) and 84 females (56%). The mean age was 69.79 ± 1.11 (Min=21, Max=91). The type of stroke was ischemic in 120 patients (80%) and hemorrhagic in the remaining 30 (20%). The means of the FOUR score and the GCS at arrival time were 13.92 ± 0.23 (Min=3, Max=16) and 11.73 ± 0.25 (min=3, max=13), respectively. The mean duration of hospitalization was 14.95 ± 1.52 days (Min=1, Max=110).

Forty-five patients (30%) died in hospital, 22 patients (14.7%) died after discharge and 83 patients (55.3%) survived after a 3-month follow-up. The results of the GCS and FOUR score at arrival, the MRS score at discharge and after the follow-up are provided in Tables I and II.

Table I. The FOUR score and the GCS at the time of arrival compared with the MRS at discharge (the data are presented as Mean ± SE)

GCS	FOUR score	MRS
13±2	16±0.001	0 (no symptoms)
13.75±1.25	15.5±0.5	1
14.8±0.42	16±1.01	2
13.83±0.61	15.67±0.22	3
13.13±0.36	15.29±0.21	4
10.72±0.43	13.72±0.35	5
9.95±0.45	11.43±0.51	6 (deceased)
<0.001	<0.001	p value

GCS: Glasgow Coma Scale, FOUR: Full Outline of UnResponsiveness, MRS: Modified Rankin Scale SE: standard error

Table II. The FOUR score and the GCS at the time of arrival compared with the MRS after a three-month follow-up (the data are presented as Mean±SE)

FOUR score	GCS	MRS
13.8±0.62	15.7±0.21	0 (no symptoms)
13.75±0.42	15.71±0.16	1
13.85±0.37	15,69±0.17	2
13.47±0.44	15.27±0.24	3
11.69±0.83	14.38±0.53	4
11±3	14.5±1.5	5
10.52±0.52	13.56±0.44	6 (deceased)
<0.001	<0.001	p value

GCS: Glasgow Coma Scale, FOUR: Full Outline of Unresponsiveness, MRS: Modified Rankin Scale SE: standard error

The FOUR score of the stroke patients had a significant converse association with hospitalization (p-value=0.01, PCC =-0.2). However, the GSC of the stroke patients had no significant association with hospitalization (p-value =0.1, PCC =-0.13).

The results of the ROC curve (Figure 1) for investigating sensitivity and specificity of the GCS and FOUR scores in the prognoses of the patients showed a significant relation between the FOUR scores and the prognosis : cut off point=9.5, sensitivity=100%, specificity=82.1%, and curved surface=-0.84 (p-value < 0.001). In addition, it showed a significant relation between the GCS and the prognosis: cut off point=10.5, sensitivity =85.5%, specificity= 68.2% and curved surface=-0.81 (p-value < 0.001).

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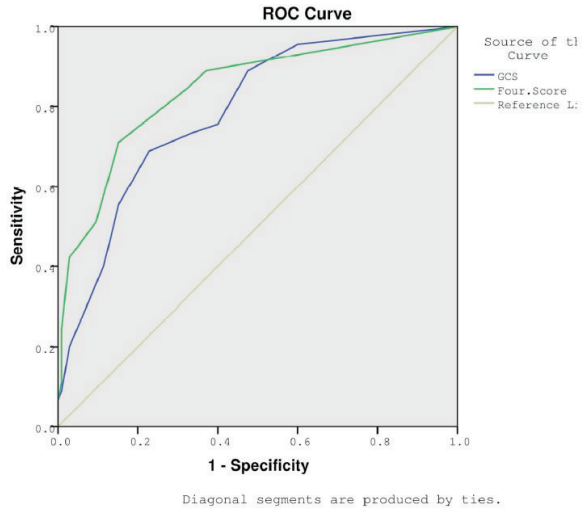

Figure 1. The receiver operating characteristic (ROC) curve for a comparison of the GCS and FOUR score in the prognosis of stroke

4. DISCUSSION

The GCS is the procedure of scoring by eye opening, motor response and verbal response and the FOUR score consists of eye reaction, motor function, brainstem reflexes and the respiratory pattern. The GCS is a simple and quickly computable method. Although, it is used widely in hospitals as a standard scale for scoring the level of consciousness [7, 8], it has defects and limitations:

1. A very important question is whether it is beneficial to use the verbal response for evaluating level of consciousness. The verbal response is investigated according to orientation in time and place, and requires a complete orientation in order to achieve the full score. But, is it necessary to be completely oriented to time and space to be considered a conscious person? We believe that confusion or stimulation or even fear or emotional stress can reduce the score of level of consciousness in the GCS evaluating. There are also other situations where a patient with no or little verbal response can lose score in measuring level of consciousness such as aphasia due to stroke. In addition, calculating the GCS score in intubated patients is more difficult than calculating the FOUR score. Hence, the latter is more appropriate in intubated patients and in Intensive Care Units (ICUs) [9, 10].

2. Another important point is that the GCS lacks the ability to evaluate brain stem reflexes and eye movements or complicated motor responses. Versus, the FOUR score can evaluate brain stem and pons reflexes and yield beneficial information about their situation [11, 12].

In the recent years, several studies had pointed the defects of the GCS [12, 13]. Considering these limitations, the FOUR score is at a higher level. It is not dependant on verbal response while it utilizes brainstem reflexes. The FOUR score can have a poor prognosis on brain death but it can differentiate the locked-in syndrome from a coma. Not only is it as easy as the GCS to utilize, but can also be used at ICUs, and thus has a wider range of usability, higher accuracy and more reliability. It has four items each ranging from 0 to 4 (Table III), whereas the GCS has three items, having 4-6 scores (Table IV). Therefore, calculation is easier in the FOUR score, reducing the probability of errors as a result [14, 15].

Table III. Glasgow Coma Scale (GCO)

Eye opening
4=spontaneous
3=due to speech
2=due to pain
1=no response
Best motor response
6=obedience
5=localization
4=withdrawal
3=abnormal flexion
2=extention
1=no response
Best verbal response
5=oriented
4=confused conversation
3=inappropriate words
2=incomprehensible sounds
1=no response

Table IV. The Full Outline of UnResponsiveness (FOUR) score

Eye response
4=eyelids open or opened, tracking, or blinking to command
3=eyelids open but not tracking
2=eyelids closed but opened due to loud voice
1=eyelids closed but opened due to pain
0=eyelids remain closed notwithstanding pain
Motor response
4=thumbs-up, fist, or peace sign
3=localizing toward pain area
2=flexion response to pain
1=extension response to pain
0=no response to pain or generalized myoclonus status
Brainstem reflexes
4= present papillary and corneal reflexes
3=one pupil wide and fixed
2= absent papillary or corneal reflexes
1= absent papillary and corneal reflexes
0=absent papillary, corneal, and cough reflexes
Respiration
4=not intubated, regular respiration
3=not intubated, Cheyne-Stokes respiration
2=not intubated, irregular respiration
1=above ventilator rate
0=at ventilator rate or apnea

We performed this study to determine a simple and fast way to make the prognosis of stroke patients and able to guide physicians to make suitable decisions. Determination of the outcome of stroke patients by the GCS or FOUR score is a new study that has never conducted before; and it provided us with a significant relation and showed its reliability. The outcomes of stroke patients were scored by the MRS (Table V) at the time of discharge and 3 months later, showing not only a good correlation between the GCS and the MRS, but also a high prognostic value of the FOUR score in patients with cerebrovascular attacks. Moreover, using this new coma scale might overwhelm the limitations of the GCS at the time of admission to an emergency department. It has also demonstrated advantages at evaluation immediately after cardiopulmonary resuscitation (CPR).

Table V. Modified Rankin Scale (MRS)

0=No symptoms at all
1=Significant disability notwithstanding symptoms; able to carry out all usual duties and activities
2=Slight disability; unable to carry out all previous activities, but able to run one's own affairs without assistance
3=Moderate disability; requiring some help, but able to walk without assistance
4=Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5=Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6= Deceased

According to our results, both of the scales were equally useful in long-term prediction of the clinical condition, evaluated by the mRS. The FOUR score and the MRS demonstrated an inverse correlation which was even stronger than that between the GCS and MRS (-0. 84 vs. –0.81; p <0.001).

Wijdicks et al., studied the reliability of the FOUR score for the first time [14]. They have demonstrated that the FOUR score can provide more neurologic information, such as brainstem abnormalities, breathing disorder or brain herniation. The FOUR score and the MRS showed an inverse correlation which was even stronger than that between the GCS and MRS (-0. 547 vs. –0.514; p <0.001). A similar difference of correlation was also seen after the three-month follow up (–0.509 vs. – 0.486). Besides, in patients with stroke, it is capable of detecting the locked-in syndrome.

Kocak et al., have investigated the predictive validity of the FOUR score in acute stroke patients in ICU [3]. The follow-up study of the admitted patients favoured our results. Our findings support the hypothesis that the FOUR score could be regarded superior to the GCS in assessing the prognosis and level of consciousness in patients with acute stroke.

Saika et al., studied on 138 patients in 2015 to investigate the prediction validity of the GCS and FOUR score in patients with brain injury [16]. The mean score of GCS and FOUR was 9.5 (3-11) and 11 (0-16), respectively. The data showed that the mean GCS and FOUR score finding were significantly lower in expired patients. The FOUR score had a cut off point of 7. 97% sensitivity, 97.5% specificity and a minister curved surface of – 0.97. The GCS had a cut off point of 6.98.3% sensitivity, 82.4% specificity and a minister curved surface of – 0.95. Hence, they mentioned that one could use both the GSC and FOUR score to predict the outcome of brain injuries.

Sadaka et al., performed a study on 51 patients in 2011 and distinguished a significant relation between the two scales and the prognoses determined by the MRS in brain injuries [9]. In this study, the minister curved surface that showed hospital mortality was equal to 0.93 for the FOUR score and 0.89 for the GCS. In addition, the minister curved surface for mortality after 3-6 months was equal to 0.85 for the FOUR score and 0.83 for the GCS. Authors pointed that the FOUR score had supremacy to the GCS at predicting the outcome of brain injuries.

There exist several other studies that show the validity of the FOUR score in predicting different brain injuries and its superiority to the GCS. Recently, Stead et al., Iyer et al. and Bruno et al., reported similar results along with others and our results are in line with these observations [12, 17-21].

We have also demonstrated an improvement in the function of the patients, corresponding to a decrease in the follow-up MRS, with a promotion in both the GCS and FOUR score grading. These results may be descriptive of the therapeutic utility in patients with acute stroke. In terms of distinction of the ischemic or hemorrhagic type of stroke, our results were unable to show a significant correlation with the MRS. It would thus be preferable to combine these prognostic indicators in a model with other components of stroke if they were to be used in patient management. The follow-up period was relatively short in our study. We suggest further studies with larger samples and longer follow-ups.

Both the FOUR score and the GCS are appropriate scales for evaluating level of consciousness in stroke patients and in addition, both have a significant relation with prognoses of stroke patients at the time of discharge and the follow-up. As a result, the GCS and FOUR score are useful predictors of the outcome of stroke and can help physicians to make decisions on patient management during initial hours.

Because of the limitations of the GCS due to dependence on verbal response and failure to consider brainstem reflexes, and additionally a stronger significant correlation between the FOUR score and the outcome of stroke according to the MRS, and also a significant association between the FOUR score and hospitalization in our study, we suggest utilizing the FOUR score instead of the GCS. The FOUR score is a reliable and valuable scale in predicting prognosis in patients wth stroke.

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

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Serum vitamin D level variation in SIRS, sepsis and septic shock

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ABSTRACT

Objectives: Vitamin D has potent immunomodulatory effects with the capability of acting as an autocrine and paracrine agent, and inhibits inflammatory signaling. In this study, our aim was to evaluate the relationship between vitamin D levels in systemic inflammatory response syndrome (SIRS), sepsis and, septic shock patients and outcomes.

Patients and Methods: A total of 45 patients whose vitamin D levels were measured within the first 48 hours of Intensive Care Unit (ICU) admission and 20 healthy controls were studied prospectively. The patients were grouped as, SIRS (Group-I,n=10), sepsis (Group-II,n=25), septic shock (Group-III,n=10) and healthy subjects (Group-IV,n=20). Serum vitamin D levels were categorized as a deficiency (≤15ng/mL), insufficiency (16-29ng/mL) and sufficiency (≥30ng/mL). Demographic characteristics, Acute Physiology and Chronic Health Assessment II (APACHE-II) scores, and biochemical parameters were noted.

Results: Vitamin D levels were significantly lower in all study groups compared to the control group ($p<0.01$), but were similar among the study groups. The hospital and ICU length of stay (LOS), and biochemical parameters were similar among the study groups. The mortality rates were 40% in Group I, 57 % in Group II, and 80 % in Group III.

Conclusion: In our study patients with SIRS, sepsis and septic shock had lower serum 25-OH vitamin D levels compared to the control group. Our results are in line with the literature that supports a relationship between vitamin D deficiency and inflammation.

Keywords: Critically ill patients, Vitamin D, Sepsis, SIRS

1. INTRODUCTION

Sepsis was defined as a clinical syndrome by the presence or suspected of both infection and systemic inflammatory response combined with organ injury in the 2001 by International Sepsis Definitions Conference [1]. The incidence of sepsis, which is one of the most important reasons for admission to the intensive care unit (ICU), has increased significantly in the last 20 years. However; although the positive effects of advances and awareness campaigns in the management of sepsis provide an increase in survival, sepsis is still the most important cause of mortality and morbidity in ICU [2].

A large amount of vitamin D, which is fat-soluble, is produced on the skin by sunlight and a limited amount is obtained from the diet. It plays an important role in the regulation of bone and calcium metabolism. Vitamin D is stored in fat cells and is converted to hydroxyvitamin D3, the so-called circulating form in the liver. Hydroxyvitamin D3 is mainly converted to calcitriol, the active metabolite in the kidneys [3,4].

Vitamin D metabolites have significant pleiotropic effects on innate and adaptive immune system cells. The calcitriol, biologically active metabolite of vitamin D, increases the production of cathelicidin and defensin and thus plays a role in the innate immune system. In addition, it exerts its effects on the adaptive immune system via vitamin D receptors on monocytes, T, and B cells. Vitamin D also affects the proliferation and differentiation of T and B cells and modulates immunoglobulin production [5-7].

It is assumed that more than 1 billion people worldwide have vitamin D deficiency. It was well-established that vitamin D deficiency is a well-known cause of musculoskeletal disorders. Recent studies have shown that vitamin D deficiency may play a role in the pathogenesis of infection, immunological, neurological, cardiovascular and respiratory diseases [4,6-8].

Its deficiency is frequently observed in critically ill patients and has been shown to be associated with increased mortality and prolonged hospital stay. Similarly, the deficiency was reported to

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be a risk factor for mortality in critically ill patients with sepsis [6,8-11].

In this study, we aimed to evaluate the relationship between the severity of sepsis and serum 25-OH vitamin D levels and its effect on survival in critically ill patients.

2. PATIENTS and METHODS

Study Design

A prospective cohort study was performed between January 2015 and July 2015 in the ICU of the Marmara University Training and Research Hospital. A total of 45 critically ill patients and 20 voluntary healthy volunteers were included in the study. The study protocol was approved by the Institutional Ethics Committee and was in compliance with the principles of the Declaration of Helsinki. The informed written consent form was obtained from patients and healthy volunteers.

Demographic data of the patients, Acute Physiology and Chronic Health Assessment II (APACHE II) scores, length of intensive care and hospital stay, serum 25-OH vitamin D, C-reactive protein (CRP), procalcitonin, parathyroid hormone (PTH) levels, hemogram, and biochemical parameters were recorded.

The patients were divided into groups as, systemic inflammatory response syndrome (SIRS) (Group I, n=10), sepsis (Group II, n=25), septic shock (Group III, n=10) and voluntary healthy subjects (Group IV, n=20) for the control group.

Definitions

The serum 25-OH vitamin D vitamin D levels were categorized as deficiency (≤20 ng/mL), insufficiency (20-29 ng/mL) and sufficiency (≥30 ng/mL) in accordance with the guidelines of the Endocrine Society. Also, the serum 25-OH vitamin D level ≤ 10 ng/mL were defined as very severe vitamin D deficiency [12].

Systemic inflammatory response syndrome; is a systemic inflammatory response to various clinical conditions. Body temperature >38°C or <36°C, heart rate > 90/min, respiratory rate >20/min or PaCO2 <32 mmHg, with the presence of at least two of the followings; leukocyte count >12000/mm3 or <4000/mm3 or > 10% of the young neutrophil [1].

According to the 2001 International Sepsis Definitions Conference, sepsis patients were defined as a clinical syndrome of systemic inflammatory response together with infection. Septic shock patients were defined as persistent arterial hypotension (systolic arterial pressure <90 mmHg, mean arterial pressure <60 mmHg, or blood pressure decreased to 40 mmHg from normal baseline value) which cannot be explained for other reasons and is unresponsive to fluid resuscitation [1].

Clinical Data

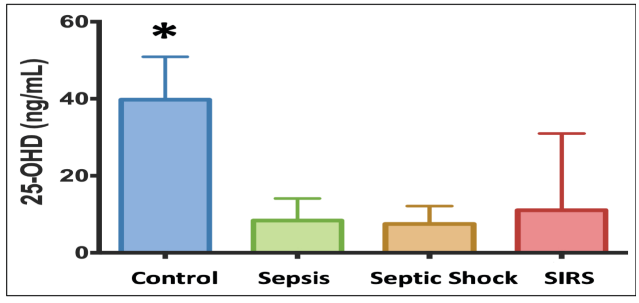
25-OH vitamin D levels and other biochemical parameters of the patients were measured within 48 hours after they were admitted to the ICU. Serum 25-OH vitamin D levels were determined by high – performance liquid chromatography method (HPLC; Recipe Chemicals, Munich, Germany), serum CRP level by BN II nephelometer method (Siemens, Erlangen, Germany), serum procalcitonin levels by electrochemiluminescence immunoassay method (Roche Cobas e411; Roche Diagnostics, Indianapolis, USA), serum PTH level was measured by chemiluminescence immunoassay method (Modular Analytics E170; Roche Diagnostics, Mannheim, Germany) and other biochemical values were measured by enzymatic colorimetric method (Roche Diagnostics, Indianapolis, USA). The complete blood count parameters were measured by Coulter STKS hematological analyzer method (Beckman Coulter Cooperation, Miami, FL, USA).

Statistical Analysis

In this study, data showing normal distribution were given as mean ± standard deviation, data not showing normal distribution were given as median (min-max) values as numbers. The One-Way Anova test was used for the analysis of distributed data. The results were evaluated with a 95% confidence interval, and $p < 0.05$ was considered as the level of statistical significance. Statistical Package for Social Sciences (SPSS) for Windows 21.0 was used for statistical analysis.

3. RESULTS

Sixty-five patients were enrolled into the study. The patients were grouped as, SIRS (Group I, n=10), sepsis (Group II, n=25), septic shock (Group III, n=10) and healthy volunteers (Group IV, n=20) for the control group. Patients with SIRS, sepsis, and the septic shock had significantly lower 25-OH vitamin D levels than healthy volunteers (p=0.001)(Figure 1). The baseline demographic characteristics, 25-OHD levels, and other clinical parameters of patients and healthy volunteers are given in Table I. The clinical and laboratory findings of patients are presented in Table II.



* p=0.001

Figure 1. Serum 25-OH vitamin D levels in patients and healthy volunteers.

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Table I. The baseline demographic characteristics, 25-OHD levels and other clinical parameters of patients and healthy volunteers

	SIRS Mean ± SD	Sepsis Mean ± SD	Septic Shock Mean ± SD	Healty Control Mean ± SD	p value
Age (years)	62.38 ± 16.69	55.74 ± 17.81	60.70 ± 11.56	44.81 ± 15.03	**0.016**
APACHE-II score	14.90 ± 5.15	22.21 ± 8.54	22.40 ± 5.04	N/A	**0.03**
ICU LOS (days)	13.80 ± 19.26	26.52 ± 54.46	16.90 ± 22.90	N/A	0.69
Hospital LOS (days)	18.50 ± 18.77	31.39 ± 60.60	18.60 ±.22.40	N/A	0.67
25-OHD (ng/mL)	8.39 ± 5.70	7.49 ± 4.64	5.16 ± 3.97	39.77 ± 11.13	**0.001**

25-OHD: 25-hydroxy vitamin D, ICU LOS: Intensive care unit length of stay, LOS: length of stay, APACHE II: Acute Physiology and Chronic Health Evaluation II, N/A: Not Applicable, SD: Standard Deviation

Table II. The clinical and laboratory findings of the patients

	SIRS Mean ± SD	Sepsis Mean ± SD	Septic Shock Mean ± SD	p value
WBC (/mm^3)	14229 ± 7013	14434 ± 7201	13127 ± 7073	0.09
25-OHD (ng/mL)	8.39 ± 5.70	7.49 ± 4.64	5.16 ± 3.97	0.19
Albumin (g/dL)	2.57 ± 0.81	3.42 ± 3.65	2.22 ± 0.60	0.66
BUN (mg/dL)	30.30 ± 28.22	28.57 ± 16.83	42.55 ± 29.10	0.44
Creatinine (mg/dL)	1.22 ± 1.24	1.02 ± 0.65	1.62 ± 1.23	0.25
Glucose (mg/dL)	143.50 ± 55.08	125.61 ± 26.32	166.73 ± 60.51	0.05
Calcium (mg/dL)	7.66 ± 1.32	8.13 ± 0.95	7.68 ± 1.18	0.40
Parathormone (pg/mL)	78.45 ± 66.36	110.44 ± 123.20	78.33 ± 108.84	0.63
CRP (mg/L)	126.72 ± 66.88	156.27 ± 106.39	184.79 ± 119.07	0.44
Procalcitonin (ng/mL)	1.96 ± 2.39	12.51 ± 27.44	5.09 ±9.54	0.35
Phosphorus (mg/dL)	2.82 ± 0.97	3.07 ± 1.31	3.52 ± 1.50	0.45

25-OHD: 25-hydroxy vitamin D, WBC: White Blood Cells, BUN: Blood Urea Nitrogen, CRP: C-reactive Protein, SD: Standard Deviation

Patient's baseline laboratory parameters at ICU admission, ICU lenght of stay (LOS), and hospital LOS were similar in patients' groups. We found lower serum 25-OH vitamin D levels in sepsis and septic shock patients compared to patients who had SIRS but without a statistical significance (p=0.19). The mortality rates were 40% in Group I, 57 % in Group II, and 80% in Group III. In addition to this, serum 25-OH vitamin D levels were lower in non-survivor patients compared with the survivors (6.9 ± 5.3 vs. 7.4 ± 3.6), but without a statistical significance (p=0.71).

