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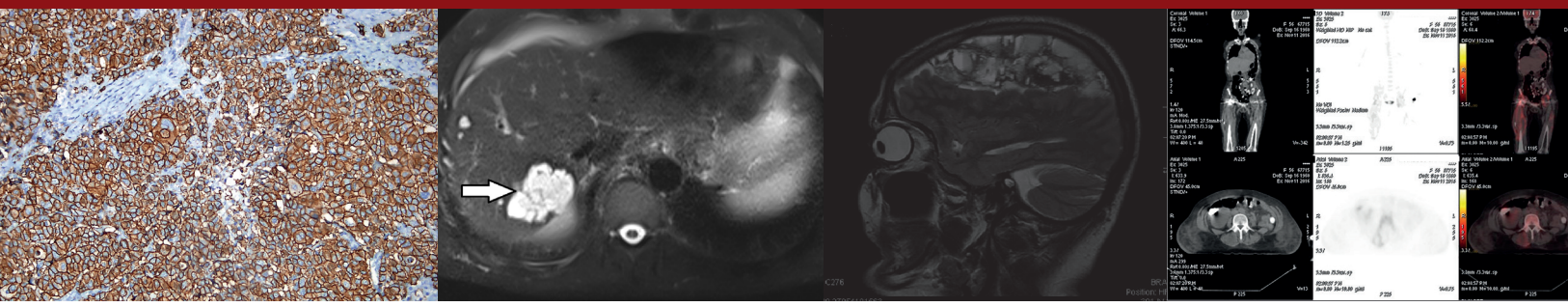

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Original Article

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The Prevalence of Sarcopenia Using Different Formulas in Patients with Prediabetes

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BACKGROUND/AIMS
Prediabetes is one of the causes of sarcopenia. In addition to the absence of a definite formula to make the diagnosis of sarcopenia, there are also limitations in assessing the muscle mass in obese subjects. The aim of the present study was to evaluate the prevalence of sarcopenia using different formulas in patients with prediabetes.

MATERIAL and METHODS
Participants with prediabetes who visited the obesity outpatient clinic between 2013 and 2015 were retrospectively evaluated. Muscle mass ratio (MMR) was calculated as the percentage of total muscle mass divided by body weight, whereas skeletal muscle index (SMI) was calculated as the total muscle mass divided by the square of the height. In addition, the percentage of appendicular lean mass (ALM) divided by body weight, the ratio of ALM divided by the square of the height, and the ratio of ALM divided by body mass index (BMI) were also evaluated.

RESULTS
A total of 524 patients with prediabetes were enrolled into the study. The prevalence of sarcopenia in female patients was 60 (18.13%) using MMR, whereas no sarcopenia was detected using ALM/BMI ratio, SMI, and ALM/height^2. Sarcopenia was detected in 63 (32.64%) male patients using MMR, in 17 (8.81%) using ALM/BMI ratio, and in 1 (0.52%) using each of SMI and ALM/height^2.

CONCLUSION
The prevalence of sarcopenia varies even using ALM/BMI ratio and MMR, which are recommended for evaluation of muscle mass particularly in obese patients with prediabetes. New formulas need to be developed for evaluation of muscle mass to prevent the concealment of sarcopenia in middle-aged patients with prediabetes.

Keywords: Sarcopenia, muscle tissue, prediabetes, body composition, obesity

INTRODUCTION

Insulin resistance plays a significant role in the etiopathogenesis of sarcopenia, which is defined as age-related progressive loss of muscle mass and muscle strength (1-3). It is estimated that sarcopenic obesity will increasingly gain importance in the next decade due to the increasing prevalence of obesity, which is one of the most important risk factors of insulin resistance (3, 4). Although previous studies about sarcopenia have usually been conducted with elder people, thinking that the loss of muscle mass starts after the third decade, the diagnosis of sarcopenia might be underestimated in middle-aged people (3, 5). In addition, there are limitations regarding the evaluation of muscle mass in obese participants due to high body mass index (BMI) and fat percentage (6-8). Another difficulty in diagnosing sarcopenia is the use of many formulas for evaluation of muscle mass and, accordingly, the variety of the prevalence of sarcopenia (1, 5). While some studies use extremity muscle mass to evaluate sarcopenia, others use total muscle mass (5). Nevertheless, some formulas calculate the ratio of muscle mass to body weight, whereas other formulas calculate the ratio of muscle mass to height (5). The aim of the present study was to evaluate the prevalence of sarcopenia using different formulas in patients with prediabetes.

This study was presented at the 16th National Family Medicine Congress, 26-29 October 2017, Ankara, Turkey

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MATERIAL and METHODS

The present study was conducted with data derived from the project entitled "Evaluation of muscle mass in obesity, prediabetes, and diabetes mellitus by different equations used for diagnosis of sarcopenia." Participants with prediabetes aged 18–65 years who visited the Kartal Dr Lütfi Kırdar Training and Research Hospital obesity outpatient clinic between 2013 and 2015 were retrospectively evaluated. In the present study, participants with fasting plasma glucose (FPG) of 100–125 mg/dL, glycated hemoglobin of 5.7%–6.4%, or 2-hour plasma glucose level of 140–199 mg/dL on a 75-gram oral glucose tolerance test were considered as prediabetics (9). The study was approved by the ethics committee of Kartal Dr Lütfi Kırdar Training and Research Hospital (approval no.: 895|3307/1009/537-118).

TABLE I. Distribution of age and bioimpedance measurements among genders

	Female (n=331)	Male (n=193)	p*
Age (year)			0.416
BMI (kg/m^2)	41.35±11.54	40.47±12.27	0.013
Total muscle mass (kg)	38.80±5.71	37.49±5.82	<0.001
ALM (kg)	29.44±4.20	43.43±6.35	<0.001
SMI (kg/m^2)	26.52±4.26	35.55±6.34	<0.001
ALM/height^2 (kg/m^2)	11.76±1.48	14.51±1.82	<0.001
MMR (%)	10.61±1.68	11.85±1.75	<0.001
ALM/BMI	30.57±3.26	39.15±4.92	<0.001
Fat percentage (%)	0.686±0.077	0.953±0.133	<0.001
	41.22±3.43	33.28±5.43	

ALM: appendicular lean mass; BMI: body mass index; MMR: muscle mass ratio; SMI: skeletal muscle index
*Student t-test
All data are presented as mean±standard deviation

TABLE 2. The frequency of sarcopenia among genders according to the formulas used for diagnosis of sarcopenia

	Female (n=331) n (%)	Male (n=193) n (%)
ALM/height^2 (kg/m^2)		
Normal	331 (100)	192 (99.48)
Sarcopenia	0 (0.00)	1 (0.52)
ALM/BMI		
Normal	331 (100)	176 (91.19)
Sarcopenia	0 (0.00)	17 (8.81)
SMI (kg/m^2)		
Normal	331 (100)	192 (99.48)
Mild sarcopenia	0 (0.00)	1 (0.52)
Severe sarcopenia	0 (0.00)	0 (0.00)
MMR (%)		
Normal	271 (81.87)	130 (67.36)
Mild sarcopenia	59 (17.83)	52 (26.94)
Severe sarcopenia	1 (0.30)	11 (5.70)

ALM: appendicular lean mass; BMI: body mass index; MMR: muscle mass ratio; SMI: skeletal muscle index

Measurement methods: After a 12-hour fasting period, all participants were evaluated by a bioelectrical impedance analysis (BIA) device (GAIA 359 PLUS; Jawon Medical, Korea; 2011) with regard to height, body weight, the sum of appendicular lean masses (ALMs) of the four limbs, impedance, and fat percentage. Thereafter, BMI was calculated as body weight in kilograms divided by the square of the height in meters, and total body mass was calculated using the formula: [(height^2 (cm)/BIA resistance×0.401)+(gender×3.825)+(age×-0.071)]+5.102 (10). A skeletal muscle index (SMI), which is calculated as the total muscle mass divided by the square of the height in meters, of ≤5.75 kg/m^2 is considered as severe sarcopenia and 5.76–6.75 kg/m^2 is considered as mild sarcopenia in female patients, whereas an SMI of ≤8.50 kg/m^2 is considered as severe sarcopenia and 8.51–10.75 kg/m^2 is considered as mild sarcopenia in male patients (11). Muscle mass ratio (MMR) is the percentage of total muscle mass divided by body weight, where <22.1% is defined as severe sarcopenia and 22.1%–27.6% is defined as mild sarcopenia in female patients and <31.5% is defined as severe sarcopenia and 31.5%–37.0% is defined as mild sarcopenia in male patients (12). With regard to the other formulas used for diagnosis of sarcopenia, the cut-off value for ALM/BMI ratio is considered to be 0.512 in female patients and 0.789 in male patients, and the cut-off value for ALM/height^2 ratio is considered to be 5.45 kg/m^2 in female patients and 7.26 kg/m^2 in male patients (13, 14). Moreover, FPG of participants was measured by hexokinase method, whereas fasting insulin level was measured by chemiluminescent immunoassay; thereafter, homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the formula: FPG (mg/dL)×fasting insulin (μU/mL)/405 (15).

Exclusion criteria: Patients with type I diabetes mellitus, hyperthyroidism, chronic renal failure, chronic liver failure, and documented neuromuscular disease and pregnant women were excluded from the study. In addition, participants >65 years were also excluded as aging is a risk factor for sarcopenia.

Sample size: The sample size was calculated using the prevalence of 50%, margin error of 5%, confidence level of 95%, and missing data of 20%. The target sample size was determined as 461 patients.

Statistical Analysis

Data were performed by Statistical Package for the Social Sciences version 22.0 program (IBM Corp.; Armonk, NY, USA).Descriptive statistics were presented as frequency, percentage, mean±standard deviation, and median (minimum–maximum). Student's t-test was used for normally distributed continuous variables. Mann–Whitney U test was used for comparison of continuous variables with non-normal distribution. Pearson's correlation test was used to assess the linear association between two variables. A p value of <0.05 was considered as significant for all results.

RESULTS

Of 524 participants with prediabetes, 331 (63.17%) were female. The mean HOMA-IR levels were 4.67 (0.78–22.77) in female and 5.53 (1.50–35.38) in male patients (p<0.001). Distributions of age and bioimpedance measurements among genders are summarized in Table I.

TABLE 3. Participants without and with sarcopenia with regard to age, HOMA-IR, and BMI values by using MMR

	Female (n=331)			Male (n=193)		
	Non-sarcopenic	Sarcopenic	p	Non-sarcopenic	Sarcopenic	p
Age (year)	41.10±11.36	42.50±12.33	0.423*	42.64±12.13	37.29±12.26	0.025*
BMI (kg/m^2)	37.56±4.80	44.37±6.21	<0.001*	35.39±4.71	42.11±6.18	<0.001*
HOMA-IR	4.67 (0.78–22.77)	4.65 (1.96–18.85)	0.625†	5.13 (1.5–35.38)	7.43 (2.47–26.89)	0.003†

BMI: body mass index; MMR: muscle mass ratio; HOMA-IR: homeostatic model assessment of insulin resistance
*Student t-test
†Mann–Whitney U test
All data are presented as mean±standard deviation or median (minimum–maximum), where appropriate

Sarcopenia was found in 60 (18.13%) female patients using MMR, whereas no sarcopenia was detected using SMI. Sarcopenia was detected in 63 (32.64%) male patients using MMR and in 1 (0.52%) male patient using SMI. The frequency of sarcopenia among genders according to the formulas used for diagnosis of sarcopenia is summarized in Table 2.

There was no significant difference between participants without and with sarcopenia of each gender with regard to age, HOMA-IR, and BMI values when sarcopenia was assessed using SMI, ALM/BMI ratio, and ALM/height^2 (p>0.05). Participants without and with sarcopenia with regard to age, HOMA-IR, and BMI values by using MMR are summarized in Table 3. Additionally, a significant relationship was observed between age and MMR in both genders (r=-0.148, p=0.040 for female and r=-0.156, p=0.004 for male).

DISCUSSION

The present study aimed to evaluate the prevalence of sarcopenia in patients with prediabetes using different formulas. While the prevalence of sarcopenia in female patients was 18.13% using MMR, no sarcopenia was determined using SMI, ALM/BMI ratio, and ALM/height^2. In male patients, the prevalence of sarcopenia was 32.64% using MMR, 0.52% using SMI, 8.81% using ALM/BMI ratio, and 0.52% using ALM/height^2.

Previous studies about sarcopenia are usually being conducted in elder people, and various formulas are used to make the diagnosis (5). In a previous study, the prevalence of sarcopenia in female patients was determined as 23.6% using MMR and 2.8% using SMI (16). In another study, the prevalence of sarcopenia in 40–59-year-old female patients was 2.5% using ALM/height^2 and 4.2% using MMR (4). The prevalence of sarcopenia in elder women was found to be 18.9% using ALM/BMI ratio, which is one of the formulas established in recent years to assess muscle mass (13). In the present study, MMR found mild sarcopenia in 17.83% and severe sarcopenia in 0.30% of female patients, whereas SMI, ALM/BMI, and ALM/height^2 determined no sarcopenia. In previous studies evaluating the prevalence of sarcopenia in male patients, it was 0.0%–56.7% using ALM/height^2 and 23.6%–68.0% using SMI (5, 17). In a previous study, the prevalence of sarcopenia in 40–59-year-old male patients was found to be 2.8% using ALM/height^2 and 1.4% using MMR (4). In another study, the prevalence of sarcopenia in male patients was determined to be 12.5% using MMR and 3.6% using SMI (16). Furthermore, the prevalence of sarcopenia in elder male patients was found to be 28.5% using ALM/BMI ratio, which is another formula (13). In the present study, MMR revealed mild sarcopenia in 26.94% and severe sarcopenia in 5.70% of male participants. Nevertheless, the prevalence of sarcopenia was found to be 0.52% using SMI and ALM/height^2 and 8.81% using ALM/BMI ratio. In the present study, the reason for low prevalence of sarcopenia detected using SMI and ALM/height^2 in both genders might be due to higher BMI level than in the literature. ALM/height^2 shows a strong correlation with BMI; therefore, it does not appear to be an appropriate method for assessment of sarcopenia in obese participants (4). Total muscle mass or ALM after adjusted to BMI, fat mass, and height is recommended for evaluation of muscle mass in obese participants (4, 5). ALM/BMI ratio, which was developed for obese participants, determined lower prevalence of sarcopenia in each gender as compared with the literature. This might be due to young and middle-aged participants of the present study, which is different from other studies. The loss of muscle mass begins from nearly 40 years old and becomes apparent from 60 years old (5). In the present study, a higher prevalence of sarcopenia determined using MMR as compared with a population-based study conducted in patients with similar age might be due to the prediabetic study population of the present study, which is a risk factor for sarcopenia (4).

Although male patients have higher muscle mass, the loss of muscle mass with aging is also higher in male patients (2). While some studies performed in older people found the prevalence of sarcopenia to be higher in female patients using MMR, a previous study found it to be higher in male patients (12, 16, 18). In addition, the prevalence of sarcopenia using SMI and ALM/BMI ratio was usually higher in male than in female patients (5, 11, 13, 16-18). In the present study, there was no difference between the genders with regard to the prevalence of sarcopenia using SMI and ALM/height^2; however, the prevalence of sarcopenia was found to be higher in male patients using MMR and ALM/BMI. This might have resulted from higher HOMA-IR in male patients, which is an indicator of insulin resistance (1, 15).

Being one of the rare studies evaluating the prevalence of sarcopenia in patients with prediabetes is the strength of the present study. One of the limitations of the present study was the fact that the duration of prediabetes among the study participants was unknown, and the other limitation is the use of the BIA method to assess particularly ALM. Although dual energy X-ray absorptiometry (DEXA) or magnetic resonance imaging is recommended for measurement of muscle mass, BIA is preferred because it is a practical, portable and low-cost method (10). There are formulas established to assess total muscle mass using BIA; however, previous studies usually prefer DEXA for measurement of ALM (5, 10, 13, 17).

In conclusion, the prevalence of sarcopenia in female participants with prediabetes was 18.13% using MMR, whereas no sarcopenia in female participants was found using SMI, ALM/BMI ratio, and ALM/height^2. The prevalence of sarcopenia in male participants was 32.64% using MMR, 0.52% using SMI, 8.81% using ALM/BMI ratio, and 0.52% using ALM/height^2. The prevalence of sarcopenia shows wide variations even using ALM/BMI ratio and MMR, which are recommended for assessment of muscle mass particularly in obese participants. As prediabetes and insulin resistance are significant risk factors for sarcopenia, new formulas are required to be developed for assessing muscle mass in young and middle-aged obese participants with prediabetes to eliminate the concealment of sarcopenia.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Kartal Dr Lütfi Kırdar Training and Research Hospital (Approval Date: 29.01.2016, Approval Number: 89513307/1009/537-118).

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Awareness of Occupational Diseases in Training Internal Medicine: The Importance of Continuing Education

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BACKGROUND/AIMS
Diagnosis of occupational diseases (ODs) by physicians is one of the most important steps in protecting the health of employees. The aim of the present study was to evaluate the awareness of residents who were training in the internal medicine disciplines on OD.

MATERIAL and METHODS
This was a cross-sectional study. The survey based on self-declaration was administered to first-year and last-year residents training in the internal medicine disciplines of a medical faculty in Ankara University School of Medicine. There was no valid survey used for the assessment of knowledge and awareness of physicians in diagnosing OD. The survey was created by utilizing the questions used in some similar studies and professional experiences of the researchers.

RESULTS
Of a total of 139 physicians, 57.6% stated that they had pregraduate OD training, and 19.3% stated that they had postgraduate OD training. Among the first-year and last-year research assistants, those who expressed that "they questioned occupations while evaluating their patients" were 34.3% and 36.8% of the participants, respectively. With regard to the primary approaches of research assistants upon confronting with rarely occurring complaints and findings, 8.7% of first-year residents and 24.6% of last-year residents stated that "they questioned the patient about environmental and occupational exposure."

CONCLUSION
Our study results indicate that training on ODs during residency and physicians' knowledge and awareness about the methods used in diagnosis, legal aspects, and notification procedures regarding ODs need to be improved and should be developed in their continuing education.

Keywords: Awareness, continuing education, occupational diseases, physicians

INTRODUCTION

According to the International Labor Organization (ILO), 160 million new occupational disease (OD) cases are expected annually, and 10% result in permanent or long-term disability (1). In general, ODs are slowly progressing diseases. The disease may occur even years after leaving the job. It has signs and symptoms that can be seen in many diseases. Therefore, it is important to establish the causal relationship between disease and occupational exposure. It is necessary to take a detailed history about the occupation of each patient to prevent misdiagnosis, process of job in which she/he works, work environment, period of employment and exposures, and intensity of exposures. Taking appropriate and adequate occupational history allows the physician to make correct and early diagnosis, to plan the correct treatment, and to conduct preventive studies in the workplace with the assessment of other employees (2). In addition, detecting OD in a workplace proves that occupational risks are not managed adequately by the employer (3). Therefore, physicians play an important role in the improvement of occupational health and prevention of ODs (2).

The institutions responsible for making the medical diagnosis of ODs in Turkey include hospitals, training and research hospitals of the Ministry of Health, and public university hospitals. Upon diagnosis of an OD, it is obligatory to notify the

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relevant authorities. This is because OD has legal consequences as well. This diagnosis brings about some compensation rights to the employee. The legal diagnosis of OD is made by the Social Security Institution (SSI) in Turkey. In short, the diagnosis of OD and notification of relevant authorities are associated with a series of medicolegal responsibilities. When physicians diagnosed OD, they contribute to the accurate analysis of data regarding occupational health and aid the government in developing the necessary measures about occupational health and safety indirectly. The level of training, knowledge, and awareness of the physicians on OD determines the diagnosis.

The aim of the present study was to evaluate the level of knowledge and awareness of research assistants who were training in the internal medicine disciplines of a public medical faculty hospital in Ankara University School of Medicine to diagnose OD.

MATERIAL and METHODS

This was a cross-sectional design study and was conducted between March 1, 2017, and April 30, 2017.

Study Population and Sample

The study population comprised first- and last-year research assistants (n=278) working in 20 internal medicine disciplines of the medical faculty of Ankara University during 2016-2017. The study sample included 139 physicians after exclusion of those who could not be reached due to pregnancy or maternity leave, annual leave, sick leave, external rotation, out-of-hospital assignment, and those who did not attend due to their excess workload. The reason for selecting the disciplines of internal medicine was that they were the departments in which the diagnoses of ODs and work-related diseases were encountered much often compared with surgical disciplines. Since the medical faculty hospital serves multiple campuses, there were some difficulties in reaching research assistants collectively.

The minimum sample volume and strength analysis of the study were calculated by two independent proportions (null case) power analysis. The study was approved by the ethics committee of Ankara University, Medical Faculty (Decision date: 02/23/2017; No.: 20 96487027-044-E.5469).

Data Collection

Data were obtained with a survey form applied to the participants. Days and hours with lower patient-load in the outpatient clinics, including lecture hours, non-visit hours, and hours with lesser workload in the in-patient clinics, were chosen for data collection to increase participation. All participants were informed about the purpose of the study. Written informed consent was obtained from the participants.

TABLE I. Distribution of some demographic characteristics of the participants according to their years in residency training*

Characteristics	Year of residency training						
	First year		Last year		Total		
	n	%	n	%	n	%	p*
Gender (n=139)							
Female	44	62.9	42	60.9	86	61.9	0.809
Male	26	37.1	27	39.1	53	38.1	
Place of graduation (n=138)							
Medical faculty inside Ankara	37	53.6	35	50.7	72	52.2	0.898
Medical faculty outside Ankara	29	42.0	72	52.2	59	41.0	
Foreign medical faculty	3	4.3	4	5.8	7	5.00	
Medical faculty graduated in Ankara (n=72)							
Ankara University, Medical Faculty	16	43.2	15	42.8	31	22.5	0.881
Hacettepe University, Medical Faculty	13	35.1	13	37.1	26	18.8	
Gazi University, Medical Faculty	5	13.5	6	17.1	11	8.0	
Private University, Medical Faculty	3	8.1	1	2.8	4	2.9	
Occupational diseases training status before graduation (n=139)							
Trained	46	65.7	34	49.3	80	57.6	0.050
Untrained	24	34.3	35	50.7	59	42.4	
Occupational diseases training status after graduation (n=138)							
Trained	21	30.4	20	29.0	41	29.7	0.852
Untrained	48	69.6	49	71.0	97	70.3	
Occupational diseases training status during residency (n=133)							
Trained	15	51.7	53	50.9	68	51.1	0.942
Untrained	14	48.3	51	49.1	65	48.9	

*Column percentage was estimated over responders, p<0.05

Data Collection Tools

Since there was no nationally or internationally valid scale for assessment of physicians' knowledge and awareness about OD, a questionnaire was created by utilizing the questions used in some similar studies and professional experiences of the researchers anonymously. The questionnaire consists of four sections: general information, awareness assessment, knowledge level, and free section.

Pilot Study

The pilot study in which the quality of questions and the duration of filling were evaluated was performed with second-year and three-year research assistants in the internal medicine disciplines of the medical faculty of Ankara University.

Statistical Analysis

The Statistical Package for the Social Sciences for Windows version 15.0 (SPSS Inc., Chicago, IL, USA) was used to calculate the chi-square test. Descriptive categorical data were expressed as count and percentage, whereas continuous data were expressed as mean and standard deviation. The chi-square test was used for comparison of categorical data. A p value of <0.05 was accepted as statistically significant.

TABLE 2. Participants' self-declarations about their statuses of having training and their educations in occupational diseases*

	n	%
Having any pregraduate training in occupational diseases during residency (n=139)		
Yes	80	57.6
No	59	42.4
Evaluation of occupational diseases training before graduation (n=80)		
Sufficient	14	17.5
Partially sufficient	44	55
Insufficient	19	23.8
I do not have any idea, I do not know	3	3.7
Having any postgraduate training in occupational diseases (n=138)		
Yes	41	29.7
No	97	70.3
Evaluation of postgraduate occupational training (n=41)		
Sufficient	8	20.5
Partially sufficient	21	53.8
Insufficient	8	20.5
I do not have any idea, I do not know	2	5.2
Having any training in occupational diseases during residency (n=133)		
Yes	29	21.8
No	104	78.2
Evaluation training in occupational training received during residency (n=29)		
Sufficient	8	27.6
Partially sufficient	14	48.3
Insufficient	6	20.7
I do not have any idea, I do not know	1	3.4

*Numbers and percentages were estimated over the responders of survey questions

RESULTS

Demographic Characteristics of Participants

The mean age of the 139 participants working in the internal medicine disciplines as first-year (n=70) and last-year (n=69) research assistants was 28.65±3.26 (range: 24–41) years, and the mean duration of employment was 4.75±2.91 (range: 1–7) years. Table 1 shows the descriptive characteristics of participants according to residency years. Of the 139 participants, 61.9% were female. There was no statistically significant difference when the residency year was compared with the medical faculties they had graduated (p=0.668). Physicians who attended to the study were from 20 different residency training departments, and most were training on internal medicine (28.8%).

Training of Participants on OD

Among all 139 participants, 57.6% had training in ODs during their medical faculty education, whereas 55% had evaluated this training as "partially sufficient." Participants who had training on OD during their residencies were low [n=29 (21.8%)] and evaluated their education as "partially sufficient" (48%) (Table 2).

Awareness of Taking Occupational History

Physicians who asked patients their professions "always definitely" while taking medical history were low (35.5% of the total participants). Of the 139 participants, 28.3% are informed "rarely/never" asked their professions. With regard to the attitudes about taking medical histories aimed at occupational and environmental exposures, those who stated that they "rarely/never" obtained medical history about occupational and environmental exposures were greater in number (n=79 (56.8%) and n=86 (62.3%), respectively). The majority of participants did not ask patients about their previous jobs. The majority of first-year and last-year residents stated that they "rarely/never" questioned their patients about their occupational and environmental exposures (Table 3). With regard to taking occupational history, 36% of the participants stated that they only asked the last job of their patients, whereas 27.9% asked in a short manner, such as worker, civil servant, retired, or housewife. The rate of participants taking occupational history according to chronological order was found to be 14.7%. Although no statistical significance was found, it was observed that physicians' attitude of inquiring, such as civil servants, workers, retired, and housewives, decreased as the number of working years and their seniorities in residency increased (Table 4).

With regard to the methods used for accessing the diagnosis generally in the daily practice, the attitudes of applying for further laboratory and radiological investigations and searching for the diagnosis through the scientific websites over the internet were higher. On the other hand, the attitudes of asking the patient's environmental and occupational exposures were in the third or fourth place (Table 5). Nevertheless, while physicians' years of seniority increased, it was observed that the number of physicians questioning occupational and environmental exposures is increased (Table 5).

Participants' Awareness of ODs

The participating physicians evaluated their knowledge level about the International Statistical Classification of Diseases system of ODs, ILO list of ODs, notification of ODs, legal aspect of ODs, and access to basic information sources about the issue as "insufficient." They considered their knowledge level about the diagnosis, treatment, social aspect, and prevention of ODs as "partially sufficient," and there was no statistically significant difference between the two groups (Table 6). There was no difference among the groups when self-declarations about knowledge level were compared according to whether they had pre-graduate and postgraduate training in ODs or according to their years in residency. The percentage of physicians who reported that OD was almost completely preventable was 87.1%.

Most of the participants reported that the specialty that was most concerned about ODs was pulmonary medicine (61.8%). Of the 139 participants, 26.6% stated that pneumoconiosis and contact dermatitis were the most commonly diagnosed ODs in Ankara University School of Medicine (Figure 1). Of those, 35.9% stated that the most common OD worldwide was "work-related stress," ranking in the first place. This was followed by contact dermatitis in the second place with 28% (Figure 2).

With regard to the physicians' knowledge about the institutions authorized to diagnose ODs and about the fact that SSI is responsible for the legal diagnosis of OD, the majority of physicians did not know these two legislations correctly. There was

TABLE 3. Asking questions about occupational and environmental exposures by the participants in their medical practices*

Frequency of the questions asked related to the occupations of patients during patient examination in daily practice	First-year assistant	Last-year assistant	
	n (%)	n (%)	p*
Questioning occupation			
Always definitely	24 (34.3)	25 (36.8)	0.700
Sometimes	24 (34.3)	26 (38.2)	
Rarely/never	22 (31.4)	17 (25.0)	
Taking medical history encompassing the occupations performed before			
Always definitely	7 (10.0)	12 (17.4)	0.374
Sometimes	21 (30.0)	22 (31.9)	
Rarely/never	42 (60.0)	35 (50.7)	
Questioning occupational exposure			
Always definitely	4 (5.7)	8 (11.6)	0.467
Sometimes	25 (35.7)	23 (33.3)	
Rarely/never	41 (58.6)	38 (55.1)	
Whether s/he has a work-related disease or not, asking occupational exposure			
Always definitely	8 (11.4)	11 (15.0)	0.232
Sometimes	31 (44.3)	21 (30.4)	
Rarely/never	31 (44.3)	37 (53.6)	
Asking/evaluating the opinion of the patient with respect to whether there is a relationship between her/his disease and occupation			
Always definitely	7 (10.0)	4 (5.8)	0.436
Sometimes	24 (37.1)	32 (46.4)	
Rarely/never	37 (52.9)	33 (47.8)	
Asking non-occupational environmental exposure			
Always definitely	5 (7.1)	9 (13.2)	0.465
Sometimes	19 (27.1)	19 (27.9)	
Rarely/never	46 (65.7)	40 (58.8)	
Asking environmental exposure			
Always definitely	7 (10.0)	9 (13.0)	0.844
Sometimes	25 (35.7)	23 (33.3)	
Rarely/never	38 (54.3)	37 (53.6)	
Asking/evaluating the opinion of the patient with respect to whether there is a relationship between her/his disease and non-occupational environment			
Always definitely	6 (8.6)	5 (7.2)	0.942
Sometimes	20 (28.6)	21 (30.4)	
Rarely/never	44 (62.9)	43 (62.3)	

*Column percentages over the responses given. There was no statistically significant difference among the groups of all participants in the evaluation of the responses to the questions, p<0.05

TABLE 4. Comparison of some characteristics of the participants with respect to making the diagnosis of occupational disease*

Some characteristics of diagnostic process	First-year assistant n (%)	Last-year assistant n (%)	p*
Whether the medical history form includes the section inquiring the occupation of the patient or not			
Present	38 (55.1)	32 (47.1)	0.634
Absent	23 (33.3)	26 (38.2)	
S/he does not know	8 (11.6)	10 (14.7)	
How the occupation of the patient is asked generally while taking medical history			
Those who ask only the last occupation s/he is working	23 (33.3)	26 (38.8)	0.554
Those who ask the occupation and exposure of each patient in chronological order	9 (13.0)	11 (16.4)	
I only ask the lines of work	2 (2.9)	3 (4.5)	
Those who make the inquiry, such as civil servant, retired, and housewife	23 (33.3)	15 (22.4)	
Those who do not make inquiry	10 (14.5)	7 (10.4)	
Others	2 (2.9)	5 (7.5)	
Follow-up of cases in which occupational disease was detected during residency			
Yes	13 (18.8)	21	0.114
No	56 (81.2)	48 (69.6)	
Making the diagnosis of occupational disease related to his/her specialty during residency up to today			
Yes	7 (10.3)	19 (27.5)	0.010
No	61 (89.7)	50 (72.5)	

*Column percentages of the responses given, p<0.05

TABLE 5. Comparisons of approaches to reaching the diagnosis depending on the year of residency in the daily practice of research assistants*

Method of reaching the diagnosis		Cases			
		Primary approach when rare complaints and findings are encountered	Primary approach to the case who cannot be diagnosed despite the investigations performed	Approach to the patient unresponsive to primary treatment	Primary approach to diseases that relapse frequently despite appropriate treatment
Requesting further laboratory and radiological investigations	First year n_1 (%)	17 (24.6)	4 (5.8)	26 (37.7)	30 (43.5)
	Last year n_2 (%)	21 (30.4)	3 (4.3)	29 (42)	39 (56.5)
Doing research on the internet through scientific websites	First year n_1 (%)	26 (37.7)	33 (47.8)	25 (36.2)	13 (18.8)
	Last year n_2 (%)	16 (23.2)	25 (36.2)	18 (26.1)	8 (11.6)
Initiating empirical therapy and reaching the diagnosis through treatment	First year n_1 (%)	14 (20.3)	5 (7.2)	2 (2.9)	3 (4.3)
	Last year n_2 (%)	10 (14.5)	6 (8.7)	3 (4.3)	3 (4.3)
Discussing in the council	First year n_1 (%)	6 (7.6)	18 (26.1)	12 (17.4)	14 (20.3)
	Last year n_2 (%)	5 (7.2)	22 (31.9)	11 (15.9)	4 (5.8)
Asking the environmental and occupational exposures of the patient	First year n_1 (%)	6 (8.7)	9 (13)	4 (5.8)	9 (13.0)
	Last year n_2 (%)	17 (24.6)	13 (18.8)	8 (11.6)	15 (21.7)

n_1: First-year research assistant, n2: Last-year research assistant

*Column percentages over the responses given

TABLE 6. Comparison of the participants' self-assessment related to their knowledge of occupational diseases*

Self-assessment of their knowledge related to the diagnosis, treatment, and notification of occupational diseases	First-year assistant	Last-year assistant	
	n (%)	n (%)	p*
Notification of occupational diseases			
Sufficient	2 (2.9)	1 (1.5)	0.267
Partially sufficient	33 (47.8)	24 (35.3)	
Insufficient	34 (49.3)	43 (63.2)	
International Statistical Classification of Diseases code list of your specialty's occupational diseases			
Sufficient	3 (4.3)	5 (7.2)	0.427
Partially sufficient	24 (34.8)	17 (24.6)	
Insufficient	42 (60.9)	47 (68.1)	
ILO list of occupational diseases			
Sufficient	1 (1.5)	6 (8.8)	0.102
Partially sufficient	23 (34.3)	17 (25.0)	
Insufficient	43 (64.2)	45 (66.2)	
Diagnosis of occupational diseases			
Sufficient	5 (7.4)	3 (4.4)	0.126
Partially sufficient	45 (66.2)	36 (52.2)	
Insufficient	18 (26.5)	30 (43.5)	
Treatment of occupational diseases			
Sufficient	4 (5.9)	3 (4.4)	0.300
Partially sufficient	37 (54.4)	29 (42.6)	
Insufficient	27 (39.7)	36 (53.0)	
Protection against occupational diseases			
Sufficient	6 (9.0)	7 (10.3)	0.115
Partially sufficient	44 (65.7)	33 (48.5)	
Insufficient	17 (25.4)	28 (41.2)	
Whether the subspecialty program of your specialty contains occupational diseases or not			
Sufficient	16 (23.5)	20 (29.9)	0.407
Partially sufficient	20 (29.4)	14 (20.8)	
Insufficient	32 (47.1)	33 (49.3)	
Access of your specialty to basic information sources related to occupational diseases			
Sufficient	9 (13.4)	11 (16.2)	0.303
Partially sufficient	25 (37.3)	17 (25.0)	
Insufficient	33 (49.3)	40 (58.8)	
Legal aspect of occupational diseases			
Sufficient	3 (4.4)	5 (7.4)	0.263
Partially sufficient	24 (35.3)	16 (23.5)	
Insufficient	41 (60.3)	47 (69.1)	
Social aspect of occupational diseases			
Sufficient	7 (10.3)	5 (7.6)	0.845
Partially sufficient	31 (45.6)	32 (48.5)	
Insufficient	30 (44.1)	29 (43.9)	

*Column percentages of the responses given, p<0.05

no statistical difference in the comparison of this knowledge among the groups (Table 7).