4. DISCUSSION

In the present study, we evaluated the relationship between vitamin D levels in SIRS, sepsis, and septic shock patients and outcomes. The main finding of this study showed that the serum 25-OH vitamin D levels were significantly lower in all study groups compared to the control group. In addition, vitamin D levels were found lower in non-survivor patients, even if it was not statistically significant. The hospital and ICU LOS, biochemical parameters were similar among the study groups.

Vitamin D is a fat-soluble steroid hormone, and it has a very important role in bone health, calcium homeostasis and also maintaining the optimal function of many organ systems [7,8]. Various clinical studies emphasize deficiency is a major healthcare problem worldwide and has a crucial role in some disease pathogenesis such as musculoskeletal diseases. Also, recent studies have shown that vitamin D deficiency is associated with systemic inflammation, infection, cancer, immunological, neurological, cardiovascular, and respiratory diseases [8,13]. Furthermore, the correlation between vitamin D deficiency and increased mortality has been shown in the general population [14,15]. Melamed et al., showed that lower serum vitamin D levels were associated with higher mortality in the general population [14]. Also, clinical studies and meta-analysis on patients with chronic kidney diseases have shown that higher serum vitamin D levels and also vitamin D supplementation were associated with improved survival [15,16].

Vitamin D deficiency in critically ill patients has been well established [7]. The prevalence of low vitamin D concentrations was more than 90% among critically ill patients and higher than in patients who were hospitalized in non-critical units [17]. Recently, published studies and meta-analysis emphasized the association between vitamin D deficiency and mortality in critically ill patients [17-20]. There was a 1.81-fold higher risk of death in critically ill patients with vitamin D deficiency [21]. Furthermore, vitamin D deficiency has been associated with longer hospital stay and ICU LOS, increased risk of ICU-acquired infections, and sepsis [7,17-19,21,22]. In our study, we found that 95.5% of patients had vitamin D deficiency (≤ 20 ng/mL) on admission to the ICU. We found that vitamin D levels were lower in non-survivor patients compared with the survivors, but it was not statistically significant.

Vitamin D deficiency is associated with an increased risk of infectious diseases and sepsis. It was considered a significant prognostic factor for mortality in patients with sepsis [6,7,11,17,20,23,24]. Its deficiency affects the development of sepsis with several mechanisms that includes adaptive and innate immune modulation, suppression of the inflammatory response, enhanced phagocytosis and chemotaxis, and increased production of antimicrobial peptide β-defensins and cathelicidins [17,25-28]. It was shown that high levels of serum 25-OH vitamin D (38 ng/mL or greater) decreased the incidences of urinary tract infections [25]. However, the association

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between respiratory tract infections (RTI) and serum vitamin D levels is controversial. Some observational and case-control studies revealed that hospital admission due to RTI is associated with lower vitamin D serum levels [26,27]. In contrast to this, some studies claimed that there was no relationship between vitamin D status and RTI [28,29]. In a prospective cohort study, including 461 patients with suspected sepsis, no statistically significant difference was found in 1,25(OH) D levels between those with or without infections [30].

Also, there is a correlation between serum levels of vitamin D and the severity of sepsis and still a contributing factor of organ dysfunction. Patients with septic shock had a lower serum level of vitamin D compared with septic patients without organ dysfunction {29,30]. Jeng et al., found that there were no significant differences in serum levels of vitamin D between patients with sepsis and critically ill patients [31]. In the present study, we found that critically ill subjects had significantly lower plasma serum levels of vitamin D compared with healthy controls even if low serum levels of vitamin D seen in septic shock, sepsis and SIRS patients but the levels were comparable among the study groups.

Limitations

The mean 25-OH vitamin D proportion of deficiency does not vary across different ages. Therefore, we did not take the age difference between the groups into account. We also did not pay attention to the comorbidities and malnutrition, which are important causes of vitamin D deficiency. In addition to this, pre-illness vitamin D levels were unknown in study groups.

Conclusion

Vitamin D deficiency is commonly seen in critically ill patients. Although, it is not an independent factor for mortality, it might be related to worse clinical status during ICU stay. In our study, we found that the serum 25-OH vitamin D level in ICU patients with SIRS, sepsis and septic shock were lower than healthy individuals, but the levels of serum vitamin D were similar among the study groups. Our results showed that the severity of the disease had no effect on the serum levels of vitamin D. Thus, inflammation mainly may cause alterations in vitamin D metabolisms. The new trials are needed to determine the role of vitamin D before or during critical illnesses, and to investigate whether it can improve clinical outcomes.

Disclosure: The authors declare that there is no conflict of interest regarding the publication of this article.

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Evaluation of residual tumors and recurrence rates of malignant melanoma and non-melanoma skin cancer of head and neck region

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ABSTRACT

Objective: In this study, we aimed to evaluate residual tumors and recurrence rates of malignant melanoma (MM) and a non-melanoma skin cancer of (NMSC) head and neck region.

Patients and Methods: Medical data of a total of 398 lesions of 323 patients who underwent surgical excision for a basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanoma (MM) were retrospectively analyzed. All patients were classified according to age, sex, location of the tumor, histopathological diagnosis, lesion diameter, excision diameter, surgical margin status, and residual lesions and recurrence rates.

Results: There were 244 lesions (61.3%) in 189 males and 154 lesions (38.7%) in 134 females. The most common type of skin cancer was BCC in 268 lesions (67%), followed by SCC in 122 (31%), and MM in eight (2%), respectively. Recurrence was seen in 3% of the NMSC cases and in 25% of the MM cases. There was a statistically significant correlation between the histopathological diagnosis and recurrence rates. Compared to NMSC, MM cases had a higher risk for recurrence (p=0.029).

Conclusion: Our study results suggest that recurrence is associated with the localization and type of the tumor, but not with the age or sex of the patient.

Keywords: Basal cell carcinoma, Squamous cell carcinoma, Malignant melanoma, Recurrence, Residual tumor

1. INTRODUCTION

Skin cancer is the leading cancer in the world [1]. In recent years, the incidence of the skin cancer has been increasing, although, the mortality rates still remain unchanged [2]. Skin cancer can be mainly divided into two groups as malignant melanoma (MM) and non-melanoma skin cancer (NMSC). There are two main subtypes of NMSC as basal cell carcinoma (BCC) in which cancer is derived from epidermal cells of the basal layer and squamous cell carcinoma (SCC) which originates from the malignant proliferation of the epidermal keratinocytes [3-5]. However, MM originates from the melanocytes which are the pigment-generating cells of the skin [6].

Basal cell carcinoma is the dominant form of skin cancer which accounts for 80% of all cases [7]. Due to its slow-growing and non-aggressive nature, metastasis is unlikely [8]. Although, BCC is not usually fatal, it may be associated with significant morbidities and complications due to its locally invasive nature [9]. As the other form of NMSC, SCC is the second most common type of skin cancer, accounting for 20% of all NMSC cases [7,10]. In contrast to BCC, SCC acts in a more aggressive manner with a higher metastasis risk [11,12]. The major factor for the development of NMSC is the cumulative exposure to ultraviolet radiation (UV) [13]. Consequently, the typical locations of NMSC are the areas of the skin which are regularly exposed to sun, such as head and neck region [14].

On the other hand, MM is the rarest form of skin malignancies, accounting for 1% of all skin cancer cases [15]. Although, MM is the least likely skin malignancy, it is associated with a high incidence of skin cancer-related mortality [16]. According to the American Cancer Society, MM is recognized as being the sixth most common cancer in the United States [17].

In the present study, we aimed to investigate the distribution of skin cancers of the head and neck and to evaluate the residual and recurrent cases.

2. PATIENTS AND METHODS

This retrospective study was conducted at Marmara University, Training and Research Hospital, Department of Plastic, Reconstructive and Aesthetic Surgery between January 2010

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and December 2015. This study included BCC, SCC, and MM cases of the head and neck region. The head and neck region were divided into eight subunits to obtain a systematic analysis of the retrieved data including the scalp, forehead, periauricular, periorbital, nose, cheek, perioral, and chin. In addition to benign tumors, tumors located in other areas, precursor lesions of the skin and rarer types of skin cancer (i.e., Merkel cell carcinoma, cutaneous lymphomas, Kaposi sarcoma, and skin adnexal tumors) were excluded.

Finally, a total of 1801 lesions underwent a surgical excision. After the pathology reports were classified for eligibility; 398 skin lesions of 323 patients were included in the study. Eligible cases were classified according to the age, sex, location of the tumor, histopathological diagnosis, lesion diameter, excision diameter, surgical margin status, and residual lesions and recurrence rates.

A residual lesion was defined as the presence of cancer cells in the surgical margins after the initial excision. A recurrent lesion was defined as histopathological evidence of tumor in the same excision area after the initial excision.

A written informed consent was obtained from each patient. Ethical approval was obtained for the present study from Marmara University Ethical Committee (approval number: …….). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using the STATA 10.0 software (STATA Corp., College Station, TX, USA). Descriptive statistics were expressed in mean ± standard deviation (SD), median (min-max), or number and frequency. The chi-square test was used to analyze statistically significant differences between the MM and NMSC groups. A p value of <0.05 was considered statistically significant.

3. RESULTS

A total of 398 skin lesions of 323 patients in the head and neck region were surgically excised. There were 244 lesions (61.3%) in 189 males and 154 lesions (38.7%) in 134 females. The male-to-female ratio was 1.41:1. The mean age of all patients was 66.3±15.55 (range, 6 to 59) years. The most common type of skin cancer was BCC in 268 lesions (67%), followed by SCC in 122 (31%), and MM in eight (2%), respectively. Following the initial excision, 333 cases (84%) were successfully removed, while there were 59 procedures (15%) resulting in tumor presence in the surgical margin. No data were available in six (2%) of the excisions. Recurrence was seen in 14 cases (3.5%) (Table I).

Sex

Among all excised BCC lesions, 152 (56.8%) were removed from males and 116 (43.2%) from females. A male dominance was observed in SCC in 86 procedures (70.5%) among male patients, while 36 procedures (29.5%) were performed in female patients. Of the total MM cases, six (75%) were males and two (25%) were females (Table I).

Age

The mean age was similar among BCC and SCC cases (66.4±13.03 years and 66.5±13.03 years, respectively). The mean age of MM cases was 59.8±20.72 years (Table I).

Table I. Demographic and clinical characteristics of patients

	Total	BCC	SCC	MM
Sex Male	244 (61.3%)	152 (57.8%)	86 (70.5%)	6 (75%)
Female	154 (38.7%)	116 (43.2%)	36 (29.5%)	2 (25%)
Tumor, n (%)	398 (100%)	268 (67%)	122 (31%)	8 (2%)
Age, years, mean±SD (range)	66.3±15.55 (6 to 95)	66.4±13.03 (17 to 90)	66.5±19.76 (6 to 95)	59.8±20.72 (27 to 84)
Surgical margin status (+)	59 (15%)	48 (18%)	11 (9%)	0
(-)	333 (84%)	216 (80.5%)	109 (89%)	8 (100%)
Missing data	6 (2%)	4 (1.5%)	2 (2%)	0
Recurrence, n (%)	14 (3.5%)	8 (3%)	4 (3%)	2 (25%)

BCC: basal cell carcinoma, SCC: squamous cell carcinoma, MM: malignant melanoma, SD: standard deviation

Location and sex

Most of the lesions were found in the nasal area in 111 cases (27.8%), followed by the perioral area in 68 cases (17.1%) and periorbital area in 65 cases (16.3%). The rate of skin malignancies was the lowest in around the chin and forehead in only six (1.5%) and 24 (6%) cases, respectively. Skin cancer appeared in the nasal region most commonly in both sexes. A total of 20.9% of the excisions were carried out in males and 38.9% in females. Around the periauricular region, the figures were much higher in males in 15.6% of the male population (n=38). On the other hand, only 0.8% of the female patients (n=2) had cancer in this area (Table II).

Table II. Sex of patients and location of tumors according to groups

	Total	M	F	BCC	SCC	MM
Cheek	33 (8.3%)	18 (7.4%)	15 (9.7%)	21 (7.8%)	8 (6.5%)	4 (50%)
Chin	6 (1.5%)	2 (0.8%)	4 (2.6%)	3 (1.1%)	3 (2.5%)	0
Forehead	24 (6%)	16 (6.5%)	8 (5.2%)	17 (6.3%)	7 (5.7%)	0
Nose	111 (27.8%)	51 (20.9%)	60 (38.9%)	91 (34%)	19 (15.6%)	1 (12.5%)
Periauricular	40 (10%)	38 (15.6%)	2 (1.3%)	28 (10.4%)	11 (9%)	1 (12.5%)
Perioral	68 (17.1%)	47 (19.3%)	21 (13.6%)	21 (7.8%)	47 (38.5%)	0
Periorbital	65 (16.3%)	38 (15.6%)	27 (17.5%)	50 (18.6%)	14 (11.5%)	1 (12.5%)
Scalp	51 (12.8%)	34 (13.9%)	17 (11%)	37 (13.8%)	13 (10.7%)	1 (12.5%)
Total	398	244	154	268	122	8

Data are given in number and percentage. M: male; F: female, BCC: basal cell carcinoma, SCC: squamous cell carcinoma, MM: malignant melanoma

Surgical margins

A total of 216 (80.5%) of 268 BCC cases were excised successfully, while in 48 cases (18%), there was a tumor presence in the surgical margins. Among 122 SCC cases, 109 (89%) were excised adequately and 11 (9%) were inadequately removed. All residual lesions were re-excised and clear margins were established. No inadequate margin was detected after the initial excision of all MM cases. There were missing data in six of the cases (Table III).

Table III. Margin status according to subunits

	Margin Status			
	Negative	**Positive**	**Missing data**	**Recurrence**
Cheek	28 (8.4%)	4 (6.7%)	1	0
Chin	4 (1.2%)	1 (1.7%)	1	0
Forehead	22 (6.6%)	1 (1.7%)	1	2 (14.3%)
Nose	91 (27.3%)	**20 (33.9%)**	0	**6 (42.9%)**
Periauricular	32 (9.6%)	7 (11.9%)	1	1 (7.1%)
Perioral	59 (17.7)	8 (13.5%)	1	2 (14.3%)
Periorbital	52 (15.6%)	12 (20.3%)	1	0
Scalp	45 (13.5%)	6 (10.2%)	0	3 (21.4%)
Total	333 (83.6%)	59 (14.8%)	6 (1.5%)	14

Data are given in number and percentage.

Recurrence rates

Recurrence was seen in a total of 3% of the NMSC cases (3% of BCC lesions, n=8 and 3% of SCC lesions, n=4), while this rate was 25% in the MM cases. There was a statistically significant correlation between the histopathological diagnosis and recurrence rates. Compared to NMSC, MM cases had a higher risk for recurrence (p=0.029), even a clear margin was achieved in all MM cases. All recurrent cases were excised and clear margins were achieved (Table IV).

Table IV. Margin status after recurrence

	Margin Status		
	Negative	**Positive**	**n**
BCC	8	0	8
SCC	4	0	4
MM	2	0	2
Total	14	0	14

BCC: basal cell carcinoma, SCC: squamous cell carcinoma, MM: malignant melanoma

4. DISCUSSION

Basal cell carcinoma is the most common type of skin cancer in Caucasians, which predominantly occurs in the exposed parts of the body, with 80% of the lesions found in the head and neck regions [18]. Our study results also showed that most of the lesions were found in the nose (27.8%), perioral (17.1%), and periorbital (16.3%) regions, which are the most prominent parts exposed to chronic sun radiation, and also in the H area.

In the literature, most of the BCC lesions are reported in the 40-79 age group with a mean age of 62 years. However, in tropical regions and in patients with a family history, BCC may occur in a younger age [19,20]. In our study, the mean age of BCC cases was 66.4 (range, 17 to 90) years with a statistically significant increase in the 60-70 and 70-80 age groups (28.9% and 27.6%, respectively). Also, in the other Mediterranean countries, it mostly affects the 70-80 age group [21-24]. Our study results regarding the age of the patients and location of the lesions were consistent with the literature.

Previous studies have demonstrated a higher rate of BCC in male patients [25-27]. In our study, there was no statistically significant difference between men (57%) and women (43%), that was consistent with previous studies [22,25,28]. According to the subunits, nose (20.9%), perioral (19.3%), periorbital (15.6%), periauricular (15.6%), and scalp (13.9%) involvement were more common in male patients. Nose (38.9%), periorbital (11%), and perioral (8.6%) involvement were more common in female patients. These can be attributed to the fact that urban life affects both sexes equally. Periauricular BCC was seen in only two female patients (0.8%), compared to male patients, which can be explained by the possible protective effect of scarf wearing in women.

The main goals of the tumor treatment are to eradicate the tumor with the safest and most cost-effective method available and to provide an aesthetically and functionally satisfactory outcome. Although, different treatment modalities for BCC have been described in the literature, surgical excision is the most effective and commonly adapted method for tumor removal [27].

In the literature, a 3-mm peripheral surgical margin is adequate for the clearance of 85% of small and well-defined BCCs, and the 4 to 5-mm margin would raise this figure to 95% [29]. This margin is 1 cm for SCCs and 1 to 2 cm for MMs, depending on the tumor depth [8]. In our study, BCCs were excised with a 3 to 5-mm clear margin, depending on the location of the tumor, while SCCs were excised with a 1-cm clear margin and MMs were excised with a 2-cm clear margin. Previous studies have shown a great variability in the re-excision rates ranging from 9% to 75% for BCCs [30]. In our study, the rate of residual tumors resulting in re-excision was 18% for BCCs, 9% for SCCs, and 0% for MMs. This relatively high rate may result from the fact that BCCs were seen around the aesthetic subunits such as nose, eyelid, and lip and there may be an effort to keep the excision margin narrow not to compromise the cosmetic results. The majority of the studies showed that BCC and SCC required a similar number of stages to obtain free margins, although some authors suggested that BCC required more stages than SCC, which was consistent with our findings [31-34]. The fewer stages required to obtain clear margins in SCC than in BCC may be due to the more aggressive behavior of the primary tumor, leading to extreme caution, and the consequent removal of more peripheral tissues per stage. Another possible explanation is that SCCS are better defined on clinical examination [35].

Review of the literature reveals that the recurrence rate for primary BCCs after surgical excision varies between 5% and 14% [29,36]. Lesions in the head and neck region are at a higher

risk for recurrence, compared to lesions in the trunk and limbs [29,36-38]. Incomplete excision has been also reported as one of the risk factors for recurrence [25,39-41]. In our series, the overall recurrence rate was 3% for NMSC (n=8 BCC and n=4 SCC) and 25% for MM (n=2). Recurrent lesions were located in the nose (n= 6), scalp (n= 3), forehead (n= 2), perioral (n=2), and periauricular (n=1) regions. The recurrence rate (2/8, 25%) seems relatively high, although this can be explained by the small number of the overall MM cases in our study. However, there was no residual tumor after MM excision in two recurrent cases. This is probably due to the aggressive behavior of MM. More interestingly, most recurrences were located in the H area which can be attributed to high recurrence rates on the embryonic fusion planes and also close margin excision tendency not to harm the aesthetic subunits [27,42-44].

Previous studies have shown a reduced residual rate of BCCs through Mohs microscopic surgery or a margin-controlled, staged surgical excision in institutions where Mohs surgery is not available [45]. In particular, in the H area where the recurrence risk is high and aesthetic subunits of the face are included, a staged surgical excision is superior to simple excision.

Nonetheless, there are some limitations to this study. Retrospective design with a relatively small sample size is the main limitation. In addition, all operations were performed by residents and specialists. Although resections were done in accordance with the current guidelines, considering the fact that learning curve may vary for each surgeon, the results might have been different for surgeons, indicating variability in surgical outcomes. Therefore, further large-scale, prospective studies are warranted.

In conclusion, our study results suggest that recurrence is associated with the localization of the tumor, but not with the age, sex, and type of the tumor. Of note, it should be kept in mind that the low recurrence rates in our series may be due to the excision with an appropriate margin and no positive surgical margins after second surgery. However, further large-scale, prospective studies are needed to establish a definite conclusion.