The rate of physicians who knew the fact that making the diagnosis of an OD correctly might have protected the physician against malpractice was 71.9%.

General Opinions, Concepts, and Suggestions

Common responses given to the open-ended questions were categorized by a biostatistician, and variables were compared.

When their "opinions about work-related diseases" were asked, 27 out of 46 responders considered that the subject was not well known, and that the awareness of it was insufficient.

The answers given by 50 doctors to the question "What are the difficulties you have experienced in the process of diagnosis, treatment, and notification of OD related to your specialty?" were categorized. Seven doctors stated that they did not have any knowledge, four doctors stated that OD did not come to their mind while examining their patients, and eight research assistants reported that they did not see enough patients about this issue. Eighteen doctors reported that they had lack of knowledge about the diagnosis, treatment, and notification of OD.

The answers of 47 doctors to the question "What are your opinions and suggestions on the development of Job and Occupational Subspecialist Programs in your country?" were categorized. Four doctors had opinion that the subspecialty program should be included in more specialties in addition to Pulmonary Medicine, Public Health, and Internal Medicine. There were 17 participants who stated that there was no academic basis, and that this subject should be supported and developed.

TABLE 7. Participants' knowledge of some issues related to the legal process and frequency of occupational diseases*

Legal process and frequently related questions	First-year assistant n (%)	Last-year assistant n (%)	p*
Institutions responsible for making the medical diagnosis of occupational disease in Turkey			
Those who know correctly	24 (34.3)	27 (39.7)	0.510
Those who misknow	46 (65.7)	41 (60.3)	
Institution making the legal diagnosis of occupational disease in Turkey			
Those who know correctly	4 (5.9)	4 (6.0)	1.000
Those who misknow	64 (94.1)	63 (94)	
The most common occupational disease announced by the official institutions in Turkey			
Those who know correctly	24 (34.3)	13 (19.1)	0.044
Those who misknow	46 (65.7)	55 (80.9)	
The most common occupational disease worldwide			
Those who know correctly	9 (13.0)	5 (7.4)	0.272
Those who misknow	60 (87.0)	63 (92.6)	

*Column percentages of the responses given, p<0.05

TABLE 8. Opinions of the participants on the training program to raise awareness of occupational diseases*

Suggestions for raising awareness	Yes n (%)	No n (%)
A postgraduate certificate program related to occupational disease for raising awareness	15 (10.9)	123 (89.1)
Presence of the occupational disease department/discipline in universities for raising awareness	34 (24.6)	104 (75.4)
Putting on a curriculum related to occupational disease in the pregraduate training for raising awareness	54 (41.9)	75 (58.1)
Putting on a specialty-specific training program related to occupational disease in the residency programs for raising awareness	23 (16.7)	115 (83.3)

*Numbers and percentages were estimated over the responders of survey questions

DISCUSSION

We determined that during the residency training, the number of physicians who have awareness and knowledge about OD is very low.

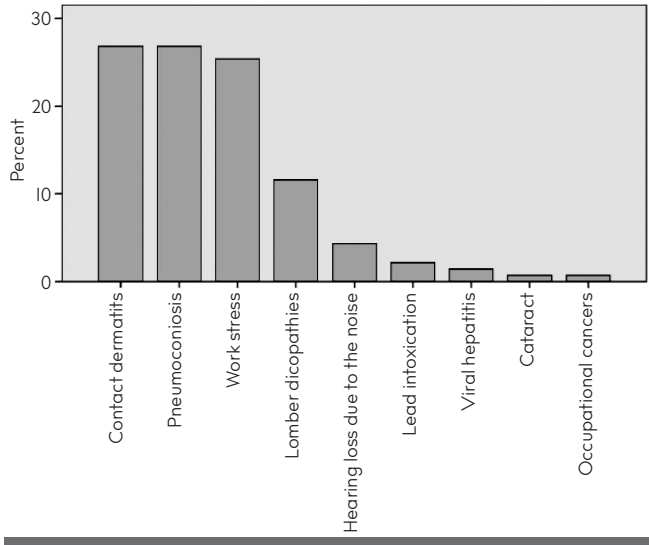

FIGURE 1. Distribution of the responses given by participants to the question "What is the most common occupational disease in Turkey according to official registrations?" (n=139)

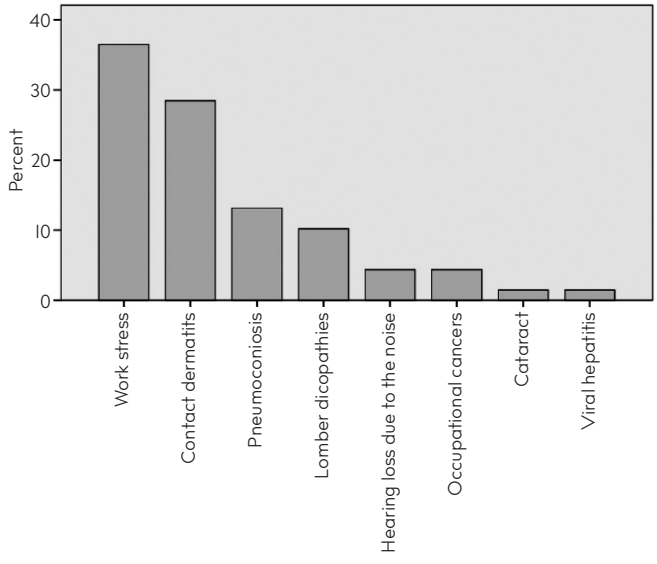

FIGURE 2. Distribution of the responses given by participants to the question "What is the most common occupational disease in the world?" (n=139)

Providing Continuing Medicine Training in OD

There is limited research evaluating the knowledge and awareness of physicians about OD during residency training. Previous studies were mostly about testing the level of awareness and knowledge of the recognition of a disease or evaluating the obstacles experienced by clinicians in notifying ODs or detecting pre-graduate education (4, 5). To our knowledge, our study is the first study evaluating the awareness of ODs in the residency training period.

Recently, the development of evidence-based medical education is important (6). The striking results of our research will be effective in identifying and improving the deficiencies in ODs in the pre- and postgraduate medical education programs in Ankara University School of Medicine.

We found that the number of physicians who had pregraduate training in ODs was low. Pregraduate training in occupational health and ODs is usually given as a theoretical course by the public health department in the third and fifth grades. Generally, the issues related to ODs are 1/10 of all public health issues. These topics are covered approximately 8 h/year in the whole training program. Workplace visits are performed in the sixth grade. However, these training and applications are not available in all medical faculties (5). In the United States and in some European countries, occupational medicine training is mandatory during the medical training program, and it is covered within general clinical specialties. The specialties that give training for becoming master and board-certified occupational medicine specialist are the clinics where occupational medicine training is given (7). OD and occupational medicine approaches in other branches are inadequate. In addition, there are very few training programs covering the relationships between environment and occupation in the medical faculties (8, 9).

Physicians find themselves insufficient regarding the diagnosis, follow-up, and notification of OD during their residency training in which they are confronted with the patient individually and make their diagnoses, treatments, and follow-ups. Although the continuity of training is very important in the profession of medicine, setbacks in the postgraduate theoretical and practical training of OD lead to inadequacies in residents' attitudes toward diagnosing ODs. However, the role of clinicians, especially that of the attending physicians working in the departments of internal medicine disciplines, is very important in being able to diagnose ODs and in the indirect prevention by making notification (10).

In Turkey, ODs are not a separate specialty branch. The Occupational Disease subspecialty program has been available in five medical faculties for the last years. It still cannot provide as a specific department. The lack of departments to improve occupational health and ODs medicine causes delays in the identification of cases with these diseases that already face a complex diagnostic process and medicolegal outcomes and even leads to deaths before they can be diagnosed.

Taking Correct Occupational History in Residency Training

The most important criterion for diagnosing OD is to determine the presence of a relationship between exposure agent and specific disease. For this, it is very important to take detailed occupational history including past exposures (2, 10, 11). The present study showed that physicians with residency training are aware of the importance of occupational history, but they have insufficient knowledge regarding the need to obtain a detailed occupational history according to chronological order to reach the source of environmental and occupational exposures. Similarly, in a research that was evaluating the knowledge, attitude, and behaviors of practitioners, pulmonologists, and rheumatologists on occupational health and ODs in France, the inquiry about the retrospective occupational exposures of patients by physicians was found to be very low (10). Although the majority of students agreed on the importance of taking occupational history, they considered that physicians did not take occupational history according to the results of a survey conducted on sixth-grade students in Ankara University School of Medicine (5). In some studies, the reasons for the inadequacy of occupational history ranked as the lack of time, lack of knowledge and motivation, and being unaware of the subject (10, 12). Although we do not focus primarily on this issue, having insufficient training on OD may have an effect.

According to a previous study conducted in a medical faculty in Ankara University, it was determined that 43.9% of the physicians did not take any information related to occupation from their patients in the patient records examined (13). In our study, physicians who took occupational history were low, but there was an increase in last-year residency physicians about following up a patient with an OD and diagnosing OD. This increase may be related to the higher number and variable of patients visited and followed up for 4 years. This may cause changes in the attitudes of last-year research assistants toward taking occupational history.

Attitudes Toward Diagnosing OD and Barriers

Diagnosis OD is quite a complex process. As in many countries, in Turkey, the legal diagnosis of OD is performed by the SSI following medical diagnosis. It carries the issue to a legal dimension where employee's financial rights are related to compensation. Not only for this responsibility of the physicians but also during the process of proceeding to the step in which treatment is provided with making the correct diagnosis and keeping away from exposure are important. It was determined that physicians generally have a lack of information about their legal responsibilities (14). Physicians during their residency training have inadequate knowledge related to the institutions playing a role in the legal process of ODs. The lack of knowledge in legal responsibilities usually causes poor quality of healthcare services and unwillingness to take legal responsibility (14).

In our study, physicians reported that the most commonly recorded ODs in Turkey were pneumoconiosis and contact dermatitis, followed by work-related stress. They had been thinking that the most common OD worldwide was work-related stress. The most commonly notified and diagnosed OD in Turkey is pneumoconiosis. It is a striking finding that physicians ranked work-related stress among the leading problems in Turkey and worldwide. Data on work-related stress are not clear in Turkey. Although work-related stress and psychological disorders are reported to increase worldwide, they are not ranked in the first three diseases (15).

Many factors such as deficiencies and difficulties brought about by legal regulations, complexities in reporting and diagnostic system, lack of diagnostic guidelines about OD, lack

of diagnostic standardization for some OD, physicians' knowledge and awareness level of OD, and avoidance of legal difficulties make it difficult to diagnose OD in Turkey as in the world. Physicians self-assessment was showed that the level of the knowledge about the medical and legal diagnostic processes were inadequate in our study. Despite this, it is a contradictory finding that participants did not want to have certification training or other postgraduate training for raising their awareness of diagnosing OD (Table 8). On the contrary, physicians in France suggest that there should be certification programs on OD (10). In our study, the reason for this contradictory finding may be associated with heavy residency training conditions and more responsibilities placed on physicians in Turkey. In addition, the initiation of a legal process with the diagnosis of OD and the employee confronted with several social problems, such as unemployment, may affect the physicians reducing willingness for being trained and being experienced. Similarly, in a previous study that investigated the barriers to diagnosing occupational asthma by pneumologists in Canada, insufficiency of physicians' awareness and knowledge, limited time allocated for the patient, and the concern that it may cause unemployment were determined as barriers (16).

Since clinicians focus primarily on treating the current disease and clinical manifestations, the patient's workplace conditions can be overlooked, and the avoidance of the exposure in the workplace cannot be ensured even if the relationship between disease and occupation is established (17). We determined that physicians who were having residency training in the internal medicine disciplines do not know that ODs are preventable diseases.

According to the data published by the Ministry of Labor and Social Security in Turkey in 2016, the number of cases in whom ODs were detected was 597, whereas the expected number of cases varied between 40.000 and 120.000 (18). The lack of knowledge and awareness of OD during the pre- and postgraduate training of physicians may be one of the reasons for the lack of these data.

When the association between disease and exposed factor cannot be established, ODs cannot be treated, and they may cause the progression of disease and permanent disability. If the physician skips the index case, it affects the ongoing insufficient hygienic conditions of the workplace indirectly.

The study was conducted on first- and last-year research assistants in the internal medicine disciplines of a single university hospital, and to the best of our knowledge, there was no such study with which we can compare our results with another hospital. Although the fact that we could not generalize our survey results to Turkey is one of the limitations of our study, the lack of any other study evaluating the resident physicians' knowledge of OD makes our study important.

Another limitation of our survey was that we did not include all research assistants including surgical disciplines, and the number of participants was limited.
Since there is no internationally valid scale for the awareness, knowledge, attitude, and behaviors of physicians about OD, using the question form created by us anonymously was another limitation of our study. However, the strong aspects of our study were utilizing the clinical observations of experienced physicians of pulmonology and ODs while creating the question form and participation of experienced researchers with public health specialty during the preparation of the questionnaire.

In conclusion, the present study emphasized that the research assistants' levels of awareness and knowledge on OD were low. The decline in the postgraduate training of physicians indicates that training in these fields is interrupted. Postgraduate and residency training programs about OD should be revised to ensure continuing education. All departments of medical sciences should be interested in OD. OD should be managed with multidisciplinary approach. In the short term, it can be possible to establish committees at departments that are frequently encountered with ODs, such as chest diseases, dermatology, and physiotherapy, and these committees can be responsible for the diagnosis, notification, and development of training programs.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ankara University School of Medicine. (Approval Date: 23.02.2017, Approval Number: 20 96487027-044-E.5469).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - D.Ç., B.Ü.; Design - D.Ç., B.Ü.; Supervision - D.Ç., M.E.O., B.Ü.; Resource - B.Ü., G.S.; Materials - B.Ü., D.Ç.; Data Collection and/or Processing - B.Ü., G.S.; Analysis and/or Interpretation - B.Ü., D.Ç., M.E.O.; Literature Search - B.Ü., G.S.; Writing - B.Ü., D.Ç., M.E.O., G.S.; Critical Reviews - B.Ü., D.Ç., M.E.O., G.S.

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Original Article

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Impact of the Mean Platelet Volume on Muscle-Invasive Bladder Cancer: An Initial Report From a Pioneer Center

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BACKGROUND/AIMS
The mean platelet volume (MPV) is used nowadays to predict the cancer prognosis. As the incidence of muscle-invasive bladder cancer (MIBC) is rising, we aimed to determine the disease-specific survival (DSS) and overall survival (OAS) rates in MIBC using MPV in patients who underwent radical cystectomy.

MATERIAL and METHODS
This is a retrospective analysis of data from an ongoing bladder cancer project. A total of 168 patients with bladder cancer who underwent cystectomy were enrolled between February 2006 and 2016. A potential prognostic value of MPV was evaluated using the receiver operating characteristic (ROC) curve analysis. We examined the impact of MPV on patients' DSS and OAS. ROC curves were drawn. A p-value <0.05 was considered to be statistically significant.

RESULTS
The mean age was 62.10±9.26 years. There were 156 (92.9%) men and 12 (7.1%) women. The optimal MPV cutoff value for DSS was 8.37fL. The group with a lower MPV showed worse progression with regard to DSS and OAS (p<0.001). Age (p=0.024), MPV (p=0.001) were independent prognostic factors for predicting DSS. Additionally age (p=0.003), and MPV (p<0.001) were determined as independent prognostic factors for predicting OAS.

CONCLUSION
A decreased MPV can be an independent prognostic factor in patients with MIBC. If the MPV is lower than 8.37fL, DSS and OAS may worsen.

Keywords: Bladder cancer, cystectomy, mean platelet volume

INTRODUCTION

Bladder cancer (BC) is the seventh most common cancer in males (1). In addition, it is the 11th most common cancer when both genders are considered worldwide (1). The age-standardized annual incidence rate for men and women is 9/100.000 and 2.2/100000, respectively (2). Nearly 75% of patients with BC are diagnosed at the stage Ta, carcinoma in situ (disease confined to the mucosa), or stage T1 (submucosa). Furthermore, many cases of the patient population (Ta, T1, and carcinoma in situ) have a high prevalence of a long-term follow-up and have a lower risk of cancer-specific mortality than T2–4 tumors (3, 4).

Surgical treatment of muscle-invasive bladder cancer (MIBC) as the radical cystectomy is considered to be standard surgical treatment modality. It is also indicated in those with high-risk and recurrent non-muscle invasive BC (5). The remaining life span depends, of course, on several factors.

On the other hand, platelets play one of the essential roles in human carcinogenesis (6). Thus, the interaction causes their involvement in many malignancies and contributes to the progression (6). The platelet count with the mean platelet volume (MPV) can measure the platelet activation (7). Recent studies suggest that the platelet activation can show a

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significant biological process related to cancer formation and metastasis (8-10). With this in view, some blood tests may help clinicians to predict the process of survival after a radical cystectomy in MIBC.

The MPV, as an early indicator of the platelet activation, is altered in several malignancies; however, there is limited information about using MPV to predict survival of patients with MIBC who underwent radical cystectomy.

In this study, we investigated the impact of MPV on survival in patients with MIBC who underwent radical cystectomy. Moreover, to the best of our knowledge, this is the first study on this issue from our community that is including a high volume patients.

MATERIAL and METHODS

The present study is a part of an ongoing study that includes a retrospective assessment of the institution's data. The board of Izmir Katip Celebi University approved the study. Signed consent forms were obtained from all patients. There were a total of 168 patients with MIBC who underwent cystectomy between February 2006 and 2016 at our department. The exclusion criteria were having another cancer, irregular follow-up, chronic drug use that could change blood parameters, any hematologic disease, and patients with incomplete transurethral resected bladder cancer.

Briefly, all blood samples were collected from the antecubital veins, after at least 12 hours of fasting, and the same laboratory checked the results. Radical cystectomies were performed 1 month after transurethral resection bladder tumor (TURBT). The medical data were obtained through the patients' medical records from the urology department and the electronic database of the hospital, and they included patient's age, gender, the pathological T stage, tumor grade, lymph node metastasis, lymphovascular invasion, white blood cell (WBC) count, and MPV. Laboratory data were collected within 1 week before radical cystectomy. A histological evaluation of BC was performed according to the World Health Organization classification (11), and BC was staged according to the TNM criteria (12).

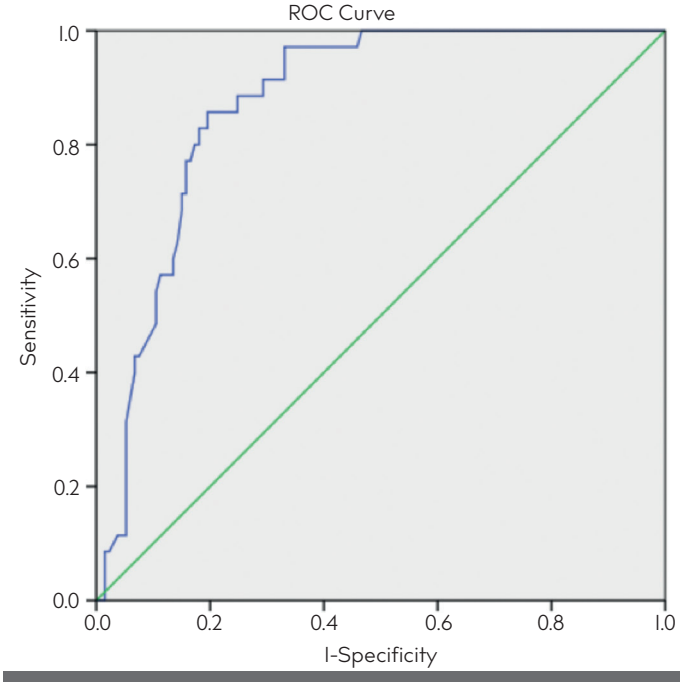

FIGURE 1. ROC analysis for disease specific survival for preoperatively measured mean platelet volume

Mortality causes were obtained by searching the computer-saved database of the institute. Disease-specific survival (DSS) and overall survival (OAS) were defined as the time from the date of surgery to the date of disease-specific mortality and as the time from the date of surgery to the date of all-cause mortality, respectively.

We used the Statistical Package for the Social Sciences software version 22 (IBM Corp.; Armonk, NY, USA). All variables were examined using visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov). A normal or abnormal distribution was determined. The descriptive analyses were used, and the results were presented as means (±standard deviations) and medians (min–max).

An optimal cutoff value of MPV was calculated using the receiver operating characteristic (ROC) curves. The Kaplan–Meier method and log rank test were performed to compare the variables and determine the impact of MPV on patient DSS and OAS. The effect of patient, age, gender, the pathological T stage, tumor grade, lymphovascular invasion, and lymph node involvement were determined by using univariate and multivariate Cox regression hazard models. A 5% Type I error level was used to obtain statistical significance. The p-value $p<0.05$ was accepted as statistically significant.

RESULTS

There were a total of 159 patients with MIBC who underwent radical cystectomy in this series. The mean age was 62.10±9.26 years (range, 37–85). There were 156 (92.9 %) men and 12 (7.1%) women. The lymphovascular invasion was positive in 51 patients (30.4%). There were 40 (23.8%) patients at the T1 stage, 43 (25.6%) patients at the T2 stage, 51 (30.4%) patients at the T3 stage, and 34 (19.5%) patients at the T4 stage. The lymph node invasion was determined in 61 (36.2%) patients. The mean WBC was 8.65±1.88 $\times 10^9$/L, and the mean MPV was 9.30±1.96 fL. The ROC analysis showed that there was an optimal threshold of MPV for DSS at the level of 8.37 fL (Figure 1). This showed us that MPV can estimate the MIBC prognosis with sensitivity and specificity of 91.4% and of 70.0%, respectively (AUC, 0.873; 95% confidence interval [CI], 0.820-0.927). Two groups were created according to the MPV levels. There were 71 (42.2%) patients with MPV<8.37 fL and 97 (57.8%) with MPV ≥8.37 fL.

In the Kaplan–Meier analyses, the group with a decreased MPV showed a worse progression in DSS and OAS ($p<0.001$, $p<0.001$) (Figure 2). The mean follow-up period was 27.59±26.7 months (range, 1–130 months), and 36 patients died during the observation period.

Age (HR, 1.039; 95% CI, 1.005–1.075; p=0.024) and MPV (HR, 17.907; 95%CI, 3.515–91.220; p=0.001) were used to predict DSS; and age

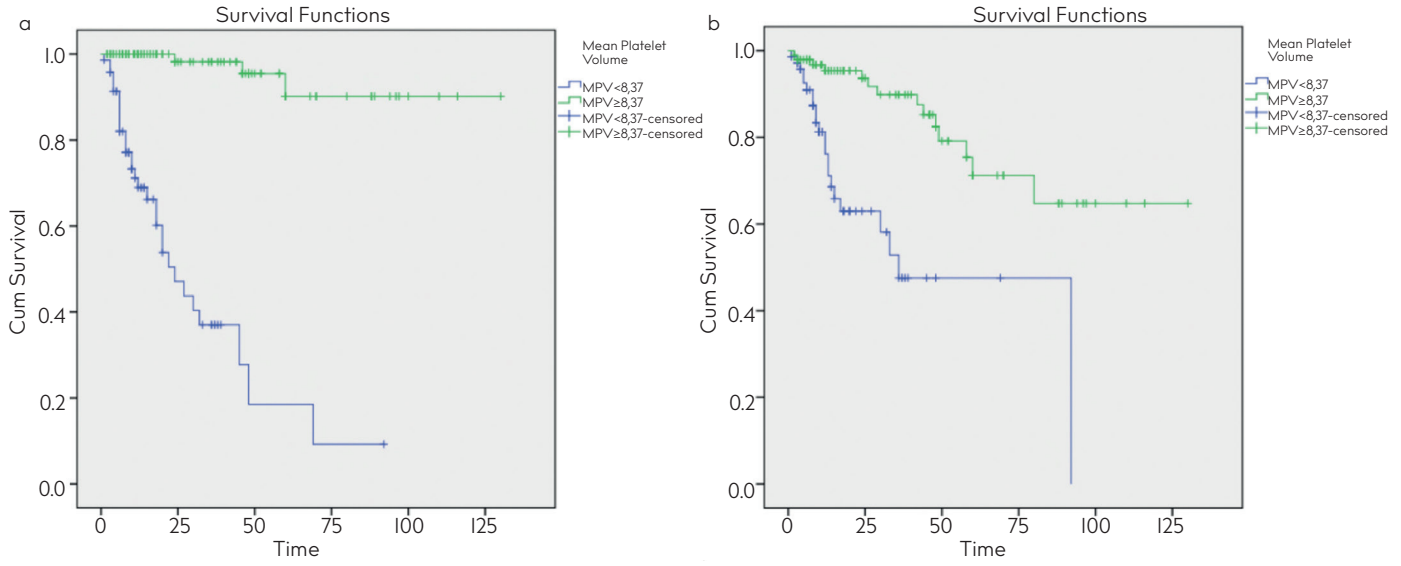

FIGURE 2. a-b. Kaplan–Meier analysis of disease-specific survival according to the mean platelet volume following cystectomy for bladder cancer (a). Kaplan–Meier analysis of overall survival according to the mean platelet volume after cystectomy for muscle invasive bladder cancer (b)

TABLE I. Univariate analyses of various parameters for disease specific survival and overall survival

Parameters	DSS HR (95%CI)	p	OAS HR (95%CI)	p
Age	1.035 (0.998–1.073)	0.066	1.054 (1.016–1.093)	0.005*
Gender (female vs. male)	0.339 (0.129–0.888)	0.028*	0.867 (0.205–3.664)	0.846
Lymphovascular invasion (negative vs. positive)	3.943 (1.955–7.951)	<0.001*	1.541 (0.719–3.306)	0.266
Pathological T stage (T1–T2 vs. T3–T4)	22.020 (6.578–73.715)	<0.001*	2.293 (1.149–4.573	0.019*
Tumor grade (G1 vs. G2–G3)	3.268 (0.445–23.971)	0.244	1.061 (0.324–3.488)	0.922
MPV (≥8.37 vs. 8.37<)	32.477 (9.658–109.210)	<0.001*	4.852 (2.378–9.898)	<0.001*
Lymph node involvement (negative vs. positive)	4.691 (2.315–9.502)	<0.001*	1.670 (0.846–3.298)	<0.139*

DSS: disease specific survival; OAS: overall survival; MPV: mean platelet volume
*Statistically significant p-value

(HR, 1.055 95% CI, 1.019–1.093; p=0.003) and MPV (HR 8.031, 95% CI, 2.930–22.012; p<0.001) were used to predict OAS. These were determined as independent prognostic factors, in terms of univariate and multivariate analyses in Cox regression models, respectively (Table I, 2).

DISCUSSION

There was a statistically significant low MPV level in patients with MIBC who underwent radical cystectomy, just before the operation. The relationship between inflammation and cancer has been identified (13). The MPV is one the markers of activated platelets. Thus, a low MPV level could be regarded as an increased consumption of large platelets during inflammation (14).

Platelets' microparticles and granules are responsible for the platelet–tumor cell interaction, as they contain some growth factors, chemokine, adhesive particles, and coagulation factors (15). Besides, platelets adhere to tumor cells and to the vessel wall after activation (16). These interactions lead to an increased tumor cell survival. Therefore, platelet aggregation to withstand the intravascular shear potency better and armor tumor cells from immune system of host (6). In addition, platelets may play an important role in the cancer progression cascade with an ultimate joint use of mediators and pathways of inflammation (17). Moreover, platelets release the transforming growth factor β1 that induces phenotypic changes of the epithelial to mesenchyme-like transition of tumor cells. Therefore, platelets facilitate their extravasation to distant sites, while metastasis is forming. The activated platelets release secretory factors that support chemokine, photolytic enzymes, and micro particles within the microenvironment to organize the tumor cell invasion (6). These results are also parallel to the current literature indicating that anti-platelet treatment is a part of cancer adjuvant therapies (18). In brief, platelets may play a major role in the tumor process. This was the starting point of our study.

Connolly et al. (19) showed an association between the platelet activities in cancer patients. Stegnet et al. (20) added to their finding and reported the platelet contribution to cancer metastasis. These studies guided us particularly in cancer processes, including cancer cells spreading to organs through blood circulation; platelets are the backbone of malign cells' aggregation (19, 20). The platelet activation parameters (soluble P-selectin, soluble CD40 ligand, and platelet factor 4) were determined to be higher in cancer patients than in cancer-free patients (21, 22). In this manner, we could show that an optimal threshold of MPV for DSS was 8.37 fL. These findings are parallel to the findings in the literature above.