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Chondroprotective efficiencies of intra-articular treatment of glucosamine sulfate and hyaluronic acid

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ABSTRACT

Objectives: The main purpose of this study was to determine a comparison between the chondroprotective efficacy of intra-articular administrations of glucosamine sulphate (GS) and hyaluronic acid (HA) in the experimental knee osteoarthritis model in rats.

Materials and Methods: Forty-five rats were assigned to the following three groups: GS group (Group 1), HA group (Group 2), control group (Group 3). The knee joints of the rats were seen macroscopically and the anterior cruciate ligaments were resected. Two weeks after surgery, an intra-articular injection was applied to each group for three weeks with 1 week interval. In the osteoarthritic rat knee joints, the histopathological effects of tissue regeneration of GS and HA applications were compared with the control group.

Results: The total Mankin Scale was 2.64±2.56, 3.58±3.9, 8.12±2.80 for the GS group, HA group and control group respectively. According to these results, the GS and HA were superior to the control group. The values of the total Mankin Scale, cartilage structure, cellular abnormality, matrix staining and tidemark integrity of the GS group were lower than the values of the HA group but this difference was not statistically significant.

Conclusion: According to the results of this study, intra-articular administration of GS for the management of osteoarthritis may be beneficial for patients with knee osteoarthritis.

Keywords: Glucosamine sulphate, Hyaluronic acid, Rat, Osteoarthritis

1. INTRODUCTION

Osteoarthritis (OA) is a noninflammatory chronic degenerative disease characterized primarily by cartilage destruction, osteophyte formation, and subchondral sclerosis, which progressively degenerate the joints [1,2]. Cartilage diseases are highly prevalant diseases with important socioeconomic problems that cause job loss and adversely affect patient comfort all over the world. There are many treatment options currently available for OA [3-5]. Chondroprotective agents such as hyaluronic acid (HA) and glycosaminoglycans are currently used in patients with OA. However, none of the treatment methods applied is sufficient to completely cure the disease. The common goal of all applications is to prevent the pathological process leading to degenerative arthritis after cartilaginous trauma, rescue the joint from arthrosis, and to create a regeneration tissue capable of fulfilling the functions of hyaline cartilage in damaged chondral areas. HA and glucosamine sulfate (GS) are drugs that are used for a long time for the treatment of OA. There are many clinical publications about the efficacy of these drugs [2-6,7-11]. Intra-articular HA application is preferred by most physicians because it shows more promising results in patients [2,3,12].

Hyaluronic acid (a glycosaminoglycan) found naturally in synovial fluid and cartilage matrix, synthesized and secreted into joints by synovial cells, fibroblasts, and chondrocytes. HA increases the viscosity and elastic nature of the synovial fluid, allowing fluid to act as a lubricant and shock absorber [13].

It has also been reported that a layer of 1-2 mm thickness on the cartilage surface contains HA [14]. Thus, HA is considered to protect the cartilage surface and soft tissues from trauma in the joints [14]. GS is a commonly used oral preparation for the treatment of OA [12]. It is a pharmacokinetic drug with positive and mild anti-inflammatory effects on cartilage and chondrocytes, depending on its pharmacological and metabolic activities [12,15]. Recently, GS has been reported to suppress the catabolic effects of pro-inflammatory molecules such as IL-1, which increase cartilage destruction in the treatment of OA[6]. There are insufficient knowledge and clinical experience on the effectiveness of intra-articular use of GS.

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Despite its controversial use, meta-analyses of clinical trials suggest that, intra-articular HA can be used in the treatment of OA. Due to this controversial situation, more work is needed and it is thought that future work will illuminate this issue [16]. This study was planned because of the widespread use of intra-articular HA despite the fact that it is controversial in the literature. Histopathologically, the effect of GS and HA on the cartilage in OA rat model were examined.

2. MATERIALS and METHODS

This study was approved by the Institutional Animal Experiments Local Ethics Committee of Düzce University, School of Medicine (number, 2010/05, date 18.02.2010). The study was performed as described in the literature [5]. A total of 45 adult male Wistar Albino rats weighing 220-275 grams and 5-7 months old were used in the study. Rats were divided into 3 groups as 15 rats in each group. The first group was GS group, 2nd group was HA group and the 3rd group was control group. During the experiment, rats were housed in 3-4 rats in each cage, fed with standard laboratory nutrients, without liquid and nutrient restriction.

One rat from the GS group, three rats from the HA group and four rats from the control group died in the experimental stage, also three rats from the control group were excluded from the study because of the obvious infection findings. A total of 34 specimens (14 rats from the GS group, 12 rats from the HA group and 8 rats from the control group) were prepared for histopathological examination.

Anterior Cruciate Ligament Transection Procedure

Animals were sedated before surgery with an intraperitoneal injection of ketamine (75 mg/kg) and metomidine (0.5mg/kg). Subcutaneous buprenorphine (0.03 mg/kg) was given immediately after surgery and twice daily for 3 days to provide postoperative analgesia.

The rats were placed in supine position and draped in a sterile manner. A longitudinal incision was made in the knees of the rats starting from the upper part of the patella and extending to the tubercle of the tibia. Arthrotomy was then performed with medial parapatellar approach. The anterior cruciate ligament was macroscopically tilted to the patella laterally and excised with the aim of creating an experimental OA model [5] (Figure 1A). Complete anterior cruciate ligament (ACL) transection was confirmed by manual test for anterior laxity of the joint. The peripatellar capsular incision was then closed using 4-0 Vicryl sutures (Ethicon, Somerville, New Jersey). After the excision, the front drawer test was performed and the operation was verified.

After the surgery, no fixation was applied to the knees of the rats and free circulation was allowed. Two weeks after the surgery, intra-articular injections were performed once a week for 3 weeks, with one-week intervals to each group. In group 1 (GS group) 12 mg / 0.06 cc GS (Dona ampul®, Abdi İbrahim, Istanbul, Turkey) was injected intra-articularly with an insulin injector. In the second group (HA group), 50µg / 0.05cc HA (Adant®, Er-Kim drug) and in the third group (control group), 50µl / 0.05cc 0.9% sodium chloride (NaCl) were applied. Rats were sacrificed by high dose ketamine (Ketalar®, Pfizer) and specimens were evaluated histopathologically after 8 weeks of surgery and 4 weeks after injection. Arthrotomy was performed on the lower right extremities of the rats. Distal femur and tuberosity were obtained by osteotomy from the tibial side (Figure 2).

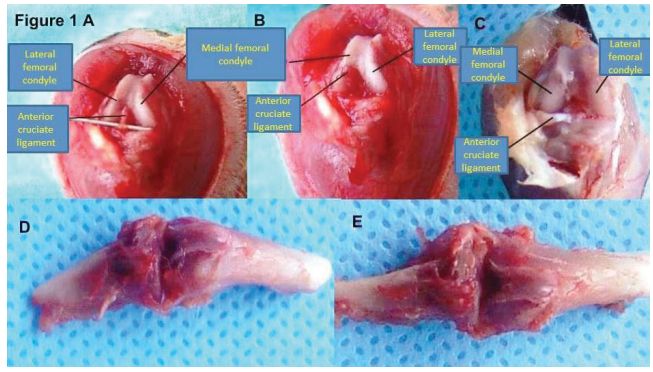

Figure 1. A: Arthrotomy performed rat knee joint, **B:** Rat knee joint with anterior cross ligation, **C:** Macroscopic image of the specimen obtained from the GS group eight weeks after the surgery. **D:** Macroscopic appearance of the specimen obtained from the HA group eight weeks after the surgery. **E:** Macroscopic appearance of the specimen obtained from the control group eight weeks after the surgery.

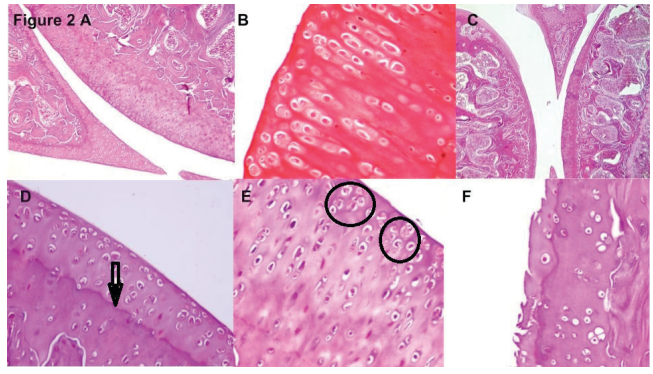

Figure 2. A: Normal articular surface, stable knee (H&E x40), **B:** Loss of total staining in cartilage matrix with safranin O dye in the control group. (Safranin-O x400), **C:** Normal articular surface in the GS group (H&E x100), **D:** Tidemark intact in the GS group. The continuity of tidemark is indicated by an arrow. (H&E x100), **E:** Cell cloning in the HA group is shown by circles (H&E x400), **F:** Tidemark intact in HA group (H&E x100).

Intra-articular Injections

Before we performed the intra-articular injections, anesthesia was induced in a chamber with 5% isoflurane. Once anesthetized, the animal was placed in supine position with its nose set in a cone and dosed with 2% isoflurane to maintain anesthesia. The knee was shaved and prepared to provide a sterile field. Intra-articular injections were then performed through the patellar tendon with the knee in flexion using a 3/10 mL, 29-gauge

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pediatric insulin syringe. Intra-articular placement of the injection was confirmed by the lack of resistance to flow with injection and by feeling the distention of the knee joint capsule, medial to the patellar tendon.

Histopathological Evaluation

Samples were randomly numbered and sent to pathology. Materials were fixed in 10% formaldehyde for one week. The tissues were decalcified for 5 days (Shandon TBD-2) following fixation. After decalcification treatment, tissues were divided into two sections, which were perpendicular to the joint space from the joint of the medial condyle and blocked. Samples were labeled according to the number, sent to the laboratory without knowing which group they belonged to. These samples were washed in the stream water for 3 hours to remove the decalcification solution and the tissues were fixed for 2 more days in formaldehyde. Later, tissues processed for 13 hours in an automatic tissue processor (Shandon Excelsior ES, Thermo Scientific, Runcorn, England). In this procedure, formaldehyde 2 times for 30 minutes, alcohol 6 times for 60 minutes, xylene 3 times for 60 minutes, and once, 60 minutes and twice, 80 minutes paraffin were applied to the tissues. Hematoxylin & eosin (H&E) and safranin-O staining were performed by taking 2 micron thick sections from the paraffin-embedded tissues. The outcome measurement was a blind assessment without knowing the groups. The sections were evaluated under a light microscope (Olympus Bx-50, Olympus Optical). The findings were evaluated according to the Mankin Scale [10]. According to the Mankin Scale, preparations were evaluated in terms of cartilage structure, cellular abnormality, matrix staining and tidemark integrity (Table I).

Table I. Mankin Scale [10]

I. Structure	
a. Normal	0
b. Surface irregularities	1
c. Pannus and surface irregularities	2
d. Clefts to transitional zone	3
e. Clefts to radial zone	4
f. Clefts to calcified zone	5
g. Complete disorganisation	6
II. Cells	
a. Normal	0
b. Diffuse hypercellularity	1
c. Cloning	2
d. Hypocellularity	3
III. Safranin-O staining	
a. Normal	0
b. Slight reduction	1
c. Moderate reduction	2
d. Severe reduction	3
e. No dye noted	4
IV. Tidemark integrity	
a. Intact	0
b. Destructed	1

In the structural evaluation, surface irregularities, pannus formation, clefts to transitional zone, clefts to radial zone, clefts to calcified zone, complete disorganization parameters were evaluated and scored. (Table I). In the cellular evaluation, diffuse hypercellularity, cloning, and hypocellularity parameters were evaluated. In the safranin-O dye, it was evaluated whether there was a decrease in matrix staining with safranin-O. In the tidemark integrity evaluation, it was evaluated whether there was a tidemark destructed or not.

It was defined as surface irregularities when there was mild tissue loss on the joint surface (Figure 3B). Grading was performed according to the depth of the existing cleft (cleft reaching transitional zone, radial zone, and the calcified zone) (Figure 3A). Complete loss of the cartilage layer was evaluated as complete disorganization (Figure 3E). When evaluating cellularity, the highest (bad) score was given to decrease cellularity. Group formation of chondrocytes in the cartilage layer was evaluated as cloning (Figure 2E).

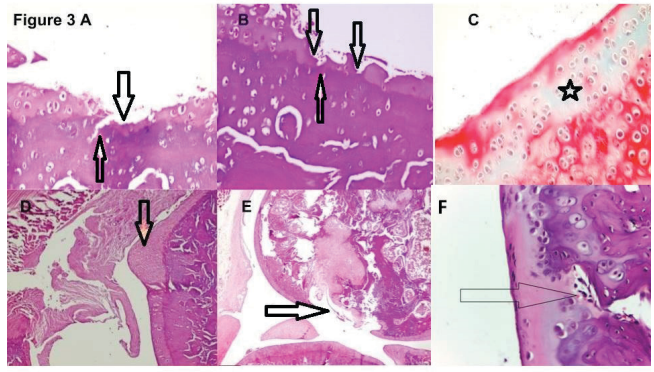

Figure 3. A; Surface irregularities (arrow pointing downwards), Cleft extending the calcified zone (arrow pointing upwards) in HA group (H&E x200), B: Loss of superficial cartilage layer in HA group is indicated by an arrow (H&E x200), C: Positive matrix stainin in GS group with safranin O indicated by a star (Safranin-O x400), D: Loss of the cartilage layer on the surface and pannus formation indicated by an arrow (H&E x100), E: Complete surface cartilage layer loss in the control group (H&E x40), F: Tidemark destruction in the control group (H&E x100).

Statistical analysis

Histological evaluation data were recorded and statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, Illinois). The distribution of continuous variables was evaluated by the one-sample Kolmogorov-Smirnov test. Kruskal-Wallis test and Mann-Whitney-U test were used in the intergroup comparisons because the distribution of the relevant variables was not normal. A p value of less than 0.05 was considered statistically significant.

3. RESULTS

Total Mankin Scale scores were 2.64±2.56, 3.58±3.9, 8.12±2.80 for GS group, HA group and control group respectively.

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The values of the total Mankin Scale, cartilage structure, cellular abnormality, matrix staining and tidemark integrity of the GS and HA groups were lower than values of the control group. These differences were statistically significant.

The values of the total Mankin Scale, cartilage structure, cellular abnormality, matrix staining and tidemark integrity of the GS group were lower than the values of the HA group but this difference was not statistically significant.

The mean results, minimum and maximum values of the histological examinations of the groups according to the Mankin Scale are given in table II.

During the histopathological examinations, the photographs of the preparations were taken (Figure 1-3).

Table II. The mean results, minimum and maximum values of the histological examinations of the groups according to the Mankin Scale.

	Structure of cartilage	Cellular abnormality	Matrix painting	Tidemark integrity	Total Mankin Scale	p
GS group	1.71±1.44 (0-5)	0.36±0.50 (0-1)	0.36±0.63 (0-2)	0.21±0.43 (0-1)	2.64±2.56	p>0.05 (GS,HA)
HA group	1.83±2.04 (0-6)	0.66±0.98 (0-3)	0.75±0.97 (0-3)	0.25±0.45 (0-1)	3.58±3.94	p<0.01 (GS Control, HA control)
Control group	3.5±1.60 (2-6)	1.88±0.64 (1-3)	1.88±0.64 (1-3)	0.88±0.35 (0-1)	8.12±2.80	

GS: Glucosamine sulphate, HA: Hyaluronic acid

4. DISCUSSION

In this study, the chondroprotective efficacy of GS and HA was compared in an experimental osteoarthritis model. The results for GS and HA groups were better than the control group. The values of the total Mankin Scale, cartilage structure, cellular abnormality, matrix staining and tidemark integrity of the GS group were lower than the values of the HA group.

Although, the histopathological effects of the GS application when compared with the HA application were quantitatively different in favor of GS, the chondroprotective effect was not statistically significant. According to this result, intra-articular use of GS was not more effective than HA, but, we can suggest that GS might also be effectively used like HA for OA.

Many conservative and surgical approaches to prevent or slow down the OA process, or to treat the osteoarthritic joint, are currently underway [17]. Research is still underway for the ideal procedure. Recently, disease-modifying agents have been emphasized [4,7,8]. Several studies have been conducted on HA and have been reported in many studies that have modified OA. In the literature, HA is routinely used in the treatment of joint OA. There are very few studies in the literature comparing the effects of intra-articular administration of GS and HA [4,8,18]. In some studies, it has been shown that GS use is more beneficial than HA use [4,7,8].

Özkan et al. have compared the chondroprotective efficacy of N-acetyl GS and HA in the treatment of early-stage OA [4]. In this study, 32 New Zealand rabbits were used and OA was formed by cutting the anterior cruciate ligaments. The animals were divided into 4 groups and 2 weeks after the operation, to the first group intra-articular N-acetyl GS, to the second group intra-articular HA, to the third group intra-articular HA and intra-muscular glucosamine and to the fourth group (control group) isotonic solution were given. Eight weeks later the rabbits were sacrificed. Macroscopically, except the control group, the other groups maintained the cartilage surface, microscopically, in groups one,two and three chondroprotective effect was demonstrated but there was no significant difference between these groups in terms of chondroprotective efficacy. In our study, GS was found to be more effective than HA in terms of chondroprotective efficacy but these results were not statistically significant.

In one study, the clinical use of GS was shown to be more beneficial than nonsteroidal anti-inflammatory agent (NSAID) use [9]. In this study, patients were compared with non-steroidal anti-inflammatory drugs for the symptomatic effect of glucosamine in 200 patients with OA. Patients were divided into two groups. Group 1 was given daily oral 1500 mg GS and the another group was given 1200 mg ibuprofen. Similar positive results were achieved (GS 48%, ibuprofen 52%). Side effects were 35% in the ibuprofen group and 6% in the GS group.

The use of HA injections is controversial but may be considered in selected patients including those with less advanced knee OA, refractory to other nonoperative treatment options and poor surgical candidate patients < 65 years old [19].

There are different recommendations on the use of HA injections from various professional organizations. American Academy of Orthopaedic Surgeons (AAOS) cannot recommend the use of intra-articular HA for patients with symptomatic OA of the knee, based on lack of efficacy with a low likelihood of achieving clinically important benefits [20,21].

Osteoarthritis Research Society International (OARSI) states use of intra-articular HA injections is of uncertain appropriateness in patients with knee-only OA with or without relevant comorbidities and is not appropriate in patients with multi-joint OA with or without relevant comorbidities [22].

According to some clinical researches, the intra-articular HA injection may modestly improve function at 6 months in adults ≥ 65 years old with knee OA. Comparing HA injection to placebo injection, HA is associated with small but significantly improved function at 6-month follow-up in the analysis of 10 trials [23].

Bannuru et al., compared intra-articular administration of HA to intra-articular placebo. At 3 months, intra-articular HA was associated with significant improvement in pain, function, and stiffness [16].

In addition, there are some researches that intra-articular HA injection may increase the risk of serious adverse events in adults with knee OA [24]. In another study, it was determined that these side effects were not very important [23].

Studies in the literature have generally achieved better results in groups using HA and GS molecules than in control groups although there are few reports showing opposite results [4,8]. GS has been used in different medical studies and the results have been reported to be most effective in these studies. Although, there are many studies in the literature on oral GS application, there are limited studies on the intra-articular application [4,8]. In our study, it was demonstrated that GS has positive effects on the experimental OA model in terms of histopathologic control and chondroprotective compared to HA groups. In our opinion, GS can be used intra-articularly, but this should be supported by more studies.

Conclusion

Although, the literature appears to support the use of intra-articular HA injections for the treatment of knee OA, according to our results GS can also be used intra-articularly.

In the future, the intra-articular use of GS may become an effective method of treatment for knee joint injuries and OA and studies will continue to be helpful to determine the most appropriate utilization in clinical practice.

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The prospective evaluation of malnutrition in hospitalized children in a pediatric urology unit

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ABSTRACT

Objective: Malnutrition has been reported to be a rather common health problem in hospitalized children and it is recommended to be evaluated in these conditions. The objective of this prospective trial was to evaluate the nutritional status of hospitalized pediatric urology patients and find the prevalence of malnutrition in this specific subgroup of patients.

Patients and Methods: All subsequent children between 1 and 18 years old who were hospitalized between January 2018 and January 2019 in the pediatric urology unit of a referral university hospital were included into the study. STRONGkids questionnaire was used to assess the nutritional status of the children. Malnutrition was evaluated according to anthropometric measurements based on the computer programs developed by the World Health Organization (WHO).

Results: A total of 76 patients were included in the study. The mean age of the patients was 8.84±4.23; mean height was 121.03±22.49 cm; mean weight was 27.8±13.41 kg. Malnutrition risk, according to STRONGkids screening tool was high in 1 patient, medium in 8 patients and low in 67 patients. According to anthropometric measurements, 6 patients (7.9%) and 25 patients (32.9%) had acute and chronic malnutrition, respectively.

Conclusion: The current prospective trial suggested that malnutrition is a significant issue also for hospitalized pediatric urology patients.

Keywords: Malnutrition, Pediatric Urology, STRONGkids, Hospital

1. INTRODUCTION

Malnutrition is a serious condition and it accounts almost half of the deaths under 5 years of age [1]. Therefore, malnutrition requires an appropriate assessment particularly in pediatric population. By definition, malnutrition denotes development of clinical problem in tissues, body shape, and functions related with inadequate intake of energy supplying foods (carbohydrates and fats), protein, and micronutrients (vitamins and minerals) [1,2]. It also adversely affects immune system and the patient may become prone to infections [2]. Consequently, infections enhance the degree of malnutrition. Particularly, malnutrition has been defined as a significant issue for hospitalized children according to a variety of different studies [3-5]. The prevalence of malnutrition for hospitalized children was observed in 6.1% to 40.9% in a multi-centric trial including different countries in 2008 [5]. In this study, malnutrition had an incidence between 6 to 14% in hospitalized children even in well-known developed countries. Two university hospitals from Turkey were included in this report. Highest malnutrition rate (ranked 1st) was reported to be 31.8% for a hospital in Izmir [6]. Second observation presented a rate of 52.4% as acute malnutrition among 585 children hospitalized at a university hospital in Istanbul [7]. A recent study reported that the prevalence of malnutrition was 13.4% in a pediatric surgery unit in Istanbul [8]. All these figures suggest that hospitalized children should be evaluated for malnutrition. Different pediatric units may have different malnutrition rates that the researchers should bear in mind. Establishment of these differences in malnutrition rates in various subgroups of pediatric patients carries clinical significance. It should be considered that an appropriate nutrition treatment is required for malnutrition particularly in hospitalized children [9]. Furthermore, hospitals should establish nutritional care teams to assess malnutrition in hospitalized children, and these teams should also provide a particular management protocol on malnourished children.

There is no particular data about the prevalence of malnutrition specific to pediatric urology in-patient clinics. These units mostly deal with vesicoureteral reflux (VUR), urinary tract infection (UTI) and urinary system stone disease in hospitalized patients. Penile or inguinal surgeries constitute the majority of remaining patient population. Therefore, determination of

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malnutrition prevalence in this specific subgroup of pediatric patients would be valuable for clinical practice.

There are different validated forms to evaluate malnutrition for pediatric hospitalized patients. Among them Screening Tool for Risk of Impaired Nutritional Status and Growth (STRONGkids) is the most widely used one [10]. This questionnaire was developed to screen malnutrition for hospitalized pediatric patients between 1 month to 18 years of age.

The objective of this prospective trial was to evaluate the prevalence of malnutrition in a pediatric urology unit of a referral university hospital.

2. PATIENTS and METHODS

This prospective trial was approved by the local ethical committee (Approval number 09.2018.402). All pediatric patients between 1 month to 18 years of age were included. The malnutrition assessment was provided by a single physician at the day of hospital admission. Demographic properties, medical history, primary diagnosis, and routine laboratory tests of all cases were recorded. The patients with diabetes mellitus, previous gastrointestinal surgery, celiac disease, neurological deficit and any pediatric malignancy were classified separately.

Weight and height were recorded as anthropometric measurements to evaluate malnutrition by the same physician. The World Health Organization (WHO) Anthro and AnthroPlus Programs were used for the assessment of these results [11,12]. For acute malnutrition (severe or moderate), "Weight-for-Height (WFH) SD (standard deviation) score" or "Body Mass Index (BMI) SD score" less than – 2 was utilized [5]. Chronic malnutrition was defined as "Height-for-Age (HFA) SD score" less than – 2 [5]. Moreover, to calculate SD different "z-scores" (WFH, BMI, HFA) were calculated using the WHO programs.

Validated STRONGkids questionnaire was applied to evaluate malnutrition risk. Similarly, all assessments were performed by the single physician without knowing the clinical diagnosis.

Statistical Analyses

Statistical analyses were performed using SPSS Software (IBM SPSS Statistics version 20.0, SPSS Inc. Chicago, IL). Categorical variables were summarized as percentages and were compared using either Fisher's exact or Chi-Square tests. A $p<0.05$ was considered statistically significant.

3. RESULTS

A total of 76 patients (36 girls, 40 boys) were included. Five patients with known concomitant diseases associated with malnutrition were excluded from the study. These diseases were type 1 diabetes mellitus in one patient, celiac disease in one patient, cerebral palsy in two patients, and recent intestinal surgey in one patient. The mean age for girls was 9.23±4.25 years, and was 8.49±4.24 years for boys. Hospitalization reasons were urinary system stone disease in 34.2% (n:26) of the cases. The remaining indications were anti-reflux surgery for vesicoureteral reflux (VUR) in 22.4% (n:17), urinary tract infection (UTI) due to neurogenic bladder in 14.5% (n:11), UTI associated with VUR in 18.4% (n:14), penile-inguinal or scrotal surgeries in 10.5% (n:8) (Table I). The assessments were performed at the day of hospital admission. The mean duration of hospitalization was 4±1.1 days.

Table I. Demographic data and hospitalization indications

	Hospitalized pediatric urology patients (n=76)
Age (Mean± SD) year	8.84 ± 4.23
Sex (Male/Female, n,%)	40/36, 52.6% /47.4%
Urinary stone surgery (n,%)	26 (34.2%)
Anti-reflux surgery (n,%)	17 (22.4%)
UTI with neurogenic bladder (n,%)	11 (14.5%)
UTI with VUR (n,%)	14 (18.4%)
Inguinoscrotal and penile surgery (n,%)	8 (10.5%)

(VUR: Vesicoureteral reflux; UTI: Urinary tract infection)

Anthropometric measurements revealed acute malnutrition in 6 cases (6/76; 7.9%), and chronic malnutrition in 25 patient (25/76; 32.9%) (Tables II and III). The overall clinical diagnosis of the children regarding acute and chronic malnutrition was urinary stone disease in 2.6% and 7.9% of the cases, respectively. Hospitalization indication was VUR with acute and chronic malnutrition in 2% and 10.5% of the patients, respectively (Table III). Remaining hospitalization indications and malnutrition rates were shown in table III. The number of patients with penile and inguinoscrotal surgeries was low. On the other hand, the rate of acute and chronic malnutrition varied with respect to the hospitalization indications (Table IV). The rate of acute malnutrition was 11.8% while chronic malnutrition was 47% in children with VUR surgery. Similarly, 9% and 45.5% of children with antibiotic treatment for UTI due to the neurogenic bladder dysfunction had acute malnutrition and chronic malnutrition, respectively. The relationship between the hospitalization indication and malnutrition was shown in Table IV. However, no statistically significant difference in acute malnutrition regarding the clinical diagnosis was observed (p=0.990). Similarly, the incidence of chronic malnutrition was not significant with respect to the hospitalization indication (p=0.432).

Table II. Anthropometric measurements

Measurement	Hospitalized pediatric urology patients (n=76) Mean (±SD)
Weight (kg)	27.8 ± 13.41
Height (cm)	121.03 ± 22.49
Body mass index (kg/m2)	17.89 ± 3.49
Weight-for-Age z score	-0.67 ± 1.11
Height-for-Age z score	-1.38 ± 1.22
Body Mass Index for Age z score	0.15 ± 1.27

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Table III. The distribution of malnutrition prevalence based on anthropometric measurements according to hospitalization indications

Hospitalization indications (n=76)	Acute Malnutrition		Chronic Malnutrition	
	+ (n, %)	- (n, %)	+ (n, %)	- (n, %)
Urinary stone surgery (n,%)	2 (2.6%)	24 (31.6%)	6 (7.9%)	20 (26.3%)
Anti-reflux surgery (n,%)	2 (2.6%)	15 (19.7%)	8 (10.5%)	9 (11.8%)
UTI with neurogenic bladder (n,%)	1 (1.3%)	10 (13.2%)	5 (6.6%)	6 (7.9%)
UTI with VUR (n,%)	1 (1.3%)	13 (17.1%)	4 (5.3%)	10 (13.2%)
Inguinoscrotal and penile surgery (n,%)	0	8 (10.5%)	2 (2.6%)	6 (7.9%)
Total	6/76 (7.9%)	70/76 (92.1%)	25/76 (32.9%)	51/76 (67.1%)

(VUR: Vesicoureteral reflux; UTI: Urinary tract infection)

Tablo IV. The respective prevalence of malnutrition according to hospitalization indications

Hospitalization indications (n=76)	Acute Malnutrition		Chronic Malnutrition	
	+ (n, %)	- (n, %)	+ (n, %)	- (n, %)
Urinary stone surgery (n:26)	2/26 (7.7%)	24/26 (92.3%)	6/26 (23.1%)	20/26 (76.9%)
Anti-reflux surgery (n:17)	2/17 (11.8%)	15/17 (88.2%)	8/17 (47%)	9/17 (53%)
UTI with neurogenic bladder (n:11)	1/11 (9%)	10/11 (91%)	5/11 (45.5%)	6/11 (54.5%)
UTI with VUR (n:14)	1/14 (7.1%)	13/14 (92.9%)	4/14 (28.6%)	10/14 (71.4%)
Inguinoscrotal and penile surgery (n:8)	0/8 (0%)	8/8 (100%)	2/8 (25%)	6/8 (75%)
Total	6/76	70/76	25/76	51/76

(VUR: Vesicoureteral reflux; UTI: Urinary tract infection)

The overall mean STRONGkids score was 0.22±0.72 in boys and 0.16±0.69 in girls. The mean STRONGkids scores were 0.23±0.6 for urinary stone disease surgery, 0.35±0.86 for VUR surgery, 0.36±1.22 for UTI due to the neurogenic bladder, 0.07±0.26 for UTI associated with VUR, and 0 for penile-inguinoscrotal surgeries (Table V). The risk assessment for malnutrition based on STRONGkids score revealed low risk in 67 patients, medium risk in 8 patients, and high risk in only 1 patient.

The incidence of malnutrition according to anthropometric measurements of STRONGkids questionnaire was shown in table VI. Six patients with acute malnutrition belonged to the low risk group based on STRONGkids score. Only 2 cases out of 25 chronic malnutrition patients were in the medium risk group according to the STRONGkids assessment. There was no correlation between the STRONGkids risk groups and anthropometric measurements.

Table V. STRONGkids risk assessments

Hospitalization indications (n=76)	Risk Groups			Acute Malnutrition	Chronic Malnutrition
	Low (0)	Moderate (1-3)	High (4-5)	BMI z score (Mean ± SD)	HFA z score (Mean ± SD)
Urinary stone surgery	22 (28.9%)	4 (5,3%)	0	0.14 ± 1.16	-1.08 ± 1.24
Anti-reflux surgery	14 (18.4%)	3 (3,9%)	0	-0.2 ± 1.46	-1.76 ± 1
UTI with neurogenic bladder	10 (13.2%)	0	1 (%1,3)	0.15 ± 1.01	-2.01 ± 1.12
UTI with VUR	13 (17.1%)	1 (1.3%)	0	0.32 ± 1.44	-1.3 ± 1.17
Inguinoscrotal and penile surgery	8 (10.5%)	0	0	0.62 ± 1.32	-0.79 ± 1.51

(VUR: Vesicoureteral reflux; UTI: Urinary tract infection; BMI: Body Mass Index; SD: Standard Deviation; HFA: Height for age)

Table VI. STRONGkids risk groups according to malnutrition based on anthropometric measurements

		STRONGkids Risk Groups		
		Low (0)	Moderate (1-3)	High (4-5)
Acute Malnutrition	+	6 (7.9%)	0	0
	-	61 (80.3%)	8 (10.5%)	1 (1.3%)
Chronic Malnutrition	+	23 (30.3%)	2 (2.6%)	0
	-	44 (57.9%)	6 (7.9%)	1 (1.3%)

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4. DISCUSSION

Malnutrition is a major public health problem with significant consequences [1]. It constitutes a remarkable risk factor for morbidity and mortality especially in children [13]. Malnutrition is also associated with several adverse events including alteration in host immune system, increased infection rate, defective tissue healing process, change in gastrointestinal tract functions, prolonged intubation and longer hospital stays [14]. Moreover, malnutrition causes muscle loss and even depression [15]. All these facts prove that malnutrition is a significant issue for health budget for governments. Besides, presence of malnutrition even in adult patients increases overall mortality rates in one year period [16]. Therefore, a relatively higher incidence of malnutrition in hospitalized children should be regarded as a frightening issue in clinical practice. A wide range of malnutrition incidence from 5% to almost 50% has been reported depending on the level of development in various countries [5]. For instance, a rate of 24.1% for malnutrition was detected in 500 hospitalized children [17]. In this study, some patient groups were defined as high risk for malnutrition. Malnutrition was seen up to 40% in mentally retarded patients. The occurrence of malnutrition was 34.5% in children with any infection and 33.3% in cases with cystic fibrosis [17]. Consequently, this study alone suggests that malnutrition prevalence varies among both different patient units and different diseases. There has been no specific report on malnutrition for pediatric urology units in the literature. Therefore, this prospective study would provide new data about malnutrition rates in this specific subgroup of pediatric patients.

All these observations clearly suggest that the physical examination of hospitalized pediatric patients should include malnutrition assessment. At least a rough nutritional evaluation should not be ignored [18]. On the other hand, a simple and easy method is required to evaluate malnutrition for busy centers. Moreover, this nutritional assessment may be performed by a specially trained nurse. For this purpose, several screening tools have been introduced [17, 19-24]. "Nutrition Risk Score (NRS)", "Pediatric Nutritional Risk Score (PNRS)", "Screening Tool for the Assessment of Malnutrition in Pediatrics (STAMP)", "Subjective Global Nutritional Assessment (SGNA)", "Pediatric Yorkhill Malnutrition Score (PYMS)", "Screening Tool for Risk of Impaired Nutritional Status and Growth (STRONGkids)" were several questionnaires developed for hospitalized children to evaluate nutritional status [24]. Among them STRONGkids is the simple and easy-to-use one requiring no anthropometric measurements. Therefore, STRONGkids is widely utilized and validated into several languages [18, 23 – 25]. In this study we then preferred STRONGkids because of these advantages. This modality was also used as a national nutritional screening modality. The Dutch national survey including 44 hospitals and 424 patients used STRONGkids [25]. In this screening study, anthropometric measurements revealed 19% of malnutrition, while STRONGkids scores proposed 62% of moderate to severe malnutrition. They also suggested a higher risk with longer hospital stays. In this study, they also defined high, moderate and low risk groups based on STRONGkids scores as 4-5, 1-3, and 0, respectively. The authors suggested a dietitian consultation for malnutrition, and nutritional management program in high risk group. For moderate risk group, a close follow-up of twice a week with weight measurement and dietary assessment plus weekly nutritional evaluation were advised. Even weekly follow-up was advised for low risk patients. In this study, 1.3% of the patients had high and 10.4% of the cases had moderate risk according to the STRONGkids scores. However, since relatively low number of patients was present in subgroups no specific risk assessment was available regarding the hospitalization indications. Similarly, no correlation between the risk groups and definitive malnutrition rates based on anthropometric measurements has been defined due to again relatively few patients in each subgroup.

In a recent trial including approximately 500 patients from the pediatric surgery unit of a university hospital, 35.7% of the cases were moderate to severe risk group based on STRONGkids scores [8]. The prevalence of malnutrition was reported to be 13.4% based on anthropometric measurements. They observed a higher malnutrition rate in children less than 60 months as 16.6% vs 10%. Overall 76% of the patients underwent surgery. Our study demonstrated 7.9% of acute malnutrition, and 32.9% of chronic malnutrition in the pediatric urology unit. This suggested that different patient groups would have different malnutrition rates. On the other hand, the mean age of the patients in pediatric surgery clinic was about 5.8 years, while in our study it was 8.8 years. Also 67% of the patients underwent outpatient surgery in the pediatric surgery study. In the current study, majority of the patients were hospitalized for at least 24 hours. About half of the surgeries were for inguinoscrotal or penile diseases in the pediatric surgery unit. This rate was only 10.5% in our study. Therefore, different characteristics of the patients in these two studies yielded different malnutrition rates. Current trial also proposed that patients with VUR and UTI associated with neurogenic bladder had higher malnutrition rates. Almost half the patients belonged to these subgroups had chronic malnutrition.

A new multi-centric trial from Turkey including 37 hospitals and 1513 patients investigated a malnutrition screening program [26]. They used STRONGkids and "Pediatric Yorkhill Malnutrition Score (PYMS)" questionnaires for screening. Anthropometric measurements were also recorded. At hospital admission, 9.5% and 11.2% of malnutrition was detected based on BMI SD scores less than – 2 and WFH SD less than – 2, respectively. STRONGkids revealed moderate risk in 747 patients and high risk in 54 cases. Presence of a chronic disorder increased the risk. A chronic disease was present in about 90% of the 54 children with high risk. However, PYMS proposed a larger high risk group as 365 patients. They concluded that the correlation of STRONGkids and PYMS results with anthropometric measurements greatly varied depending on age groups. They calculated different specificity and sensitivity values for malnutrition screening tools for different subgroups. Therefore, they suggest that every pediatric patient should have a comprehensive evaluation for malnutrition including anthropometric measurements and a screening tool. In current trial there was no correlation between

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anthropometric measurements and STRONGkids risk groups due to low number of subgroup patients. Nevertheless, we also detected chronic malnutrition in 1/3 of the cases. Moreover, some disorders such as VUR constitute a risk factor for malnutrition based on anthropometric measurements. Therefore, we also strongly advised a malnutrition evaluation that must include anthropometric measurements besides a screening tool. On the other hand, it was shown that there is a probability of significant discrepancy between the WHO and Turkish specific growth charts [27]. This problem may cause a major defect for the evaluation of malnutrition. Therefore, prospective trials are required to develop country specific growth charts and to detect malnutrition rates in different patient subgroups. This current study presented a relatively high rate of malnutrition for hospitalized pediatric urology patients. Screening questionnaires alone may be insufficient to investigate malnutrition for these patients. Therefore, anthropometric measurements should not be ignored in particularly certain patient subgroups such as VUR.

In conclusion, the current trial demonstrated that appropriate research should be conducted on malnutrition in hospitalized pediatric urology patients. Malnutrition was seen in approximately 30% of children admitted to our pediatric urology clinic. Particularly, children having VUR or UTI with neurogenic bladder had a higher malnutrition prevalence.

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Diagnostic accuracy of noncompacted-to-compacted wall ratio criteria on CMRI for the diagnosis of left ventricular noncompaction

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ABSTRACT

Objectives: To investigate the diagnostic accuracy of the current criterion, noncompacted-to-compacted (NC/C) wall ratio > 2.3 on cardiac magnetic resonance imaging (CMRI) for the diagnosis of left ventricular noncompaction (LVNC).

Materials and Methods: We retrospectively enrolled 37 patients as an LVNC group and a total of 97 participants with ischemic, hypertrophic, and dilated cardiomyopathy and healthy controls as a control group. The NC/C ratio was measured perpendicularly on short-axis cine images for segments 1-16 and four-chamber cine images for the apex during the end-diastole. The sensitivity, specificity, and diagnostic accuracy of NC/C ratio > 2.3 for the diagnosis of LVNC were calculated.

Results: LVNC patients comprised 24 males (64.8%) and 13 females (35.2%) with the mean age of 29.24 ± 11.79 years. The NC/C ratio > 2.3 detected in all but one of the LVNC patients (97.3%). On the other hand, the specificity of NC/C ratio > 2.3 was 79.4% for the diagnosis of the LVNC patients. Using NC/C ratio > 2.66 and > 2.8 yielded 91.9% sensitivity and 97% specificity, and 81% sensitivity and 100% specificity, respectively.

Conclusion: NC/C ratio > 2.3 might lead to overdiagnosis of LVNC. We suggest using higher NC/C cut-off value in individuals without high clinical suspicion of LVNC.

Keywords: Accuracy, Cardiomyopathy, CMR, Cut-off, Noncompaction

1. INTRODUCTION

Left ventricular noncompaction (LVNC) is a type of cardiomyopathy with an estimated prevalence ranging from 0.014% to 0.17, and up to 1.3% [1-3]. LVNC is currently recognized as "unclassified cardiomyopathy" by the European Society of Cardiology while the American Heart Association classified the disease as "distinct cardiomyopathy" [4,5]. Several genetic and environmental factors have been identified as the risk factors for LVNC development; however, the precise mechanisms leading to LVNC remains poorly understood [6-8]. The disease is principally delineated by increased endocardial trabeculations, deep intertrabecular recesses communicating with the left ventricular cavity, and thin compacted myocardium [9]. LVNC manifests with a heterogeneous clinical spectrum ranging from systemic thromboembolism, arrhythmia, and heart failure to asymptomatic course for a lifetime [10].

Echocardiography is a first-line imaging modality in case of LVNC suspicion whereas cardiac magnetic resonance imaging (CMRI) is being increasingly employed for the diagnosis of LVNC given to its higher spatial and contrast-resolution, multiplanar imaging capability, and higher inter-observer variability [9,11,12]. Various diagnostic criteria have been proposed for the diagnosis of LVNC on CMRI. Petersen et al., introduced a practical semi-quantitative criterion, noncompacted-to-compacted (NC/C) myocardium threshold ratio > 2.3 at end-diastole on CMRI for the diagnosis LVNC [12]. Despite it is being extensively used in daily practice owing to its practicality and high inter-observer variability, several authors have raised concerns regarding the reliability of Petersen's criteria [13,14]. Much of the debate has gathered around the specificity of the approach, and several authors claimed that using NC/C > 2.3 might lead to overdiagnosis of LVNC [13,14].

The aim of the study was twofold: (1) to test the diagnostic accuracy of the criterion, NC/C wall threshold ratio > 2.3 on CMRI for the diagnosis of LNVC, and (2) to explore whether a refined cut-off value with higher diagnostic accuracy could be established on CMRI.