TABLE 2. Multivariate cox models of clinicopathological parameters as predictors of bladder cancer

Parameters	DSS HR (95%CI)	p	OAS HR (95%CI)	p
Age	1.039 (1.005–1.075)	0.024*	1.055 (1.019–1.093)	0.003*
Gender				
Female	1 (referent)	0.815	1 (referent)	0.593
Male	0.890 (0.335–2.367)		1.492 (0.344–6.472)	
Lymphovascular invasion				
Negative	1 (referent)	0.315	1 (referent)	0.728
Positive	1.527 (0.669–3.486)		0.846 (0.330–2.169)	
Pathological T stage				
T1–T2	1 (referent)	0.15	1 (referent)	0.306
T3–T4	3.466 (0.639–18.797)		0.565 (0.189–1.687)	
Tumor grade				
G1	1 (referent)	0.317	1 (referent)	0.588
G2–G3	0.305 (0.030–3.121)		0.700 (0.192–2.549)	
Lymph node involvement				
Negative	1 (referent)	0.319	1(referent)	0.446
Positive	1.543 (0.657–3.620)		1.419 (0.576–3.492)	
MPV				
8.37 or greater	1 (referent)	0.001*	1 (referent)	<0.001*
Less than 8.37	17.907 (3.515–91.220)		8.031 (2.930–22.012)	

DSS: disease specific survival; OAS: overall survival; MPV: mean platelet volume

Xin Wang et al. (23) showed that a decreased MPV was associated with the T stage and histology in the Kaplan–Meier analysis, with a poorer overall survival in patients with MIBC. Our results are parallel to theirs. However, our database belongs to a single pioneer center from our community pointing that the results should be repeated in other nationalities. In addition, both the present study and Xin Wang et al. (23) reported a similar finding that activated platelets could play a major role in MIBC. An easily performed blood test including MPV can tell clinicians more in terms of predicting DSS and OSS in MIBC. In another work, Seles et al. (24) similarly reported that a low platelet volume was associated with large tumors, a high Fuhrman grade, sarcomatoid components, histologic tumor necrosis, and vascular invasion. In univariate and multivariate analyses, a small platelet volume was able to correctly predict the renal cell carcinoma recurrence and cancer-specific survival (24).

On the other hand, studies investigating the platelet activity in gastrointestinal cancers indicate the opposite: high MPV levels showed a poor prognosis (25). Nevertheless, according to our study if the preoperative MPV is less than 8.37 fL, a decreased DSS and OAS could be determined in patients with MIBC. These are in compliance with the literature.

Our study had some limitations. Its retrospective nature is the first one. The second one are limited MIBC patient numbers. The absence of molecular studies is another limitation. However, our data belong to a single pioneer center, and novel results are supporting the current literature.

The MPV can be measured by a simple blood test before the operation. Results of the test can show clinicians valuable data on DSS and OSS in MIBC. Therefore, clinicians can inform patients more accurately indirectly. Our findings should be examined in future molecular studies.

Considering the above discussion, a decreased preoperative MPV can be an independent prognostic factor in patients with MIBC who underwent radical cystectomy. If the preoperative MPV is less than 8.37 fL, DSS and OAS could be worse. However, our results should be confirmed by large and properly designed prospective, randomized trials with molecular studies for revealing the tangible effects of MPV on MIBC.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of İzmir Katip Çelebi University School of Medicine (Approval Date: 21.03.2018, Approval Number: 101).

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Importance of Thiol/Disulfide Homeostasis and the Neutrophil-to-Lymphocyte Ratio in Diagnosing of Urinary Tract Infections in the Emergency Department

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BACKGROUND/AIMS
The aim of this study was to investigate the diagnostic utility of thiol/disulfide homeostasis parameters, which are oxidative stress markers, together with the neutrophil-to-lymphocyte ratio (NLR) in diagnosing of urinary tract infections (UTI) in the emergency department (ED).

MATERIAL and METHODS
The study was conducted prospectively and included 63 patients who were admitted to the ED between May 15th, 2018, and October 1st, 2018, and 59 healthy subjects included as the control group. A new method developed by Erel and Neşelioğlu was used to measure the thiol/disulfide homeostasis parameters (thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol), and the NLR was studied in the patient and control groups. In addition, the relationship between these parameters and the body temperature and urinary cultures in patients with UTI was investigated as well.

RESULTS
The NLR, disulfide/native thiol, and disulfide/total thiol mean values were found to be significantly higher ($p<0.001$, 0.013 and 0.009, respectively) in patients with a UTI compared to the control group. Disulfide, disulfide/native thiol, and disulfide/total thiol mean values were found to be significantly higher ($p<0.001$, <0.001 and <0.001 respectively) in the group with a high body temperature compared to the group without high temperature. Native thiol and total thiol values were found to be significantly lower ($p=0.048$ and 0.020, respectively) in the group with a positive urine culture compared to the group with a negative urine culture.

CONCLUSION
Thiol/disulfide homeostasis parameters together with the NLR values are diagnostically useful in patients with UTI.

Keywords: Thiol/disulfide homeostasis, urinary tract infection, neutrophil-to-lymphocyte ratio

INTRODUCTION

Urinary tract infection (UTI) is a clinical condition that affects approximately 150 million people annually across the world (1). UTI may occur in a wide range of clinical manifestations, from asymptomatic bacteriuria to urosepsis. Nevertheless, it may be seen frequently at any age (newborns to elderly). UTI can be caused by microorganisms such as bacteria, fungi, protozoa, and viruses, and bacterial infections often originate from the enteric flora. The most commonly detected bacteria is Escherichia Coli (2-4).

To diagnose UTI, an anamnesis, medical examination, laboratory examinations, and if needed radiological examinations, are used. Burning and pain while urinating, the feeling of compression in the suprapubic area, and an increase in the urinary frequency are seen frequently in patient's history. The leukocyte count in a complete blood count, C-reac-

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tive protein, and the erythrocyte sedimentation rate are used as blood laboratory examinations. Measuring 5–10 leukocytes in each microscopic field of a centrifuged urine sample or positive leukocyte esterase are commonly used in urinary laboratory examinations. In addition, the urine culture is also used in diagnosis (5-7).

Tissue damage may develop in UTI, and inflammatory mediators are released. Acute phase reactants, cytokines, and oxidation products are the mediators that reveal this damage. Especially reactive oxygen products that emerge due to oxidative stress in bacterial infections play a key role in the cell and tissue damage (8, 9). The release of cytokines and proteolytic enzymes in addition to the level of leukocyte infiltration and inflammation are important factors in the scar formation and renal fibrosis (10, 11). Many biochemical parameters that show a systemic inflammatory response in UTI have been studied, but none of them is safe and correct enough for making diagnosis (12, 13).

The neutrophil-to-lymphocyte ratio (NLR) is a strong predictor of an inflammatory response; it is inexpensive and can easily be obtained. The NLR has been studied in many clinical scenarios, from infections to chronic diseases, and it is used as an indicator of systemic inflammation and infection (14-16).

Mediators that are released as a result of oxidative stress cause many systemic diseases and this diseases courses with inflammation. Thiol/disulfide homeostasis parameters are used in the detection of oxidative stress, and it has been measured since 1979 single directionally. Today, these parameters can be measured both separately and also collectively by a new method that was developed by Erel and Neşelioğlu (17-19).

To the best of our knowledge, no study that investigated thiol/disulfide homeostasis parameters and the relation between the NLR and UTI has been published by now. The aim of this study was to investigate the diagnostic utility of thiol/disulfide homeostasis parameters together with the NLR in diagnosing of UTI in emergency department (ED) patients.

MATERIAL and METHODS

Approval for this study was received from the Ankara Yıldırım Beyazıt University Ethics Committee according to the latest version of the Helsinki Declaration. The study was conducted between May 15th, 2018 and October 1st, 2018, in an urban ED with approximately 150,000 patient visits per year. Sixty-three patients who were diagnosed with a UTI and were followed up and discharged from the ED were included in the study prospectively. Fifty-nine healthy subjects who shared similar characteristics with the patient group regarding age and gender were identified as the control group. Informed consent was obtained from both the patients and healthy subjects. In addition, the patient group was divided into two subgroups according to the body temperature (high/not high), and the results of urine culture (positive/negative). Thiol/disulfide homeostasis parameters and NLR values were compared between the groups.

Age, gender, presenting complaint, medical background, accompanying systemic diseases, symptoms, and findings at the time of presenting to the emergency department (dysuria, thamuria, urinary urgency, abdominal and/or flank pain, high temperature [≥38.3 ^{0}C]), physical examination findings, laboratory results, and imaging results of the patients were registered on the standard study forms. Patients ≤18 years of age, pregnant patients, patients with chronic and inflammatory diseases, patients who used drugs or had substance-use disorders, who used antibiotics, and who had an infectious disease episode in the past month were excluded from the study. Patients who were hospitalized were also excluded.

Blood samples were drawn from each patient to study a complete blood count, liver and renal function tests, electrolytes, and thiol/disulfide homeostasis parameters (thiol, disulfide, native thiol, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol) levels. Urine samples were obtained to examine the urine microscopy and cultures.

Native thiol and total thiol values were measured using a new method developed by Erel and Neşelioğlu (17), and disulfide, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol values were calculated. The NLR ratio was calculated by proportioning the neutrophil and lymphocyte values.

Statistical Analysis

Results were presented as the mean±standard deviation. The normality analysis was made using the Kolmogorov–Smirnov test. A p-value >0.05 was accepted as normally distributed data. Univariate statistical analyses were made using the chi-square test for categorical valuables and Student's t test for continuous variables. A p-value <0.05 was accepted as statistically significant.

RESULTS

Thirty-four out of 63 patients who were included in the study were male. The mean age of the patient group was 40.86±18.36 years. There were 33 male and 26 female subjects in the control group, and the mean age was 36.03±8.93 years. There was no statistically significant difference between the patient group and the control group regarding age and gender (p=0.066 and 0.266, respectively).

When the patient and control groups were compared according to the NLR and thiol/disulfide homeostasis parameters, NLR, disulfide/native thiol and disulfide/total thiol, the average values were found to be significantly higher (respectively, p<0.001, 0.013 and 0.009) and the native thiol and total thiol average values were found to be significantly lower (respectively, p=0.004 and 0.001) in the patient group compared to the control group. NLR and thiol/disulfide homeostasis parameters of the patient and control groups are presented in Table I. When the high body temperature group was compared to the group without a high body temperature, the NLR and thiol/disulfide homeostasis parameters; disulfide, disulfide/native thiol, and disulfide/total thiol average values were found to be significantly high (respectively, p <0.001, 0.001, and 0.001), and native thiol/total thiol average values were found to be significantly low (respectively p=0.017 and <0.001) in the group with a high temperature. A comparison

of thiol/disulfide homeostasis parameters and NLR values according to body temperature in the patient group is presented in Table 2. When the patient group was divided into a culture negative and positive, and the NLR and thiol/disulfide homeostasis parameters were compared, native thiol and total thiol values were found to be significantly lower (respectively, p=0.048 and =0.020) in the group with microbial growth in culture compared to the group without the growth. The comparison of thiol/disulfide homeostasis parameters and NLR values according to microbial growth in culture in the patient group is presented in Table 3.

TABLE I. Comparison of thiol/disulfide homeostasis and NLR levels of the patient group and the control group

	Patient Group (n=63)	Control Group (n=59)	p
Native thiol (μmol/L)	405.25±106.00	450.14±54.32	0.004
Total thiol (μmol/L)	447.06±106.72	499.53±53.97	0.001
Disulfide (μmol/L)	25.48±12.40	22.92±11.06	0.231
Disulfide/native thiol	0.068±0.044	0.052±0.027	0.013
Disulfide/total thiol	0.059±0.032	0.045±0.023	0.009
Native thiol/total thiol	0.903±0.069	0.901±0.052	0.867
NLR	5.42±2.31	1.47±0.67	<0.001

NLR: neutrophil-to-lymphocyte ratio

TABLE 2. Comparison of thiol/disulfide homeostasis parameters and NLR values in the patient group with high body temperatures and without high body temperatures

	With High Temperature (n=33)	Without High Temperature (n=30)	p
Native thiol (μmol/L)	379.09±104.97	442.49±97.69	0.017
Total thiol (μmol/L)	428.73±107.50	473.14±102.00	0.102
Disulfide (μmol/L)	32.01±11.24	16.19±6.81	<0.001
Disulfide/native thiol	0.090±0.045	0.037±0.014	<0.001
Disulfide/total thiol	0.076±0.029	0.034±0.014	<0.001
Native thiol/total thiol	0.879±0.080	0.936±0.024	<0.001
NLR	5.41±1.66	5.43±3.04	0.971

NLR: neutrophil-to-lymphocyte ratio

TABLE 3. Comparison of thiol/disulfide homeostasis parameters and NLR values in the patient group with microbial growth and without microbial growth

	With Microbial Growth (n=19)	Without Microbial Growth (n=44)	p
Native thiol (μmol/L)	363.40±108.19	423.33±100.96	0.048
Total thiol (μmol/L)	400.23±98.99	467.28±104.56	0.020
Disulfide (μmol/L)	25.42±11.20	25.51±13.01	0.978
Disulfide/native thiol	0.082±0.059	0.063±0.035	0.191
Disulfide/total thiol	0.069±0.041	0.054±0.026	0.163
Native thiol/total thiol	0.895±0.089	0.906±0.059	0.610
NLR	5.80±1.33	5.25±2.62	0.277

NLR: neutrophil-to-lymphocyte ratio

DISCUSSION

There is an increase in the production of pro-inflammatory cytokines associated with increased oxidative stress mediators in many diseases where inflammation is in the forefront. Thiol/disulfide homeostasis is also one of the oxidative stress markers (19). Thiol/disulfide homeostasis plays a significant role in detoxification, signal transduction, apoptosis, and the regulation of enzyme activities. Disturbance in the thiol/disulfide balance plays a part in the development process of many inflammatory diseases such as diabetes mellitus, cardiovascular diseases, rheumatoid arthritis, chronic renal failure, cancer, and Alzheimer's and Parkinson's (20-22). The NLR is a systemic inflammation parameter that is being investigated to be used in the diagnosis of inflammatory and infectious conditions. It is an indicator with a predictive value in the conditions that cause inflammation, especially bacterial infections (23, 24).

Changes in thiol/disulfide homeostasis have been investigated in some studies, especially with regard to infections, in the literature (25, 26). We found that the NLR was also investigated in the infection setting in medical literature (24, 27). A correlation between the NLR and UTI was detected in a study by Han et al. (28). Similar results were found in the study by Lee et al. (29). In our study, NLR values were significantly higher in the group with UTI compared to the control group, consistent with the literature.

In our literature review, we could not find any studies investigating the relationship between thiol/disulfide homeostasis and a UTI infection. Our study was, to the best of our knowledge, the first one that investigated this relationship in the literature. Disulfide/native thiol and disulfide/total thiol average values were found to be significantly higher in the patient group with UTI compared with the control group; contrary to this, native thiol and total thiol average values were found to be significantly lower. This result is in line with the studies in the literature showing the increase of thiol/disulfide homeostasis parameters in cases of infection and inflammation (30).

Disulfide, disulfide/native thiol, and disulfide/total thiol average values were detected as significantly high in the group with high temperature compared to the group without temperature. As for native thiol and native thiol/total thiol average values, they were detected as significantly low. No significant difference was observed between the two groups regarding the NLR. This result was deemed compatible with the low thiol-to-disulfide ratio, as shown in the studies that were made in conditions with inflammation (25, 31).

A direct correlation between the NLR and bacterial infections was shown by some studies (32, 33). Sarier et al. (34) stated that they found the culture positivity at approximately 30% in their study in patients with urethral stent, and they detected a significant correlation between increasing the duration of stent and a high NLR. The culture positivity was found similarly at approximately 30% in our study too, but no significant correlation between the NLR and culture positivity was detected. Regarding thiol/disulfide parameters, only low native and total thiol values were found to be significant in the group with microbial growth in culture. We think that the inflammation degrees may be higher

in bacterial infections (35, 36), but in our study, a low number of patients with microbial growth in culture might be the reason for our results.

We think that studying thiol/disulfide homeostasis parameters together with the NLR may be diagnostically useful in UTI diagnosing, and more large-scale studies with a higher number of cases are needed in this respect. In our study, the gender distribution is not compatible with the literature, and this can be explained by the low number of our patient population.

We could not find any studies investigating UTI and NLR and thiol/disulfide homeostasis together in the literature. To the best of our knowledge, our study is the first one in this respect.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ankara Yıldırım Beyazıt University School of Medicine (Approval Date: 07.05.2018, Approval Number: 104).

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Original Article

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MitoTEMPO Increases the Gastrointestinal Motility in Aged Rats

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BACKGROUND/AIMS
During the process of aging, many physiological functions in organs change. Mitochondria play an important role in aging. In addition, there are several important gastrointestinal system dysfunction and intestinal problems occur in aging. The aim of this study is to examine how aging affects the gastrointestinal motility and to investigate the effects of the (2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride monohydrate (mitoTEMPO) in adult and aged rats' gastrointestinal contractility, on the isolated ileal and duodenal segments.

MATERIAL and METHODS
In this work, 24-month old male was used as aged-rats and was compared with those of 6-month old as adult-rats. The isolated duodenal and ileal segments were suspended in isolated tissue-bath. The contractile responses induced with acetylcholine (ACh) and the relaxation responses induced with phenylephrine were recorded. In order to study the effects of mito-TEMPO, the segments were incubated for one hour with I μm mito-TEMPO and the responses of acetylcholine and phenylephrine were recorded.

RESULTS
The results indicate that the responses of the ACh-induced contraction were significantly lower in aged rats as compared to adult animals in the ileal and duodenal segments, and phenylephrine-induced relaxations were higher in the duodenal segments.

CONCLUSION
Treatment with the antioxidant mitoTEMPO has significantly affected the responses to the ACh-induced contraction and phenylephrine-induced relaxation that occurs with aging.

Keywords: Aging-contraction, aging-relaxation, mitoTEMPO, isolated ileum, isolated duodenum

INTRODUCTION

Aging is one of the greatest risk factors for many organ dysfunctions and physiological functions in organs and tissues. Thus, the number of nerve cells in the human colon decreases with age (1). The time for food to pass through the colon is prolonged in aged rats, and therefore the colonic motility may be also affected by aging (2). Reports imply that more frequent chronic functional constipation in elderly people has been observed (3). In aging, the gradual loss of the Ca^{+2} balance in cells alters cellular physiological functions (4, 5). Reports showed that during aging, there was a decrease in the Ca^{+2} release according to Ca^{+2}-induction (6) in addition to nitric oxide, the most vital mediator in the longitudinal muscle of young individuals, which losses its significance with aging (7).Mitochondria are exposed to oxidative stress, which is involved in the normal aging process. Under pathological conditions, reactive oxygen species are over produced by the mitochondrial electron-transport chain uncoupling, the activation of the xanthine–oxidoreductase system, or by excessive stimulation of the NAD(P)H, which can overwhelm the endogenous antioxidant defense mechanisms (8). However, the role of mitochondrial dysfunction still remains unclear (9).

The mitoTEMPO has been used as an antioxidant. It is a nitroxide, conjugated to a triphenylphosphonium moiety, which is mitochondrion targeted. As nitroxides are the superoxide-dismutase mimetics, mitoTEMPO could act as a mitochondrial superoxide scavenger and therefore protect mitochondria from the age-induced oxidative damage (10). Previous studies showed that mitoTEMPO lowers mitochondrial superoxide levels and increases the survival rate in septic mice (11).

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In this work, we report that aging might have been associated with the reduction of the contractile effects of acetylcholine and relaxation effects of phenylephrine in isolated ileal and duodenal segments.

MATERIALSAND METHODS

This study has received the approval by the local ethics committee of Ankara University (reference number, 2016-18-165).Healthy adult male Wistar rats (150–250 g) were housed under standard conditions. Two experimental sets were designed. The first set of animals was 6 month old (10 male rats weighing 340±11g) and was considered the adult set. The second experimental set was 24 months old (10 male rats weighing 350±8 g), and it was considered the aged set. The blood glucose level of the adult group compared to the aged group was 80.5±1.4 mg/dL and 83.9±2.5 mg/dL, respectively.

Pentobarbital (30 mg/kg intraperitoneally) was used for anesthetizing. The ileum and duodenum segments (0.3–0.5-mm-long pieces) were removed. Then, the tissue segments were suspended in an isolated tissue bath containing 15 mL of the Krebs–Henseleit solution (mM NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 11.1), and a 95% O_2, 5% CO_2 mixture at 37°C, pH 7.4, was passed into the solution. The segments were brought into equilibrium for 60 min under an optimal resting tension of 1g. After equilibration, the ileum and duodenum segments were contracted with acetylcholine (ACh) (10^{-4}–10^{-7},6 M) and relaxed with phenylephrine (10^{-4}–10^{-7},6 M). These doses were considered as maximal doses, after the cumulative addition of ACh and phenylephrine into the control group. Contraction and relaxation changes were recorded in the isometric tension recorder on the Biopac version 3.7.0. Then, mitoTEMPO (SML 0737, Sigma) was added into the perfusion medium. The isolated ileal and duodenal segments were incubated for 1 h in the medium, and then the contraction and relaxation responses were recorded.

Statistical analyses were performed with an unpaired t-test. Significance values were accepted as *p<0.0 5vs. adult and p<0.05 vs. aged. Values were presented as mean±standard error of mean.

RESULTS

The effects of mitoTEMPO on the gastrointestinal contractility of the isolated ileal and duodenal segments of adult and aged rats were examined.

From Figure 1, it can be observed that no significant differences between the responses of the ACh-induced contractions in the adult and aged duodenal segments were found. When the adult and aged duodenal segments were incubated with mitoTEM-

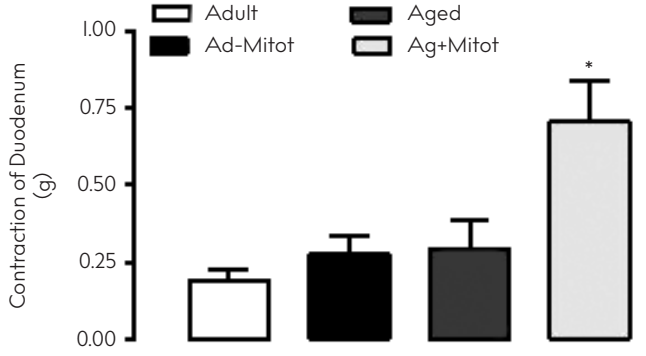

FIGURE 1. Effects of mitoTEMPO on the ACh-induced contraction in an adult and aged isolated duodenum segments. Data are presented as the mean±standard error of mean.
N=8-12, *p<0.05 vs. aged

FIGURE 3. Effects of mitoTEMPO on the phenylephrine-induced relaxation in an adult and aged isolated duodenum segment. Data are presented as the mean±standard error of mean
N=7-10, *p<0.05 vs. aged

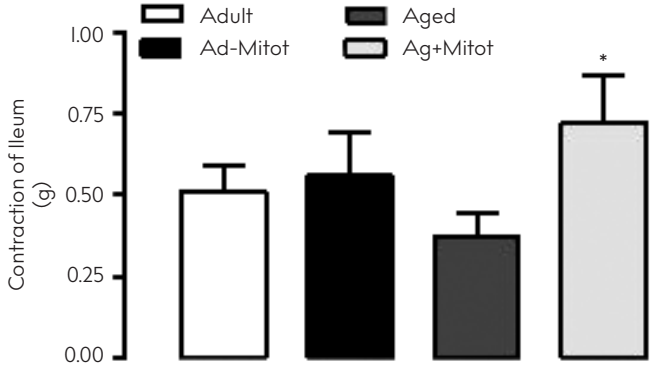

FIGURE 2. Effects of mitoTEMPO on the ACh-induced contraction in an adult and aged isolated ileum segment. Data are presented as the mean±standard error of mean
N=8 for each group. *p<0.05 vs. aged

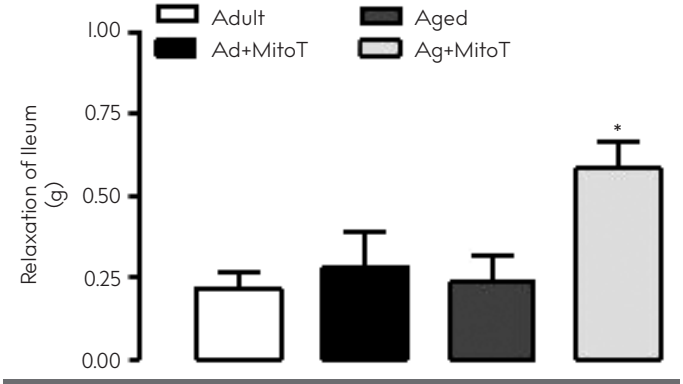

FIGURE 4. Effects of mitoTEMPO on the phenylephrine-induced relaxation in an adult and aged isolated ileum segment. Data are presented as the mean±standard error of mean
N=7 for each group, *p<0.05 vs. aged

PO, significant increases in aged-doudenal ACh-induced contractions compared to adult rats were observed.

When comparing the ACh-induced contraction of the ileal segments of adult and aged rats, it was shown that the responses of adult ileal segments were higher when compared to the aged rats. When the adult and aged ileal segments were incubated with mitoTEMPO, the responses of the ACh-induced contractions in aged ileal segments were significantly higher when compared to the adult segments, as shown in Figure 2.

Figure 3 shows that the responses of the phenylephrine-induced relaxations were lower in adult duodenal segments compared to the aged duodenal segments. When the adult and aged duodenal segments were incubated with mitoTEMPO, the responses of phenylephrine-induced relaxation in aged ileal segments were significantly higher when compared to the adult duodenal segments.

As shown in Figure 4, the responses of the phenylephrine-induced relaxations were similar in adult and aged ileal segments. But when the ileal segments were incubated with the mitoTEMPO, the values were significantly higher in the aged ileal segments when compared to the adult ileal phenylephrine-induced relaxations.

DISCUSSION

The effects of mitoTEMPO on the contractility of adult and aged isolated ileal and duodenal segments in rats were investigated in this study. Our results indicate that the contractility in the aged group decreased and that the relaxation was greater than in the adult group. The results also showed that with the incubation for I h with mitoTEMPO, ileal and duodenal contractility increased significantly in aged rats.

Acetylcholine is an important neurotransmitter, and it induces the contraction in the gastrointestinal tract. Our results showed that the ACh-induced contraction decreased in aged rats; however, these data conflict with Tezuka et al. (12) who report that the contractile response to acetylcholine has no effect on the jejunum and colon in aged rats.

The processes of aging are not well defined. More than 300 theories have been introduced since Medvedev, but none was accepted (13). In 1954, the free-radical theory of aging was proposed by Harman who stated that "aging results from imperfect protection against tissue damage by free radicals in addition to factors like genetic, activity, nutrition and temperature." Despite many investigations on the relationship between lipid peroxidation and aging, the results were conflicting because aging changes are small early in life but increase quickly with age (14). However, many investigators have accepted that biological aging is the failure of an organism to maintain homeostasis (15). In aging of the submucous plexus of the jejunum–ileum, there is a loss of 38% of the neurons, which is probably due to degenerated mitochondria in old animals (16). However, histological experiments carried out by Lee et al. (17) reported that during aging, a significant loss in the thickness of the intestinal mucosa is accompanied by higher reactive species.

In this work, 24-month-old animals were used and showed that contractility in the ileum decreased. According to reports, aging is characterized by a decrease in glutathione concentrations and an increase in the oxidation of protein with a decrease in the mitochondrial NO content in 28-month-old rats (18). During the aging process, the oxidative stress takes place, and endogenously produced oxidants in the cells are usually increased (19, 20).

Lopes et al. (4) reported that the activation of muscarinic receptors induces more contractions in aged rats than in adult animals, which conflicts with our data. They showed that the changes in intracellular Ca^{2+} stores affect contraction and may cause dysfunction during the cell-aging damage.

In aging, several aspects of Ca^{2+}homeostasis might be affected, such as the Ca^{2+} influx, release of Ca^{2+} from stores, and Ca^{2+}uptake process by the sarcoplasmic reticulum and mitochondria (21). It is unknown whether similar changes occur in the gastrointestinal system. Although functional and structural changes are known to occur in many functions, the mechanisms responsible for these changes are unclear. Many investigators claimed that the oxidant stress increases, due to an elevated reactive oxygen, species production, or to decrease the function of natural antioxidant pathways (22-24).

mitoTEMPO was used as the antioxidant factor in our experiments. Mitochondria are the most important sources of reactive oxygen species in the body. We assumed that the therapeutic inhibition of mitochondria by a mitochondrial targeted antioxidant mitoTEMPO might be beneficial in the setting of the gastrointestinal dysmotility. mitoTEMPO is a physicochemical compound that has the ability to pass through lipid bilayers easily and accumulate in the mitochondria selectively (10). Both *in vitro* and *in vivo* studies have confirmed that mitoTEMPO is a mitochondria-targeted antioxidant with superoxide and alkyl radical scavenger properties (10, 25). It is unknown if there are any off-target effects of mitoTEMPO when it is accumulated in mitochondria, and it is also unclear how much of mitoTEMPO has actually gone to the organs (26).

In this study, we have also demonstrated that therapeutic inhibition of mitochondrial ROS using mitoTEMPO prevented oxidative stress and reduced the gastrointestinal dysmotility in aged animals. Our data strongly indicated that mitochondria-targeted antioxidants have therapeutic effects on aged-related dysmotility; thus, a form of antioxidant therapy such as mitoTEMPO may be useful for treating age-related gastrointestinal dysmotility. To the best of our knowledge, this study is the first to demonstrate that antioxidant treatment, such as mitoTEMPO, can significantly reverse the response to acetylcholine and phenylephrine in isolated gastrointestinal segments in aged rats.

In conclusion, we demonstrated that the treatment with the antioxidant mitoTEMPO can significantly affect the responses to the ACh-induced contractions and phenylephrine-induced relaxations that occur with aging. The results are consistent with the hypothesis over the production of ROS with aging.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ankara University School of Medicine (Approval Date: 2016, Approval Number: 18-165).

Informed Consent: N/A.

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

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Original Article

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Implementations Related to the Use of Antibiotics and Data Sources Used by Community Pharmacists in the Northern Cyprus

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BACKGROUND/AIMS
As the bacterial resistance to antibiotics became one of the major problems in today's world, a solution to this problem is possible through a common awareness of the doctor who prescribes antibiotics, the pharmacist who sells them, and the patient who consumes them. The aim of this study was to determine the sales ratio of non-prescription antibiotics in pharmacies, which is the most common category of the sold antibiotic group according to the pharmacists' records due to the received prescriptions, and to detect the relationship between the resources of drug information and the sociodemographic data.

MATERIAL and METHODS
A total of 84 pharmacies out of 168 pharmacies located in the Northern Cyprus were included in the study with a 50% stratified systematic sampling.

RESULTS
This study was carried out in the first and the second trimester of 2014 (01 January-30 June), and the daily sale of antibiotics was found to be 41.5%. In addition, the most purchased antibiotics were discovered to be the penicillin with 76.2%, and on the other hand, the disease for which antibiotics were most commonly prescribed was found to be the upper respiratory tract infection with 86.9%. When the level of self-awareness of the pharmacists was examined, it was discovered that they were highly knowledgeable about drug indications, as well as their side effects.

CONCLUSION
The rate of the use and sale of non-prescribed antibiotics in the Northern Cyprus has been found to be at a higher level compared to the rates in many developed countries. According to the new legislation for 2016, the rate of the use and sale of non-prescribed antibiotics is expected to be decreasing in the future.

Keywords: Community pharmacist, sources of drug information, knowledge of drugs, Cyprus

INTRODUCTION

As the bacterial resistance to antibiotics became one of the major problems in today's world, a solution to this problem is possible through a common awareness of the doctor who prescribes antibiotics, the pharmacist who sells them, and the patient who consumes them. The rate of non-prescribed sales differs from one country to another, and it is known that the worldwide rate of non-prescribed antibiotics sales from public pharmacies is approximately 50% (1-5). Except in the North America and countries in the Northern Europe, accessing non-prescription antimicrobial drugs is possible throughout the rest of the world. Studies in developed European countries, including the United Kingdom, where the use of antibiotics is under strict control, have shown that the use of non-prescribed antibiotics is 14%–37%. According to the same study, it was emphasized that 47% of the non-prescribed antibiotics were maintained from the public pharmacies.

The use of non-prescribed antibiotics arises substantially from the lack of laws and deficiencies in regulations, especially when the rates are compared between the developed and developing countries. The use of non-prescribed antibiotics in developing countries like Nigeria (76%) is high due to lack of laws and inadequate regulations (6-11). The main prob-

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lem that arises as a result of non-prescribed antibiotic use is increased antibiotic resistance, which triggers the existing problems (12). In addition, an increased cost of treatment and the side effects of the drugs are known to be important consequences of the non-prescribed antibiotic use (13).

The aim of this study is to determine the rate of the non-prescribed antibiotic sales in pharmacies, the mostly sold antibiotic group according to the pharmacists' records, and to detect the level of general knowledge as well as the self-assessment of pharmacists' own levels of knowledge; also the study intends to investigate the relationship between non-prescribed sales and the knowledge levels together with the use of data sources, if any.

MATERIAL and METHODS

Study Design

A list of 84 pharmacies out of 168 pharmacies located in the Northern Cyprus (Nicosia, Kyrenia, Famagusta, and Morphou) was obtained from the 2014 Turkish Medical Association Health Guide published by the Cyprus Turkish Medical Association. Those 84 pharmacies were selected according to the 50% stratified systematic sampling, and a questionnaire was distributed to all.

Necessary approval were received from the Near East University Medical Research Ethics Committee the pharmacists concerned were asked to fill out and sign a consent form by one of the researchers of the study, a specialist in infectious diseases and clinical microbiology, in the period from June to September 2014.

Methods

The sociodemographic characteristics, non-prescribed antibiotics sold, the most sold antibiotic group, the data sources, and the self-declaration of their own levels of knowledge were included in the questionnaire. The self-assessed knowledge levels of the pharmacists were evaluated by the 5-pointLikert Scale, from *very poor* (1) to *excellent* (5). Data sources used by the pharmacists were the following: Vademecum, RxMediaPharma, British National Formulary (BNF), Drug information prepared by the Turkish Pharmacists Union (TEBRP), pharmacology books, colleagues, the Internet, lecture notes, and consulting colleagues and other professionals.

Statistical Analysis

For statistical analysis, Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA) was used, and the chi-squared test was applied. A p-value <0.05 was accepted as significantly different.

RESULTS

Considering the distribution of pharmacies in the Northern Cyprus, it can be seen that the most pharmacists are located in Nicosia (42.9%). A total of 86.9% of the pharmacists graduated in the Republic of Turkey, 67.9% received only a bachelor's degree, and 82.1% were female. In addition, 87.3% of pharmacists had been working as pharmacists for over 10 years (Table 1).

When asked if they gave drugs to patients without any prescription, 82 (97.6%) pharmacists replied yes. When the daily antibiotic sale rates were examined, it was determined that the pharmacists were selling more than 10 antibiotics per day. Pharmacists selling less than 10 antibiotics a day had an antibiotic prescription rate of 56.2%, while pharmacies selling more than 10 antibiotics a day had an antibiotic prescription rate of 67.5%.

The sale rate of penicillin-derived drugs was 76.2%, and the sale of penicillin among other antibiotic strains was identified as the most common drug. Moreover, it was found out that antibiotics have been used most often in the treatment of upper respiratory tract complaints 86.9% (Table 2).

Pharmacists' self-assessment of their own level of knowledge on drugs was investigated through the 5-pointLikert Scale. None of the questioned parameters were selected as *very bad* (1) (Figure 1). Generally, pharmacists had a positive view of themselves with regards to medical knowledge of antibiotics. Their strong areas were selected to be issues relating to indications and side effects. Pharmacists determined that they felt they were weak

TABLE 1. Sociodemographic Characteristics of Pharmacists

Parameters (n=84)				
Location	Nicosia	Kyrenia	Famagusta	Morphou
n (%)	36 (42.9)	16 (19.0)	26 (31.0)	6 (7.1)
Age in years	<50	≥50		
n (%)	42 (50.0)	42 (50.0)		
Gender	female	male		
n (%)	57 (67.9)	27 (32.1)		
Educational level	BSc[a]	MSc[b]		
n (%)	69 (82.1)	15 (17.9)		
Country where the bachelor's degree was obtained	NC[c]	Turkey	UK[d]	
n (%)	3 (3.6)	73 (86.9)	8 (9.5)	
Years of experience as a community pharmacist	<10 years	≥10		
n (%)	19 (12.7)	65 (87.3)		

MSc[a]: Master of Science; BSc[b]: bachelor's degree; NC[c]: Northern Cyprus; UK[d]: United Kingdom

TABLE 2. Group of the Most Sold Antibiotics and Indications

	n	**%**
The group of antibiotics		
Penicillin derivate	64	76.2
Cephalosporins	17	20.2
Macrolides	2	2.4
Quinolones	1	1.2
Indications		
Urinary tract infections	5	6.0
Dermatologicalinfections	1	1.2
Upper respiratory tract infections	73	86.9
High fever	5	6.0

in the areas of pharmacological properties and drug interactions, again according to their own statements (Figure 1).

TABLE 3. Sources of Drug Information Used by Community Pharmacists (n=84)

Sources of Drug Information (n=84)	n	%
Vademecum medication guide[1]	74	88.1
RxMediaPharma[2]	31	36.9
BNF[3]	13	15.5
TEBRP[4]	4	4.8
Pharmacologybooks	19	22.6
Colleague	24	28.6
Internet	57	67.9
Lecture notes	12	14.3
Otherhealth personnel	13	15.5

Vademecum[1]: CD and book; RxMediaPharma[2]: source of drug information; BNF[3]: British National Formulary; TEBRP[4]: drug information prepared by the Turkish Pharmacists Union

When drug information sources used by pharmacists were examined, it was seen that the Vadamecum medical guide was the most frequently used printed (88.1%) information source, while the Internet took the second place with 67.9% (Table 3).

When considering the impact of the independent variable on the level of knowledge, it can be observed that indications, pharmacological properties, contra-indications, side effects, drug interactions, and warnings were not affected by the level of knowledge in specific situations or bioequivalence issues. The level of education affects the variable posology and the method of administration regarding the level of knowledge only. Nevertheless, those with a master's degree (Master of Science, MSc) evaluated their knowledge level as perfect compared to those without it (Table 4).

Statistically, there was no relationship between the use of Vademecum, BNF, TEBRP, RxMediaPharma, Internet, lecture notes, and consulting colleagues or other health personnel and the level of education. It was determined that pharmacology refer-

TABLE 4. Dependent Variables Affected by the Level of Education, Seniority, and the Age of Pharmacist

			Level of Education				
Dependent Variable	**BSc (n=69)**	**%**	**MSc (n=15)**	**%**	**Total**	**x^2**	**p**
Posology and the method of administration regarding the level of knowledge							
Excellent	29	42.0	2	13.3	31		
Good and ↓*	40	58.0	13	86.7	53	4.357	0.037
Use of pharmacologybooks as a source							
Use	14	20.3	7	46.7	21		
Not use	55	79.7	8	53.3	63	-	0.048
			Seniority as a community pharmacist				
Dependent variable	**<10 years (n=21)**	**%**	**≥10 years (n=63)**	**%**	**Total**	**x^2**	**p**
Level of knowledge related to pharmacological properties							
excellent	2	9.5	24	38.1	26		
good and ↓**	19	90.5	39	61.9	58	6.016	0.014
Use of RxMediaPharma							
Use	12	57.1	20	31.7	32		
Not use	9	42.9	43	68.3	52	4.308	0.038
Use of lecture notes							
Use	6	28.6	5	7.9	11		
Not use	15	71.4	58	92.1	73	-	0.025
			Age				
Dependent variable	**<50 (n=42)**	**%**	**≥50 (n=42)**	**%**	**Total**	**x^2**	**p**
Use of lecture notes							
Use	10	23.8	1	2.4	11		
Not use	32	76.2	41	97.6	73	8.473	0.004
Use of RxMediaPharma							
Use	21	50.0	11	26.1	32		
Not use	21	50.0	31	73.9	52	5.048	0.025

*Seven community pharmacists interpreted it as average (3), and one community pharmacist interpreted it as bad (2); therefore, those answers were added to the good (4) category
** Since 14 community pharmacists interpreted it as average (3), their answers were added to the good (4) category
MSc: Master of Science; BSc: Bachelor's degree

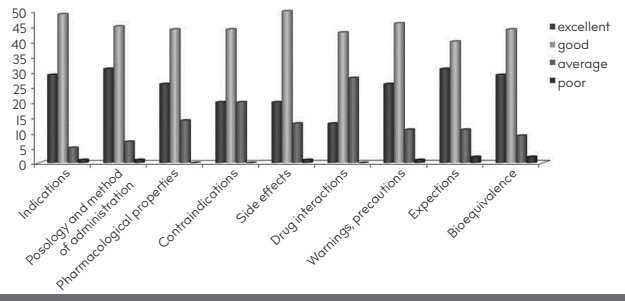

FIGURE I. Pharmacists' self-assessed knowledge of drugs

ence books were the only variable among the sources used to access the information that was affected by the level of education. It was clearly seen that the use of a pharmacology book as a source by pharmacists with a master's degree was twice as frequent as in those without it (Table 4).

The level of knowledge related to pharmacological properties seems to be only affected by the seniority as an independent pharmacy. According to the statements by the pharmacists who participated in the survey, there is a statistically significant relationship between the knowledge level regarding the pharmacological properties and the seniority as pharmacist. In pharmacists' own words, as the years of being in the independent pharmacy practice increase, the level of knowledge related to the pharmacological properties of drugs increases accordingly. Pharmacists who had been working as community pharmacists for a period greater than10 years evaluated their knowledge level regarding the pharmacological features as *perfect*, approximately 4 times higher than those who had been working as community pharmacists for a period shorter than 10 years (Table 4).

Statistically, no significant relationship was found between the use of Vademecum, BNF, TEBRP, the Internet, pharmacology reference books, and consulting colleagues or other health personnel and seniority as a community pharmacist. However, data indicated that there was a clear relationship between lecture notes and the use of RxMediaPharma with the seniority of community pharmacists. This revealed that the pharmacists who worked as community pharmacists for less than 10 years referred to RxMediaPharma and lecture notes as sources of information more than those with less experience in terms of the years of practice (Table 4).

The age group showed no major relationship between the use of Vademecum, BNF, TEBRP, the Internet, and pharmacology reference books and consulting with colleagues or other health personnel, yet there was a significant relationship between lecture notes and the use of RxMediaPharma with the age group. Lecture notes and RxMediaPharma were used more frequently by the pharmacists who were younger than 50 than those who were older (Table 4). According to pharmacists' own assessments, age had no effect on variables regarding the pharmacists' level of knowledge.

DISCUSSION

Every country has its own national health system that reflects its history, economic development, and the political ideology within its territory. In this respect, it would be possible to say that there is no health system in the Northern Cyprus that could provide adequate basic health services and health insurance encompassing everyone (14). The rate of non-prescribed antibiotics purchased from public pharmacies is found to be 41.5% in the Northern Cyprus, compared with the studies conducted in Greece, Italy, Malta, and Spain (47%), and Egypt (50.4%) (15, 16).

If we consider countries such as Israel, with a rate of 4%, and Nigeria, with a much higher rate (76%), it would be possible to think that the type of health system in these countries that are welfare-oriented plays a significant role in providing more efficient health services (14, 17, 18).

In pharmacies selling less than 10 antibiotics (n=79), the sale rate of non-prescription drugs is 42.82%, and in pharmacies selling 10 antibiotics and more (n=5), the daily sale rate is 32.5%. This situation highlights that pharmacies that have existed for a longer time and selling 10 antibiotics or more have a greater reputation and have an established clientele of patients as customers.

According to the Malaysia Medical Statistics 2005, among the most widely prescribed drugs, the penicillin group of antibiotics came at the top with 47% (19, 20). In the study conducted in primary health care units in Malaysia in 2004 by Teng et al. (21), it was determined that 50% of antibiotics were prescribed for the treatment of upper respiratory tract infections. In another study, it was observed that amoxicillin, which is a derivative of penicillin, was prescribed for common cold at a rate of 54% (22). Similarly, in our study, we found that the most applied group of antibiotics in the first 6 months of 2014 were penicillin derivatives at 76.2% for the treatment of upper respiratory tract complaints at the rate of 86.9%. In this regard, the rate of prescription of penicillin in our research group was found to be more than one and a half times higher than the rate in Malaysia.

According to the data from the European Surveillance of Antibiotics Consumption (ESAC) Project carried out in 2003 and including25 European countries, the most used antibiotics for the upper respiratory tract infections is penicillin, which is derived from antibiotics (23). It has been reported that the most common causative agents of the upper respiratory tract infections are viruses, and even though it is a known fact that antibiotic treatment is has no clinical benefit in such cases, antibiotics remain overconsumed (24). The reasons for which the rates are so high in our study can be identified as prescribed and non-prescribed antibiotic sales. Moreover, when all types of penicillin derivatives are examined as a whole, we can also observe that doctors may prescribe antibiotics unnecessarily and that the pharmacists sell non-prescribed antibiotics. In France, one campaign aimed to reduce the use of antibiotics for upper respiratory tract infections across the country, which resulted in a 26.5% decrease in the use of antibiotics between the years 2002 and 2007 (25).

In the study conducted by Jawla et al. (26), which investigated data on drug interactions and drug dosages, it was specified that 73% of the pharmacists had information concerning drug interactions. The same study specified that 35% of the pediatric and geriatrics doses were calculated by pharmacists. A total of 66.7% of pharmacists in the Northern Cyprus assessed their level of knowledge on the drug interactions as *excellent* or *good*.

Pharmacists stated that they calculated the drug doses themselves in pregnant, pediatric and geriatric patients. And in the calculation of drug doses, 84.5% of the pharmacists stated that they were either excellent or good. Northern Cyprus pharmacists indicated that their knowledge about the side effects of the medication was *excellent* or *good* at the rate of 83.3% and as average at the rate of 15.5%. Chan et al. (13) stated in their study that 67% of the independent pharmacists specified the importance of having a place to get information about the adverse drug reactions, and 28% of them specified it as average. This result confirms our research concerning the self-confidence in the field of drug side effects.

In a study conducted in Hong Kong, the rate of independent pharmacists consulting pharmacist colleagues *very frequently* to get information on drugs was 14%, whereas *frequent* consultation remained at 38%. This method of access to information is lower in the Northern Cyprus (28.6%) (21). Chen et al. (13) reported that the rate of *very frequent* consultation of other health personnel was at 5% and *frequent* consultation was at 13%. Independent pharmacists consulting other health personnel in the Northern Cyprus had similar rates as the study in Hong Kong (15.5%).

Among the reasons as to why pharmacists have a higher preference for consulting other health personnel and pharmacist colleagues to access information rather than consulting Vademecum or the Internet, contrary to hospital pharmacists, are various issues such as the absence or restricted numbers of easily accessible doctors to consult, as well as their workload.

In addition, it would be possible to think that hospital pharmacists receive significantly different types of questions in terms of drug information from other health workers, whereas it is generally considered that answering questions from the community is the role of an independent pharmacist.

Answers to questions relating to renewed data sources by Jawline et al. (26) showed that 64% of pharmacists preferred books and magazines, 31% medical representatives, and 5% of the pharmacists stated that they used other sources.

The Vademecum medical guide was determined to be the first data source at 88.1% compared to the books and magazines used by the Northern Cyprus pharmacists as a source of information. The study determined that the utilization rate of the BNF in the Northern Cyprus is (15.5%), contrary to the rate of BNF used at a rate of 67% in the Northern Cyprus (13). In the study conducted in Hong Kong, the use of Goodman and Gilman's *The Pharmacological Basis of Therapeutics* book as a source of information was 5%, compared to the use of pharmacology books in the Northern Cyprus at 22.6% (13). As the Internet is one of the easiest and fastest ways to access information in today's world, it appeared as the second most common source with 67.9% preferred by the Northern Cyprus pharmacists to access information. The majority of pharmacists in the Northern Cyprus work as independent pharmacists, and therefore journal and periodicals on pharmacy or pharmacotherapy sources are not easily accessible. Also, the use of sources such as the Internet and Vademecum medication guide instead of standard texts are more popular as they are considered to be updated and revised. Medical representatives rarely visit pharmacies in the Northern Cyprus because of the scarcity of population. For this reason, medical representatives are not seen as a drug information source in this country.

Pharmacists with an MSc degree evaluated their knowledge levels on posology and administrative methods as *excellent* at a low rate of 13.3% compared to those without the degree. This situation mostly depended on the increase of awareness with regards to education. As the seniority increases, the level of knowledge on the pharmacological features of drugs is thought to be increasing as well.

A limited use of RxMediaPharma as an interactive drug reference source by the pharmacists who are aged ≥50 years, as well as those with ≥10 years of experience as independent pharmacists, is thought to be due to the fact that RxMediaPharma is more up to date and hence may not be as known as the other reference sources.

We believe that pharmacists with an MSc degree using pharmacology books two times more frequently than those with a BSc degree are more likely to be effective in raising awareness due to the education they have received.

We discovered that independent pharmacists who are aged <50 years have worked as independent pharmacists <10 years use their lecture notes to access the information concerning drugs at a higher rate than the pharmacists whose work experience is ≥10 years and are aged ≥50 years. This is probably because young graduates keep and use their lecture notes and have up-to-date knowledge from their university course.

Unnecessary and excessive use of antibiotics creates a problem that is not simply a personal problem, but a global one. The problem primarily encompasses providing more effective health services, limiting doctor prescriptions of antibiotics, and preventing pharmacists from selling any kind of non-prescription antibiotics. Overuse and sale of antibiotics can be prevented by modifying and restricting the existing regulations and training the public (16, 17). Campaigns can be arranged as in France to reduce the inappropriate use of antibiotics throughout the country. Such a campaign could also aim at reducing the prescription of antibiotics by doctors, as well as veterinarians according to the updated guidelines by the country's surveillance results (14).

For pharmacies, the law that no antibiotics could be sold without a prescription came into force on April 1st, 2016. To determine the attitudes and behaviors of pharmacist in the sale of antibiotics in the Northern Cyprus, new studies should be conducted to compare the pre- and post-law conditions.

Our findings signify the indiscriminate and high rate of antibiotic use in the Northern Cyprus. Considering a high number of students and tourists coming from various parts of the world to Northern Cyprus, it may be considered that the antibiotic resistance is effective in a much wider geographical area.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Near East University Medical Research Ethics Committee (Approval Date: 14.11.2013, Approval Number: NEU/2013/23/094).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Forecasting Measles in the European Union Using the Adaptive Neuro-Fuzzy Inference System

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BACKGROUND/AIMS
Measles is one of the diseases that cause child mortality. The measles forecasting is essential in planning the fight against the disease and reducing the risk of the vaccine stocks expiration. Governments and health institutions estimate the measles vaccine requirements using certain equations, which are generally based on the size of the target population and the past consumption records. There are several studies that have examined the measles forecasting and conducted a vaccine requirement assessment.

MATERIAL and METHODS
This study uses a forecasting model that employs an adaptive neuro-fuzzy inference system (ANFIS) based on clustering. In this study, the measles data were derived using the World Health Organization (WHO) Measles and Rubella Surveillance Data, which cover the period from January 2011 to March 2018 and include 28 European Union member countries. Out of total 87 monthly measles cases, 80% were used for training, and 20% were chosen for testing.

RESULTS
In addition to the mean square error, the root mean square error, normalized root mean square error, mean absolute error, and mean absolute percentage error were calculated.

CONCLUSION
The model created for this purpose has demonstrated that the predictions made for the data collected between January 2011 and March 2018 were successful.

Keywords: Measles, forecasting, European Union

INTRODUCTION

Measles is still one of the leading causes of child mortality. The measles forecasting is essential in planning the fight against the disease and reducing the risk of the vaccine stocks expiration.

Governments and health institutions estimate the measles vaccine requirements using certain equations, which are generally based on the size of the target population and the past consumption records. There are several studies that have examined the measles forecasting and conducted a vaccine requirement assessment using tools, such as the Statistical Analysis System (1), the World Health Organization (WHO) Measles Programmatic Risk Assessment Tool (2-7), Statistical Package for the Social Sciences (8), nonlinear forecasting and chaos (9), and Markov Chain Monte Carlo methods (10).

Measles cases in the European Union (EU)/European Economic Area principally occur in unvaccinated populations, affecting both adults and children (11). The European Centre for Disease Prevention and Control annual report shows that as many as 80% of teenagers and young adults who contracted measles in 2017 had not been vaccinated (11, 12).

MATERIAL and METHODS

According to the WHO Measles and Rubella Surveillance Data showing the distribution of measles cases by country and by month between January 2011 and March 2018 (Table 1), there were a total of 1,353,222 measles cases worldwide,

This study was presented at the Second International Biomedical Engineering Congress 2018 (IBMEC-2018), 24-27 May 2018, Nicosia, Cyprus.

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TABLE 1. Part of the World Health Organization measles and rubella surveillance data distribution of measles cases by country and month, 2011–2018

				Month											
Region	**ISO3**	**Country**	**Year**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
EUR	AUT	Austria	2011	13	11	10	19	34	61	41	7	4	1	12	6
EUR	AUT	Austria	2012	6	8	5	2	3	4	2	2	1	2	0	0
EUR	AUT	Austria	2013	4	8	8	13	10	4	2	4	7	8	2	9
EUR	AUT	Austria	2014	32	13	6	4	12	16	4	0	0	4	11	17
EUR	AUT	Austria	2015	33	29	49	65	69	40	9	2	0	0	3	1
EUR	AUT	Austria	2016	0	0	0	2	1	2	9	1	5	3	1	4
EUR	AUT	Austria	2017	33	29	7	2	7	0	2	2	1	2	8	1
EUR	AUT	Austria	2018	7	6	0	0								
EUR	BEL	Belgium	2011	18	46	291	212	250	184	88	20	20	6	25	3

EUR: European Region; ISO3: International Standards Organization 3 - digit country code - AUT: ISO3 of Austria; BEL: ISO3 of Belgium

TABLE 2. Total monthly number of measles cases in the European Union between January 2011 and March 2018

	Month											
Year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2011	2407	3441	5860	6246	5770	3208	1873	919	752	567	911	927
2012	1439	1281	1422	1420	1352	1072	817	481	376	612	617	432
2013	651	793	905	1130	1365	1398	1394	704	656	662	447	
435												
2014	1270	761	1027	641	388	245	185	142	104	104	147	303
2015	586	588	801	717	586	296	146	54	41	48	62	79
2016	117	181	237	276	326	364	377	412	377	588	761	635
2017	997	1739	2825	2525	2290	975	871	537	439	493	593	743
2018	1137	1305	63									

EUR: European Region; ISO3: International Standards Organization 3 - digit country code - AUT: ISO3 of Austria; BEL: ISO3 of Belgium

TABLE 3. Results

	Train	**Test**	**Overall**
MSE	79671.423	253835.159	114918.846
RMSE	282.261	503.821	338.997
NRMSE	0.045	40.908	18.520
MAE	196.618	352.406	228.146
MAPE	47.503	136.486	65.512

MSE: mean square error; RMSE: root mean square error; NRMSE: normalized root mean square error; MAE: mean absolute error; MAPE: mean absolute percentage error

as reported by the member countries (13). The WHO indicates that many cases are not reported. For the same period, a total of 86.246 measles cases (Table 2) were reported by the EU member states (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom).

This study uses a forecasting model in MATLAB (MathWorks Inc., USA) (14) that employs an adaptive neuro-fuzzy inference system (ANFIS) based on clustering that was applied to the EU measles cases. Fuzzy clustering is a data-clustering technique wherein each data point belongs for a cluster to some degree that is specified by a membership grade. ANFIS uses a hybrid learning algorithm to tune the parameters of a Sugeno-type fuzzy inference system. The algorithm uses a combination of the least-squares and back-propagation gradient descent methods to model a training dataset. ANFIS also validates the models using a checking dataset to test for overfitting of the training data.

Out of the total 87 monthly EU measles cases (Table 3), 67 (80%) were used for training, and 17 (20%) of them were chosen for testing. In this study, the mean square error (MSE), root mean square error (RMSE), normalized root mean square error (NRMSE), mean absolute error (MAE), and mean absolute percentage error (MAPE) were calculated using the following equations, which are the indexes of forecasting accuracy:

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\left(Y_i - P_i\right)^2, \quad (1)$$

$$RMSE = \sqrt{\left(\frac{1}{N}\sum_{i=1}^{N}\left(Y_i - P_i\right)^2\right)}, \quad (2)$$

$$NRMSE = \frac{RMSE}{Y_{\max} - Y_{\min}}, \quad (3)$$

$$MAE = \frac{1}{N}\sum_{i=1}^{N}|Y_i - P_i|, \quad (4)$$

$$MAPE = \left(\frac{1}{N}\sum_{i=1}^{N}\frac{|Y_i - P_i|}{Y_i}\right)\times 100\%, \quad (5)$$

where Y_i is an actual value, P_i is the forecasted value of the i-th data obtained, and N is the number of data. In our forecasting model, we assumed the relationship as

$$y(k) = F(y(k-3), y(k-2), y(k-1)), \quad (6)$$

where *y(k)* are the measles cases by *k*-th month, *y(k-1)* is the measles cases by *(k-1)*-th month, *y(k-2)* is the measles cases by *(k-2)*-th month, and *y(k-3)* is the measles cases by *(k-3)*-th month.

Since this study was based on merely a statistical dataset freely available online (13), we did not use any human materials. So, an ethic committee approval and informed consent were not necessary. This study was performed in accordance with the principles of the Declaration of Helsinki.

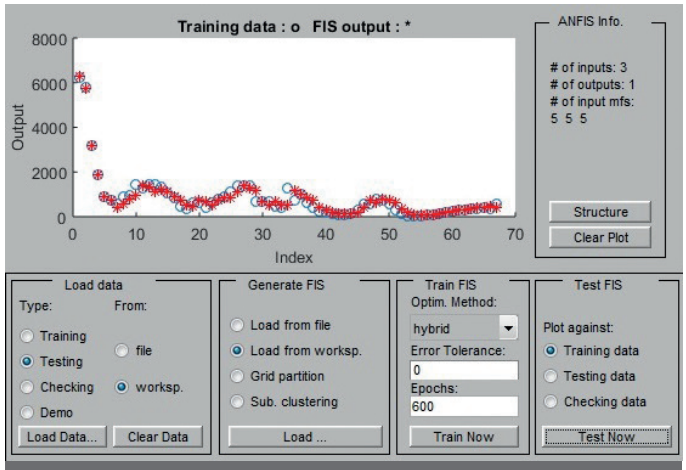

FIGURE I. Adaptive neuro-fuzzy inference system training

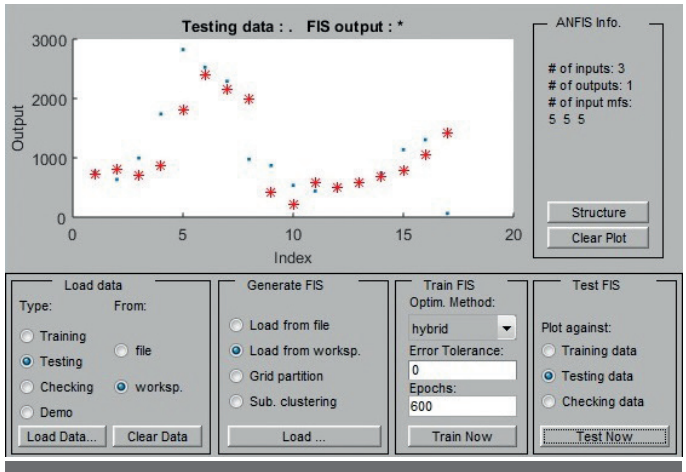

FIGURE 2. Adaptive neuro-fuzzy inference system Testing

RESULTS

The study was aimed at predicting the number of measles cases expected in the following month given the data for previous three months. The data cover all measles cases in the EU area between January 2011 and March 2018 by month. The data collected for March 2018 are not complete, but we included them because they were listed.

The client data were subjected to the subtractive clustering procedure using MATLAB. The algorithm was repeated for the cluster radii 0.1 through 0.9, while keeping the accept ratio, reject ratio, and squash factor constant at 0.5, 0.15, and 1.25, respectively. The training and testing of the FIS with the radius 0.2 at 600 epochs resulted in the lowest training error of 282.261 and testing error of 503.821. The ANFIS graphical outputs show the training points in Figure 1 and testing points in Figure 2.

The input membership functions obtained from the subtractive clustering using MATLAB are of the Gaussian type. Each input space shown in Figure 3 generalizes the input data submitted to the ANFIS training.

The model is constructed with five clusters, hence five rules as shown in Figure 4. The model structure has three inputs, each made of five Gaussian membership functions. The five rules determined as a result of the training of the network. The first order Sugeno-type reasoning was conducted on the forth layer where the firing strengths were obtained.

The crisp output was received on the 6th layer after the aggregation process. Figure 5 shows the crisp input data and the crisp

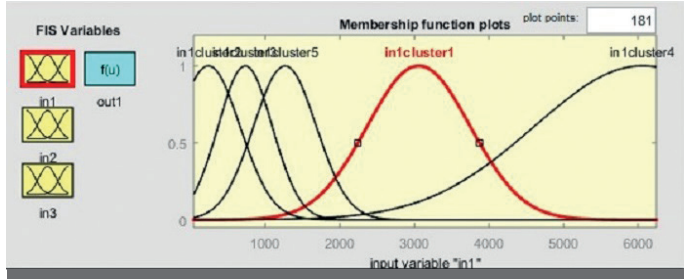

FIGURE 3. Membership Functions

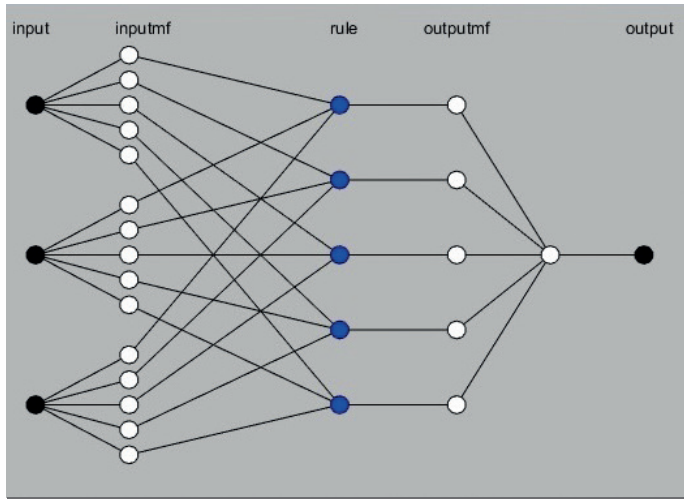

FIGURE 4. Adaptive neuro-fuzzy inference system Model

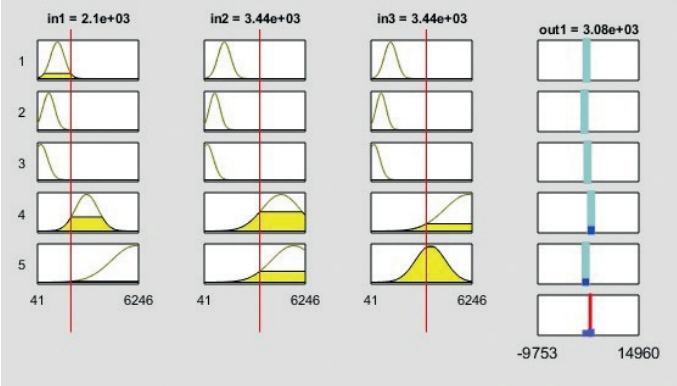

FIGURE 5. Fuzzy Rules

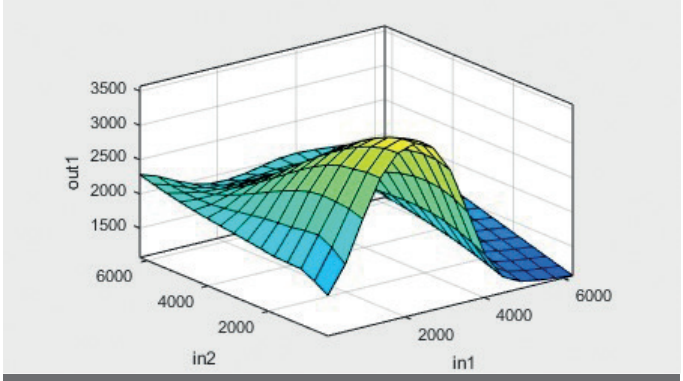

FIGURE 6. Surface

output of the number of expected measles cases for the following month.