2. MATERIALS and METHODS

The Institutional Ethics Committee of Istanbul Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Research and

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Training Hospital approved (registration number 2017-34) this retrospective study conducted between January 2018 and May 2019 and waived the need for informed consent for the investigation and presentation of deanonymized medical data. We retrospectively evaluated our picture and archive communicating system (PACS, ExtremePacs, Ankara/Turkey) to identify patients scheduled for CMRI examination on a provisional diagnosis of LVNC. The diagnosis of LVNC was established according to the recommendation of Jenni et al. [9] on echocardiographic imaging. To enhance the reliability of LVNC diagnosis, we have implemented several additional clinical and imaging criteria, and patients had to meet at least one of these criteria to be included in the final LVNC cohort: (1) having one first-degree relative with a diagnosis of LVNC, (2) having associated neuromuscular disorder, (3) having ventricular tachyarrhythmia detected on 24-hours Holter examination, (4) history of systemic thromboembolic events, and (5) appearance of distinct double layered ventricle composed of NC/C on CMRI [9, 10, 15,16]. We did not use the criterion introduced by Petersen et al. since the primary purpose of the current work was to test the diagnostic value of the criteria [12]. Exclusion criteria for LVNC cohort were: (1) having a diagnosis of coronary diseases, (2) having a diagnosis of other cardiomyopathies, and (3) having congenital heart disease. As control group, potential mimickers such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and ischemic cardiomyopathy and healthy controls without any ischemic, valvular, autoimmune disease or known cardiomyopathy were included in the present work.

CMRI acquisition

All MRI studies were acquired with a 1.5 T scanner (Aera, Siemens Medical Systems, Enlargen/Germany). All CMRI acquisitions were performed using phased-array body coils. All of the sequences were acquired using prospective cardiac gating. Our CMRI protocol in the order of first to latest consisted of breath-hold black-axial blood fast spin-echo (SE), multiple breath-hold long-axis four-chamber, long axis two-chamber, and a 9-12 stack of short axes cine images breath-hold using balanced steady-state free precession imaging (SSFP). The parameters for SSFP cine images were: TR/TE = 3.8/1-3 ms, slice thickness = 5 mm with 5 mm interslice gap, temporal resolution = 35 m.

Image Analysis

The CMRI images of the patients were retrieved from our hospital picture archiving and communicating system (PACS, ExtremePacs system, Ankara/Turkey).

A single radiologist (D.A) with over four years of CMRI interpretation experience assessed all the CMRI images. First, the observer assessed the left ventricular functions by calculating ejection fraction (EF) using modified Simpson's method on short-axis cine images with the software (ARGUS, Siemens, Erlangen/Germany). The observer semi-automatically traced the endocardial and epicardial borders as demonstrated in several previous works [9,11,12]. The left ventricular myocardium divided into 17 segments as six regions at the basal level, six regions at the midventricular level, four regions at apical level, and apex according to the American Heart Association segmentation model for the left ventricle [17].

The NC/C myocardium was perpendicularly measured on short-axis cine images for segments 1-16 and measured on four-chamber cine images for segment 17, as recommended in the previous works [15]. All measurements were performed at end-diastole using digital clippers. The segment, which the maximum ratio was achieved was determined, and the maximum NC/C myocardium ratio was noted for each patient. The maximum ratios of NC/C myocardium were separately calculated for segments 1-16 and segment 17. Figure 1 shows measurement of NC/C ratio in a LVNC patient.

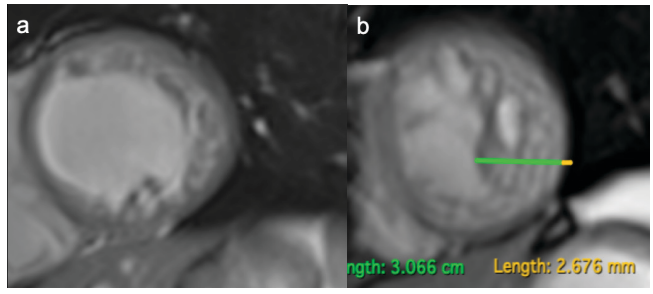

Figure 1. Short-axis cine images of a patient with myocardial noncompaction show deep trabecular recesses, double layer appearance in segment 7,8 (a) and 13,14,15 (b). The maximum NC/C ratio was measured as 11,23 in segment 14 in this patient.

Statistical analyses

Statistical analyses were performed using the SPSS software version 21. The variables were investigated using Kolmogorov-Simirnov test to determine whether or not they were normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed variables and median and interquartile ranges for non-normally distributed variables. The Chi-square test was used to compare proportions of mitral regurgitation, and gender between two groups. Patients were classified using the cut-off value for NC/C ratio > 2.3. LVNC patients with NC/C > 2.3 were accepted as true positive (TP) cases, while LVNC patients with NC/C < 2.3 were accepted as false negative (FN) cases. In control group, participants with NC/C > 2.3 were accepted as false positive (FP), while participants with NC/C < 2.3 were accepted as true negative (TN). The sensitivity was calculated as TP / (TP + FN), the specificity was calculated as TN/ (TN + FP), and diagnostic accuracy was calculated as (TP +TN)/ (TP+TN+FP+FN). The segments with NC/C ratio > 2.3 in LVNC patients were demonstrated using visual graphs according to the American Heart Association segmentation model for the left ventricle. The proportion of segments with NC/C wall ratio > 2.3 in LVNC group and controls were compared using Man-Whitney-U

test. A P value of less than 0.05 was used to infer statistical significance.

3. RESULTS

Consecutive 37 patients with LVNC, 24 males (64.8%) and 13 females (35.2%), with a mean age of 29.24 ± 11.79 years were enrolled in the final study cohort. The control group consisted of 21 patients with ischemic cardiomyopathy, 21 patients with HCM, 38 healthy controls, and 17 patients with dilated cardiomyopathy. The mean age of LVNC patients, healthy controls, and DCM patients was comparable (p>0.05) while the mean age patients with ischemic cardiomyopathy and HCM exceeded the mean age of LVNC patients (p<0.0001). The detailed CMRI findings of each group are listed in table I. Figure 2 shows the NC/C wall ratio of each group using violin plot graphs.

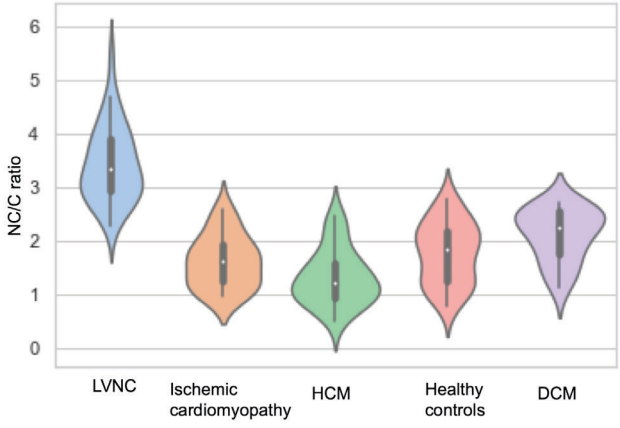

Figure 2. Violin-plot graph shows the NC/C ratio of each group

For the whole study cohort, a total of 56 patients had NC/C wall ratio > 2.3. Among 56 patients, 8 patients had 1 segment (14.3%), 16 had 2 segments (28.6%), 13 had 3 segments (23.2%), 7 had 4 segments (12.5%), and 12 had 5 segments or more (18.4%) with NC/C wall ratio > 2.3. In LVNC patients, 36 patients had NC/C wall ratio > 2.3. In LVNC group, non of the patients had 1 segment with NC/C wall ratio > 2.3, while 5 patients had 2 segments (13.9%), 12 had 3 segments (33.3%), 7 had 4 segments (19.4%), and 12 had 5 segments or more (33.4%) with NC/C wall ratio > 2.3. Notably, in control group, among 20 patients with NC/C wall ratio > 2.3, 8 patients had 1 segment (40%), 11 had 3 segments (55%), and 1 had 3 segments (5%) with NC/C wall ratio > 2.3. No patients in the control group had 4 or more segments with NC/C wall ratio > 2.3. The proportion of the segments with NC/C wall ratio > 2.3 in LVNC group was statistically higher compared with the control group (p<0.0001). Figure 3 shows the segments with NC/C ratio > 2.3 for LVNC patients using a bull-eye graph.

Table I. CMRI findings of LVNC patients and control group and diagnostic metrices of different cut-off values for LVNC

Variables	LVNC (n=37)	Ischemic cardiomyopathy (n=21)	HCM (n=21)	Healthy controls (n=38)	DCM (n=17)
Age (years)	29.24 ± 11.79	57.76 ± 13.17	46.95 ± 17.01	25.13 ± 7.61	33.88 ± 17.12
Gender					
Male	24 (64.8%)	13 (61.9%)	12 (57.1%)	27 (71.1%)	10 (58.8%)
Female	13 (35.2%)	8 (38.1%)	9 (42.9%)	11 (28.9%)	7 (41.2%)
Noncompacted myocardium (mm)	12.59 ± 1.15	9.17 ± 2.22	9.19 ± 8.80	7.54 ± 2.16	10.65 ± 3.1
Compacted myocardium (mm)	3.72 ± 0.73	6.01 ± 1.84	7.33 ± 2.49	6.48 ± 2.26	5.05 ± 1.18
Noncompacted-to-compacted myocardium	3.42 ± 0.69	1.60 ± 0.48	1.32 ± 0.53	1.77 ± 0.57	2.11 ± 0.48
Ejection fraction (%)	48.45 ± 9.69	39.04 ± 6.20	70.09 ± 5.56	62.57 ± 3.25	35.52 ± 8.36
Noncompacted-to-compacted myocardium >2.3					
Yes	36 (97.3%)	3 (14.3%)	2 (9.5%)	6 (15.8%)	9 (52.9%)
No	1 (2.7%)	18 (85.7%)	19 (90.5%)	32 (84.2%)	8 (47.1%)
Noncompacted-to-compacted myocardium >2.66					
Yes	34 (91.9%)	0	0	2 (94.7%)	1 (5.9%)
No	3 (8.1%)	21 (100%)	21 (100%)	36 (94.7%)	16 (94.1%)
Noncompacted-to-compacted myocardium >2.8					
Yes	30 (81.1%)	0	0	0	0
No	7 (18.9%)	21 (100%)	21 (100%)	38 (100%)	1 (100%)

LVNC=Left ventricular noncompaction, HCM=Hypertrophic cardiomyopathy, DCM=Dilated cardiomyopathy

The mean noncompacted myocardium was 12.59 ± 1.15 mm, and the mean compacted myocardium was 3.72 ± 0.73 mm in LVNC group. The mean NC/C ratio was 3.42 ± 0.69 ranging from 2.27 to 5.43 in LVNC patients. We identified no correlation between the NC/C and EF in LVNC. The cut-off threshold value > 2.3 was able to identify all but one LVNC patient (97.3%). In this patient, the NC/C ratio was 2.27, which was close to the cut-off threshold value of > 2.3. Nevertheless, the LVNC diagnosis in this particular patient was verified by other clinical and echocardiographic findings. On the other hand, 20 of 97 (20.6%) patients in the control group had NC/C ratio > 2.3, which resulted in 79.4% specificity for the diagnosis of LVNC. Of these 20 patients, 2 had HCM, 3 had ischemic cardiomyopathy, 6 were healthy controls, and 9 were DCM patients. To identify a cut-off

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threshold value with higher specificity, receiver-operating curve (ROC) was implemented using the NC/C ratio as a test and having LVNC as a state variable (Figure 4). Using the cut-off threshold value of > 2.66 for the ratio for NC/C layer as a semi-quantitative criterion resulted in 91.9% sensitivity and 97% specificity and the cut-off threshold value of > 2.8 resulted in 81% sensitivity and 100% specificity (p>0.05) (Table I).

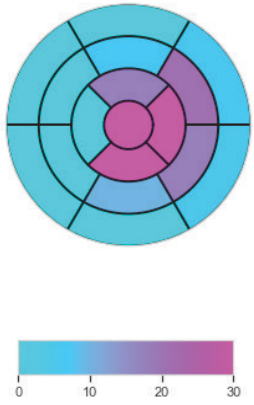

Figure 3. Bull-eye illustration shows the segments with NC/ C ratio > 2.3 in LVNC patients

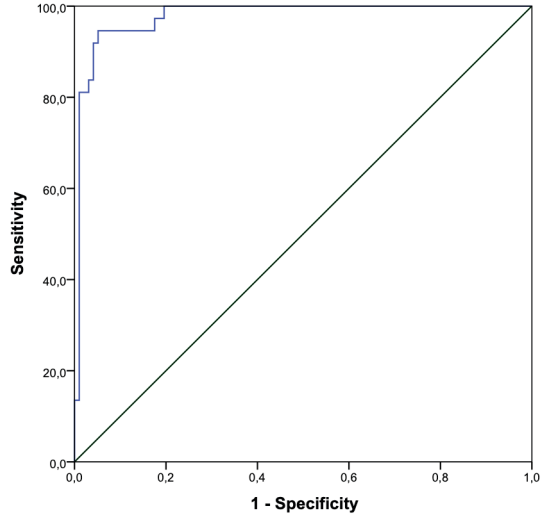

Figure 4. ROC curve analysis showing NC/C ratio > 2.3 in predicting the presence of LVNC in the whole study cohort

Additionally, the control group was further subclassified according to their diagnosis as ischemic heart disease (n=21), HCM (n=21), healthy controls (n=38), and DCM (n=17) to explore the diagnostic performance of the cut-off threshold value > 2.3 in discriminating LVNC patients comparing each subgroup. The subgroup analysis revealed that the cut-off threshold value of > 2.3 had the worst diagnostic accuracy in discriminating LVNC patients from DCM patients (81.4%) while had the best diagnostic accuracy in differing LVNC patients from HCM patients (94.8%). The cut-off threshold value of > 2.3 showed diagnostic accuracy of 90.6% with excellent sensitivity (97.3%) despite relatively lower specificity (84.2%) in discriminating LVNC patients from healthy controls. Table II depicts the detailed diagnostic metrices of the cut-off threshold value of > 2.3 in discriminating LVNC patients from other subgroups.

Table II. The diagnostic performances of cut-off threshold value > 2.3 in discriminating LVNC vs. each study subgroup.

Conditions	Sensitivity	Specificity	NPV	PPV	Diagnostic accuracy
LVNC vs. ischemic heart disease	97.3%	85.7%	94.7%	92.3%	93.1%
LVNC vs. HCM	97.3%	90.5%	95%	94.7%	94.8%
LVNC vs. healthy controls	97.3%	84.2%	97%	85.7%	90.6%
LVNC vs. DCM	97.3%	47.1%	88.9%	80%	81.4%
LVNC vs. Whole study cohort	97.3%	79.4%	98.7%	64.3%	86.5%

LVNC= Left ventricular noncompaction, HCM=Hypertrophic cardiomyopathy, DCM= Dilated cardiomyopathy,
NPV = Negative predictive value, PPV = Positive predictive value

4. DISCUSSION

The findings of the present study indicated that utilizing NC/C wall threshold ratio > 2.3 as recommended by Petersen et al. had excellent diagnostic sensitivity reaching up to 97.3%, yet had rather a low specificity (79.4%), hence, suggesting that it might lead to overdiagnosis. Notably, 6 of 38 healthy controls (15.8%) in the present work had NC/C wall ratio > 2.3. We identified two different cut-off threshold values for NC/C ratio for the diagnosis of LVNC, 2.66 and 2.8, which resulted in 91.9% sensitivity and 97% specificity and 81% sensitivity and 100% specificity, respectively.

In 2005, Petersen et al., introduced a cut-off value for the diagnosis of LVNC, NC/C wall ratio > 2.3 by appraising 177 individuals with and without cardiac disease [12]. The cut-off value yielded excellent specificity (99%) in their work, which was higher than the specificity value of 79.4% in the present work. Notably, we demonstrated higher sensitivity compared with their work (97.2% vs. 86%). The mean NC/C myocardium ratio identified in their study was substantially lower than the present work (3.0 ± 1.5 vs. 3.72 ± 0.73). However, the study by Petersen et al., substantially suffered from the fact that their study cohort only had seven patients with a diagnosis of LVNC [12]. Notwithstanding this limitation, this cut-off value gained popularity in clinical applications while also had been subjected to considerable criticism by many authors. The study by Boban et al., supported the reliability of the initial criteria proposed by Petersen et al., [12, 18] and also suggested measuring noncompacted myocardium blood flow using T2 sequences and geometric eccentricity of the ventricle as supplementary diagnostic markers on CMRI for the diagnosis of LVNC. Boban

et al., assessed the diagnostic value of NC/C threshold ratio > 2.3 in 71 LVNC patients and 129 controls [18]. In their work, the cut-off threshold value revealed equal sensitivity (97.2%) and comparably higher specificity (96.3%) compared with the present study.

Contrarily, the authors of The Multi-Ethnic Study of Atherosclerosis (MESA) found that 140 of 323 healthy individuals (43%) had NC/C wall threshold ratio > 2.3 at least in one segment of the myocardium, and questioned the reliability of the cut-off value [13]. The authors suggested the re-evaluation of the criteria [13]. A further study by the MESA investigators showed that using NC/C threshold ratio >2.3 led to an incorrect diagnosis of LVNC in 706 of 2742 (25.7%) participants without any known cardiac disease [14]. Furthermore, only in the number of negligible patients, noncompacted myocardium headed to changes in left ventricular parameters over ten years [13,14]. Fazio et al., also questioned the over sensitivity of the NC/C wall threshold ratio and proposed to use > 2.5 cut-off threshold value for the diagnosis of LVNC on CMRI [19]. Grofthoff et al., explored the reliability of the cut-off threshold value of 2.3 in their study that consisted of 12 patients with LVNC and 24 controls [15]. The authors demonstrated that NC/C ratio >2.3 had 100% sensitivity while 80% specificity, which was comparable with our findings [15]. The authors demonstrated that using the NC/C threshold ratio >3 yielded 100% sensitivity and 93% specificity [15]. In the present work, the determined cut-off threshold value, NC/C ratio > 2.66, resulted in 91.9% sensitivity and 97% specificity, which had higher specificity despite lower sensitivity than their study.

In line with the aforementioned works, the findings of the present work highlighted that using NC/C wall threshold ratio > 2.3 might lead to overdiagnosis of LVNC in an important amount of patients. The misdiagnosis of LVNC might cause severe and unnecessary burdens for the psychical and mental well-being of the individuals [20,21]. We suggest that NC/C wall threshold ratio > 2.3 should be used only in patients with a high clinical suspicion of LVNC, and in patients without any specific symptoms, echocardiographic or electrocardiographic findings suggesting LVNC, and the clinicians should keep in mind that using NC/C wall threshold ratio > 2.3 for the diagnosis of LVNC might result in overdiagnosis. In such instances, other proposed methods such as measuring trabeculated ventricular mass, fractal analyses, or higher cut-off values, which yielded higher specificity as proposed in the present work might be used [15,22,23]. Furthermore, the number of the segments with NC/C wall threshold ratio > 2.3 in LVNC patients was substantially higher than the control group in the current work. Notably, no patients in control group had 4 or more myocardial segments with NC/C wall ratio > 2.3, and only 1 patient had 3 segments with NC/C wall ratio > 2.3. All other participants in the control group had only 1 or 2 segments with NC/C wall ratio > 2.3. Contrarily, with no LVNC patients had only 1 segment NC/C wall ratio > 2.3 and approximately 86% of LVNC patients meeting the diagnostic CMRI criteria had 3 or more segments with NC/C wall ratio > 2.3. Hence, we suggest that the number of the segments with NC/C wall ratio > 2.3 might be also beneficial in identifying LVNC. Nevertheless, we acknowledged that there is a pressing need to address the issue of overdiagnosis of LVNC by CMRI, and besides criteria relying solely on imaging modalities, diagnostic workflow combined with clinical and imaging data as proposed by several authors might also be beneficial to reduce the number of incorrect diagnoses.

Finally, some important limitations of this present work need to be viewed. First, and foremost, the retrospective nature of the study inherently limits the generalizability of our results. Second, there was a selection bias since the study and control cohort was derived from a tertiary referral center; hence, the cohort in the study might have differed from the general population in several aspects. Third, we had a relatively small number of patients with LVNC given to scarcity of the disease. Nevertheless, our sample size was higher than those of several similar works [12,15,19]. Fourth, we did not assess inter-observer variability of NC/C ratio measurements; however, previous studies demonstrated that this measurement technique had a good inter-observer reliability [16]. Finally, we did not investigate several other quantitative methods proposed for the diagnosis of LVNC [15,22-24].

In conclusion, using NC/C wall threshold ratio > 2.3 for the diagnosis of LVNC had excellent sensitivity, yet rather a low specificity. Our findings indicated that NC/C threshold ratio > 2.3 might lead to overdiagnosis of LVNC in the healthy population. Furthermore, using NC/C threshold ratio > 2.3 might also lead to misdiagnosis of other cardiomyopathies, particularly DCM, as LVNC. Therefore, we suggest that using higher NC/C cut-off threshold values or integrating other measures such as measuring trabeculated ventricular mass, fractal analyses, or taking the number of the segments with NC/C wall ratio >2.3 might be implemented in daily clinical practice to avoid incorrect diagnoses.

Conflict of interest statement: None

Ethical Statement: 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. Institutional Ethics Committee approval was obtained.

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Health beliefs, behaviour and determining factors in breast self – examination among a group of university students

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ABSTRACT

Objectives: Breast cancer is the most frequent malignancy in both Turkey and the World. Breast self-examination (BSE) is the cheapest and the easiest method for early detection. According to Turkey's Ministry of Health, 65.1 percent of women have never performed BSE. Only 10.1 percent of women perform BSE monthly. The purpose of this study was to examine health beliefs, behaviour and determining factors in BSE among a group of university students.

Materials and Methods: This cross-sectional analytical study was carried out with 15,940 female university students in 2016. Sample size was 912. The research data was collected using a self-report questionnaire form and the adapted Turkish version of Champion's Health Belief Model Scale (CHBMS).

Results: Ten point four percent of students (n=91) reported that they performed BSE monthly. Monthly BSE rates were higher in the students who were educated about BSE, who knew BSE timing and technique, who had been performed clinical breast examination and whose confidence subscale of CHBMS was higher.

Conclusion: In conclusion, health education programs should include BSE education in high schools and universities. Also, these education programs should include all women and relate to the confidence subscale of CHBMS.

Keywords: Health behaviour, Breast self-examination, Breast cancer

1. INTRODUCTION

Breast cancer is the most common type of cancer among women in both the World and Turkey. Age standardized frequency of the disease is 43.1/100.000 in the World and 45.9/100.000 in Turkey [1,2].

Early diagnosis of breast cancer improves the efficacy of treatment and extends the life span of the patient. Mammography, breast ultrasonography, clinical breast examination, and breast self examination (BSE) may be performed for early detection of breast cancer. The cheapest and most effortless method is BSE.

Breast self-examination, is the regular, monthly inspection-palpation examination of one's own breast. After twenty years of age every women should perform BSE regularly between 7th – 10th days of their menstrual period every month [3]. Regularly performed BSE is the simplest, most economical method for early detection of breast cancer and can be easily applied at home alone.

According to Turkish Ministry of Health data, 65.1% of women over the age of 18 in Turkey never performed BSE. The prevalence of monthly and regularly performed BSE was 10.1% [4].

The Health Belief Model is one of the psychosocial models that explains the attitudes and beliefs that affect individuals' health behaviours. According to this model: the main factors affecting the health behaviours of individuals are: perceived susceptibility, perceived severity, perceived benefits, perceived barriers, confidence and general health motivation. It can be used to guide health promotion and disease prevention programs.

The purpose of this study is to determine the prevalence of BSE and the health beliefs related to BSE among female university students over 18 years of age and examine the factors affecting the outcome.

2. MATERIALS and METHODS

Type of Research, Sample (Universe) and Sampling Method

The cross-sectional analytical type of work was carried out with 15,940 female university students over the age of 18 during the 2015-2016 academic year.

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We selected the number of female students to be evaluated from the total number of female students attending 17 faculties at our university. The selected female students represented the total number of female students at the 17 faculties, in other words, they represented the total universe. The sample of the research was selected using stratified and simple random sampling methods. A total of 877 students were reached because of lack of attendance to school and not accepting to participate in the survey (Level of access 96.2%).

Data Collection Method

The data was collected through the application of Socio-demographic Data Form prepared by the researchers and the Champion's Health Belief Model Scale (CHBMS) under observation.

Socio-demographic Data Form consisted of 26 questions about socio-demographic characteristics of the students (age, marital status, faculty, maternal age, maternal education, maternal employment status, family economic status, smoking and alcohol intake status) and information related to BSE (health related course attendance, BSE related education, interest for breast cancer / examination, status for having clinical breast examination, mammography and breast ultrasonography, knowing the best time for BSE, the right technique for BSE and the knowledge of risk groups, to whom BSE should be performed, presence of first degree relatives with breast cancer diagnosis, status for performing BSE and frequency).

Champion's Health Belief Model Scale, which is used to determine students' health beliefs about BSE, is a scale developed by Champion and revised in 1999 and adapted to Turkish by Karayurt and Dramalı [5,6]. The scale is a 42-item form consisting of six sub-dimensions (perceived susceptibility, perceived seriousness, perceived benefits, perceived barriers, confidence and general health motivation). The answers given to each question are of 5 likert type ranging from "absolutely disagree" (1 point) to "strongly agree" (5 points) and each of the subscales of the scale is evaluated within itself. Perceived susceptibility subscale consists of 3 questions, perceived seriousness subscale consists of 7 questions, perceived benefits subscale consists of 4 questions, perceived barriers subscale consists of 11 questions, confidence subscale consists of 10 questions and general health motivation subscale consists of 7 questions.

The scores that correspond to answers given to a questions in each subscale were collected. As the scores increased, the level of perception in that subscale increased.

Variables of Research

Dependent variables of the study were scores obtained from the health beliefs subscale of BSE and regular BSE practice every month.

Independent variables of the study included: age, marital status, study in a health related faculty, mother's age, educational level and working status, economic status of the family, smoking and alcohol intake, attending courses on health sciences, education on BSE, interest in breast cancer and BSE, having clinical breast examination, having mammography and breast ultrasonography, knowing the right time for BSE, knowing the right technique for BSE, knowing the risk groups for BSE, having first degree relatives or close relatives with breast cancer diagnosis. Performing BSE at least once a month was considered as regular BSE. Subscales of CHBMS were taken as independent variables while variables related to regular BSE status were examined.

Ethical Permissions

Ethical approval was given by Suleyman Demirel University School of Medicine Clinical Research Ethics Committee (Approval number: 113). Before the start of the study, students were informed about the research and informed consent was obtained, and necessary permissions were obtained from the university in order to carry out the research.

Evaluation of Data

Data was assessed using descriptive statistics (number, percentage, mean, standard deviation), t-test in independent groups, Mann-Whitney U test, Pearson correlation and chi-square tests in the statistical package program (SPSS, Version 17.0). Statistical significance level was accepted as $p < 0.05$. In the analysis of the data, parametric tests were used where the variables provided parametric conditions ($n \geq 30$ and normal distribution was appropriate). The normal distribution of variables was evaluated using Kolmogorov Smirnov test.

Logistic regression analysis was used to identify independent predicators for regular performance of BSE. Variables that affected regular BSE and differed significantly in univariate analyses were age, education in a health-related faculty, mother's age and employment status, smoking status, attending courses on health sciences, education about BSE, interest in breast cancer and BSE, having clinical breast examination, having mammography and breast ultrasonography, knowing the right time for BSE, knowing the right technique for BSE, and knowing the risk groups for BSE. CHBMS subscale scores were entered into a logistic regression model. In logistic regression analysis, it is suggested to examine the correlations between variables to avoid multiple linkage, and to model only one of the variables that correlates with each other at a high level [those with correlation coefficients (r or rho) above 0.80] [7]. Since, there was no independent variable with correlation coefficient over 0.80 in the correlation analysis of the variables to be included in the logistic regression model, all variables listed above were left in the model in our study. Logistic regression analysis was performed by the "enter" method and model fit was assessed by the Hosmer and Lemeshow test. As a high p value was obtained from the Hosmer and Lemeshow test, it was decided that the model was a high predictive model ($X^2=4.020$, Degrees of Freedom (df)=8, $p>0.05$).

3. RESULTS

The descriptive characteristics of the group are presented in Tables I and II. Fifty-six point one percent (n = 492) of the group were under 21 years of age, 97.8% (n = 858) were single and 15.1% (n = 132) were educated in a health related faculty. The average age of the study group was 21.3 ± 2.2 and the mean age of the mothers was 47.0 ± 5.7. Sixty-five point one percent of the mothers were 45 years old or over, 64.9% (n = 569) had primary education and 25.9% were working (n = 227). Among the study group, 97.4% (n = 854) of the families had medium or good economic status. Forty point nine percent of the students (n=359) were smoking and 43.1% (n=378) were alcohol intaker.

The characteristics of the study group regarding breast cancer family history and early diagnosis are shown in Table II. Among individuals 23.7% (n = 208) were taking health related courses and 36.1% (n = 317) were trained in BSE. Seventy one point four percent (n = 626) of the group stated that they were interested in BSE and breast cancer. Clinical breast examination was performed by a physician in 16.0% (n = 140) of individuals, mammography was performed in 3.4% (n = 30) and breast ultrasonography was performed in 9.9% (n = 87) of the individuals. Among the study group; 29.4% (n = 258) knew the most suitable time for BSE, 17.2% (n = 151) knew the technique and 71.8% (n = 630) knew risky groups correctly. There was a first-degree relative in 1.7% of individuals in this study (n = 15) with a diagnosis of breast cancer and on the other hand 21.3% (n = 187) of the participants had a relative or a friend with breast cancer diagnosis.

In the study group, 10.4% (n = 91) of the individuals were regularly performing BSE every month (Figure 1). Students who were over the age of twenty-two, those who were educated in a health-related faculty, those with a mother aged 45 or over, those whose mothers were working and non-smokers were significantly more likely to perform BSE regularly (p<0.01, p<0.001, p<0.01, p<0.05 and p<0. 05, respectively) (Table I).

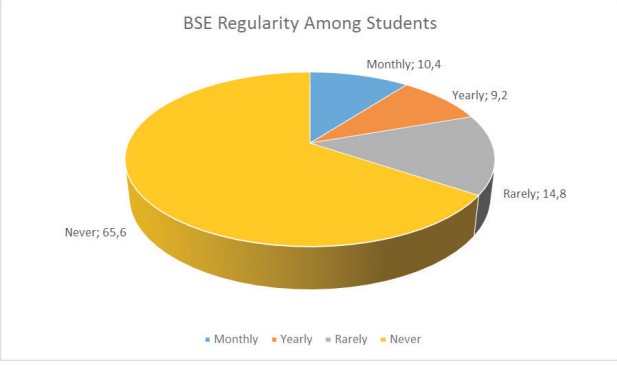

Figure 1. Breast self-examination regularity among students

The prevalence of regular BSE in individuals who attended courses on health sciences, had education on BSE, had interest in breast cancer and BSE, had clinical breast examination, had mammography and breast ultrasonography and those who knew the right time and the right technique for BSE, knew the risk groups for BSE was significantly higher when compared with the findings of others (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001 and p<0.001, respectively) (Table II).

Champion's Health Belief Model Scale point averages for the subscales (±Standard Deviation) in our study were found as follows: 8.1±2.7 for the perceived susceptibility subscale (lowest and highest possible scores 3-15), 22.7±5.7 for perceived seriousness subscale (lowest and highest possible scores 7-35), 15.3±3.7 for perceived benefits subscale (lowest and highest possible scores 4-20), 28.1±7.6 for perceived barriers subscale (lowest and highest possible scores 11-55), 31.1±8.8 for confidence subscale (lowest and highest possible scores 10-50) and 25.1±5.4 for general health motivation subscale (lowest and highest possible scores 7-35). Correlations between CHBMS subscale scores for our study group are presented in Table III.

In individuals who performed BSE regularly: perceived benefits, confidence and general health motivation subscale scores were significantly higher than the others and on the other hand, perceived barriers subscale score was significantly low (p<0.001, p<0.001, p<0.001 and p<0.001, respectively) (Table IV).

Perceived susceptibility subscale scores of students whose mothers aged 45 or over and economic condition was bad, who were smokers and alcohol intakers, who had BSE education before, who were interested in BSE and breast cancer, who knew the correct time for BSE and who had first degree relatives or close relatives with breast cancer diagnosis, were significantly higher than the scores of others, (p<0.01, p<0.01, p<0.05, p<0.05, p<0.05 p<0.001, p<0.001, p<0.01 and p<0.01, respectively) (Tables I and II).

The average of the perceived seriousness subscale score of those who knew the most suitable time for BSE was significantly higher than the scores of the other students (p<0.05) (Table II).

The average perceived benefits subscale scores of the individuals who were trained in a health related faculty, attended courses on health sciences, had education about BSE, had interest in breast cancer and BSE, had clinical breast examination, had mammography and breast ultrasonography, who knew the right time for BSE, knew the right technique for BSE, the risk groups for BSE, were significantly higher than the scores of other attendants (p<0.001, p<0.001, p<0.001, p<0.001, p<0.05, p<0.05, p<0.01, p<0.001, p<0.001 and p<0.01, respectively) (Tables I and II).

Perceived barriers subscale averages of the students who were under 21 years old, single, trained in a faculty rather than in a health related one, and who attended courses on health sciences, and who did not have training on BSE, who had interest in breast cancer and BSE, who had clinical breast examination, mammography and breast ultrasonography, and who did not know the right time for BSE, the right technique for BSE, the risk groups for BSE and who did not have any first-degree relatives with breast cancer diagnosis, were significantly higher than others (p<0.01, p<0.05, p<0.001, p<0.01, p<0.001, p<0.001, p<0.001, p<0.01, p<0.05, p<0.01, p<0.01, p<0.001, p<0. 001 and p<0.05, respectively) (Tables I and II).

Table I. The relationship between descriptictive characteristics and regular BSE and CHBMS subscale points among research group

Descriptive Characteristics		n	Percent	Regular BSE [n (Percent)]	CHBMS Subscale Points (Mean±Standard Deviation)					
					Susceptibility Perception	Seriousness Perception	Benefits Perception	Barriers Perception	Confidence	General Health Motivation
Age	Under 21	492	56.1	37 (7.5)	8.1±2.7	22.5±5.8	15.1±3.7	28.8±7.4	30.4±8.4	24.7±5.5
	Above 22	385	43.9	54 (14.0)**	8.1±2.6	22.8±5.5	15.5±3.7	27.3±7.9**	32.1±9.2**	25.7±5.2**
Marital status	Single	858	97.8	87 (10.1)	8.1±2.6	22.6±5.7	15.3±3.7	28.2±7.6	31.1±8.8	25.1±5.4
	Married	19	2.2	4 (21.1)	7.4±2.7	23.7±6.1	16.3±4.4	24.8±9.9*	30.1±8.8	26.2±5.5
Faculty	Health Related	132	15.1	44 (33.3)	8.3±2.8	22.6±5.6	16.6±3.3	24.2±7.8	38.1±7.9	26.8±5.3
	Others	745	84.9	47 (6.3)***	8.1±2.6	22.7±5.7	15.0±3.8***	28.9±7.4***	29.9±8.3***	24.8±5.4***
Mother's age	Under 44	306	34.9	17 (5.6)	7.7±2.4	22.4±5.3	15.1±3.9	28.1±6.9	29.8±8.8	24.6±5.3
	Above 45	571	65.1	74 (13.0)**	8.3±2.6**	22.8±5.9	15.4±3.7	28.2±8.0	31.9±8.7**	25.4±5.5*
Mother's educational status	Middle School	569	64.9	51 (9.0)	8.1±2.7	22.6±5.5	15.2±3.7	28.1±7.5	30.8±8.7	25.3±5.3
	Higher Education	308	35.1	40 (13.0)	8.1±2.6	22.7±6.1	15.4±3.9	28.1±7.9	31.8±8.9	24.9±5.7
Mother's working status	Working	227	25.9	32 (14.1)	8.4±2.7	22.7±6.0	15.3±3.8	28.2±8.0	31.7±8.6	24.9±5.5
	Not Working	650	74.1	59 (9.1)*	8.0±2.6	22.7±5.6	15.2±3.7	28.1±7.5	30.9±8.8	25.2±5.4
Economic status of the family	High or Middle	854	97.4	87 (10.2)	8.0±2.6	22.6±5.7	15.3±3.7	28.1±7.6	31.1±8.8	25.2±5.4
	Low	23	2.6	4 (17.4)	9.8±3.0**	24.0±6.3	15.1±3.7	31.2±9.4	33.1±8.6	24.2±4.9
Smoking	Not Smoking	518	59.1	64 (12.4)	7.9±2.7	22.6±5.7	15.3±3.8	27.5±7.6	31.2±9.1	25.6±5.5
	Smoking	359	40.9	27 (7.5)*	8.3±2.6*	22.8±5.7	15.3±3.6	29.1±7.6**	31.1±8.2	24.4±5.3**
Alcohol intake	Not Drinking	499	56.9	53 (10.6)	7.9±2.7	22.8±5.7	15.2±3.9	28.0±7.6	30.8±8.8	25.4±5.6
	Drinking	378	43.1	38 (10.1)	8.3±2.6*	22.5±5.7	15.4±3.5	28.4±7.7	31.6±8.7	24.8±5.1
Total		**877**	**100.0**	**91 (10.4)**	**8.1±2.7**	**22.7±5.7**	**15.3±3.7**	**28.1±7.6**	**31.1±8.8**	**25.1±5.4**

*p<0.05, ** p<0.01, ***p<0.001, BSE: Breast Self Examination, CHBMS: Champion's Health Belief Model Scale, n: Number

Table II. The relationship between characteristics about breast cancer and regular BSE and CHBMS subscale points among research group

Factors		n	Percent	Regular BSE [n (Percent)]	CHBMS Subscale Points (Mean±Standard Deviation)					
					Susceptibility Perception	Seriousness Perception	Benefits Perception	Barriers Perception	Confidence	General Health Motivation
Taking courses on Health Sciences	Taking	208	23.7	53 (25.5)	8.3±2.7	22.7±5.7	16.1±3.5	25.3±7.9	35.5±9.1	26.3±5.6
	Not	669	76.3	38 (5.7)***	8.0±2.6	22.7±5.7	15.0±3.8***	29.0±7.3***	29.8±8.2***	24.8±5.3***
BSE related education	Taken	317	36.1	76 (24.0)	8.3±2.8	22.8±5.8	16.2±3.5	26.3±8.3	35.9±8.4	26.1±5.4
	Not	560	63.9	15 (2.7)***	8.0±2.6*	22.6±5.6	14.7±3.8***	29.2±7.0***	28.4±7.7***	24.6±5.4***
Interest in breast cancer and BSE	Interested	626	71.4	84 (13.4)	8.3±2.6	22.9±5.7	15.6±3.7	27.5±7.8	32.2±8.7	25.6±5.4
	Not	251	28.6	7 (2.8)***	7.6±2.8***	22.2±5.7	14.4±3.8***	29.7±6.9***	28.5±8.3***	23.9±5.4***
Clinical breast examination	Done	140	16.0	33 (23.6)	8.1±2.8	22.6±5.8	15.9±3.5	26.5±8.3	34.2±8.9	25.8±5.4
	Not	737	84.0	58 (7.9)***	8.1±2.6	22.7±5.7	15.2±3.8*	28.5±7.4**	30.5±8.6***	25.0±5.4
Having mammography	Done	30	3.4	4 (13.3)	7.9±2.9	22.9±5.6	16.9±2.5	25.3±7.4	33.7±9.7	27.0±5.0
	Not	847	96.6	87 (10.3)	8.1±2.6	22.7±5.7	15.2±3.8*	28.2±7.6*	31.0±8.7	25.1±5.4
Having breast ultrasonography	Done	87	9.9	21 (24.1)	8.3±2.8	22.4±5.8	16.4±3.5	25.8±8.3	35.4±8.2	25.8±5.6
	Not	790	90.1	70 (8.9)***	8.1±2.6	22.7±5.7	15.2±3.7**	28.4±7.5**	30.7±8.7***	25.1±5.4
Knowing the right time for BSE	Knowing	258	29.4	60 (23.3)	8.6±2.8	23.4±5.7	16.4±3.4	26.9±8.7	34.2±9.2	25.9±5.2
	Not	619	70.6	31 (5.0)***	7.9±2.5***	22.4±5.7*	14.8±3.8***	28.7±7.1**	29.8±8.3***	24.8±5.5**
Knowing the right technique for BSE	Knowing	151	17.2	53 (35.1)	8.3±2.8	22.7±6.0	17.0±3.2	25.3±8.5	37.2±8.6	26.6±5.6
	Not	726	82.8	38 (5.2)***	8.0±2.6	22.6±5.6	14.9±3.7***	28.7±7.3***	29.9±8.2***	24.8±5.3***
Knowing the risky groups for BSE	Knowing	630	71.8	82 (13.0)	8.1±2.6	22.6±5.7	15.5±3.7	27.2±7.4	31.8±8.7	25.5±5.3
	Not	247	28.2	9 (3.6)***	8.0±2.8	22.7±5.6	14.7±3.8**	30.5±7.6***	29.3±8.8***	24.2±5.7**
First degree relatives with breast cancer	Yes	15	1.7	2 (13.3)	10.4±2.6	23.7±6.9	15.5±3.6	24.3±5.8	34.9±6.9	25.3±4.9
	No	862	98.3	89 (10.3)	8.1±2.6**	22.6±5.7	15.3±3.7	28.2±7.6*	31.1±8.8	25.1±5.4
Relatives or friends with breast cancer	Yes	187	21.3	26 (13.9)	8.5±2.4	22.9±5.5	15.5±3.5	28.1±7.7	31.4±8.9	24.8±5.9
	No	690	78.7	65 (9.4)	8.0±2.7**	22.6±5.7	15.2±3.8	28.2±7.6	31.1±8.7	25.2±5.3
Total		**877**	**100.0**	**91 (10.4)**	**8.1±2.7**	**22.7±5.7**	**15.3±3.7**	**28.1±7.6**	**31.1±8.8**	**25.1±5.4**

*p<0.05; ** p<0.01; ***p<0.001, BSE: Breast Self Examination, CHBMS: Champion's Health Belief Model Scale, n: Number