The surface plot is a three-dimensional graphics tool in MATLAB that shows the shape of the FIS with three-dimensional plots at a time. A particular instance of the three consecutive months is shown in Figure 6. A general idea can be obtained from the surface plot by observing the shape of the relationship between the given parameters.

DISCUSSION

Measles cases are considered to be epidemiologically linked, confirmed by laboratory findings and clinical cases as reported to the WHO. Some countries report measles cases at irregular intervals, providing multiple months of data in a single month period, and missing future months are reported as no cases; hence, they are expected to be updated as data becomes available (11).

In this study, a forecasting model was created using ANFIS to predict the number of future measles cases in the EU area. Such a work was particularly required to control the vaccine stocks and organize an effective distribution through the member countries. The model created for this purpose demonstrated that the predictions made for the data collected between January 2011 and March 2018 were successful.

Ethics Committee Approval: The authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (amended in October 2013).

Informed Consent: N/A.

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Original Article

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Is There Any Correlation Between Preoperative Platelet Indices and Surgical Prognostic Factors in Patients with Cervical Cancer?

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BACKGROUND/AIMS
To evaluate the variations in platelet indices (PIs), including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), in cervical cancer (CC) and to evaluate the relationship between these PIs and pathological features of tumor (i.e., tumor stage and size, depth of stromal invasion, lymphovascular space invasion (LVSI), and histological type).

MATERIAL and METHODS
This retrospective, comparative study included 40 patients with CC who underwent complete clinical staging and 80 healthy controls at a tertiary center between 2007 and 2017. Groups were compared using independent sample t-tests and Mann–Whitney U tests. Variables with a $p<0.05$ were included in the receiver operating characteristic curve analysis to determine cut-off values. The associations of PIs with pathological features were analyzed using the chi-square test.

RESULTS
The mean age of patients with CC was 56.9 years old. Demographic characteristics (i.e., age, gravidity, and parity) did not differ between the groups ($p>0.05$). Mean MPV and PCT were significantly lower ($p=0.002$ and $p=0.026$, respectively), and PDW was significantly higher ($p=0.049$) in the CC group than in the control group. The platelet count did not differ significantly between the groups ($p=0.558$). The optimal cut-off points of MPV, PDW, and PCT levels for discriminating between the groups were 10.45 fL, 13 fL, and 0.28%, respectively. Except a significant relationship between low PCT level and positive LVSI ($p=0.01$), there was no significant relationship between PIs and pathological features.

CONCLUSION
Low MPV and PDW and high PCT may be considered as a useful additional tool for preoperative diagnosis of CC. However, the usability of these markers for predicting pathological features is unsatisfactory.

Keywords: Mean platelet volume, platelet count, prognosis, uterine cervical neoplasms

INTRODUCTION

Cervical cancer (CC) is the third most common malignancy of women worldwide, and approximately 266,000 patients die from this disease annually (1). However, the survival of patients with CC is well-correlated with the stage of tumor and other prognostic factors (i.e., tumor size, histological type and grade, depth of stromal invasion, lymphovascular space invasion (LVSI), and lymph node involvement status) (1).

Cervical cancer is staged clinically. The current staging procedure includes histological diagnosis by biopsy or cold-knife conization, pelvic examination under anesthesia, and related imaging techniques (i.e., cystoscopy, proctoscopy, and chest radiography). If needed, magnetic resonance imaging and computerized tomography can also be used. On the other hand, the uses of tumor markers (i.e., serum squamous cell carcinoma antigen, tissue polypeptide antigen, carcinoembriyonic antigen, and carcinoma antigen 125) for diagnosing, monitoring response to therapy, and detecting recurrence in CC have been investigated (2, 3). Nevertheless, none of them has achieved widespread acceptance, yet.

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It is well-known that inflammation influences the tumor microenvironment and plays a central role on the cancer development process (i.e., initiation, promotion, progression, and metastasis) (4). Simultaneously, tumor-induced inflammatory response may lead to change in complete blood count (CBC) parameters (5). In addition, some studies about CC revealed that various CBC parameters have a significant relationship with CC and its pathological features (5-15). Furthermore, the utility of platelet indices (PIs) for patients with CC remains to be determined.

The aim of the present study was to evaluate the differences in PIs, including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), in CC. Furthermore, we examined whether there was any relationship between these indices and various pathological features of tumor (i.e., tumor stage and size, depth of stromal invasion, LVSI status, and histological type).

MATERIAL and METHODS

This was a retrospective, case–control study conducted at a tertiary hospital. The research was conducted in accordance with the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh. The study was approved by the Ankara Atatürk Training and Research Hospital ethical review board committee of the institution (approval no.: 117). Written informed consent was obtained from all participants.

The medical records of 55 patients with CC who underwent complete clinical staging and the diagnosis of cancer as confirmed by histology in our institution between 2007 and 2017 were analyzed to form the CC group. The medical records of 80 healthy controls who underwent surgery for interval tubal sterilization or vaginal reconstruction at the same institution during the same time period were analyzed to form the control group. Exclusion criteria were as follows: concomitant malignancy, acquired or congenital hematological disease, acute or chronic inflammatory disease, use of any drug that may influence the coagulation cascade (e.g., anticoagulants, hormonal contraceptives, and steroids), and smoking. Fifteen patients from the CC group were excluded from the study. A total of 40 patients with CC and 80 healthy control patients were enrolled in the study.

Demographic (i.e., age, gravidity, and parity) and pathological (i.e., tumor stage and size, depth of stromal invasion, LVSI status, and histological type) features and preoperative PIs (i.e., platelet counts, MPV, PDW, and PCT levels) were obtained from the patients' medical records.

Blood samples were collected while patients were admitted to the hospital and before receiving any medications. Specimens were analyzed within 2 h using a Sysmex XE-2100 Automated CBC Analyzer (Sysmex Europe, Germany).

The CC and control groups were compared with regard to the examined demographic characteristics and PIs. Once variables significantly associated with CC were determined, correlations between these variables with tumor stage (stage <IIB vs. ≥IIB), tumor size (<4 cm vs. ≥4 cm), depth of stromal invasion (<50.0% vs. ≥50.0%), and LVSI status (positive vs. negative) were investigated.

Statistical Analyses

Statistical analyses were conducted using Statistical Package for the Social Sciences for Windows, software ver. 21.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test was used to assess the normality of data. Normally distributed data were expressed as mean and standard deviation. Nonparametric data were expressed as median and interquartile range (IQR). The independent samples *t*-test and Mann–Whitney *U* test were used for comparison of the groups. Variables with a $p<0.05$ were included in the receiver operating characteristic (ROC) curve analysis to determine the cut-off values. Then, the largest Youden Index (sensitivity+specificity-1) was selected as the optimal cut-off point. The chi-square test or Fisher's exact test was used to analyze the associations of PIs with pathological features. Odds ratios and 95.0% confidence intervals were determined. A p value <0.05 was considered statistically significant.

RESULTS

The mean age of patients with CC was 56.9±15 years. Medians of gravidity and parity were 4 (IQR: 3) and 3 (IQR: 3), respectively. Demographic characteristics (age, gravidity, and parity) did not differ significantly between the CC and control groups ($p>0.05$ for all) (Table 1).

Among the preoperative PIs, mean MPV and PCT were significantly lower ($p=0.002$ and $p=0.026$, respectively), and PDW was significantly higher ($p=0.049$) in the CC group than in the control group. The mean platelet count did not differ significantly between the groups ($p=0.55$) (Table 1). Furthermore, ROC curve analyses revealed that the optimal cut-off points of MPV, PDW, and PCT levels for discriminating between the CC and control groups were 10.45 fL, 13 fL, and 0.29%, respectively. ROC curve analyses of MPV, PDW, and PCT are shown in Figure 1.

The pathological features of patients with CC and the relationship of MPV, PDW, and PCT with these are summarized in Table

TABLE 1. Demographic features and platelet indices of patients in the cervical cancer and control groups

Variable	Cervical cancer *n*=40 (33.3%)	Control *n*=80 (66.6%)	P*
Demographic characteristic			
Age (years), mean±SD	56.9±15	51.9±9.8	0.066
Gravidity, median (IQR)	4 (3)	4 (2.75)	0.053
Parity, median (IQR)	3 (3)	3 (2)	0.165
Platelet indices[a]			
Platelet count ($\times 10^3/\mu L$)	291.1±91.6	300.4±77.5	0.558
MPV (fL)	10.0±1.3	10.6±0.8	0.002
PDW (fL)	13.5±2.6	12.7±1.7	0.053
Plateletcrit (%)	0.28±0.07	0.31±0.07	0.026

IQR: interquartile range; MPV: mean platelet volume; PDW: platelet distribution width; SD: standard deviation
*p values by independent samples t-test and Mann–Whitney *U* test

2. Of the 40 patients, 26 (65.0%) were at early stage (International Federation of Gynecology and Obstetrics stage ≤IIB). However, tumor size was ≥4 cm, and depth of stromal invasion was ≥50.0% in 18 (45.0%) patients, and LVSI was positive in 13 (32.5%) patients. The majority of histological type was squamous cell carcinoma (87.5%). Except a significant relationship between lower PCT level and positive LVSI (p=0.01), there was no significant relationship between PIs and CC's pathological features.

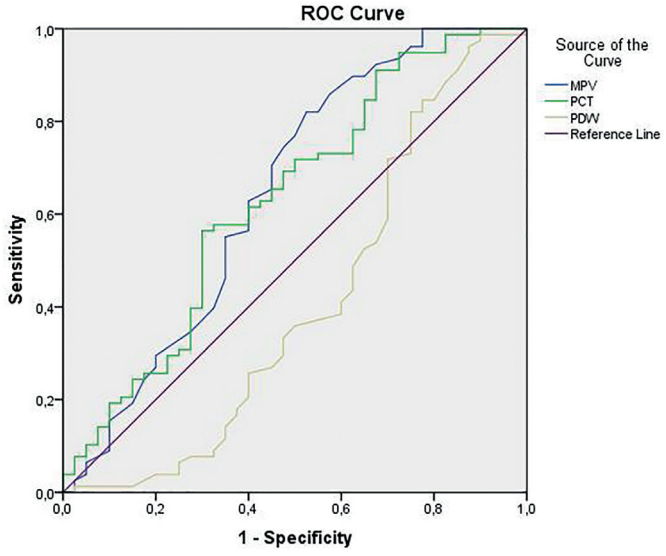

FIGURE I. Receiver operating characteristic curve analyses of mean platelet volume, platelet distribution width, and plateletcrit levels for discriminating patients with cervical cancer from control patients (area under curves were 0.64, 0.59, and 0.63, respectively)

DISCUSSION

The present study has shown that some PIs, including MPV and PCT, were significantly lower, and that PDW was significantly higher in the CC group than in the control group. Nevertheless, it revealed that except a significant relationship between lower PCT level and positive LVSI, there were no significant relationships between PIs and CC's pathological features.

Cervical cancers occur and are diagnosed at an earlier age than other gynecological malignancies (1). In population-based studies from different regions worldwide, the median age of the patients has been reported to be between 44.7 and 52 years (16-18). In our study, the mean age of patients with CC was 56.9 years and a little more than these data. This may be because of the small sample size of our study population or may be a result of representing a single-center experience.

Platelets are dynamic cytoplasmic fragments of bone marrow megakaryocytes. In addition to their role in hemostasis and thrombosis, recently, increasing evidence displayed that platelets play a role on inflammatory processes, host defense, wound healing, angiogenesis, and remodeling (19). In addition to platelet count, PIs, including MPV, PDW, and PCT, are potential biomarkers of platelet activation. Various studies investigating the relationship between CCs and PIs have shown that these markers may correlate with the prognosis of disease (20-24).

High platelet count is a well-known systemic inflammatory response to the development of cancer (20). Various studies with CC showed that platelet count is significantly higher in patients with CC than in healthy controls (6). Furthermore, in a comprehensive review, Cheng et al. suggested that high platelet count may reflect poor prognosis (i.e., higher clinical stage, positive pelvic lymph node involvement, and a larger tumor size) (20). In

TABLE 2. The relationship of MPV, PDW, and plateletcrit with clinicopathological features of cervical cancer

Characteristics		MPV (fL), n			PDW (fL), n			Plateletcrit (%), n		
	n (%)	<10.45	≥10.45	P*	<13	≥13	P*	<0.29	≥0.29	P*
FIGO stage										
<IIB	26 (65)	13	13	0.10	10	16	0.98	14	12	0.27
≥IIB	14 (35)	11	3		6	8		10	4	
Tumor size										
<4 cm	22 (55)	12	10	0.52	9	13	0.89	12	10	0.52
≥4 cm	18 (35)	12	6		7	11		12	6	
Depth of stromal invasion										
<50.0%	22 (55)	12	10	0.52	7	15	0.30	15	7	0.33
≥50.0%	18 (35)	12	6		9	9		9	9	
Lymphovascular space invasion										
Positive	13 (32.5)	9	4	0.51	7	6	0.30	4	9	<0.01
Negative	27 (67.5)	15	12		9	18		20	7	
Histological type										
Squamous cell carcinoma	35 (87.5)	21	14	0.9	12	23	0.10	12	13	0.33
Adenocarcinoma	5 (12.5)	3	2		4	1		2	3	

FIGO: International Federation of Gynecology and Obstetrics; MPV: mean platelet volume; PDW: platelet distribution width
*p values by chi-square test

contrast to these studies, in the present study, we did not find a relationship between high platelet count and CC. This may be a result of the relatively small sample size of our study, as well as a consequence of methodological differences between the studies (i.e., inclusion and exclusion criteria).

Mean platelet volume represents the average volume of platelets found in blood. PDW is an indicator of volume variability in platelet size, and its increase shows that abnormally large and small platelets are in the circulation. Both of them are an index of platelet activation and are associated with different inflammatory conditions. Studies with non-gynecological (i.e., gastric, colon, and lung cancers) and gynecological cancers (i.e., endometrial, ovarian, and CCs) revealed that both MPV and PDW may have differed significantly (i.e., increase or decrease) in patients with cancer compared with healthy controls (3,20-22,24,25). Similar to these studies, in the present study, we did find that both MPV and PDW were significantly lower in patients with CC than in control patients. Nevertheless, studies investigating the relationships between these markers and pathological features of CC have yielded inconsistent results (3, 25). Similar to two previous studies, in the present study, we did not find any significant relationships between CC's pathological features (i.e., tumor stage and size, depth of stromal invasion, LVSI status, and histological type) and neither MPV nor PDW, respectively (3, 25).

Plateletcrit is the volume occupied by platelets in the blood as a percentage and is considered as analogous to hematocrit, which used to evaluate red cell mass. In healthy subjects, the range of PCT varies between 0.22% and 0.24%, but has no clinical meaning by itself (26). However, limited studies with inflammatory diseases, coronary heart diseases, and various pregnancy-related conditions, such as preeclampsia and hyperemesis gravidarum, displayed that PCT may significantly correlate with the prognosis of these diseases (27-30). Furthermore, studies with various cancer types, such as lung and endometrial cancers, revealed that PCT increased significantly in these cancer types and was related with poor prognosis (23, 24). This may be the result of platelet number and functional changes that occurred as part of paraneoplastic syndrome. In the present study, PCT was significantly higher in patients with CC than in healthy controls, and high PCT level (≥29.0%) was significantly correlated with positive LVSI status. To our knowledge, this is the first report that identified a relationship between PCT and CC.

In conclusion, although the present study has its limitations (e.g., its retrospective single-center design and relatively small sample size), it revealed that serum MPV, PDW, and PCT levels of <10.35 fL, <13 fL, and ≥0.29%, respectively, were significantly associated with CC. On the other hand, the present study revealed that except a significant relationship between high PCT level and positive LVSI, there was no significant relationship between PIs and CC's pathological features. However, further descriptive and comparative studies are needed to determine the clinical utility of PIs in the preoperative diagnosis of CC and its pathological features.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Review Board of Ankara Atatürk Training and Research Hospital. (Approval Date: 11/7/2018, Approval Number: 117).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

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Original Article

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Can Liver Imaging for Focal Lesions be Limited with Only Diffusion-Weighted Sequences in Patients without Known Malignancy?

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BACKGROUND/AIMS

Our purpose is to investigate whether the diffusion-weighted imaging (DWI) can be used as an initial and decisive sequence to shorten liver magnetic resonance imaging (MRI) study, rather than being complementary to conventional sequences especially in patients without known malignancy.

MATERIAL and METHODS

The MRI characteristics of 105 focal liver lesions (FLLs) were classified as benign or malignant by visually assessing the DWI features, and the lesions were compared with a complete liver MRI protocol. Hyperintensity or isointensity of a lesion in apparent diffusion coefficient map by visual assessment was accepted as unrestricted diffusion and benignity, while hypointensity in any part of a lesion was accepted as restricted diffusion and malignancy. Specificity, sensitivity, positive predictive value, negative predictive value, and accuracy of DWI were calculated.

RESULTS

The visual assessment of DWI alone had 74.29% sensitivity, 94.29% specificity, 86.67% positive predictive value, 88.00% negative predictive value, and 87.62% accuracy in differentiating malignant lesions from the benign ones. In 28 of 32 patients without malignancy, DWI results were in concordance with the final diagnosis of benign lesions. The misinterpreted lesions in these patients were three abscesses and a hemorrhagic adenoma.

CONCLUSION

Visual DWI characteristics of FLLs in patients without known malignancy can accurately classify the lesions as benign or malignant. In appropriately selected patients, a liver MRI examination can be completed with only DWI, resulting decrease in time and cost related to intravenous contrast media usage.

Keywords: MRI, diffusion-weighted imaging, liver, tumor, intravenous contrast, abdomen

INTRODUCTION

Characterizing focal liver lesions (FLLs) is an important part of the radiology practice. Ultrasonography (US), computed-tomography (CT), and magnetic resonance imaging (MRI) can be used for this; however, intravenous (iv) contrast-enhanced liver MRI has the highest accuracy. Relatively costly and time-consuming conventional abdomen MRI requires the use of iv contrast agents. Diffusion-weighted imaging (DWI) has long been used as a complementary sequence in abdominal MRI. DWI is a noninvasive imaging method based on the Brownian motion of water molecules. Diffusion of water molecules is quantitatively expressed by apparent diffusion coefficient (ADC). Low ADC values are associated with high cellularity and viscosity suggesting malignancy or abscess, respectively (1). DWI has begun to play an important role in the detection and characterization of FLLs in conjunction with evolving hardware and software technologies (2). Many studies investigate the role of DWI in discrimination of malignant abdominal tumors from the benign ones. Most of these studies compare quantitative ADC values (3-9). A systematic database review study conducted by Vermoolen et al. (10) reported that mean ADC values of malignant lesions and benign lesions ranged from 0.86±0.11 to 1.52±0.55×10^{-3} mm^2/s and 1.94–2.86×10^{-3} mm^2/s, respectively. Bharwani et al. (11) reported that the threshold of 1.7×10^{-3} mm^2/s ADC value had a significantly higher diagnostic sensitivity and specificity in differen-

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tiation of malignant and benign FLLs. In an another study that examined 215 FLLs, the optimal ADC threshold value generating 79% sensitivity and 82.6% specificity was found to be 1.25×10^{-3} mm^2/s (4). As shown in the abovementioned studies, the wide range of ADC values can be because of scanners and the b-values. A group of researchers compared visual evaluation of the DWI and ADC maps with measured ADC values in FLLs, and they found high sensitivity and accuracy rates of visual assessment in both adult and pediatric abdominal tumors (12, 13). We hypothesized that beginning the liver imaging with DWI and visual assessment of its results eliminates an iv contrast-enhanced abdominal MRI protocol and decreases time and cost. Our purpose is to investigate whether the DWI can be used as an initial and decisive sequence to shorten liver MRI study, rather than being complementary to conventional sequences especially in patients without known malignancy.

MATERIAL and METHODS

Patients

Ethics committee approval was received for this study from Near East University Ethics Committee for Scientific Researches (Approval Date: 04.26.2018). In total, 213 upper abdominal MRI studies performed in our department between January 2016 and February 2018 were retrospectively evaluated. FLLs were found in 65 patients. Patients without known primary malignancy were referred to MRI examination for lesions determined in US or in CT examination, while patients with known primary malignancy were referred for screening or follow-up. Institutional review board approval was obtained for this retrospective study. FLLs smaller than 1 cm, non-iv contrast studies, ADC map with motion or pulsation artifacts, and cases with nine or more liver lesions were excluded. Twelve of these patients were excluded because of lack of a contrast-enhanced study or an artifact-free ADC map. Three patients were excluded for having nine or more lesions. Only the first MRI study of a patient was included if they had multiple. Finally, 105 FLLs of 50 patients were included in the statistical analysis.

MRI Protocol

The MRI studies were carried out with a 1.5-T MR scanner (Magnetom Aera, Siemens Healthcare, Erlangen, Germany). The standard imaging protocol included axial and coronal T2-weighted half-Fourier acquisition single-shot turbo spin echo (HASTE) sequences [repetition time (TR)/echo time (TE), 1200/91 ms; flip angle (FA), 169°; slice thickness (THK), 6 mm; and number of excitations (NEX), 1], axial fat-saturated T2-weighted HASTE sequence (TR/TE, 1200/94 ms; FA, 160°; THK, 6 mm; and NEX, 1), axial fat-saturated T1-weighted fast low-angle shot (FLASH) sequence (TR/TE, 126/2.38 ms; FA, 70°; THK, 6 mm; and NEX, 1), axial in-phase and opposed-phase T1-weighted FLASH sequences (TR/TE, 119/4.76 and 2.38 ms, respectively; FA, 70°; THK, 6 mm; and NEX, 1), axial T2 and heavily T2-weighted HASTE sequence (TR/TE, 1600/118 and 445 ms, respectively; FA, 157°; THK, 6 mm; and NEX, 1), pre-contrast axial, fat-saturated T1-weighted volumetric interpolated breath-hold examination (VIBE) sequence (TR/TE, 4.36/1.91 ms; FA, 10°; THK, 4 mm; and NEX, 1) and contrast-enhanced fat-saturated T1 weighted VIBE sequences with same parameters at 30 s (arterial phase), 60 s (portal phase), and 150 s (venous phase) after injection of 0.1 mmol/kg of gadoterate meglumine (Dotarem; Guerbet Group, France) bolus tracked visually by a real-time sequence, and a post-contrast axial T1-weighted fat-saturated FLASH sequence at 300 s (late venous phase) after the injection. Diffusion-weighted images were obtained in axial plane with b-values of 50, 400, and 800 s/mm^2. Respiratory-triggered single-shot echo-planar images had these parameters: TR/TE, 8000/61 ms; FA, 90°; THK, 6 mm; NEX, 3. The ADC maps were automatically created.

MRI Analysis

Histopathologic results or clinical and radiographic follow-up and typical imaging findings were accepted as reference standard. A radiologist with more than 10 years of experience in abdominal imaging read the whole data for each patient including physical examination findings, medical history, all MRI sequences, findings in other modalities (CT, US), pathology reports, and follow-up imaging findings to classify the lesions as malignant or benign. Lesions with peripheral globular contrast enhancement in the arterial phases and being hyperintense in the late venous phases compared to the normal liver parenchyma were evaluated as hemangioma (14). Lesions with peripheral rim-type contrast enhancement and having different enhancement pattern from normal liver parenchyma in patients with known primary tumors were evaluated as typical metastasis (15, 16). The diagnoses of metastases were also confirmed by decrease in size in patients receiving chemotherapy and increase in size in untreated patients who have follow-up imaging. Lesions being hyperintense on T2-weighted images and showing no contrast enhancement were evaluated as cysts (16). Lesions being mildly hypointense to moderately hyperintense on T1-weighted images, mildly hyperintense on T2-weighted images, showing homogeneous enhancement in the arterial phases, and becoming nearly isointense in venous phases were evaluated as adenomas (16, 17). Lesions with heterogeneous internal structure and rapid contrast enhancement in the arterial phases and wash-out in venous phases were interpreted as hepatocellular carcinoma (HCC) (16-18). Pathological diagnoses of HCCs were also present. The abscesses showed peripheral enhancement of capsules and central restriction of diffusion in the ADC maps (19). Abscess material was evacuated with percutaneous drainage from two lesions. Transient hepatic attenuation differences (THAD) were found in a patient who showed wedge-shaped focal areas of hyperintensity in arterial phase and isointensity to normal liver in other phases and sequences and did not have mass effect (20). A lesion without a mass effect, showing signal drop-out in opposed-phase T1-weighted sequence and similar contrast enhancement compared to normal liver parenchyma was diagnosed as focal fatty infiltration (21, 22).

An another radiologist with six years of experience in general radiology interpreted only the diffusion-weighted and ADC images of the patients who were included in the study, and

classified the lesions as being benign or malignant. He was blinded to the data and images other than DWIs and ADC maps. As in previous studies, hyperintensity or isointensity of a lesion in ADC map by visual assessment was accepted as unrestricted diffusion and benignity, whereas hypointensity in any part of a lesion was accepted as restricted diffusion and malignancy (13, 23).

Statistical Analysis

Diagnostic performance of DWI-alone interpretation was made by identifying true benign, false benign, true malignant, and false malignant lesions. Specificity, sensitivity, positive predictive value, negative predictive value, and accuracy were calculated from these results.

RESULTS

Eighteen of the patients included into the study had at least one known primary malignancy. Thirty-two of the patients did not have any known history of malignancy. Among the patients included in statistical analysis, 13 had malignant and

TABLE I. Distribution of the lesions and patients according to the diagnosis and counts of total lesions and patients

Type of lesion	Number of lesions	Number of patients	Number of patients with known malignancy	Number of patients without known malignancy
Hemangioma	47	23	4	19
Metastasis	32	11	11	0
Cyst	10	7	1	6
Adenoma	7	3	1	2
HCC	3	2	1	1
Abscess	3	2	0	2
THAD	2	1	0	1
Focal fatty infiltration	1	1	0	1
Total	105	50	18	32

HCC: hepatocellular carcinoma; THAD: Transient hepatic attenuation differences

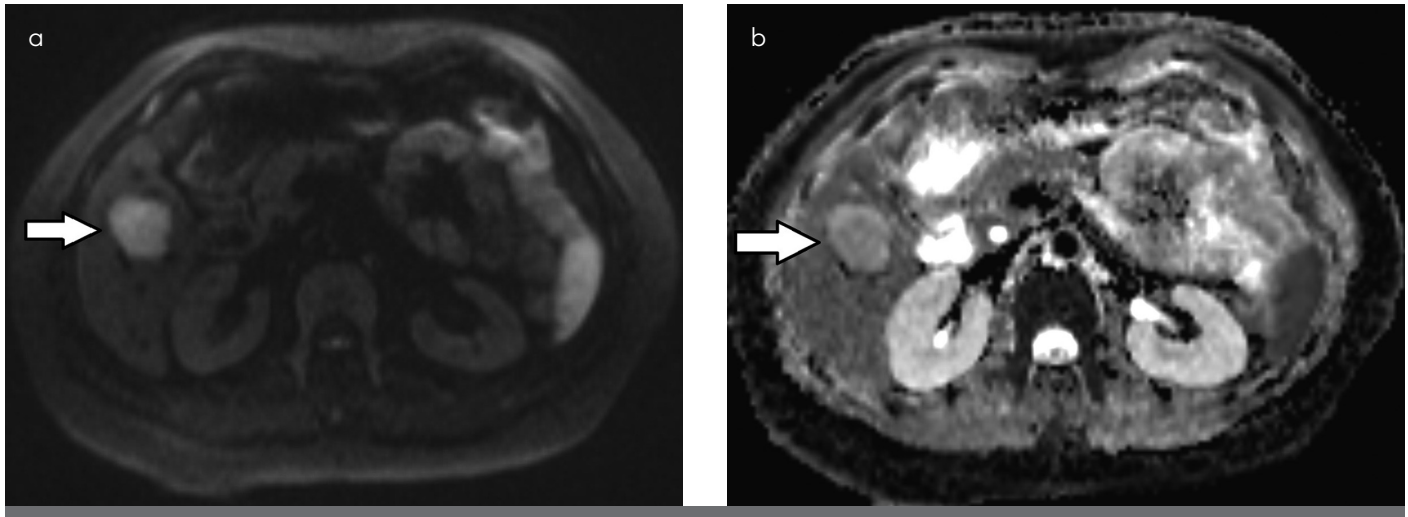

FIGURE 1. a, b. A hemangioma in the right liver lobe. The lesion is hyperintense both on the diffusion-weighted image (a) with b value of 800 s/mm² and on the ADC map (b)

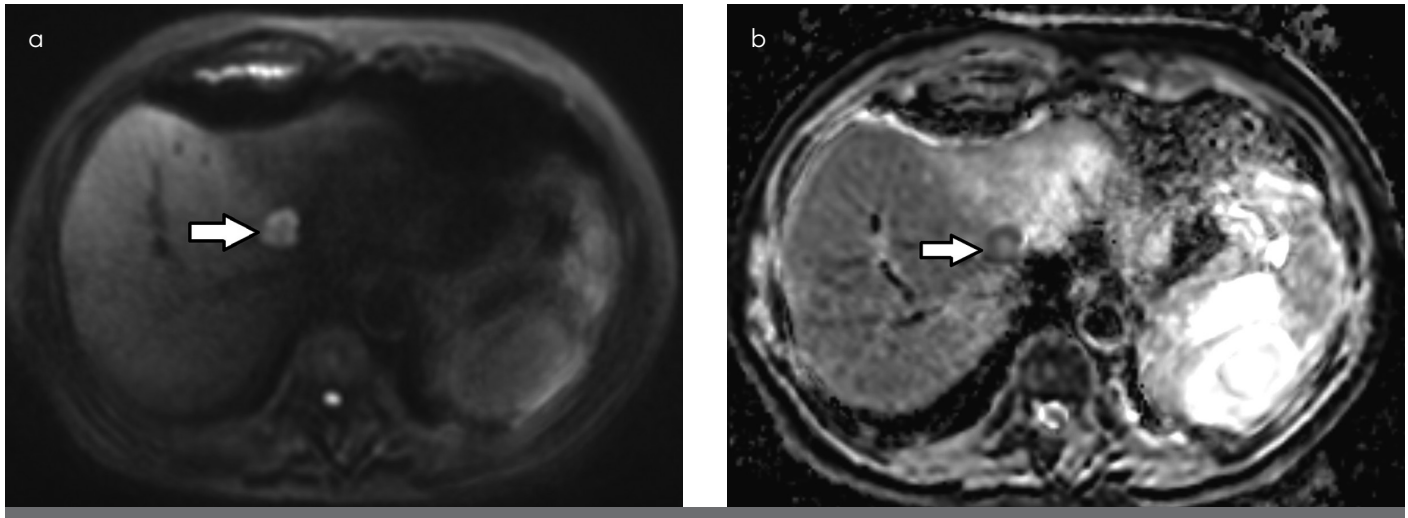

FIGURE 2. a, b. The periphery of a metastasis is seen hyperintense on b800 (a) and hypointense on ADC map (b)