Table III. Correlation between CHBMS subscale points

CHBMS Subscale Points (r)	CHBMS Subscale Points (r)					
	Perceived Susceptibility	Perceived Seriousness	Perceived Benefits	Perceived Barriers	Confidence	General Health Motivation
Perceived Susceptibility	1.000	**0.277*****	0.056	**0.128*****	**0.118*****	-0.033
Perceived Seriousness	**0.277*****	1.000	**0.195*****	**0.138*****	0.038	**0.106****
Perceived Benefits	0.056	**0.195*****	1.000	**-0.215*****	**0.302*****	**0.353****
Perceived Barriers	**0.128*****	**0.138*****	**-0.215*****	1.000	**-0.209*****	**-0.254*****
Confidence	**0.118*****	0.038	**0.302*****	**-0.209*****	1.000	**0.287*****
General Health Motivation	-0.033	**0.106****	**0.353****	**-0.254*****	**0.287*****	1.000

*p<0.05, ** p<0.01, ***p<0.001, CHBMS: Champion's Health Belief Model Scale, r: Pearson's Corelation Coefficient

Table IV. The relationship between CHBMS subscale points and regular BSE performing

CHBMS Subscales	Regular BSE Performing	
	Regular (Mean±Standard Deviation)	Not Regular or None (Mean±Standard Deviation)
Perceived susceptibility	8.5±2.9	8.0±2.6
Perceived seriousness	23.1±6.1	22.6±5.6
Perceived benefits	17.0±3.4***	15.1±3.7
Perceived barriers	23.9±8.9***	28.6±7.3
Confidence	40.0±7.2***	30.1±8.3
General health motivation	27.7±4.9***	24.8±5.4

***p<0.001, CHBMS: Champion's Health Belief Model Scale, BSE: Breast Self-Examination

Table V. Logistic regression analyses of the factors for regular BSE

Factors	OR (95% CI)
Age (Above 22)	0.7 (0.4 – 1.2)
Faculty (Health Related)	0.8 (0.3 – 2.2)
Mother's age (Above 45)	1.6 (0.8 – 3.1)
Mother's working status (Working)	1.6 (0.9 – 2.9)
Smoking (Not Smoking)	1.4 (0.8 – 2.5)
Attending courses on Health Sciences	2.1 (0.8 – 5.4)
BSE related education (Taken)	2.4* (1.2 – 5.0)
Interest in breast cancer and BSE	2.1 (0.9 – 5.1)
Clinical breast examination	3.2** (1.4 – 7.6)
Having breast ultrasonography	1.0 (0.4 – 2.7)
Knowing the right time for BSE	1.9* (1.0 – 3.5)
Knowing the right technique for BSE	3.0** (1.5 – 5.7)
Knowing the risky groups for BSE	1.7 (0.8 – 4.0)
Perceived Susceptibility	1.1 (0.9 – 1.2)
Perceived Seriousness	1.0 (1.0 – 1.1)
Perceived Benefits	0.9 (0.9 – 1.0)
Perceived Barriers	1.0 (0.9 – 1.0)
Confidence	1.1*** (1.0 – 1.1)
General health motivation	1.0 (1.0 – 1.1)

*p<0.05, **p<0.01, ***p<0.001, BSE: Breast Self Examination, OR= Odds Ratio, CI= Confidence Interval

Confidence subscale averages of individuals who were 22 years old and over, were trained in a health related faculty, whose mothers were 45 years old and over, who were taking lessons about health sciences, who had education on BSE, who had interest in breast cancer and BSE, who had clinical breast examination, who had breast ultrasonography, who knew the right time and the technique for BSE and who knew the risk groups for BSE, were significantly higher than the findings of other students (p<0.01, p<0.001, p<0.01, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001 and p<0.001, respectively) (Tables I and II).

General health motivation subscale averages of individuals who were 22 years old or over, trained in a health related faculty, whose mother was 45 years old or over, were taking lessons about health sciences, and who had education on BSE, who had interest in breast cancer and BSE, who had clinical breast examination, breast ultrasonography, and who knew the right time and the technique for BSE and the risk groups for BSE, were significantly higher than the values of other participants (p<0.01, p<0.001, p<0.05, p<0.01, p<0.001, p<0.001, p<0.001; p<0.01, p<0.001 and p<0.01, respectively) (Tables I and II).

The results of the logistic regression analysis that we conducted to determine the independent predictors of the prevalence of regular BSE are presented in Table V. Having education on BSE (OR=2.4, p<0.05), having clinical breast examination (OR=3.2, p<0.01), knowing the right time for BSE (OR=1.9, p<0.05), knowing the right technique for BSE (OR=3.0, p<0.01), and trust / self efficacy subscale scores affected BSE (OR=1.1, p<0.01).

4. DISCUSSION

The study was conducted in order to find out the prevalence of BSE among university students. Regularly performed BSE every month was found to be 10.4%. Thirty two percent of Arabic refugee women in Canada performed BSE regularly. [8] In previous studies conducted in Turkey, higher ratios were found especially in nursing students (31%, 32%, 53%) but our ratio is similar with Karayurt, et al., who conducted a study on 193 university students from various faculties (6.2%) [9-12].

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Sapountzi-Krepia et al., also found that the ratio of nursing students in Cyprus who performed regular BSE for more than a year as 10.9% [13]. Besides, in 2014 Health Statistics Yearbook, published by Turkish Ministry of Health, it was reported that 10.1% of women regularly performed BSE every month [4].

Subscale point averages of CHBMS (±Standard Deviation) in our study were found as follows: 8.1±2.7 for the perceived susceptibility subscale, 22.7±5.7 for the perceived seriousness subscale, 15.3±3.7 for the perceived benefits subscale, 28.1±7.6 for perceived barriers subscale, 31.1±8.8 for confidence subscale and 25.1±5.4 for general health motivation subscale. Karayurt et al., studied the effects of peer and group education on knowledge and belief on breast cancer and BSE. In their study, CHBMS subscale point averages for peer and group education were found as follows: 8.6±2.9 and 8.1±1.6 for the susceptibility perception subscale, 20.5±7.2 and 21.0±5.1 for seriousness perception subscale, 14.1±5.9 and 14.5±5.7 for benefits perception subscale, 24.6±4.8 and 24.5±5.3 for barriers perception subscale, 26.8±9.6 and 28.2±7.5 for confidence subscale 24.3±5.3 and 24.3±5.6 for general health motivation subscale, respectively [10]. Karayurt et al., performed this study in Izmir, a city in the West part of Turkey, 10 years ago. The scores for barriers perception and confidence subscale scores in our study were higher than those obtained in Karayurt et al's study. This may be due to the date of the study or regional differences. Erbil and Bölükbaşı studied a group of policlinic patients and found subscale points as follows: susceptibility perception score (7.5±2.5), seriousness perception score (21.2±5.5), benefits perception score (15.0±3.8), barriers perception score (27.0±6.7), confidence score (32.0±6.7), and general health motivation score (25.1±5.6) [14]. Lower susceptibility perception score, in the study of Erbil and Bölükbaşı, when compared to those in our study might be due to the population of university students in our study. The average educational level of our universe was higher than the related study. This may have led to a high awareness of breast cancer in our universe that it may be seen in everyone so that the perceived susceptibility subscale may be high.

As a result of our logistic regression analysis, we determined that breast self-examination training was a predictor that increased the rate of regular BSE. Aker et al., also found in their logistic regression analysis that, being educated on breast health, increased BSE rate by 3.81 (95% GA, 2.16-6.72) times [15]. Uzun et al., showed an increased ratio of performing BSE in undergraduate nursing students after education [12]. Özkahraman et al., conducted a study with trainee women in a public education center and detected that having information on BSE increased the ratio of performing BSE significantly [16]. Tuyen et al., reported that performing monthly BSE was negatively associated with receiving information on BSE [17]. Lee, in his study showed increased BSE training ratio but in regression analysis BSE training was not significant [18]. In conclusion, these results demonstrated that, education can positively change individuals' health beliefs.

Our regression analysis showed that clinical breast examination was a predictor of BSE performance ratio. In a study of Aksoy et al., on women over 40 years of age it was found that the rate of having a clinical breast examination was higher in regular BSE performers [19]. Clinical breast examination and information given by physicians on BSE might increase the awareness of the individuals on regular BSE.

In our logistic regression analysis, it was found that knowing the most appropriate time for BSE and the BSE technique correctly increased the BSE rate. Uncu, did not detect a significant relation between knowing the appropriate BSE technique and performing BSE. On the other hand, Lee showed in logistic regression analysis that BSE rate increasesd as BSE knowledge score increased [18,20]. Our findings showed that those who regularly performed BSE, knew the right time and technique for BSE. These results were similar with the above studies.

In our logistic regression analysis, it was determined that the BSE rate increased as the confidence / self-efficacy score increased. Spountzi-Krepia et al., Aker at al., Lee, and Lavdaniti showed that the ratio of BSE increased as trust/self efficiacy increased in logistic regression models [13,15,18,21].

Although, the CHBMS perceived barriers subscale was found to be significant in univariate analysis in our study, it was found that regular regression model did not significantly affect BSE performance. In the studies of Aker et al., and Lee it was determined that the score of perceived barriers was a variable affecting the BSE ratio in the negative direction [15,18]. As seen in our univariate analysis results, as the barriers subscale scores increased, the rate of BSE was expected to increase.

By using the CHBMS for assessment, educators and primary health care providers can more easily understand the beliefs that influence women on breast cancer and BSE.

In conclusion,we detected in our regression model that being educated on BSE, knowing the best time for BSE and appropriate BSE technique and having a clinical breast examination regularly, increased the ratio of performing BSE.

We can conclude that education is the most appropriate tool that can be used to raise awareness on BSE. Considering the necessity of performing BSE starting from young ages, inclusion of BSE education in the curriculum of high schools and universities will also increase the rate of performing BSE. Also, we recommend that planned interactive training programs for breast cancer and BSE should be implemented for larger groups of women at Women's Health Centers and Family Centers. Further research is needed in order to measure the effectiveness of these trainings.

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The susceptibility of *Proteus mirabilis* and *Enterococcus faecalis* to various antimicrobial agents in polymicrobial biofilms formed using a drip flow reactor

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ABSTRACT

Objectives: Interspecies interactions in poly-species biofilm are substantial. Our aim is to set up dynamic biofilm models of *Enterococcus faecalis* and *Proteus mirabilis* using Drip Flow Biofilm Reactor (DFR) and to evaluate the effect of these dual population on anti-biofilms of some antimicrobials.

Materials and Methods: *E.faecalis* and *P.mirabilis* biofilms were formed in a DFR. Influences of the dual interactions on their susceptibilities to antimicrobial agents (disinfectants, antibiotics and probiotic strains) were determined.

Results: Gluteraldehyde and quaternary ammonium compounds (QAC) effectively killed the cells in both biofilms of *E.faecalis* and *P.mirabilis*. However, the efficacy of hydrogen peroxide (H_2O_2) was dependant on the microbial species present. *P. mirabilis* was less susceptible to the ampicillin and ciprofloxacin in co-culture compared to when cultured alone. Here, the influence of the presence of *E.faecalis* on *P.mirabilis* susceptibility was determined. For high concentrations of ciprofloxacin (1024 and 512 µg/ml), the log reduction in *P.mirabilis* cells was determined as approximately 4.5 and 3.5 in mono and dual-species biofilms respectively. Compared to *B.lactis, L.acidophilus* was found to be more effective both on single and dual species.

Conclusion: The effect of antimicrobial agents on microbial cells in a polymicrobial biofilm may depend on the composition of the biofilm.

Keywords: Polymicrobial biofilm, *E. faecalis*, *P. mirabilis,* Antimicrobial, Drip flow reactor, Probiotic strains

1. INTRODUCTION

Cells in biofilms are embedded in a self-produced extracellular polymeric matrix (EPM) that mainly consist polysaccharides, DNA, proteins, and dead cells [1]. In biofilms, the limited penetration of antimicrobial agents and accumulation of antibiotic-degrading enzymes are observed due to EPM production. EPM formation and the transfer of resistance genes in the presence of high cell density lead to an increased biofilm resistance [1,2].

Multi-species biofilms that exist in the human body are complex communities in which cells of various microorganisms are present and live together [1]. These biofilms are commonly encountered clinical concern and are found in multiple body sites (such as the skin, teeth and mucosa) in chronically infected wounds and on indwelling medical devices such as prostheses, stents, implants, catheters and endotracheal tubes [3].

P. mirabilis is associated with a large number of human infections such as catheter-associated urinary tract infection (CAUTI), cystitis, pyelonephritis, wound, eye and burn infections [4]. It is frequently found as coisolates with other pathogens, such as *Enterococcus* species, *Candida* spp. in the samples of patients with biofilm related infections [5]. It was shown that expression of the virulence factors increased and resulted in greater tissue damage in parallel with the presence of polyspecies pathogens in the biofilm environment [5,6].

Enterococcus species are gram positive bacteria and the harmless members of gut flora in humans and animals. They are among the most frequent causes of nosocomial infections since they have the ability to create colony in different human body sites as well as on the surface of medical devices and may exhibit resistance to many antibiotics [7]. It is now well known that these infections are mainly associated with biofilm formation and are difficult to treat [8].

Interspecies interactions in biofilms have been extensively studied in human body and environments [9-11]. Different microbial interactions could effect the biofilm composition. A study reported that the biofilm cell counts of *Escherichia coli* decreased when co-cultured with *Pseudomonas aeruginosa*, but *P.aeruginosa* obtained some advantages when grown in

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dual-species biofilms [12]. However, there is little information about antimicrobial susceptibility profile (such as antibiotics, disinfectants etc.) of polymicrobial biofilms. The results of a study showed that a multispecies biofilm with six different bacterial species including *Acinetobacter calcoaceticus* had the highest resistance to sodium hypochlorite (NaOCl), while a multispecies biofilm without *A.calcoaceticus* was more susceptible to NaOCl compared to the monospecies biofilms. No significant difference was reported in a previously published study in susceptibility to ethanol between mono – and multispecies biofilms of *Candida albicans* and *Staphylococcus aureus* [13]. Kart et al. concluded that the effect of the disinfectants tested in a multispecies biofilm depends on the type of the strain and the kind of the disinfectant used [14].

Dynamic mono and dual species biofilm models of *E. faecalis* and *P.mirabilis* which were reported as frequently isolated species from catheter-associated polymicrobial infections were developed in this study in a repeatable style using Drip Flow Biofilm Reactor (DFR) [15].

The aim of this study is to evaluate the effect of dual species interactions between *E.faecalis* and *P.mirabilis* in the developed biofilm models on individual susceptibility against various antimicrobial agents such as antibiotics (ampicillin and ciprofloxacin), disinfectants (Quarternery Ammonium Compound (QAC), hydrogen peroxide, glutaraldehyde) and probiotic strains.

2. MATERIALS and METODS

Bacterial strains

P. mirabilis ATCC 29906, *E. faecalis* ATCC 47077/OG1RF, *B. lactis* ATCC 27536 and *L. acidophilus* ATCC 4356 were used in the study. *P.mirabilis* ATCC 29906 and *E.faecalis* ATCC 47077/OG1RF were grown overnight at 37°C in brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK).

B.lactis ATCC 27536 and *L.acidophilus* ATCC 4356 were cultured in Man-Rogosa-Sharpe broth (MRSB; Merck, Darmstadt, Germany) and incubated at 37 °C in an anaerobic jar for 18 h and maintained on MRS agar plates (MRSA; Merck, Darmstadt, Germany).

Preparation of cell-free culture supernatant of probiotic strains

After incubating *L. acidophilus* and *B.lactis* strains in MRS broth at 37 °C for 18 h, the cultures were centrifugated at 8000xg for 20 min at 4 °C to obtain the cell-free supernatants. Then, the supernatants were filtered through 0.2-µm syringe filter (Millipore, Bedford, MA, USA) [9].

Antimicrobial agents

Disinfectants, antibiotics and probiotic strains tested in the study were shown in Table I. All disinfectant solutions were prepared using water of standard hardness (WSH), filter-sterilized and stored at 4°C for up to 1 month. Sterile WSH distilled water and MRS broth were used as positive controls for antimicrobial activities of the disinfectants, antibiotics and probiotic supernatants, respectively.

Table I. Antimicrobials used in the study

Disinfectants/ Other antimicrobials	Concentration (%/mg/ml)	Contact Times (minutes/ hours)
Quarternery Ammonium Compound (QAC)	3%	15 min.
Quarternery Ammonium Compound (QAC)	1.5%	15 min.
Hydrogen peroxide (H_2O_2)	1.5%	5 min.
Hydrogen peroxide (H_2O_2)	0.75%	5 min.
Gluteraldehyde	2%	15 min.
Gluteraldehyde	1%	15 min.
Ampicillin	32-1024 µg/ml	24 h.
Ciprofloxacin	32-1024 µg/ml	24 h.
Probiotic supernatants (*B. lactis* ATCC 27536 and *L. acidophilus* ATCC4356)	–	24 h.

Development of mono – and dual-species biofilms in Drip Flow Reactor Model

Biofilms consisting of *P.mirabilis* and *E.faecalis* were grown in DFR (Bio Surface Technologies Corp, Montana, USA) with low shear and continous flow approved by the American Society for Testing and Materials International (ASTM) standard setting organization [15]. Final inoculum suspensions (10^6 CFU/ml) of both bacteria were prepared in 10 ml BHI broth and placed into the DFR, which comprises six individual, parallel test channels including sterile slide (Figure 1). After operating the reactor in batch mode for 6 h, the flow was started and maintained for another 48 h with a continuous flow rate of 0.82 ml/min per channel [16]. During continuous flow, the media was dripped onto the surface of slides set at a 10° angle and mature biofilms were formed on these slides. The microorganisms were cultured alone (for mono species biofilms) and together (for dual-species biofilms).

Figure 1. Drip flow reactor used in the study

Anti-biofilm evaluation

The prescribed concentrations and contact times of each agents were applied to the slides which mono and dual-species biofilms of the strains were formed on (Table I). After the contact time, the disinfectant was neutralized with Dey-Engley neutralizing broth, as described previously [13,14]. For the ampicillin and ciprofloxacin, all concentrations (ranging from 1024 to 32 mg/ml) were tested in each of the six channel, individually, at same time. The supernatants of *B. lactis* ATCC 27536 and *L. acidophilus* ATCC 4356 were obtained after centrifugating the overnight cultures of both strains in MRS broth (Merck, Darmstadt, Germany) and were transferred onto the pre-formed mature biofilms to evaluate their anti-biofilm effects.

Colony counting

After treatments, the slide samples were removed from the reactor channels and rinsed with sterile buffered saline solution to remove planktonic cells. Biofilms were scraped from the slide surfaces with the cell scrapper and clumps were disaggregated by vortexing and sonication steps (at a frequency of 42 kHz (model 2510 sonicating water bath; Branson, New Hampshire,USA) according to the published protocols [17]. After dissagregating, the biofilm suspensions were serially diluted in sterile buffered saline solution and plated triplicate on selective growth media; ie tryptic soy agar (containing 1.5% agar) supplemented with vancomycin (16 µg/ml) for *P.mirabilis* and bile esculin azid agar for *E.faecalis*, respectively. Following 24 h of incubation time, the colonies were counted as colony forming units per milliliter (cfu/ml).

Scanning electron microscopy (SEM)

After the mature biofilm formations, the slides were removed from DFR, rinsed with 1 ml of buffered saline solution and cutted with glass diamond. Subsequently, all parts were washed with sterile distilled water and fixed in a buffer containing 2% glutaraldehyde and 0.1 M cacodylate for 30 min followed by rinsing three times for 10 minutes in 0.2 M cacodylate buffer. After passing them through serial ethanol solutions, samples were dried, then coated with gold-palladium and examined by a scanning electron microscope [18].

Statistical analysis

The colony counts were recorded for each treatment as log10. All statistical calculations were performed on the log density values. Statistical analysis was performed using two-tailed t-test assuming unequal variances with $\alpha = 0.5$ and a p value < 0.05 was considered to be significant.

3. RESULTS

Single and dual-species inoculum suspensions containing 10^6 cfu/ml of *E. faecalis* and *P. mirabilis* in BHI was added to each channel of the DFR to form mature biofilms. In single and dual-species biofilms *E. faecalis* and *P. mirabilis* were grown up to ~10^7 **cfu/ml** and ~10^8 cfu/ml respectively. Compared to single species biofilm, the results demonstrated that the survival rate of each species was not affected with the other one. The cell numbers in the biofilm following treatment with the disinfectants were determined by plating onto the selective media concerning the results (Table II).

All cells were death after treatment with two other disinfectants. Except H_2O_2, all other disinfectants led to cell death in all biofilm formations with either *P. mirabilis* or *E. faecalis* in single species and in dual-species biofilm (Table II).

Table II. Efficacy of disinfectants on biofilm

Disinfectant			*P.mirabilis*		*E.faecalis*	
	Conc. (%)	Time (min.)	Mono	Dual	Mono	Dual
H_2O_2	2	5	95.71±0.4	99.93±0.01	99.47±0.1+	88.42±1.2
H_2O_2	1	5	86.91±1.5=	98.28±0.3	93.12±0.7+	73.26±1.7
QAC*	3	15	99.99±0.0	99.99±0.0	99.99±0.0	99.99±0.0
QAC*	1.5	15	99.99±0.0	99.96±0.01	99.99±0.0	99.99±0.0
GA*	2	15	100±0.0	100±0.0	100±0.0	100±0.0
GA*	1	15	100±0.0	100±0.0	100±0.0	100±0.0

The results are expressed as hundred percent effective and are shown as the average ± standard error (n = 3). *Statistically significant reduction for all biofilm conditions compared to untreated control ($p < 0.05$). +Significantly more cells of this organism are killed in a single-species biofilm than in a dual-species biofilm ($p < 0.05$). =Significantly more cells of this organism are killed in the dual-species biofilm than in single-species biofilm ($p < 0.05$). H2O2 : hydrogen peroxide, QAC: quarternery ammonium compounds, GA: Gluteraldehyde

The ampicillin and ciprofloxacin susceptibilities of *P. mirabilis* and *E. faecalis* cells both in single and dual species biofilms were determined individually. In comparison to untreated controls, none of the tested concentrations of ampicillin against *E. facealis* showed anti-biofilm activity both in single and dual-species biofilms (Figure 2). In contrast, a significant decrease in cell survival of *P. mirabilis* was determined only with 1024 µg/ml among all the tested concentrations of ampicillin both in single and dual species biofilms (Figure 2). Statistically significant difference was obtained between susceptibilities of single and dual species biofilms formed by *P. mirabilis*. Accordingly, in the presence of *E. faecalis*, the biofilm cells of *P.mirabilis* were found to be less susceptible to ampicillin (1024 µg/ml).

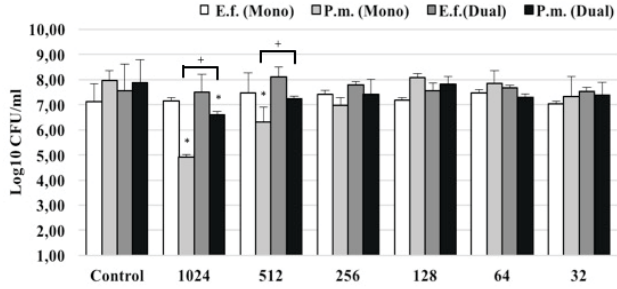

Figure 2. The effect of ampicillin on mono and dual species biofilms of *E.faecalis* and *P.mirabilis*

*Statistically more significant decrease in the cell counts than control ($p < 0.05$)
+Significantly more cells of this organism were killed in a monospecies biofilm than in a dual-species biofilm ($p < 0.05$)

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The significant decrease in the growth of *E. faecalis* cells both at 512 and 1024 mg/ml concentrations of ciprofloxacin were obtained in single and also in dual-species biofilm when compared to the untreated control (Figure 3). It was concluded that the ciprofloxacin showed more inhibitory activity against *P. mirabilis* than *E. faecalis* as the significant decrease in cell growth in *P. mirabilis* single species biofilm was obtained even at the lowest tested concentration (32 mg/ml) (Figure 3). Furthermore, the cells in single species biofilm formed by *P.mirabilis* were more susceptible to both tested antibiotics when compared to dual species biofilm (Figure 3).

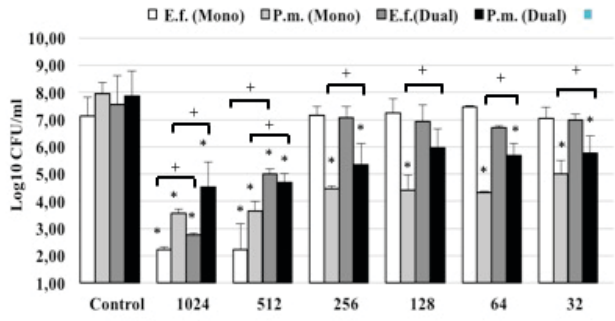

Figure 3. The effect of ciprofloxacin on mono and dual species biofilms of *E.faecalis and P.mirabilis*

*Statistically more significant decrease in the cell counts than control ($p < 0.05$)
+Significantly more cells of this organism were killed in a monospecies biofilm than in a dual-species biofilm ($p < 0.05$)

When the results of the effect of two probiotic supernatants on the cell survival were reviewed, *L. acidophilus* was found to be more effective both on single and dual species compared to *B. lactis*. None of the probiotic supernatants showed anti-biofilm effect on *E. faecalis*. cells in dual species biofilm. However the significant decrease in the cell growth *of E. faecalis* was obtained by *L. acidophilus* supernatant treatment in the single species biofilm (Figure 4). The dense cell populations of *P. mirabilis* and *E. faecalis* were observed in mono and also in dual-species biofilms by SEM images (Figure 5).

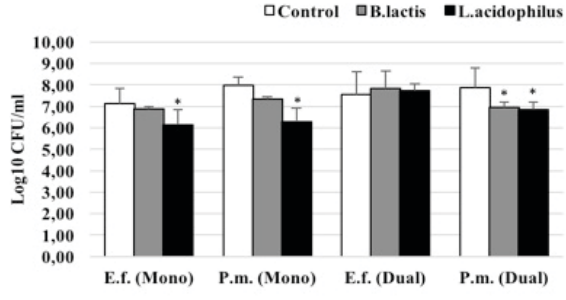

Figure 4. The antimicrobial effects of probiotic supernatants on the biofilms of *E.faecalis* and *P.mirabilis.*

*Statistically more significant decrease in the cell counts than control ($p < 0.05$)

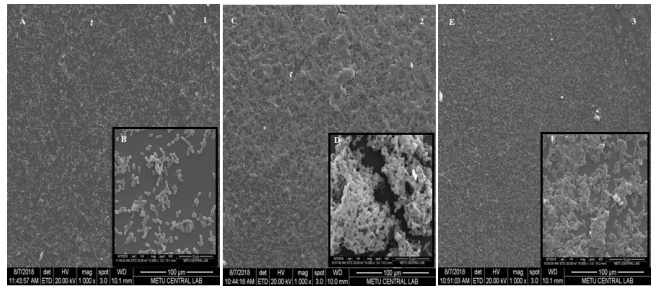

Figure 5. Scanning electron microscopy (SEM) images of biofilms

1. Dual-species biofilm of *E. faecalis* and *P.mirabilis* 2. *E. faecalis* 3. *P. mirabilis* (A,C,E): SEM images magnified by 1000X. (B,D,F): SEM images magnified by 10000X

4. DISCUSSION

The anti-biofilm susceptibilities of *E. faecalis* and *P. mirabilis* cells against three different disinfectant were determined both in single and dual species biofilms, individually. Except for H_2O_2, our results demonstrated that all tested concentrations of glutaraldehyde and QAC effectively kill more than 99.999 % of cells both in mono and dual-species biofilms. In previously published reports it was reported that monospecies biofilms are often more susceptible to antimicrobial agents than polyspecies biofilms [3,19]. On contrary, our results clearly suggested that the efficacy of H_2O_2 was dependent on the type of microbial species present in the biofilm environment. Compared to its mono species biofilm *P. mirabilis* was found to be more susceptible to H_2O_2 when it was co-cultured with *E. faecalis*. On the other hand, *E. faecalis* was found to be less susceptible to H_2O_2 in the dual species biofilm with *P. mirabilis* compared to its mono species biofilm. These results support our previously published data indicating that the antimicrobial susceptibility of biofilm cells was dependent on both the nature of microbial species and properties of the selected disinfectant [14]. In our previous study, H_2O_2 was found to kill 96.94 % of *P. aeruginosa* cells in a triple-species biofilm consisting of *P. aeruginosa, S. aureus* and *C. albicans*, whereas *P. aeruginosa* cells in a single-species biofilm were not affected [14]. Alfa et al. evaluated the ability of different high-level disinfectants, frequently used for endoscope reprocessing to remove the single-species biofilms of *E. faecalis* and *P. aeruginosa*, separately. They have concluded that high-level disinfectants such as glutaraldehyde and H_2O_2 were insufficient to completely kill the biofilm cells when the high numbers of cells were found in the environment [20].

The results of glutaraldehyde activity obtained from the presented study were not consistent with the literature. This difference may be caused by using different protocols (flow based system versus static conditions) in the studies. In a study reported by Bock et al, the efficacy of H_2O_2 based disinfectants was investigated against biofilms formed by multi-drug resistant *Acinetobacter* spp., *Klebsiella pneumoniae* and *P. aeruginosa*. In comparison to planktonic forms the efficacies of recent working concentrations of disinfectants were not found to be susceptible

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[21]. Majority of the previously reported studies on biocide susceptibility of planktonic or single-species biofilm cells have not taken into consideration the effect of the co-existence of bacteria in a mixed biofilm on individual biocide susceptibility. In the literature the reduced susceptibility to antibiotics in biofilm related infections formed by *E. faecalis* was attributed to biofilm-specific antimicrobial tolerance mechanisms [22]. Penetration limitation is one of these mechanisms and basically defined as the limited diffusion of the antibiotics into the biofilm matrix thus only the surface of the biofilm can get into contact with the antibiotic [23,24].

The MIC values of the antibiotics commonly used in treatment of the planktonic form of pathogen microorganisms are insufficient to eradicate the biofilm-embedded counterparts [25]. Due to the lack of standards and specific breakpoints for anti-biofilm assays biofilm susceptibility tests are not currently used in clinical diagnostic routines [26]. In this study ampicillin was found to be insufficient to eradicate the *E. faecalis* biofilm cells both in mono and dual-biofilms. For high concentrations of ciprofloxacin (1024 and 512 µg/ml), the log reduction in viable population was determined as approximately 4.5 and 3.5 in mono and dual-species biofilms respectively. These results were found to be in accordance with the results of two recently published studies [26].

In the present study the influence of coexistence of *P. mirabilis* with *E. faecalis* in dual-species biofilm on the susceptibility of *P. mirabilis* against ciprofloxacin and ampicillin was evaluated. Compared to its single-species biofilm, *P. mirabilis* showed less susceptibility to both antibiotics in dual-species biofilm (Figure 2 and 3). The interaction of two bacteria was resulted with the decrease of *P.mirabilis* susceptibility to the tested antibiotics with an unknown mechanism. Ampicillin significantly decreased the number of *P. mirabilis* viable cells in mono and also in dual-species biofilms at 1024 and 512 mg/ml concentrations (Figure 2). Statistically significant decrease was determined in mono and dual-biofilm cells of *P.mirabilis* at 32 µg/ml concentration of ciprofloxacin.

In a previously published study concerning dual species biofilm formed by *P.aeruginosa* and *P. mirabilis*, the inhibitory activity of *P. mirabilis* on *P. aeruginosa* has been reported. By the authors this inhibition was attributed to less biofilm biomass of *P. aeruginosa* in dual species biofilm than the single species biofilm [27]. The susceptibilities of *P.mirabilis* biofilm cells against eight different antimicrobial agents including amoxicillin, erythromycin, gentamicin, nitrofurantoin, ciprofloxacin, trimethoprime-sulfamethoxazole, ceftriaxone and chloramphenicol were tested by Wasfi et al. Among these agents ciprofloxacin and ceftriaxone both at 8 µg/ml concentration were found to be the most effective agents which removed up the pre-formed biofilms by 34-55% and 33-69%, respectively [28]. Another study determined the eradicating effect of ciprofloxacin against the biofilm forming isolates of *P. mirabilis* at four antibiotic concentrations, corresponding to 0.125 MIC, 0.25 MIC, 0.5 MIC and 1 MIC values [29].

Previously, it was shown that lactobacilli could break down cells of opportunistic pathogenic bacteria [30]. However, there was limited information about the interactions between *Lactobacillus* and other bacteria that occurred during mixed-biofilm formation. Compared to *B. lactis, L. acidophilus* was found to be more effective on *P.mirabilis* cells both in single and dual-species biofilms than *E.faecalis*. Based on our findings, it can be concluded that probiotics may display distinct antimicrobial effects on individual species of mixed-biofilms. Rybalchenko et al. concluded that a probiotic strain, *L. fermentum* 97, suppressed the growth of *Staphylococcus* spp., Enterobacteriaceae and *C. albicans* biofilm cells [30]. In another study, the growth inhibition of *Streptococcus mutans* by *L. acidophilus* LA-5 was observed significantly [31].

In conclusion, the cells in dual-species biofilm may display different responses to antimicrobial agents than their counterparts in single-species biofilm. This study investigated the effects of microbial interactions in dual-species biofilms of *P. mirabilis* and *E. faecalis* on their responses to various antimicrobial agents in a flow-based biofilm reactor model. Single or dual-species biofilm models were successfully developed by *P. mirabilis* and *E. faecalis* which were frequently co-isolated from the samples of patients with CAUTI. Findings of this study demonstrated that glutaraldehyde and quaternary ammonium compounds are the agents that might be effectively used to eradicate biofilms in the hospital cleaning settings. Ampicillin and ciprofloxacin are antibiotics used to treat the urinary system infections caused by urinary pathogens such as *E. faecalis* and *P. mirabilis*. In the study, concentrations lower than 512 µg/ml were found to be insufficient to kill *E. faecalis* biofilm cells in single and dual-species biofilms for both antibiotics. However, ciprofloxacin was able to decrease the number of viable cells of *P. mirabilis* at 32 µg/ml both in mono and dual-species biofilms significantly. The presence of *E. faecalis* in the dual-species biofilm decreased the susceptibility of *P. mirabilis* to ciprofloxacin. Although, further studies are required, this study emphasized the importance of the microbial interactions in polymicrobial biofilms, especially in response to antimicrobials for the treatment of biofilm-related diseases. Although, the effect of probiotic supernatants were found to be dependant on the nature of biofilm strain, *L. acidophilus* was more succesfull in killing bacterial cells when grown alone.

Conflict of Interest: The author declares that she has no conflict of interest.

Ethical Issues: According to the Institutional Ethical Committee this study did not require ethics approval as it was conducted on reference stains and the data did not contain patient-specific information.

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Case Report

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Renal involvement in diffuse large B-cell lymphoma: A case report

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ABSTRACT

Diffuse large B-cell lymphoma is the most common subtype of non-Hodgkin lymphoma. Approximately 40% of cases have extranodal involvement. Renal involvement is rare, however, it is associated with poor prognosis. Furthermore, renal involvement increases the risk of central nervous system (CNS) recurrence. Therefore, it is recommended that CNS prophylaxis should be included in lymphoma treatment in cases of renal involvement.

Keywords: Diffuse large B-cell lymphoma, Extranodal, Kidney, Involvement

1. INTRODUCTION

Lymphomas are malignant neoplasms originating from lymphoid cells. They are classified under two categories as Hodgkin lymphoma and non-Hodgkin lymphoma (NHL). Diffuse large B-cell lymphoma (DLBCL) constitutes 30%–58% of all NHLs. There are 3-4/100,000 new cases per year in the European Union, and its incidence increases with age [1]. Extranodal involvement occurs in approximately 40% of DLBCL cases (with 36% in the gastrointestinal tract, 20% in the head and neck region and 14% in the bone marrow) [2]. Renal involvement, which is rare, occurs in 1%–2% of DLBCL patients [3-5].

2. CASE REPORT

A 74-year-old female with a history of hypertension, diabetes mellitus and coronary artery disease presented at our hospital with complaints of weight loss and abdominal pain that had been persisting for a month. Physical examination revealed tenderness in the abdomen. An abdominal ultrasound scan showed a vascularised heterogeneous hypoechoic lesion of approximately 7.5 × 5.5 cm in the left kidney and two hypoechoic lesions with a diameter of 8.5 cm that surrounded the vascular structures in the pancreatic duct and adjacent to the left iliac artery. Positron emission tomography/computed tomography (PET/CT) scan showed a pathological increase in 18-fluoro-2-deoxyglucose (18F-FDG) uptake, lymphadenopathies in the right mediastinal region (SUV_{max}: 8.9), a mass with a diameter of approximately 9 × 6.5 × 10.5 cm in the pancreatic duct (SUV_{max}: 27.9) and a mass with a diameter of 9 × 6 cm in the left kidney (SUV_{max}: 26.5). Conglomerate lymph nodes, the largest one being 5 × 3.5 cm, were observed on the mesenteric, para-aortic, left main iliac and right iliac chain (SUV_{max}: 26.0) (Figure 1). The tru-cut biopsy results performed at the pancreatic duct and left kidney indicated non-germinal centre diffuse large B-cell lymphoma [vimentin (+), LCA (+), CD20 (+), CD10 (–), BCL-6 (+), BCL-2 (–), MUM-1 (+), p53 (+) and KI67 index of 98%] (Figure 2). Laboratory test results showed that hemoglobin level was 13.48 g/dl, leukocyte count was 7.95 mm^3, platelet count was 377,000, creatinine level was 0.79 mg/dl, LDH level was 510 U/L, Eastern Cooperative Oncology Group (ECOG) performance score was 3, Ann Arbour stage was 4 and international prognostic index (IPI) score was 5 (high). Owing to old age and poor performance, the patient was initiated on R-mini-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone) treatment. Cerebrospinal fluid test results did not reveal any involvement. Intrathecal methotrexate was administered for CNS prophylaxis.

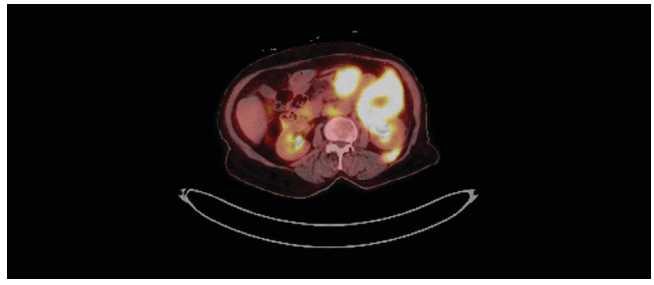

Figure 1. In the left kidney, peripheral and pathological increase in 18F-FDG uptake is observed in a soft tissue mass that has a hypodense hypometabolic area (necrosis) at its center.

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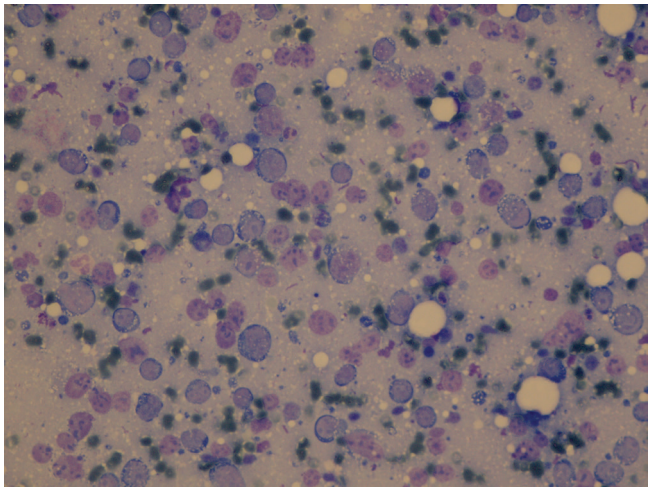

Figure 2. Renal biopsy findings were compatible with DLBCL: Atypical lymphocytes singly scattered on a base consisting of erythrocytes and lymphoglandular bodies, having a cytoplasm with vacuoles, nuclear pleomorphism and irregular nuclear contours and prominent nuclei, are observed in the smear (MGG staining, original magnification 40× with objective lens).

3. DISCUSSION

In a study with 821 patients diagnosed with DLBCL, renal involvement was noted in 22 (3%) of them at the time of diagnosis. Among the patients with renal involvement, 86% had advanced stage DLBCL with high IPI scores and 32% had renal insufficiency. It was demonstrated that renal involvement leads to a 3-year progression-free survival (PFS) rate of 44% and a 3-year overall survival (OS) rate of 52%, indicating that the survival rates are worse with renal involvement than without renal involvement. In addition, it was observed that the rate of CNS recurrence was high (36%) [6]. In other studies, renal involvement was found to be an independent risk factor for CNS recurrence [7-9]. Intrathecal methotrexate administration is the most widely used prophylaxis method for the risk of CNS in high-risk patients. However, recent studies have demonstrated this method to be insufficient in reducing the risk of CNS recurrence [10,11]. Conclusions concerning the insufficiency of intrathecal treatment for the prevention of parenchymal CNS recurrence have led to the administration of intravenous high-dose methotrexate as CNS prophylaxis. The positive effect of intravenous high-dose methotrexate administration in patients with a high risk for CNS recurrence has been demonstrated in many studies [12,13]. Our patient had no CNS involvement at the time of diagnosis. Owing to old age and a low-performance score, intrathecal methotrexate was included in our patient's treatment regimen instead of high-dose methotrexate for CNS prophylaxis.

Several published studies on DLBCL patients treated with R-CHOP-like regimens have reported the 3-year PFS rate to be 62%–79% and the 3-year OS rate to be 72%–93% [14-17].

Extranodal involvement at multiple sites has been identified as an independent risk factor for NHL [18]. Additionally, renal involvement has been demonstrated to be associated with poorer survival [6]. It is known that acute kidney damage adversely affects survival in patients with hematological malignancy [19]. In one study, one-third of patients with DLBCL with renal involvement had renal failure. The presence of renal failure was associated with poor outcome [6]. In our patient, renal failure was not identified at the time of diagnosis.

Following the discovery of rituximab, the R-CHOP chemotherapy regimen (rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone) has been accepted as the standard treatment approach in DLBCL [15, 20-23]. However, it is recommended that primary mediastinal DLBCL and high-grade B-cell lymphomas should be treated with more intense chemotherapy regimens, such as DA-EPOCH – R (dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab) [24,25]. There is still no standard approach regarding the treatment regimen to be followed for cases with renal involvement. However, successful cases of treatment with the DA-EPOCH-R regimen have been reported in the literature [26,27].

In conclusion, renal involvement in DLBCL is rare, however, it is associated with poor prognosis. Moreover, it involves an increased risk for CNS recurrence. Therefore, CNS prophylaxis is recommended for such patients. However, randomized controlled trials are required to establish a standard treatment approach.

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