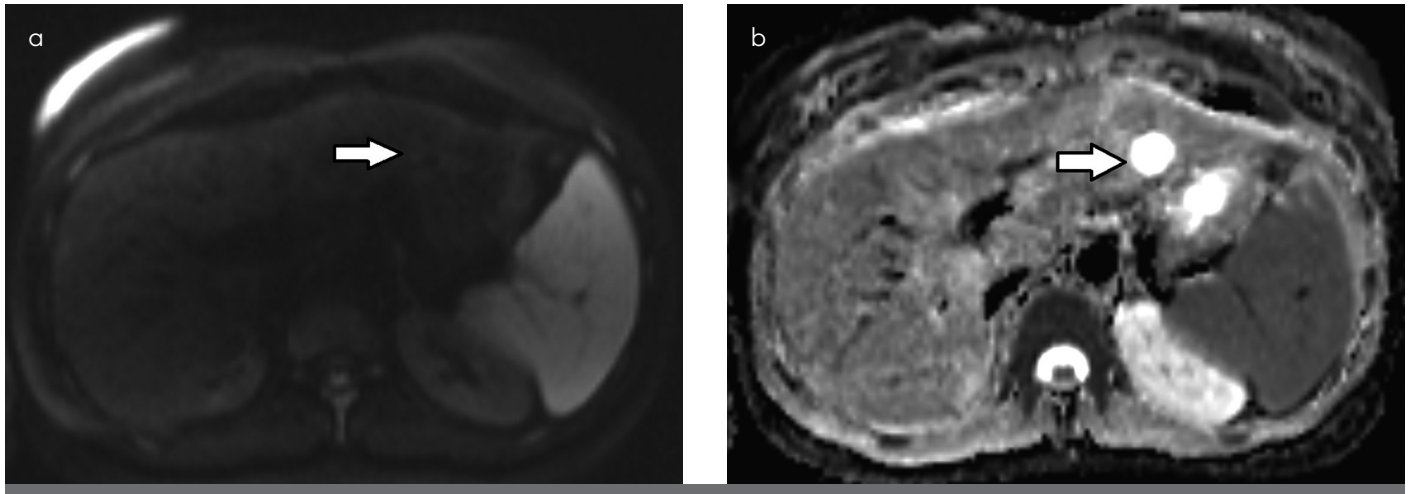

FIGURE 3. a, b. A cyst is totally suppressed on b800 image (a). Hyperintensity of the lesion is seen on ADC map (b)

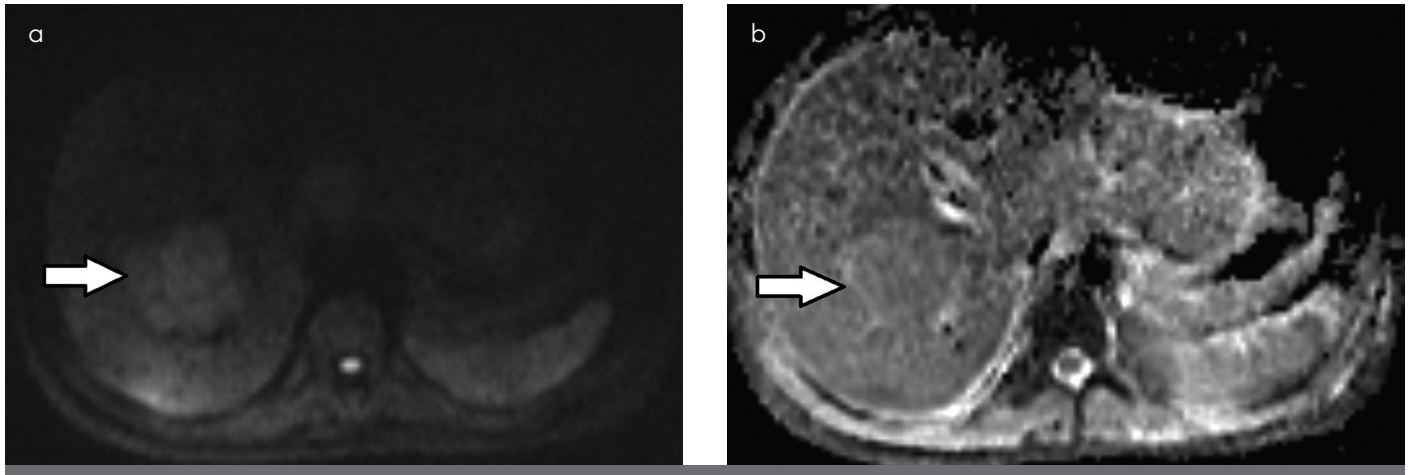

FIGURE 4. a, b. An adenoma is slightly hyperintense on b800 image (a) and slightly hyperintense on ADC map (b)

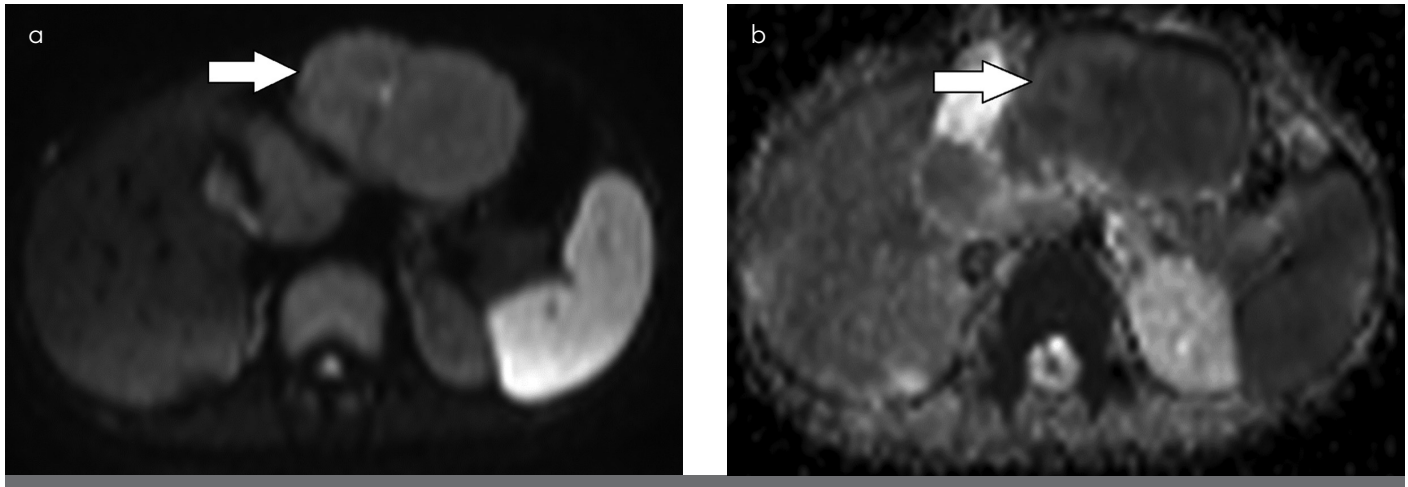

FIGURE 5. a, b. A fibrolamellar HCC located in the left liver lobe shows restriction of diffusion on b800 image (a). The lesion is hypointense on ADC map (b) compared to normal liver parenchyma

37 had benign lesions in MRI scans. The average age of patients with and without known primary malignancy was 58.3 (range 30–81) years and 49.4 (range 16–86) years, respectively. There were 32 female and 18 male patients. Eleven female patients had known malignancy, and seven had malignant liver lesions. Seven male patients had known malignancy, and six had malignant liver lesions. Number of lesions included in statistical analysis was 105. None of the patients had both malignant and benign FLLs. The average number of benign and malignant lesions in a liver was 1.89 (range 1–6) and 2.69 (range 1–6), respectively. Most common lesions were hemangiomas and metastases (Table 1). The DWI and ADC features

TABLE 2. Signal characteristics of the lesions in the DWI-800 and ADC maps. (↑↑: hyperintense, ↑: mildly hyperintense, —: isointense, ↓: hypointense). Numbers in the boxes are the count of lesions

Type of lesion	DWI-800 ↑↑	DWI-800 ↑	DWI —	ADC ↑↑	ADC ↑	ADC ↓	ADC —
Hemangioma	46	1	0	47	0	0	0
Metastasis	25	4	3	4	4	23	1
Cyst	0	0	10	10	0	0	0
Adenoma	1	2	4	0	2	1	4
HCC	3	0	0	0	0	3	0
Abscess	3	0	0	0	0	3	0
THAD	0	0	2	0	0	0	2
Focal fatty infiltration	0	0	1	0	0	0	1

DWI: diffusion-weighted imaging; ADC: apparent diffusion coefficient; HCC: hepatocellular carcinoma; THAD: Transient hepatic attenuation differences

TABLE 3. Distribution of all lesions according to the reference standard and DWI evaluation

		Reference standard		
		Benign	Malignant	Total
DWI evaluation	Benign	66	9	75
	Malignant	4	26	30
	Total	70	35	105

DWI: diffusion-weighted imaging

TABLE 4. Distribution of the lesions found in the patients without known malignancy according to the reference standard and DWI evaluation

		Reference standard		
		Benign	Malignant	Total
DWI evaluation	Benign	52	0	52
	Malignant	4	1	5
	Total	56	1	57

DWI: diffusion-weighted imaging

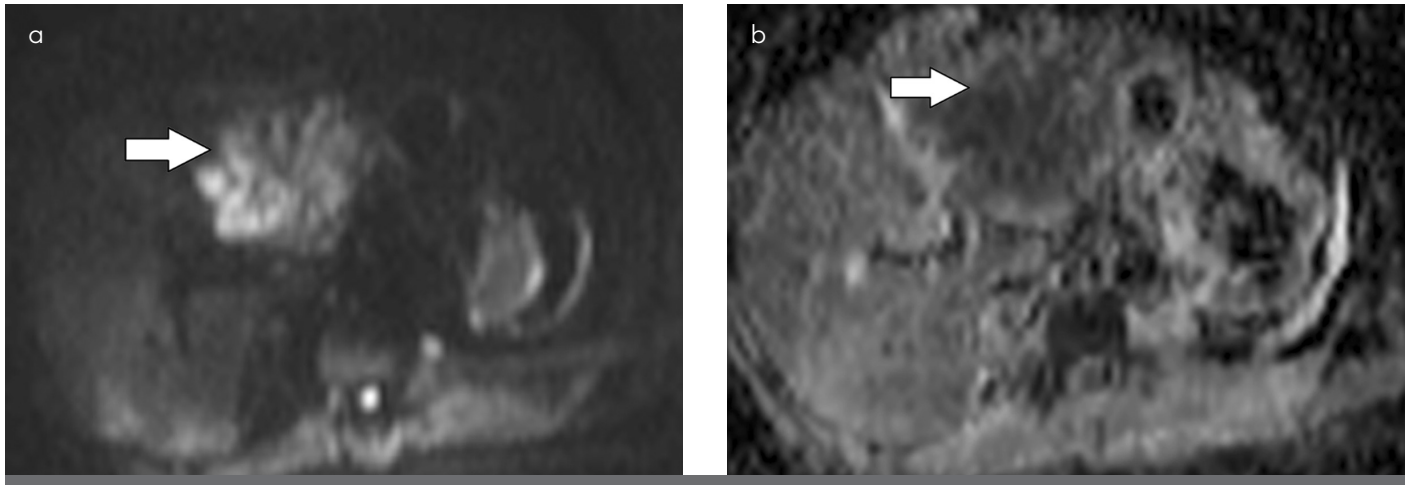

FIGURE 6. a, b. An abscesses in the left liver lobe of a patient shows restriction of diffusion on b800 image (a). The lesion is hypointense on ADC map (b) compared to normal liver parenchyma

of common lesions are demonstrated in Figure 1-5. All the hemangiomas and cysts were hyperintense in the ADC maps. The cysts were isointense, and the hemangiomas were either hyperintense or mildly hyperintense in the diffusion-weighted images with b value of 800 s/mm² (DWI-800). Except one, lesions evaluated as adenomas were seen as isointense or mildly hyperintense in the ADC maps and in the DWI-800. All of the HCCs showed hypointensity in the ADC maps and hyperintensity in the DWI-800. Twenty-three of the metastases showed peripheral hypointensity in the ADC maps and hyperintensity in the DWI-800. Nine of the metastases did not show hypointensity in ADC maps, and they were almost entirely necrotic. The abscesses were hypointense in the ADC maps and hyperintense in the DWI-800. The THADs and the focal fatty infiltration were isointense in the ADC maps and in the DWI-800. Features of all lesions in DWI evaluation are presented in Table 2.

According to the reference standard, 35 (33.33%) of all lesions were malignant, and 70 (66.66%) were benign. According to the DWI evaluation, 30 (28.5%) of all lesions were malignant, and 75 (71.4%) of them were benign (Table 3). The results derived from this table were as follows (the values in brackets are 95% confidence interval): sensitivity: 74.29% (56.75%–87.51%), specificity: 94.29% (86.01%–98.42%), positive predictive value: 86.67% (71.10%–94.50%), negative predictive value: 88.00% (80.63%–92.81%), and accuracy: 87.62% (79.76%–93.24%).

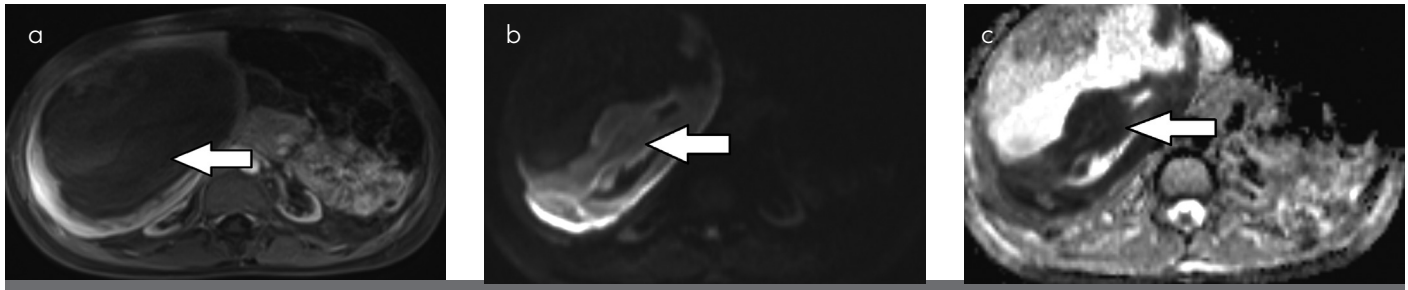

FIGURE 7. a-c. A very large lesion measuring 14 cm in the right liver lobe of a patient does not show contrast enhancement in the portal venous phase (a) where there is hyperintensity on b800 image (b) and hypointensity on ADC map (c), because of the effects of blood products

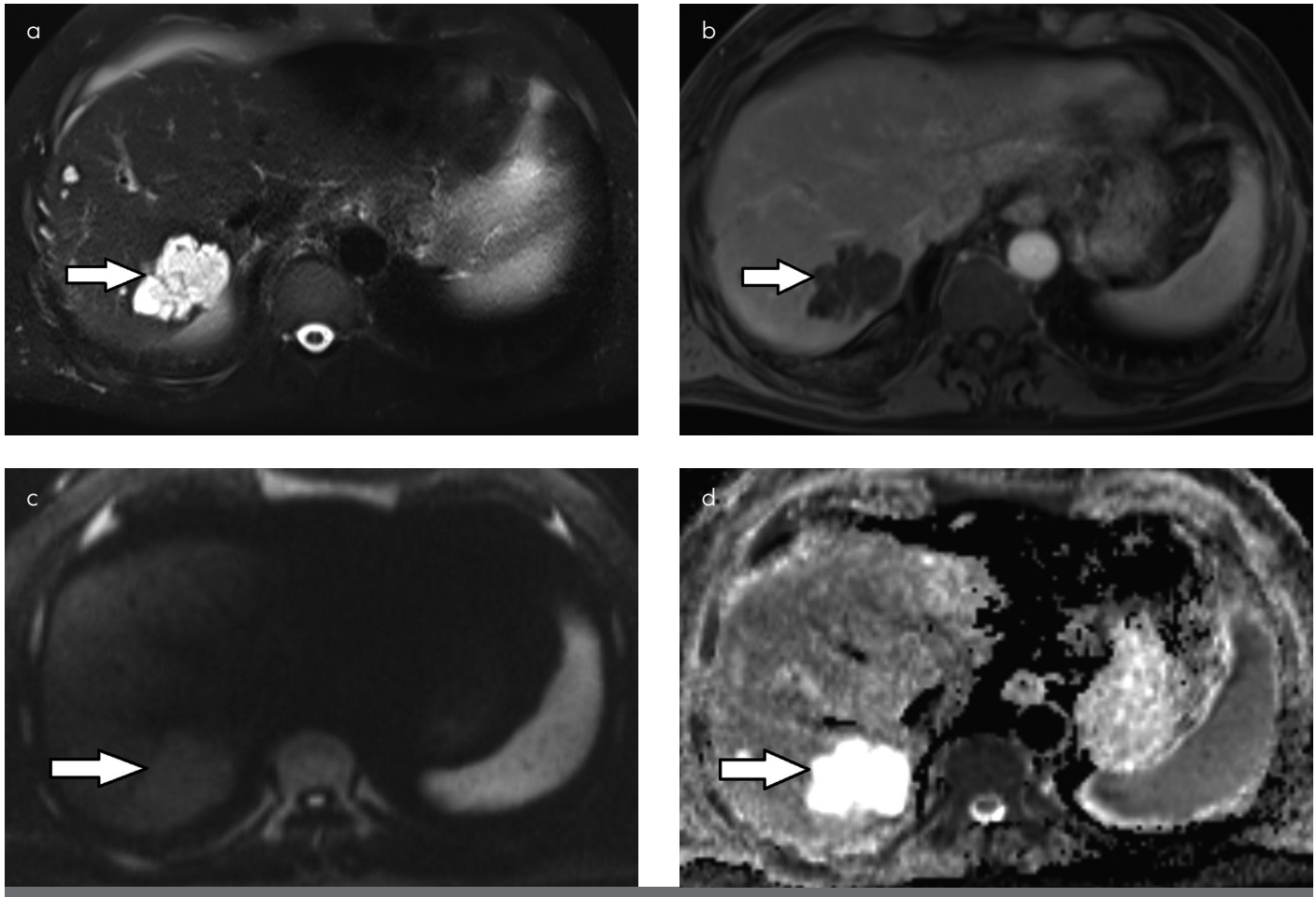

FIGURE 8. a-d. A necrotic metastasis. The lesion has septa on the T2-weighted image (a) and slight contrast enhancement on these septas on venous phase (b). It is slightly hyperintense on b800 (c) and obviously hyperintense on ADC map (d)

No malignant FLL was found in six patients with primary malignancy. Four of them had hemangiomas, one had a simple cyst, and one had an adenoma. In this respect, the DWI-alone interpretation was adequate to classify benign FLLs in patients with malignancy. In 28 patients without known malignancy, all FLLs were accurately interpreted as benign in the DWI-alone interpretation. The DWI-alone interpretation was accurate about 52 lesions in this group (Table 4). Only four lesions were misinterpreted as malignant in the DWI-alone interpretation. Three of these lesions were abscesses, and one of them was a hemorrhagic adenoma (Figure 6, 7). Only one patient without known malignancy had a malignant lesion, and it was an HCC. The DWI-alone interpretation was able to detect this HCC and classified as a malignant lesion. In the DWI-alone interpretation, nine lesions were misinterpreted as benign although they were malignant. All of these lesions were found in the livers of previously known malignancy patients, and they were diagnosed as necrotic metastases (Figure 8).

DISCUSSION

According to our results, upper abdominal MRI studies of 28 of 32 patients (87.5%) without known primary malignancy could be classified as benign or malignant with only signal characteristics on DWI sequences. All benign lesions in these patients were accurately classified by the DWI, which means that these MRI studies could have been completed without additional sequences and iv contrast media. These lesions were either

hyperintense or isointense in the ADC maps because of the absence of diffusion restriction and were classified as benign by visual assessment. In the formation of the DWI, density of atoms and T1 and T2 time differences of a tissue are influential (19, 24). In diffusion-weighted images with low b-values, the cystic or liquid components of tissues may have a high T2 effect (T2 shine-through effect). But in images with high b-values, this effect diminishes and the signal intensity highly depends on cellularity (25). Factors causing restriction of diffusion have been implicated to differences in cellularity, necrosis, nucleus/cytoplasm ratios, viscosity, and perfusion status (26-30). Tightly and randomly arrayed cells hinder the movement of extracellular water molecules. The increased cellularity of a tissue and the integrity of cell membranes are inversely proportional to the free diffusion of water molecules (26, 27). In addition, the movement of intracellular water molecules is restricted in tumors with high nucleus/cytoplasm ratio (26, 31). In the remaining four patients without known malignancy, the lesions were a hemorrhagic adenoma, three abscesses, and one HCC. These lesions were hypointense in the ADC maps suggesting restricted diffusion. Diffusion restriction of malignant tumors is the result of their high cellularity and smaller cell size as in the HCCs (32). Despite being benign, diffusion restriction in abscesses is associated with high viscous fluid containing large proteins, bacteria, and inflammatory cells that resist movement of molecules (30). Partial hypointensity of the hemorrhagic adenoma in the ADC map may be attributed to magnetic susceptibility effects of hemoglobin products (33). In our study, detection of malignant lesions by DWI evaluation was performed with 74.29% sensitivity and 88% negative predictive values. These values are relatively low for a screening test for patients with cancer. When we examined the cause of this, we found that false negative lesions according to the DWI-alone interpretation were the necrotic metastases under chemotherapy. These lesions were hyperintense or isointense in the ADC maps. Solid areas of these lesions that could restrict diffusion were almost vanished (34). They had only thin enhancing walls or septa. For this reason, we think that DWI imaging alone is not adequate especially in patients with known malignancy under treatment.

There are articles suggesting to use liver DWI for the detection of lesions and then subsequent characterization of lesions with iv contrast-enhanced T1-weighted sequences rather than using DWI alone (35). In this study, we did not intended to characterize the FLLs but classifying them as benign or malignant to eliminate iv contrast use and additional sequences.

The main difficulty in classifying FLLs with DWI is the differentiation of solid benign lesions (especially FNHs and adenomas) from malignant lesions (8, 36). FNHs and adenomas of liver are relatively rare according to the simple hepatic cysts and hemangiomas (37, 38). According to the data obtained from our study, the diagnosis of liver cysts and hemangiomas can mostly be made with DWI. In a study conducted by Girometti et al. (23) comparing visual assessment of ADC maps and quantification of ADC value to differentiate benign and solid lesions, accuracy of both methods was limited. We consider that the reason for this result is excluding hemangiomas and cysts from the study and analyzing only solid lesions. We included hemangiomas and cysts in our study since these lesions are common and may not be able to precisely diagnosed by US in patients with fatty liver or in conditions such as obesity and meteorism (39). Kenis et al. (40) found that visual assessment of DWI alone had the same performance as contrast-enhanced MRI in their study investigating the diagnosis of liver metastases in 68 patients. We think that visual assessment of DWI alone is sufficient for demonstrating benign features of common incidental FLLs in majority of cases. To do this, a radiologist must initially interpret the DWI images, and a decision must be made whether the examination should continue.

The limitations of our study are relatively low benign solid lesion diversity and lack of pathology reports in all solid lesions. Except one, biopsy was not required in patients without known malignancy because MRI findings and follow-up examinations were enough to show that the lesions were benign. In a patient with cirrhosis, the diagnosis of an HCC was straightforward depending on the detection of wash-out in contrast-enhanced sequences. In patients with previously known malignancy, the metastatic lesions were diagnosed by detecting peripheral halo type enhancement, contrast loss in late phases compared to normal liver parenchyma, nuclear medicine imaging results, and size changes because of treatment status. The diagnosis of benign lesions in these patients was made as in the patients without known malignancy, and no biopsy was required.

In summary, benign features of an FLL detected in a patient without known malignancy in modalities other than MRI can be shown using DWI alone. DWI is also capable of detecting malignant lesions in patients with or without known malignancy. However, it is not a suitable method for screening necrotic metastases under treatment. In appropriately selected patients, a liver MR examination can be completed with only DWI. This will contribute to reducing workload, allocating more time to the requiring patients, and reducing contrast agent usage and costs. A disadvantage of this practice is the need for a radiologist's interpretation for the decision whether to continue scan.

In conclusion, starting the upper abdominal MRI studies with DWI seems to be a practical approach to the management. We believe that researches from this perspective having more patients and more diverse lesions will make this opinion more acceptable.

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Review

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Brucella canis and Public Health Risk

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Pregnancy losses in dogs are associated with many types of bacteria. *Brucella canis* is reported to be one of the most important bacterial species causing pregnancy loss in dogs. Dogs can be infected by 4 out of 6 Brucella species (*B. canis, B. abortus, B. melitensis*, and *B. suis*). *B. canis* is a Gram-negative coccobacillus first isolated by Leland Carmichael and is a cause of infertility in both genders. It causes late abortions in female dogs and epididymitis in male dogs. Generalized lymphadenitis, discospondylitis, and uveitis are shown as the other major symptoms. *B. canis* infections can easily be formed as a result of the contamination of the oronasal, conjunctival, or vaginal mucosa . Infection can affect all dog breeds and people. While morbidity may be high in infection, mortality has been reported to be low. Most dogs are asymptomatic during infection, and it is difficult to convince their owners that their dogs are sick and should not be used in reproduction. The diagnosis of the disease is quite complex. Serological tests may provide false results or negative results in chronic cases. Therefore, diagnosis should be defined by combining the results of serological studies and bacterial studies to provide the most accurate result. No antibiotic treatment is reported to be 100% effective in B. canis infections, and infected animals are recommended to be removed from other animals and not be used for reproduction for a prolonged period.

Keywords: *Brucella canis*, humans, pathogen, zoonotic

INTRODUCTION

Brucella infection may form invaginal discharge during abortion or estrous. It may form as a result of the presence of $>10^{10}$ bacteria/mL in postnatal fetus, placenta, or lochia. It may form as a result of receiving these bacteria from oro-nasal, conjunctival, or vaginal mucosa contact (1-6). Bacteria are spread with sperm in male dogs (7). The infection may develop after copulation of a non-infected female dog with an infected male dog (8). Bacteria are also spread via urine in both genders. Male dogs can have high levels of bacteria in their urine, and this is the reason why male urination can be dangerous. *Brucella* infection begins to spread via urine after 4–8 weeks of infection (7).

Bacterial concentration in the milk of infected animals is reported to be high (9). The idea of disease spreading with high levels of bacteria in the milk has been a matter of debate (4). While some researchers are defending that milk is insignificant in disease transmission because puppies are infected in the uterus (6), some researchers report that infected milk may be dangerous for environmental contamination (4). Bacterium is isolated in low concentrations from saliva, nose and eye, and feces (10).

The aborted material of dogs infected with *Brucella canis* in dog shelters or in co-cultivated environments is very dangerous for non-infected dogs. Waste placenta tissue and fluids may contain high levels of microorganisms. Infection after contamination in shelters can be spread very quickly (5).

Huts, equipment, and people permanently in contact with infected dogs are also reported as sources of infection (11). Infections in humans are usually reported in laboratory workers and shelter workers exposed to sustained or major exposures. Owners who are in constant contact with infected dogs are also reported to be infected. As a result, animal owners should be informed about the potential risk but not alarmed about this zoonotic disease (12).

CLINICAL SYMPTOMS

Non-Specific Symptoms

B. canis-infected dogs may not always show serious clinical symptoms (5). Breeders provide barely or unidentifiable information about the clinical symptoms in *B. canis* infections. These clinical symptoms are weak hair

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structure, poor, exhausted, overworked, drowsy movements during exercise, weight loss, lameness, lymphadenitis, and changes in behavior (loss of appetite, poor performance) (2). Death has not yet been reported as a primary cause of death in *B. canis* infections (5).

Fever is rarely seen in *B. canis* infections, which is explained by the lack of endotoxin-producing lipopolysaccharides in the organism (2, 13, 14).

Decrease in pregnancy rate, early embryonic death, fetal resorption, and late-period abortions can be seen in female dogs, whereas painful scrotal growth, testicular atrophy, moist scrotal dermatitis, decrease in ejaculatory volume, loss of libido, reluctance to breed, poor quality sperm with white blood cells, and increase in morphological deformation in sperm can be observed in male dogs in examinations usually during the first 3 months of infection (2, 15). Discospondylitis, meningoencephalitis, or uveitis may also develop as a symptom in both genders (2).

B. canis affects the active macrophages (intracellular) during infection, causing the growth of lymph node nodules and reticuloendothelial cell hyperplasia. In addition to lymph nodes, spleen and liver growth can be observed. A hardening and a nodular structure in the spleen have also reported (2).

Specific Symptoms

Reproductive failure has reported to mean abortion, epididymitis, orchitis, and testicular atrophy. The most obvious clinical symptom that can be seen and reported by patient owners is the spontaneous abortions formed in dogs that are assumed to be healthy. These abortions usually occur between days 45 and 59 of pregnancy. Brucellosis can also result in resorption or early embryonic death after mating. Infants born as a result of pregnancies formed in infected animals may die in 20 days on average. Infected dogs have been reported to be able to give birth normally in the following pregnancy, as well as localized autolyzed fetuses, fetal mortality, or normal fetus that die within a few hours may be born. Puppies that are able to survive are bacteremic for several months (2, 4).

Long-term, viscous, serosanguinous vaginal discharge following abortion may last for 1–6 weeks. This discharge may contain high amounts of bacterial colonies and can be a major problem in infecting patient owners and other dogs. Necessary precautions and warnings must be made against contagion that can occur as a result of contamination with mouth, respiratory tract, or waste material and liquid to protect people and other dogs. Brucellosis does not affect estrus symptoms and mating. Dogs can continue to mate after two or three abortions and then have a normal birth (2, 4).

B. canis affects androgen-related organs in male dogs. Acute inflammation associated with pain and swelling can cause orchitis or epididymitis, which can also be determined by hand examination (2, 16, 17). Scrotal dermatitis can be formed as a result of licking by male dogs for comfort (2, 18). Chronic or prolonged infections can cause unilateral or bilateral testicular atrophy in breeding dogs (17, 19). Atrophy can result in reluctance to mate or loss of libido resulting from pain.

The systems in the body are also affected by bacteria. Discospondylitis in the thoracic vertebrae and lumbar vertebrae can be observed by radiography. Ophthalmologic examinations may result in endophthalmitis and recurrent uveitis (20-22). Non-suppurative meningitis is also reported at low rates (13).

Clinical Signs in Humans

A small number of studies regarding clinical cases caused by *B. canis* have been published. The consequences of infection with other zoonotic brucellosis range from asymptomatic infections to various syndromes that can appear insidiously or suddenly. Acute brucellosis is a febrile disease with non-specific influenza-like symptoms, such as fever, chills, headache, weakness, backache, muscle aches, and lymphadenopathy (growth in the lymph nodes) and accompanying splenomegaly and/or hepatomegaly. Patients may experience sweating, especially in the evening. Humans acquire *B. canis* infection through direct contact with infected dogs or their reproductive or blood products. Clinical signs and symptoms include undulate fever, chills, malaise, splenomegaly, and peripheral lymphadenomegaly. Diagnosis is often complicated in humans because of the non-specific signs and symptoms coupled with a low index of suspicion by many physicians. If the disease is part of the differential diagnosis, culture is the only test available for diagnosing *B. canis* infection in humans, and confirmation is problematic because of low-level and intermittent bacteremia. Even if physicians suspect brucellosis, diagnoses may be missed because the commercially available serological tests screen for smooth *Brucella* species and will not detect antibodies against *B. canis*. Canine serological tests for *B. canis* infection have been adapted for use in humans, but test results should be interpreted with caution (23-28).

Published clinical cases associated with *B. canis* contain various representations suitable for this description. They vary from fatigue, nausea, tremor, night sweats, and fever with headaches to one symptom, such as mild tiredness or fatigue and intermittent fever. There is sometimes a long-standing syndrome of unknown fever in some people. Spleen and/or liver enlargement and increased liver enzymes have been reported in various cases. Weight loss, anemia, enlarged lymph nodes/ovaries, and abdominal pain have also been documented. Nausea, vomiting, and diarrhea have been described especially in children, and constant cough, sore throat, and conjunctival burning, in addition to night sweats, headache, numbness, and muscle pain, have been reported in one person. Serious complications have been reported, including endocarditis in a few cases. *B. canis* was associated with aortic valve and lower extremity aneurysm in one child and associated with calvarial osteomyelitis, epidural abscess, pleural effusion, and

pulmonary nodules in another child. *B. canis* bacteremia and peritonitis were observed in an adult with concurrent hepatitis C infection and cirrhosis. Liver disease is also a predisposing factor in rare cases of peritonitis associated with other *Brucella* species. Very few *B. canis* infections have been described in immunosuppressed individuals; however, this organism causes non-specific febrile syndromes in two individuals who are simultaneously infected with human immunodeficiency virus-I (28-32).

A laboratory worker exposed to a less virulent M-strain of *B. canis* developed symptoms similar to the symptoms caused by *Brucella's* wild-type strains (24, 33).

Incubation Period

There is less information about the incubation period of brucellosis caused by *B. canis*. Acute symptoms caused by other *Brucella* species usually occur within 1–4 weeks. However, it may be insidious at first, and some cases are diagnosed 6 months after exposure (28, 31).

Diagnosis

Infertility, abortion, enlarged lymph nodes, swollen scrotum, and testicular atrophy are reported as clinical symptoms in *B. canis* infections; however, no clinical symptoms have been reported in some cases (34).

Bacterial isolation has been reported to be required from the tissue, discharge, blood, sperm, vertebra, or eye for a definitive diagnosis of *B. canis* cases. The results obtained should also be supported by positive serological agglutination tests, other serological titers, and hemoculture results. The variable amount and duration of bacteria in dogs make diagnosis difficult (35). Owing to this, a single negative blood culture does not show that there is no *B. canis* infection.

Diagnostic Tests

It can be difficult to diagnose brucellosis, which is caused by *B. canis*, in humans. The serological tests used to diagnose infections of the most commonly isolated zoonotic species (*Brucella abortus, Brucella suis*, and *Brucella melitensis*) do not detect antibodies against *B. canis*, and the tests used to detect *B. canis* antibodies are not usually available in diagnostic laboratories. In some studies, antibody reactions have been identified with the tests developed for this purpose or identified from analyses of dogs in the institute. These tests include microagglutination, tube agglutination, rapid slide agglutination test, and enzyme-linked immunosorbent assay (28, 36, 37).

Morbidity and Mortality

There is very less information about *B. canis* infections in humans. Since relatively few clinical cases have been documented (<100 since 2018) and most reported cases are mild, the virulence of this organism for humans may be low. However, the diagnosis of this disease is also insufficient given the low clinical suspicion and the difficulties of making a definite diagnosis among physicians. In a limited number of disease studies, some individuals who have been exposed to infected dogs have developed clinical findings or have had subclinical infection findings, such as laboratory abnormalities in liver function tests, whereas no evidence of disease was observed in others despite antibody detection. Nowadays, no deaths have been reported resulting from *B. canis* infections. The case mortality rate for untreated diseases caused by other *Brucella* species, including the highly virulent organism *B. melitensis,* is generally estimated to be 1%–2% or less. In serological studies, mostly conducted during the 1970s and early 1980s, <2% of the studied populations were reported to have antibodies against *B. canis*, and the seroprevalence of 68% in dogs, 73% in veterinarians, and 57% in male blood donors was observed in people who are in contact with dogs at average levels in a study conducted at the Oklahoma Health Sciences Center in 1975. A previous study in the 1970s reported that 13% of the hospitalized patients due to various diseases in Mexico are seropositive. During the last decade, two studies, one of which was published in the United States and the other in Turkey, found seroprevalence rates of ≤4% even during regular exposure to dogs. However, some studies indicate that *B. canis* infections may occur in some weakened/impoverished areas where dogs roam freely. In a previous study of a child with dog brucellosis in Argentina, 19% of the people in the neighborhood were seropositive (31, 38-43).

Treatment

Brucellosis is usually treated with long-standing antibiotics, and usually two or more drugs are combined in some part or all of the treatment in humans. Different antibiotics may be recommended depending on the patient's age, gestational status, and syndrome. Monotherapy has reported high levels of recurrence. If treatment is inadequate, recurrence may occur. Most recurrence often occurs within 3–6 months. Surgery may sometimes be required for localized focus. It has limited experimentation with *B. canis* in particular. However, standard antibiotic treatments for brucellosis appear to be effective in the cases reported. Several patients relapse as result of poor treatment (24, 25, 28, 31).

Future study is required to improve diagnostic assays for humans and animals and to generate policies to prevent the spread of disease (23, 28, 44-46).

CONCLUSION

Brucellosis in dogs remains endemic to many areas worldwide and without stronger intervention measures will probably remain an under-recognized threat to human health and animal welfare. Potential hazards for humans should be discussed when a dog is diagnosed with brucellosis because antibiotics do not eliminate *B. canis* safely, and the level of risk in humans is now uncertain. Euthanasia in infected animals is often recommended in shelters and is also a choice in pets. Some authors suggest follow-up for periodic serological testing of pets starting treatment.

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Case Report

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Tonsillar and Parotid Gland Metastases of Breast Cancer: Two Cases and A Literature Review

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Breast cancer is the most common tumor in women. One in eight women is affected by this disease in her lifetime. Both parotid gland and tonsillar metastases of breast cancer are extremely rare. Here, we present two separate cases of parotid gland and tonsillar metastases of breast carcinoma mimicking second primary tumors. The patient having parotid gland metastasis had disseminated disease and received palliative chemotherapy and hormone therapy. The other patient who had tonsillar metastasis underwent surgery without significant morbidity. Surgical resection of isolated tonsillar metastasis appears to have an influence on survival as the patient is free of disease at 12 months post-surgery.

Keywords: Breast neoplasms, neoplasm metastasis, palatine tonsil, parotid gland

INTRODUCTION

Breast cancer is one of the most common neoplasms in women. The most common sites of metastasis are the bone, lungs, liver, lymph nodes, and brain. Metastasis to the head and neck region, such as tonsil and parotid gland, is uncommon. As the parotid gland includes the lymph nodes inside the gland, metastasis to the parotid gland from the head and neck region is not rare; however, parotid gland metastasis of malignant tumors of infraclavicular origin is uncommon. Unlikely, tumor metastasis to the tonsil is extremely rare, accounting for only 0.8% of all tonsil tumors (1).

Herein, we present two separate cases of parotid gland and tonsillar metastases of breast carcinoma mimicking second primary tumors and a literature review.

CASE PRESENTATIONS

Case 1

A 54-year-old woman was presented to our department with a complaint of progressive painless swelling in the left parotid gland since the previous 3 months. Upon examination, a solitary swelling of approximately 3×2 cm was observed on the left parotid region. Fistulization of the mass to the skin was observed, and the overlying skin was hyperemic. Facial nerve functions were normal. She had a history of grade 3 invasive ductal carcinoma of left breast with multiple bone metastases at the vertebrae, costas, right iliac bone, and cranium. She underwent surgery, radiotherapy, and chemotherapy for breast carcinoma.

Computed tomography (CT) and ultrasound examination of the parotid region revealed a 3×2.5 cm mass in the left parotid gland. Pathological examination of fine needle aspiration biopsy revealed a malignant epithelial tumor. Both primary parotid malignancy and metastasis from breast cancer were considered in differential diagnosis. An incisional biopsy was performed, and pathological examination revealed a poorly differentiated estrogen receptor-positive adenocarcinoma

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and a progesterone receptor-negative adenocarcinoma with focal cytoplasmic staining with mammaglobin, supporting the possibility of parotid gland metastasis from breast carcinoma (Figure 1). The test for c-erbB-2 was negative according to immunohistochemical examination. The patient was treated with chemotherapy and hormone therapy after diagnosis. She was alive with disease 8 months after the diagnosis of parotid metastasis.

Case 2

A 60-year-old woman presented to our department with sore throat and swelling of her left tonsil since the previous 2 months. She had a history of grade 2 invasive ductal carcinoma of right breast treated using modified radical mastectomy, postoperative radiotherapy, and chemotherapy 2 years ago. Physical examination showed a unilateral enlargement of the left tonsil. CT of the oropharynx and the neck revealed a 5×3-cm mass in the left tonsillar region. Incisional biopsy was performed, and pathological examination revealed poorly differentiated carcinoma. Positron-emission tomography/CT demonstrated an increased uptake (SUV_{max}=29) at the left tonsil and left deep cervical lymph nodes that indicated a second primary tonsillar carcinoma. There was no evidence of distant metastasis.

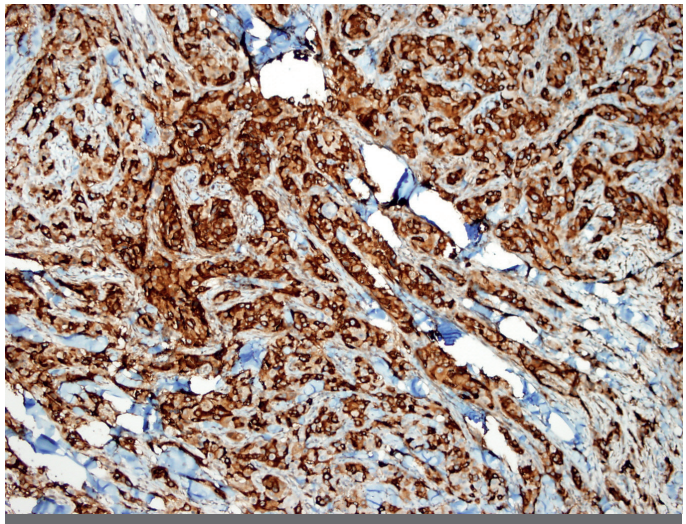

FIGURE 1. Photomicrograph of a section through the parotid tumor revealing a metastatic invasive lobular carcinoma of the breast with focal cytoplasmic staining with mammaglobin (×400 magnification)

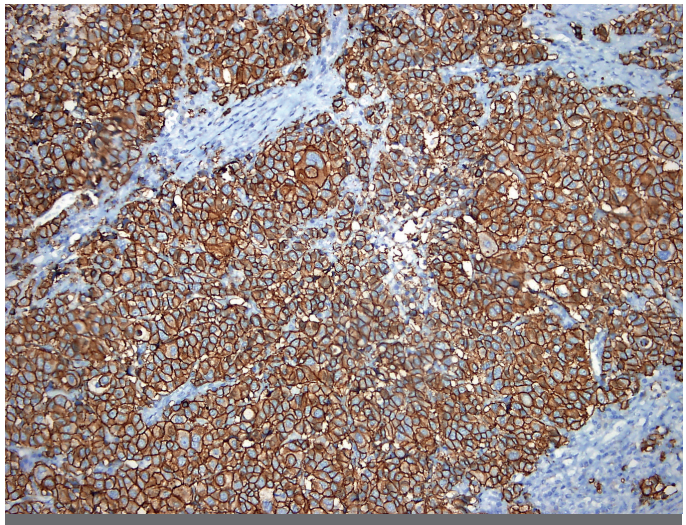

FIGURE 2. Photomicrograph of a section through the tonsil tumor revealing a metastatic invasive lobular carcinoma of the breast with c-erbB-2 expression (×200 magnification)

Based on these results, the tumor was considered a second primary tonsillar carcinoma. Intraoral resection of the tumor combined with left functional neck dissection was performed. Pathological examination of the specimen revealed a ductal carcinoma infiltration of the tonsil according to diffuse cytoplasmic staining with CK7 and focal cytoplasmic staining with mammaglobin. Some positive reactions were observed with c-erbB-2 and gross cystic disease fluid protein 15 (GCDFP-15); however, no staining was observed with CK20 (Figure 2). In immunohistochemical examination, tumor was negative for estrogen and progesterone receptors and positive (score 3) for HER2. Similar histological findings were observed in eight lymph nodes. The patient received hormone therapy post-surgery and was free of disease at 12 months post-surgery. Written informed consent was obtained from both patients who participated in the study.

DISCUSSION

Breast cancer is the most common tumor in women. One in eight women is affected by this disease in her lifetime. The median survival of metastatic breast cancer is 12 to 24 months (2). Tumor stage and lymph node involvement are the most accurate prognostic indicators for a patient with breast cancer. Metastasis of breast carcinoma is rarely observed in the head and neck region; the most common areas are the brain and skull bones.

Metastasis from distant tumors accounts for <1% of all malignancies of the oral, maxillofacial, and upper neck regions (3). Metastatic tumors of the head and neck region usually originate from the lungs, breast, kidneys, thyroid gland, and prostate gland (3).

Metastatic involvement of the parotid gland is often secondary to squamous cell carcinoma of the head and neck and melanomas of the neighboring skin. Additionally, primary tumors other than the head and neck region, such as breast, prostate, kidneys, and gastrointestinal tumors, have the capability to metastasize to the parotid gland through the thoracic duct or the Batson's paraspinal venous plexus, bypassing the pulmonary vascular filtration (4). The treatment options for metastatic disease of the parotid gland include parotidectomy, radiation therapy, chemotherapy, and hormone therapy (5). Furthermore, all the current therapeutic procedures for metastatic disease of the parotid gland are palliative in advanced disease.

The status of estrogen and progesterone receptors can vary between primary sites to metastasis for breast carcinoma. This suggests a dedifferentiation of the tumor (6). Therefore, hormone receptor analysis of the metastasis is warranted by surgical biopsy. HER2 testing of metastasis does not appear to have any influence on treatment decisions (6).

Perez et al. (7) reported a patient with parotid gland metastasis that occurred 5 years after the treatment of breast carcinoma. They suggested that the immunohistochemical study of estrogen receptor is fundamental for diagnosis of parotid gland metastasis. Dangore et al. (8) reported a patient with swelling in the

left parotid gland, who was previously diagnosed with breast cancer. Based on the clinical findings, second primary malignancy of the parotid gland was suspected, and parotidectomy was performed (8). Additionally, the patient received chemoradiotherapy. They suggested that swelling in the parotid gland needed to be examined very carefully. Ando et al. (5) reported a patient with advanced breast cancer and swelling in the parotid gland. They also performed parotidectomy for diagnosis. Their patient received capecitabine in addition to trastuzumab postoperatively, which is one of the treatments used for patients with HER2-positive breast cancer.

In the literature, to the best of our knowledge, 9 out of 17 patients presenting with parotid gland metastasis underwent parotidectomy. Most of the cases were performed due to suspicion of second primary tumor. In the case of disseminated metastasis of breast cancer, resection of parotid metastasis does not improve survival rates (5). In the case of isolated parotid gland metastasis, parotidectomy could be the preferred choice of treatment, and it may indicate the percentage of hormone receptors requiring adjuvant hormone therapy.

Metastatic tumors involving the tonsils are rare. In the literature, only a few cases of tonsillar metastasis as a presenting feature have been reported (9). Metastasis to the tonsil is more often reported unilaterally with a preference for the left side, although primary tumor is in the right breast (9). In general practice, late-onset metastasis has better prognosis than early metastasis in breast cancer. Patients with tonsillar metastasis of breast cancer have poor prognosis regardless of the location of the primary tumor, its histological type, and the treatment modality (9).

Tonsillar metastasis is often associated with multifocal disseminated disease. However, the patient in our report with tonsillar metastasis had no evidence of recurrent tumor or other distant metastasis. Pathological evaluation of preoperative tonsillar biopsy did not help us distinguish between primary and metastatic tumor of the tonsil. The tumor was considered to be a second primary tonsillar carcinoma, and surgical treatment was performed with curative intent. Breast was confirmed as the origin of metastasis by the presence of GCDP-15 in the postoperative pathological examination of the tonsil and lymph nodes of the neck.

Patients with tumors that overexpress HER2 protein are candidates for therapies that significantly reduce mortality (10). In our case study, immunohistochemical analysis was negative for estrogen and progesterone receptors and positive for HER2. Therefore, trastuzumab therapy was continued postoperatively. The presence of disseminated disease is probably the main factor in determining prognosis. Surgical resection of tonsillar metastasis appears to have an influence on survival because our Case 2 patient is free of disease at 12 months post-surgery.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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Case Report

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Pulmonary Embolism Mimicking Community-Acquired Pneumonia: A Case Series

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Pulmonary embolism (PE) is a severe medical condition that requires a high degree of clinical suspicion to prevent mortality and morbidity. PE can mimic pneumonia due to its clinical and laboratory findings. Here we report of three cases without an identifiable thrombotic source, who first presented with more likely pneumonia and were later diagnosed with and treated for PE.
Keywords: Pulmonary embolism, community-acquired pneumonia, lung

INTRODUCTION

Pulmonary embolism (PE) is a life-threatening clinical condition, and it is the third most common cause of cardiovascular death in the United States (1). Mortality is related to comorbidity factors and the time from diagnosis to treatment. When PE is accompanied by pulmonary infiltrates in a chest x-ray, most of the pathologies in differential diagnosis include pneumonia, tuberculosis, pulmonary fungal disease, pulmonary vasculitis, etc. (2). Different clinical presentations make its diagnosing difficult (3). Community-acquired pneumonia (CAP) is one of the most common pulmonary syndromes, and it has similar symptoms to PE. A delayed PE diagnosis, due to clinical and radiological similarity with CAP, may cause increased mortality and morbidity. Herein, we report three cases of PE initially diagnosed and treated as CAP. Written informed consent was obtained from all patients.

CASE PRESENTATIONS

Case 1

A 52-year-old male patient presented to the emergency department with a 3 days history of high fever, dry cough, and pleuritic right-sided chest pain. The patient had a fever of 38.8°C, a heart rate of 108 beat/minute, an oxygen saturation of 95% (room air), and the blood pressure of 130/80 mmHg. A physical examination revealed decreased breath sounds in the right lower zone of the lung. Laboratory tests showed an increase in the white blood cell (WBC) count (14,900 μL) and C-reactive protein (CRP; 12.24 mg/dL). A chest X-ray (CXR) showed the right lower lobe consolidation obscuring the right hemidiaphragm (Figure 1). The patient was hospitalized with a diagnosis of CAP, and empirical antibiotherapy was initiated. The following day, he suffered from the exercise-induced shortness of breath. The oxygen saturation was 82% at room air. Subsequently, an echocardiography was performed by the cardiologist revealing a right heart chamber enlargement, right ventricular systolic disfunction, and an increased pulmonary artery pressure (50 mmHg) that suggested pulmonary embolism. Diagnosis was confirmed by a computed tomography (CT) angiography of the thorax. As a result, thrombus formation was detected in the right pulmonary artery, predominantly on the bifurcation level. The lower limb Doppler ultrasound was performed, and it was negative for thrombus. We initiated low-molecule-weight heparin (LMWH) therapy. On the third day of hospitalization, patient's body temperature was normal, there was no dry cough, the CRP level significantly decreased (2.52 mg/dL), and the sputum culture was negative. Thus, we excluded the CAP and discontinued the empirical antibiotic therapy. One week later, we switched the LMWH to Rivaroxaban 20 mg twice a day. On the 10th day of the anticoagulation therapy, the patient completely recovered.

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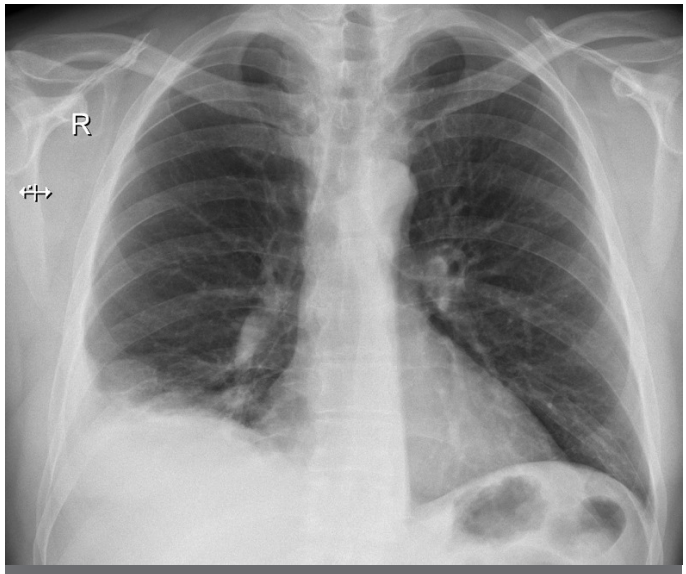

FIGURE 1. Right lower lobe consolidation obscuring the right hemidiaphragm

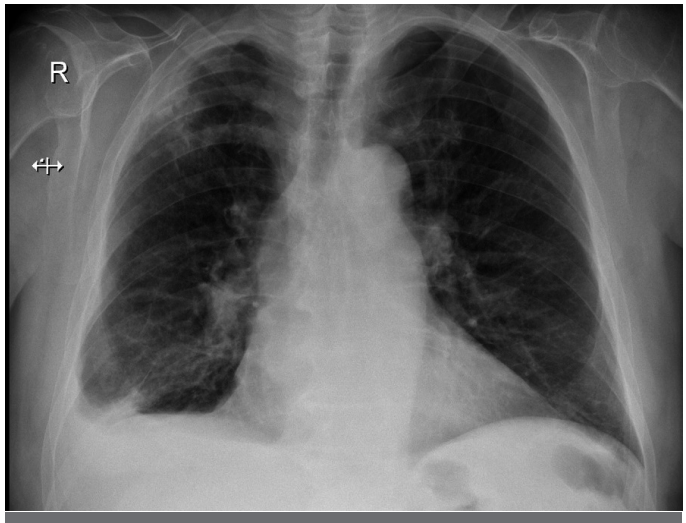

FIGURE 2. Right lower Lobe infiltration with effusion

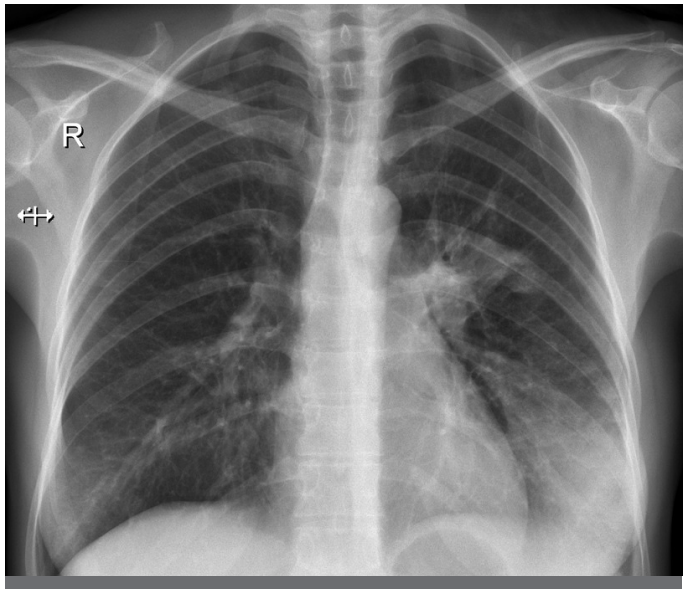

FIGURE 3. Left lower and mid-zones consolidation

Case 2

A 79-year-old male with a history of coronary artery disease presented to our clinic with complaints of high fever, cough, shortness of breath and back pain for 2 days. He was a smoker (30 packs a year, quit 20 years ago). The patient had a fever of 38 0°C, a heart rate of 96 beats/minute, an oxygen saturation of 93% (room air), blood pressure of 145/85mm of Hg. The lung auscultation revealed rales in the right lower lung. Laboratory tests showed the WBC count of 18,400 μl and CRP of 15.69 mg/dL. The CXR PA revealed an infiltration in the right lower lobe with effusion (Figure 2). The patient was initially treated with nonspecific antibiotherapy. Three days after the antibiotherapy, the patient suffered from sharp chest pain with worsening shortness of breath, and the SpO2 level was 82% at room air. The patient was evaluated at the cardiology clinic. Echocardiography revealed a severe right heart chamber enlargement, right ventricle basal segments hypokinesia, and a significantly increased pulmonary artery pressure. Diagnosis was confirmed by a CT angiography of the thorax, which revealed acute thrombus formation in the right main pulmonary artery. The LMWH was initiated. After the anticoagulation treatment, the patient was screened with echocardiography every day to see if there were any improvements in the right ventricular systolic function and pulmonary artery pressure. The patient continued with the LMWH and supportive treatment, he improved gradually within 5 days, and his pulse, respiratory rate, and sufficient oxygen saturation on room air values returned to normal.

Case 3

A 35-year-old female presented to our clinic with fever, cough, sputum, and hemoptysis that lasted for the past 4 days. She had small amounts of blood-streaked sputum. Her past medical history included asthma for 5 years. The patient had a fever of 38.1°C, heart rate of 91 beats/minute, an oxygen saturation of 97% (room air) and blood pressure of 100/65 mm of Hg. The lung auscultation revealed mild crackles in the left lower lung. Laboratory studies showed the WBC count at 12,700 μL and CRP at 7,69 mg/dL. The CXR PA showed an obvious infiltration and consolidation in the lower and mid-zone of the left lung (Figure 3).

Levofloxacin 1x500 mg tablets were prescribed. Five days after the visit, the patient presented to our emergency department with an abrupt onset of chest pain and significantly increased bloody sputum. To exclude other medical conditions, a cardiology consultation was requested. Echocardiography revealed a moderate right heart chamber enlargement, moderate right ventricle systolic dysfunction, and markedly increased pulmonary artery pressure. A CT angiography of the thorax was performed, and it revealed multifocal embolus in the posterobasal and laterobasal branches of the left pulmonary artery. The LMWH was initiated, and close monitoring of coagulation parameters and vital signs has been done. Within four days of the LMWH therapy, the oxygen saturation returned to normal.

DISCUSSION

Community-acquired pneumonia is an acute infection of the lung. It was reported that 5%–17% of patients with diagnosed pneumonia actually suffer from non-infectious CAP that is mimicked. In addition, CRP and procalcitonin may be elevated

in many of such cases. Usually, CAP shows significant improvement in vital signs within 48–72 hours following the antibiotic therapy initiation (4). Even if there are different reasons of the CAP treatment failure, in this study, we focused on PE.

Clinical suspicion of PE is not a frequent consideration in patients who present with classical infectious diseases symptoms. Furthermore, the prevalence of CAP complicated with PE is very rare (3). Cough, fever, dyspnea, sputum, and pleuritic pain are the main symptoms of CAP, as well as PE. Coexistence may also occur rarely. However, if physicians were aware of PE, further investigation for diagnosis would be performed correctly (2).

Imaging tests are important in confirming the diagnosis. Contrast-enhanced computed tomography pulmonary angiography (CTPA) is the standard imaging modality for the diagnosis of PE. Ventilation/perfusion lung scanning is an alternative to CTPA and useful for patients in whom CTPA is contraindicated. However, it should be kept in mind that nearly two-thirds of the patients have a non-diagnostic result, which is neither normal nor high probability (4). There are many risk factors for PE, such as recent surgery, pregnancy, oral contraceptives, immobilization, and trauma (5). The only relative risk that we suspected was physical inactivity in the first case due to patient's profession. Other cases did not have any remarkable risk. All of these patients had negative hypercoagulability screen tests.

Pulmonary embolism is treated by anticoagulants in all patients who do not have a significant contraindication (1). If there is no response to anticoagulant therapy, pulmonary embolectomy surgery can be applied (6). In our cases, we started the LMWH treatment and continued with oral anticoagulants.
In conclusion, PE mimicking CAP is not uncommon. Therefore, clinicians must be aware of this medical condition, particularly in patients who do not respond to antibiotic treatment.

Informed Consent: Informed consent was obtained from the patients who participated in this study.

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Case Report

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Emergency Surgical Intervention to Treat a Wide Septated Subdural Hematoma in a Patient with ITP: A Case Report

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Immune thrombocytopenic purpura (ITP) is a syndrome that reduces platelet levels, and it is caused by the development of autoantibodies against thrombocytes. The ITP cases that present with subdural hematoma are rarely seen. It is necessary to start corticosteroid and intravenous immunoglobulin (IVIg) treatment early in the emergency cases, until a definite diagnosis is made. A 54-year-old female patient applied to the neurosurgery department with the complaint of dizziness that lasted for a week. The patient indicated that she had an upper respiratory tract infection a month ago. On her computed tomography examination, she was diagnosed with a large and septated subdural hematoma with different components of acute, subacuteand chronic bleeding with the ITP diagnosis. The patient had to undergo an urgent surgical procedure due to right hemiparesis and confusion caused by the finding of increased intracranial pressure. During pharmacotherapy for ITP, the patient was re-operated due to recurrent bleeding. The patient was discharged without any deficiency after the second operation. Thrombocytopenia cases are challenging with regard to surgical intervention. It is necessary to start corticosteroid treatment early in emergency cases, until the definite diagnosis is made. Corticosteroids inhibit the development of autoantibodies in the treatment of ITP but also increase the release of platelets from the vascular wall. Corticosteroid treatment together with IVIg treatment should be initiated, and in the case of an emergency surgical intervention, a thrombocyte replacement needs to be initiated immediately.

Keywords: Septated subdural hematoma, immune thrombocytopenic, purpura thrombocytopenia, multicomponent subdural hematoma

INTRODUCTION

Immune thrombocytopenic purpura (ITP) is a syndrome that reduces platelet levels, and it is caused by the development of autoantibodies against thrombocytes. The ITP cases that present with subdural hematoma are rarely seen (1). It is necessary to start corticosteroid and intravenous immunoglobulin (IVIg) treatment early in emergency cases, until a definite diagnosis is made (2, 3). In this paper, we aimed to present a wide septated subdural hematoma that occurred in a case diagnosed with ITP and an emergency surgical intervention.

CASE PRESENTATION

A 54-year-old female patient was admitted to the hospital complaining of dizziness that lasted for a week. The patient did not have any complaints of headache, vomiting, epileptic seizures, and neurological deficit; however, purpural rash was observed on the examination and was more prominent in the upper limbs. Frust hemiparesia was present on the right side on the neurological examination. Bilateral papillary edema was detected in the fundus oculi upon the ophthalmic examination. The patient indicated that she had an upper respiratory tract infection a month ago and that her blood test results were as follows: hemoglobin, 11.4; leucocytes, 8500; and the platelet value, 5000. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined, respectively, as 12 and 29. Liver and kidney function tests were within the normal limits. Splenomegaly was not detected upon an abdominal ultrasound examination. Serologic tests were negative. Peripheral smear was compatible with thrombocytopenia, and atypical cells were not detected. The ANA (hep2) and Coombs tests were negative. The patient did not mention any use of antiaggregants or anticoagulants.

The cranial magnetic resonance imaging (MRI) examination showed a subdural hematoma in the left fronto-parietal area, which was 6 cm thick in the largest part, was causing a 2-cm-thick midline shift, and also contained a large number

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of septations (Figure 1). An underlying vascular pathology was not detected in the MR angiography. The patient was given intravenous methylprednisolone 1 g/day and IVIg (1 g/kg/day) as treatment. One day later, the number of platelets increased to 11.000 in control blood tests. On the same day, the patient had to undergo an urgent surgical procedure due to right hemiparesis and confusion caused by the finding of increased intracranial pressure. The preoperative Glasgow coma scale was evaluated as 10/15.

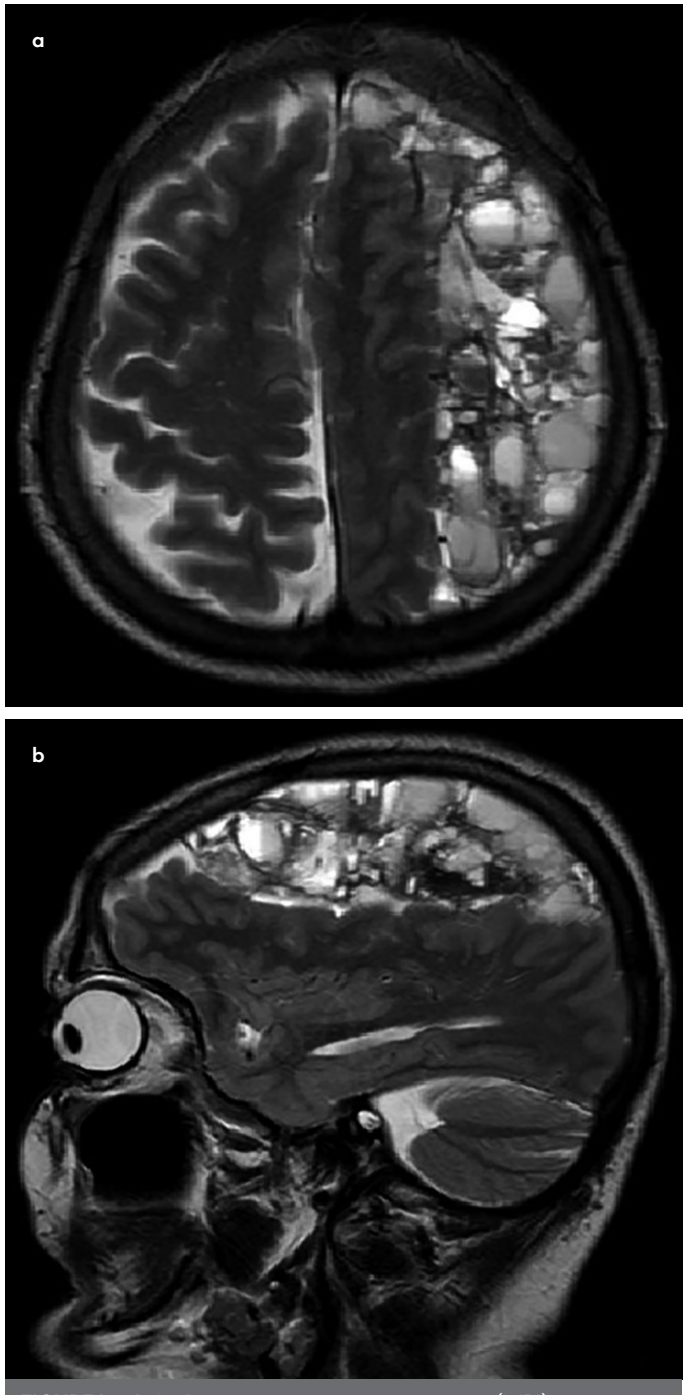

FIGURE 1. a, b. In the magnetic resonance imaging (MRI) examination, a wide septated subdural hematoma with different components can be seen (a) in the left fronto-parietal area, 6 cm thick in the largest part, causing a 2-cm-thick midline shift, and also containing a large number of septations (b)

The patient was operated with left fronto-parietal craniotomy, and subdural hematoma was evacuated. Levetiracetam treatment was started as an antiepileptic treatment in the postoperative period. The platelet count was 18.000 on the first postoperative day, and the corticosteroid treatment continued. The patient was re-operated upon the findings of increased intra-

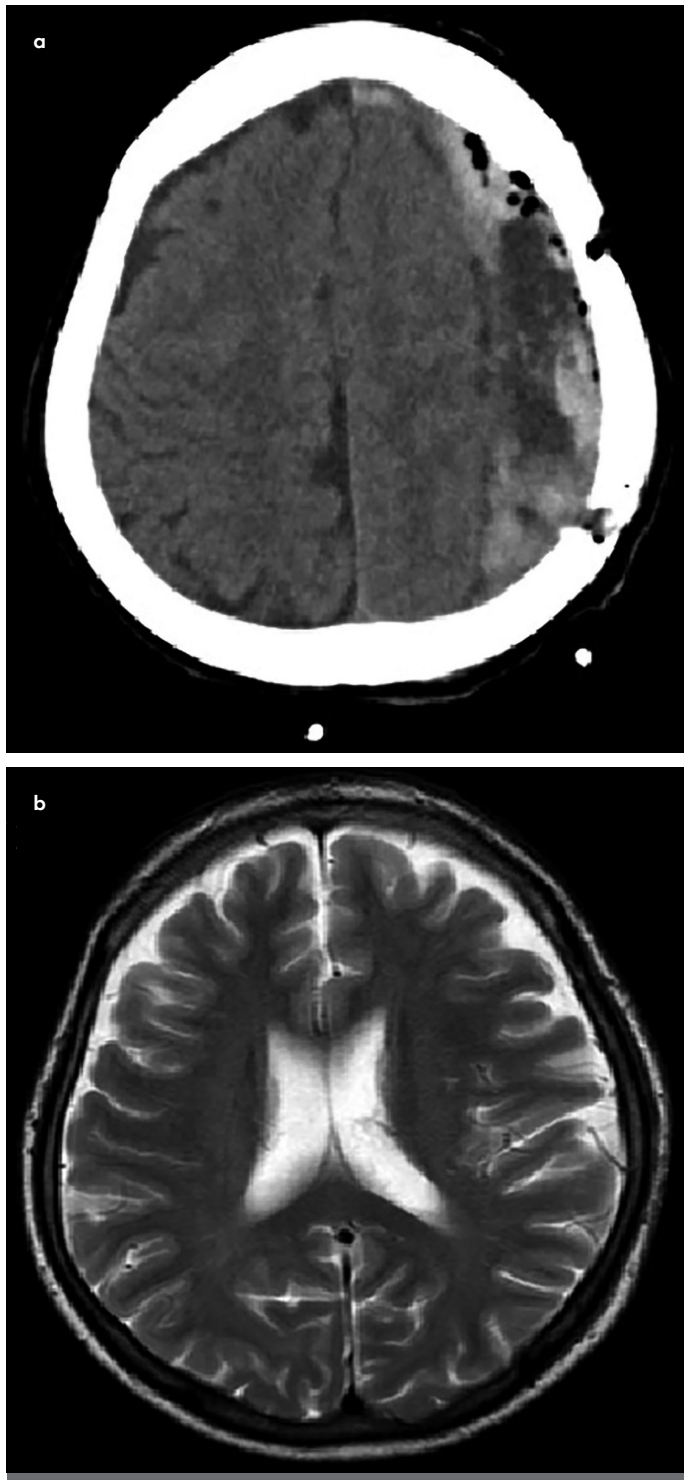

FIGURE 2. a, b. On the postoperative 3rd day computed tomography (CT) scan, it can be seen that the bleeding occurred again in the operation area (a). On the MR examination 5 months after the operation, it can be seen that the subdural space was completely closed, and there were no pathologies that could lead to spontaneous bleeding (b)

cranial pressure, and hemorrhage was detected again in the same region on the computed tomography (CT) scan (Figure 2a). During surgery, the acute subdural hematoma in the same region was evacuated. No additional neurological deficits were detected in the postoperative early period, except the right frust hemiparesis. On the fifth postoperative day, the patient's platelet count was 23.000, and the patient was discharged without any neurological deficits 8 days after the second operation, because the cranial CT revealed that the blood in the subdural space was reabsorbed. Eight months after the operation, the MRI examinations revealed that the subdural space was completely closed and that there was no any other pathological formation (Figure 2b). Informed consent form was obtained from the patient.

DISCUSSION

Immune thrombocytopenic purpura is a syndrome that reduces platelet level sand it is caused by the development of autoantibodies against thrombocytes. Coagulation parameters are within the normal range. The most serious and life-threatening complication of ITP is intracranial hemorrhage (1). A reduced platelet count is a risk factor for the development of spontaneous intracranial hemorrhage, and in addition, minor traumas can lead to intracranial hemorrhages.

In the case of ITP, the PT and aPTT values are within the normal limits because there is no factor deficiency. Bleeding and coagulation times are prolonged due to a low platelet count. Therefore, there is no efficacy of plasma replacement when surgical intervention is to be applied to ITP cases.

For these cases, corticosteroid treatment is administered to stop the development of autoantibodies (2). Sole thrombocyte replacement is not curable because the autoantibody presence persists. A continued autoantibodies presence leads to the destruction of replaced platelets. Therefore, corticosteroid and IVIg treatment is applied to prevent the autoantibody development during the treatment (2). Corticosteroids are the first drug of choice in the treatment; however, if the use of corticosteroids is contraindicated and if an emergency intervention is needed, IVIg is preferred. However, the efficacy of the IVIg treatment is temporary and lasts for 2-4 weeks (3).

Subdural hematomas are the most commonly seen intracranial hemorrhages; however, subdural hematoma cases caused by ITP are rarely seen. A low platelet level is a risk factor for the subdural hematoma formation, but it is not associated with an increase in the hematoma size (1). It is thought that the hematoma volume increases due to the fibrinolytic activity in hematomas that are larger in volume and that were early asymptomatic and were detected in the chronic phase.

Septated subdural hematomas are rarely seen. The presence of a large number of septations and the presence of acute, subacute, and chronic components of the hematoma at the same time suggest that the bleeding repeated many times at different times. Due to the presence of numerous septations, the craniotomy method was preferred during surgery.

Timing of the surgical treatment is vital in ITP cases. The platelet count needs to be at the acceptable levels as much as possible in surgical timing. In these cases, the number of platelets lower than 20,000 is a treatment indication (1). The main goal of treatment in adult ITP is to achieve a safe number of platelets, and not to completely normalize the platelet value.

A thrombocyte replacement solely may not be enough in cases that need an emergency surgical intervention. The main underlying pathology is the presence of autoantibodies, and thrombocyte replacement is not enough alone to increase the platelet count due to the presence of autoantibodies (1). For this reason, a platelet replacement together with IVIg and corticosteroid treatments should be performed (1).

Thrombocytopenia cases are challenging for surgical intervention. It is necessary to start the corticosteroid treatment early in the emergency cases until definite diagnosis is made. Corticosteroids inhibit the development of autoantibodies in the treatment of ITP, but also increase the release of platelets from the vascular wall. Corticosteroid treatment together with IVIg treatment should be initiated, and in emergency surgical interventions, thrombocyte replacement needs to be initiated immediately.

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

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Author contributions: Concept - A.K., Z.A.; Design - A.K., Z.A.; Supervision - A.K., Z.A.; Resource - Z.A.; Materials - A.K., Z.A.; Data Collection and/or Processing - Z.A., A.K.; Analysis and/or Interpretation - A.K., Z.A.; Literature Search - Z.A.; Writing - Z.A., A.K.; Critical Reviews - Z.A., A.K.

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

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Case Report

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Effectiveness of FDG PET/CT in Metastatic Infections

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Prosthesis infections that develop two years after the operation or later occur completely hematologically. In some patients, systemic inflammatory response syndrome or metastatic infection can occur. Diagnosing metastatic infections is time consuming and difficult. All the infection foci cannot be found even with the use of many imaging techniques. But F-18 fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) imaging allows whole body scan anatomically and functionally. Here, we aimed to discuss the role of F-18 FDG PET/CT imaging in detecting and diagnosing multiple abscess foci secondary to *Staphylococcus aureus* bacteremia in a case with late prosthesis infection and atypical complaints.

Keywords: FDG PET/CT, prosthetic joint infection, pyogenic abscess

INTRODUCTION

Prosthetic infections are difficult to diagnose and treat, and treatment costs are high. Infections are usually due to polymicrobial, coagulase negative Staphylococci and *Staphylococcus aureus (S. aereus)*. The time since implantation of the prosthesis is of concern. In early prosthetic infections (<3 months), nonspecific complaints such as fever, tachycardia, and joint pain may develop. Infections that develop between 3 and 24 months cause more joint pain. Whereas, infections that develop 2 years after the operation or later occur completely hematologically (1). In some patients, systemic inflammatory response syndrome or metastatic infection can occur. Early detection of metastatic infection enables the identification of appropriate treatment. There are several useful serologic tests and imaging methods for diagnosis. Ultrasonography (USG) and magnetic resonance imaging (MRI) are extremely sensitive and reliable methods to diagnose regional infection foci, but their use is limited in assessing multifocal infection. F-18 fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) imaging has an important role in the detection of multifocal infections. F-18 FDG PET/CT whole body imaging allows examining all organs and tissues from the vertex of head to the foot. It is extremely useful in the detection of infection focus and foci in patients with infection history and predisposing factors such as prosthesis. Here, we aimed to discuss the role of F-18 FDG PET/CT imaging in detecting and diagnosing multiple abscess foci secondary to *S. aureus* bacteremia in a case with late prosthesis infection and atypical complaints.

CASE PRESENTATION

A 56-year-old female patient was admitted to the outpatient clinic with complaints of right leg swelling and pain, weakness, and high fever. On physical examination, increase in the diameter and temperature, hyperemia, and tenderness were detected in the right leg. Also, left hip joint movements were painful. The patient had a 16-year-old left hip and 12-year-old right hip prosthesis. Doppler ultrasonography showed abscess foci with septations and deep femoral vein thrombosis in the right leg. Treatment was started. During follow-up, increased temperature and hyperemia developed in both wrists, elbows, and right ankle. Hyponatremia and respiratory distress occurred. The CT images showed multiple nodules and ground glass appearance in the lungs. Retroperitoneal lymph nodes were also detected. On the same day, blood cultures were taken because of suddenly rising fever; and atypical pneumonia therapy was started with levofloxacin from the quinolone group. On the second day of treatment, respiratory distress disappeared;

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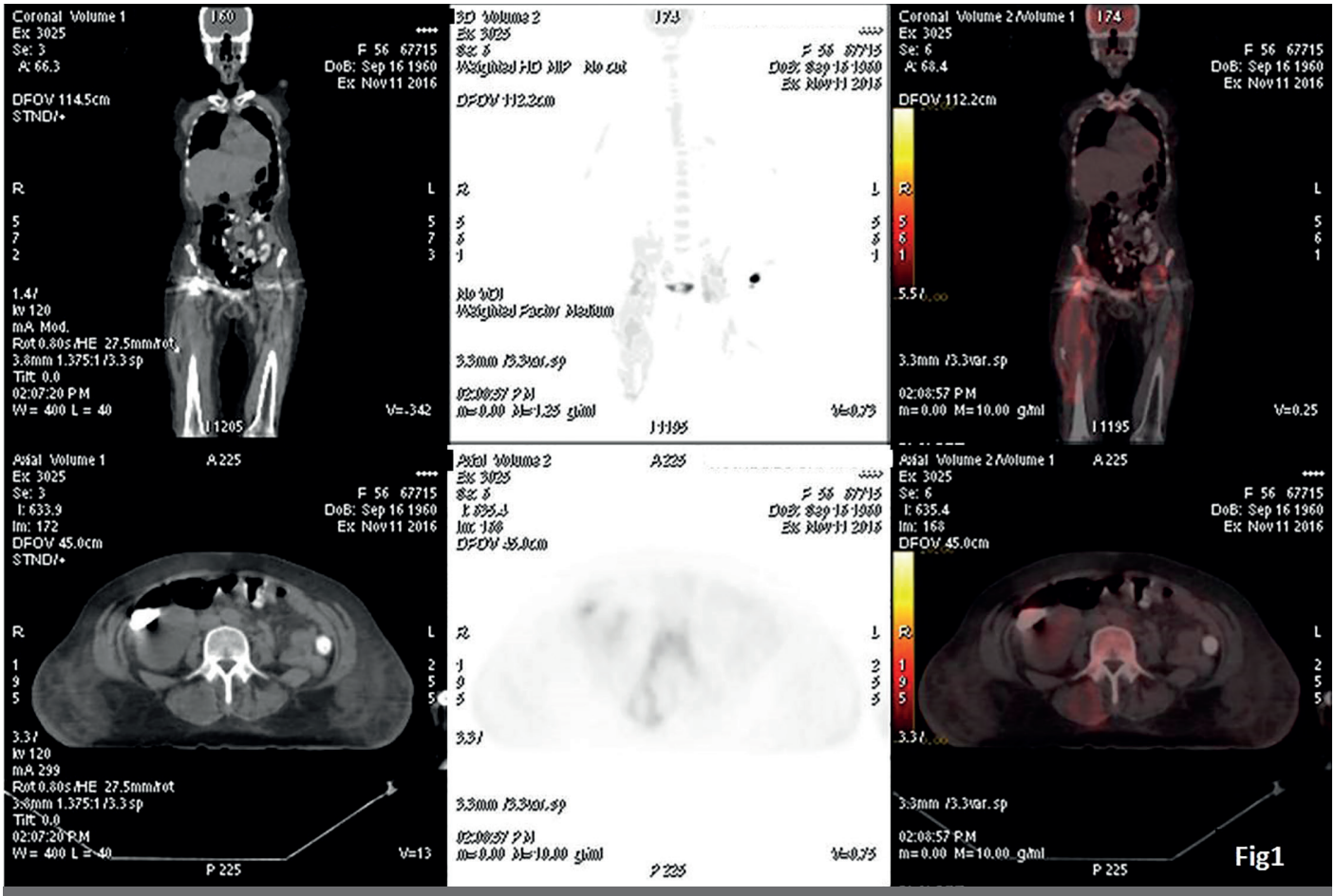

FIGURE I. F-18 FDG PET/CT imaging; abscesses in all paravertebral muscles between the T12 and L5 vertebrae and in the anterior and lateral group muscles of the right thigh

but there was no regression of the arthritis findings, and high fever persisted. On the fifth day of treatment, *S. aureus* was detected in blood cultures; antibiotic therapy was reorganized to teicoplanin from the glycopeptide group. Bilateral hip prostheses are considered to be the source of bacteremia. Hip MRI was performed. It revealed abscesses extending to the femur shaft around the bilateral prosthesis. Surgical treatment was planned for the patient. However, because the patient had pneumonia and multiple arthritis findings, F-18 FDG PET/CT imaging was performed because of the suspicion of bacterial metastatic infection (Figure 1). Whole body F-18 FDG PET/CT images showed abscesses in all paravertebral muscles between the T12 and L5 vertebrae and in the anterior and lateral group muscles of the right thigh. Increased F-18 FDG uptake was detected around both hip prostheses (Figure 2). Inflammatory F-18 FDG uptake was observed in the lower extremity veins. However, there was no evidence of active endocarditis in the images.

The abscess foci identified in the F-18 FDG PET/CT images were drained; and antibiotic therapy continued for 14 days after surgery. Rapid healing was observed in follow-up exams. Arthritis findings improved, hip pain reduced, and high fever was not observed again.

Written informed consent was obtained from the patient who participated in this study.

DISCUSSION

S. aureus, which may be part of normal flora in some of the healthy people, is a frequently found microorganism in late prosthetic infections. It can cause bacteremia, especially in diabetics, in intravenous drug users, and in those with leukocyte dysfunction. The development of metastatic infection (endocarditis, vasculitis, spondylodiscitis, or pulmonary abscess) is a major complication of gram-positive bacteremia. Early detection of metastatic infection has critical importance. Long-term antibiotic treatment and drainage should be applied in some cases. Metastatic infections can occur in one-third of bacteremia; and they most commonly involve bone, joint, kidney, and lungs. In metastatic infections, the detection of all foci is sometimes impossible; so treatment is often carried out on a determined single focus. In asymptomatic cases, inadequate treatment increases the morbidity and mortality because the infection cannot be eradicated. This situation creates difficulties in treatment.

Whole body F-18 FDG PET/CT imaging is useful for early and accurate diagnosis of metastatic infection foci in patients with prosthesis and for the detection of focus in the case with fever of unknown origin. F-18 FDG PET/CT imaging within the first two weeks after detection of bacteremia is beneficial in directing treatment. In their study, Hussman et al. (2) used F-18 FDG PET/CT imaging following vascular grafts, and reported that it is very valuable in managing the treatment of patients. In the study of Kouijzer IJ et al. (3), focus of infection was identified in 38% of 13 pediatric pa-

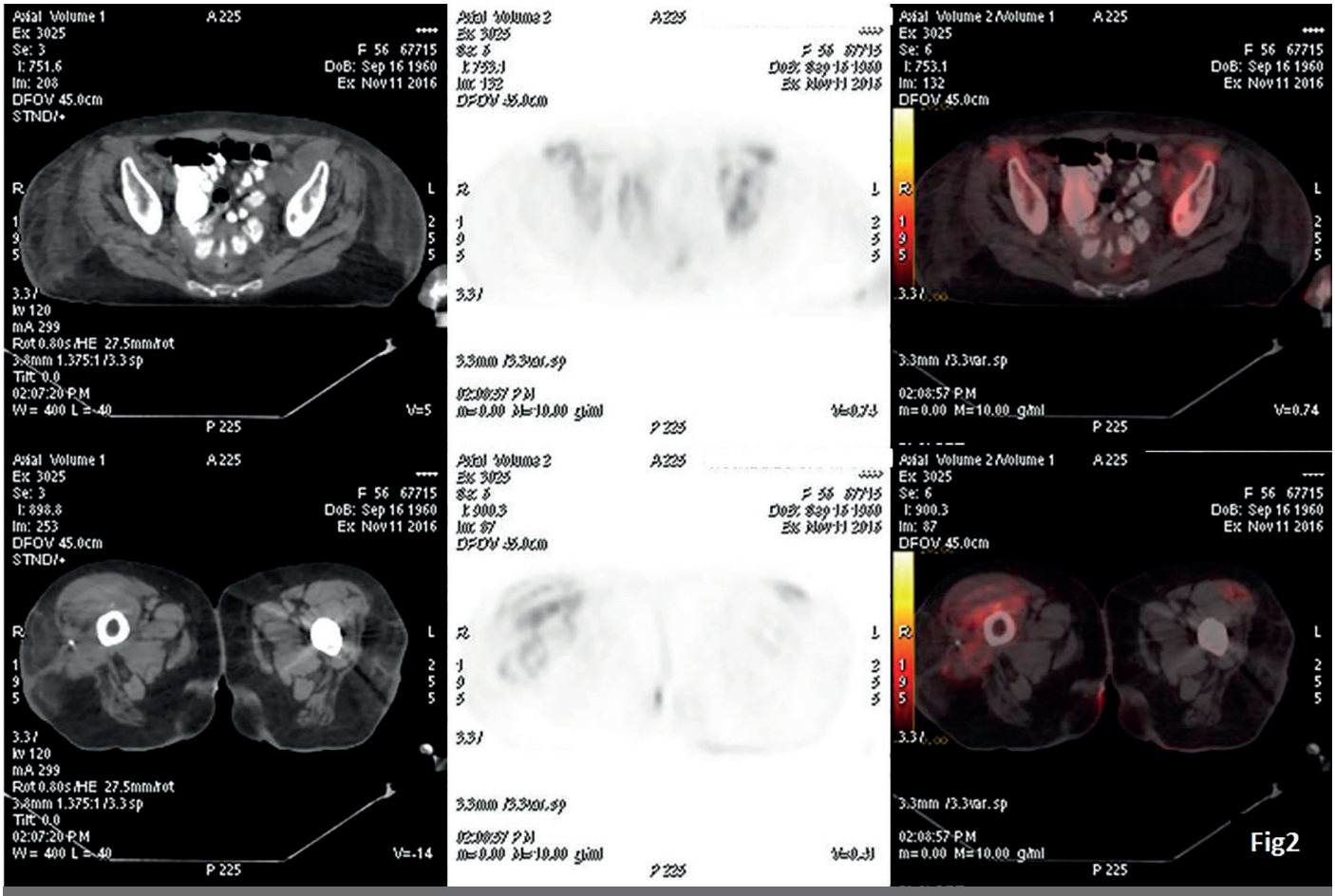

FIGURE 2. F-18 FDG PET/CT imaging; increased F-18 FDG uptake detected around both hip prosthesis

tients who were suspected of metastatic infection. The positive predictive value was calculated as 71%, and the negative predictive value as 100% (4). Vos FJ et al. (4) reported a sensitivity of 100%, a specificity of 87%, and a negative predictive value of 89% for F-18 FDG PET/CT imaging in the detection of metastatic infections in patients with gram-positive bacteremia (3).

In our case, presence of multiple infection foci revealed difficulties in treatment planning. We could try to detect infection foci by doing many MR/CT imaging, but this option would be time consuming and expensive. Therefore, we preferred to do F-18 FDG PET/CT imaging; and in this way, we detected all the foci of the infection even also evaluated endocarditis.

F-18 FDG PET/CT imaging also has an important role in the detection of infection focus in fever of unknown origin. Tseng JR et al. (5) reported that positive FDG PET/CT findings were detected in 35 (66%) of 53 patients with fever of unknown origin; and the treatment of 13 patients was rearranged according to this result. Determining the focus and extent of the disease in metastatic infections is often difficult and time consuming. There are delays in the diagnosis because MRI or CT imaging is performed on too many regions in the patient. This situation negatively affects the prognosis. Whereas, F-18 FDG PET/CT imaging provides important findings in the determination of foci that cannot be detected by standard examinations, and helps to establish the diagnosis earlier. So, morbidity and mortality decrease. It seems to be more effective in reducing treatment cost and loss of labor since it allows us to choose appropriate therapy (6).

In conclusion, whole body F-18 FDG PET/CT imaging is useful in the early and accurate diagnosis of metastatic infection foci in patients with prosthesis, and in detecting fever of unknown origin. F-18 FDG PET/CT imaging performed especially within the first two weeks after detection of bacteremia is of great benefit in directing therapy.

Informed Consent: Written informed consent was obtained from the patient who participated in this study

Peer-review: Externally peer-reviewed.

Author contributions: Concept - O.S., D.B., S.K.; Design - O.S., D.B., E.S.; Supervision - Ö.S., D.B., S.K.; Resource - O.S., D.B., E.S.; Materials - O.S., D.B., E.S.; Data Collection and/or Processing - O.S., D.B., S.K.; Analysis and/or Interpretation - O.S., D.B., S.K.; Literature Search - O.S., D.B., E.S.; Writing - O.S., D.B., E.S.; Critical Reviews - O.S., D.B., S.K.

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

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Case Report

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A Case of Hydatid Cyst Mimicking Kidney Tumor

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A 45-year-old male patient presented with left flank pain that started a month ago. There was no history of fever or pyuria. Vital signs were normal. The rest of the systemic examination was unremarkable. There were no pathological findings on urinalysis or urine cytology. Ultrasonography revealed an 8×6.2×6 cm heterogeneous exophytic trending lesion at the lower pole of the left kidney. The lesion's walls were seen to be calcified in places, and the margin between the kidney and lesion was indistinct. It could not be differentiated by ultrasonography if the lesion is a complicated cyst or a mass. Magnetic resonance imaging scan of the upper abdomen also revealed a 9×7.5×7 cm cortical–parapelvic localized lesion at the lower pole of the left kidney, extending exophytically to the inferior. Given the possibility that the lesion is malignant, nephrectomy was planned. A laparoscopic approach was performed. Histopathological diagnosis was hydatid cyst. Enzyme-linked immunosorbent assay test for hydatid disease was negative. Albendazole 10 mg/kg twice a day was administered postoperatively for 3 weeks.

Keywords: Kidney, cancer, hydatid cyst

INTRODUCTION

Hydatid cyst disease, also known as echinococcosis, is a parasitic infestation caused by Echinococcus granulosus, a cestode from the Taeniidae family (1). Carnivores, such as wolves, foxes, and especially dogs, are definitive hosts for the parasite, and herbivores, such as sheep, cows, and goats, are intermediate hosts. Humans are incidental hosts. They do not play a role in the transmission cycle and may be infested through contact with a definitive host or oral ingestion of water or vegetables contaminated by the eggs of the parasite. Hydatid disease may affect many parts of the human body. Approximately 40%–80% of patients with primary hydatid disease have single-organ involvement, and it commonly affects the liver (61%) and lungs (18.7%) in single-organ involved cases. However, primary kidney hydatid cysts are extremely rare (2.6%) (2).

Cysts in the kidney can cause hematuria or flank pain (3). In some patients, as a result of the rupture of the cyst in the collecting system, hydatiduria may occur. The diagnosis is based on imaging and serological tests. In radiological and serological studies, renal hydatid disease cannot be diagnosed preoperatively in 1 out of 3 patients (4). Here, we present a case of a 45-year-old man with primary renal hydatid disease.

CASE PRESENTATION

A 45-year-old male patient presented with left flank pain that started a month ago. There was no history of fever or pyuria. His vital signs were normal. Costovertebral angle tenderness was revealed on physical examination. The rest of the systemic examination was unremarkable. He had a history of Behcet's disease, but there was no oral or genital ulcer on admission. Laboratory studies presented normal eosinophil rate (2.7%), normal renal functions (blood urea 12.71 mg/dL and creatinine 0.74 mg/dl), and normal liver function tests. There were no pathological findings on urinalysis or urine cytology. Ultrasonography revealed an 8×6.2×6 cm heterogeneous exophytic trending lesion at the lower pole of the left kidney. The lesion's walls were seen to be calcified in places, and the margin between the kidney and lesion was indistinct. It could not be differentiated by ultrasonography if the lesion is a complicated cyst or a mass. Magnetic resonance imaging (MRI) scan of the upper abdomen also revealed a 9×7.5×7 cm cortical–parapelvic localized lesion at the lower pole of

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the left kidney, extending exophytically to the inferior (Figure I, 2). There was no significant contrast enhancement on post-contrast study. There was no lymphadenopathy on the para-aortic areas.

Given the possibility that the lesion is malignant, nephrectomy was planned. A laparoscopic approach was performed. Under the left flank position, trocars were placed. After entering the abdominal cavity, the colon was medialized, and the left kidney was dissected from the surrounding tissue. The pedicle was released and clamped using a linear stapler. The kidney was removed through paramedian incision by retrieval pouch.

The specimen measured 13×10.5×9 cm and weighed 519 g. Adjacent to the upper pole of the kidney, a single, 8×8.5×9 cm necrotic lesion containing membranes in it was present. Histopathological diagnosis was hydatid cyst. Enzyme-linked immunosorbent assay (ELISA) test for hydatid disease was negative. Albendazole 10 mg/kg twice a day was administered postoperatively for 3 weeks.

DISCUSSION

Primary renal hydatid disease is very rare, mostly solitary, and unilateral. The initial phase of primary infection is always asymptomatic. Patients may remain asymptomatic for years (5).

FIGURE I. MRI scan of the upper abdomen also revealed a 9×7.5×7 cm cortical–parapelvic localized lesion at the lower pole of the left kidney

FIGURE 2. MRI scan of the upper abdomen also revealed a 9×7.5×7 cm cortical–parapelvic localized lesion at the lower pole of the left kidney

Morbidity mainly depends on different factors, such as dimensions, quantity, and localization of the cyst/cysts. Factors, such as the immune response of the patient and the pressure of the cyst on its surrounding, also affect morbidity. Clinical symptoms, such as flank pain, fever, hematuria, abdominal pain, and weight loss, are non-specific, except gross hydatiduria. Hydatiduria is a pathognomonic sign for the disease and seen in 28%–29% of patients (6, 7). It was not detected in our patient.

The diagnosis of hydatid disease is based on the identification of cyst structures by imaging techniques, such as ultrasonography, computed tomography, and magnetic resonance imaging (MRI), and confirmation by immunodiagnostic studies (1). Although its effectiveness depends on the operator, diagnosis is mainly based on ultrasonography due to its advantages, such as cost effectiveness and non-invasiveness. On ultrasonography, renal hydatid cysts are usually detected as a single cystic structure that has a floating membrane and daughter cyst. In our patient's ultrasonography, daughter cyst was not detected, and cyst or mass discrimination of the lesion could not be done. To determine daughter cysts and see the location of the hydatid cyst and its relationship with the surrounding tissue more accurately, computed tomography or MRI can be performed. We performed upper abdominal MRI, and also by MRI, it could not be distinguished if the lesion is a complicated cyst or renal mass.

Serological studies, such as indirect hemagglutination test, ELISA, and indirect fluorescent antibody, are useful in the diagnosis. Fekak et al. (8) demonstrated that serological tests are positive in 55% of patients. Eosinophilia is reported in 25%–50% of patients (9). Hydatid cysts rarely affect renal function. Our patient's blood creatinine was normal, eosinophilia was not present, and ELISA for hydatid disease was also negative.

Surgical modalities are the main treatment for renal hydatid disease. Surgical procedure should be considered after evaluating the size and localization of the cyst, the relationship with the surrounding tissue, the status of the renal parenchyma, and renal function. Total nephrectomy or kidney-sparing modalities are available techniques, and laparoscopic approach is also an alternative. If preoperative diagnosis is possible, partial nephrectomy can be performed for localized cysts. In our case, owing to the suspicion of renal mass, laparoscopic total nephrectomy was performed.

Primary renal hydatid disease is extremely rare. However, especially in endemic areas, hydatid disease should be taken into consideration for the differential diagnosis of complicated renal cysts. If preoperative diagnosis is possible by imaging and laboratory studies, the preoperative administration of albendazole could be helpful for preventing complications of the probable rupture of the cyst during surgery.

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