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From the Editor

Dear readers,

In the second issue of 2022, we have prepared 10 original research articles and a review for you. We hope that these articles will be a guide for healthcare professionals, especially primary care physicians.

We are proud that our journal has the highest citation rate among the primary care journals published in Turkey. Thank you for your growing interest in our journal.

Please stay tuned for the next issue.

Assoc. Prof. Dr. Ahmet Keskin

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Research Article

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THE EFFECTS OF INTUITIVE EATING ON MENTAL WELL-BEING AND EATING BEHAVIORS OF HEALTH WORKERS

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Abstract

Objectives: Intuitive eating is identified as eating by hearing to and adapting to the physical hunger, satiety and satisfaction reactions given by the body naturally. The aim of this study was to research the effects of intuitive eating on mental well-being and eating behaviors in healthcare workers.

Materials and Methods: The plan of this study included all health workers in the state hospital in Edirne. The questionnaires were filled by the researchers according to the answers given by the participants to the questions. The five sections in the questionnaire consist of demographic information, anthropometric measurements, Beck Depression Inventory (BDI), The SCOFF Questionnaire to screen for eating disorders and the Intuitive Eating Scale-2 (IES-2). Health workers were separated into two groups according to the intuitive eating scale-2 median score (Group 1: below 3.60; Group 2: 3.60 and above).

Results: The participants' IES-2 mean score was 3.50 ± 0.59 (1.50 - 4.80). The BDI mean score of the participants in Group 1 was higher than that of those in Group 2. The number of participants with normal mental well-being according to the BDI score was lower in Group 1 than in Group 2. The number of participants exhibiting risky eating behavior according to the SCOFF score was higher in Group 1 than in Group 2.

Conclusion: The health workers who ate intuitively had better mental well-being and fewer eating disorders than those who did not intuitively eat.

Keywords: Intuitive eating, mental well-being, eating behaviors, health worker.

Introduction

Adequate and balanced nutrition constitutes the basis of health in every period of life. Adequate and balanced nutrition is only possible with good eating habits. Eating disorders are problems that occur in eating behavior, usually based on emotional problems. People with eating disorders have an obsession with food and excess weight. They think about which food they eat, how much they eat and the calories they take for a significant part of the day.[1,2]

The concept of "intuitive eating" was first introduced in 1995 by Tribole and Resch.[1] It is based on three basic approaches: unconditional allowance to eat, eating based on physical causes rather than emotional reasons, and eating based on physical hunger and satiety signals. Intuitive nutrition is a holistic alternative approach to diet and restrictive nutrition methods.[1,3,4] Tribole and Resch[1] formed the intuitive eating concept after observing that participants who lost weight with dietary restriction resorted to increased body weight after a diet, and those with eating behavior disorders resorted to impaired eating behaviors after reaching a healthy body weight with diets with energy intensity. Studies propose that more intuitive eaters have preferable behavioral health, lower body mass index (BMI), and more healthy weight histories than less intuitive eaters.[3,5-8] More importantly, it has been suggested that high intuitive eating, beyond its contributions to diet and nutritional disorders, is uniquely beneficial for individuals' psychological states.[9-11] The results of Hazzard et al.'s study showed that intuitive eating estimates better psychological and behavioral health also in the long term.[12]

Although there are few studies examining the effects of intuitive eating on nutritional behaviors, there is no study examining the interrelation between intuitive eating and mental well-being in Turkey.[13,14] The goal of this study was to research the effects of intuitive eating on mental well-being and eating behaviors in healthcare workers.

Materials and Methods

Study Design and Sample Selection

This cross-sectional study included all health workers working in the state hospital in Edirne between January and February 2020. Out of the 269 health workers in the hospital, 200 (74.35%) had agreed to attend and were included in the study. The informed consent form was distributed to the participants before starting the study, and a questionnaire was applied to those who agreed to attend the study. The questionnaires were filled by the researchers according to the answers given by the participants to the questions. The five sections in the questionnaire consist of demographic properties (age, gender, educational status, marital status),

anthropometric measurements, Beck Depression Inventory (BDI), The SCOFF Questionnaire to screen for eating disorders and Intuitive Eating Scale-2 (IES-2). Participants were separated into two groups according to the IES-2 median score (Group 1: below 3.60; Group 2: 3.60 and above).

Anthropometric Measurements

The body weight of the participants was measured on an empty stomach, in light clothing and without shoes, using a digital scale with a weight measurement sensitivity of 0.10 kg. Height measurement was measured using a stadiometer with the feet side by side and the head on the Frankfort horizontal plane. BMI was calculated by the formula "weight (kg)/[height (m)]^2". BMI scores are classified into four groups: underweight (≤18.50); normal weight (18.60–24.90); overweight (25–29.90); and obese (≥30).[15]

Beck Depression Inventory

The BDI, advanced by Beck et al., was employed to define the risk in terms of depression and to evaluate the level and severity of depressive symptoms.[16] This form, which includes a total of 21 self-assessment items, ensures a four-point Likert-type measurement with each item scoring between 0 and 3. The total high score indicates the high severity of depression. 0-9 points are interpreted as no depression, 10-16 points as mild, 17-29 points moderate, and 30-63 points as severe depressive symptoms.[16,17] The Turkish validity and reliability study was performed by Hisli[17,] and the Cronbach alpha reliability coefficient was found to be 0.80.

SCOFF Questionnaire

The SCOFF (Sick, Control, One, Fat, Food) Questionnaire advanced by Hill et al. in 2010 was used to investigate the risk of eating disorders.[18] The Turkish validity and reliability study was performed by Aydemir et al.[19] The Cronbach alpha reliability coefficient was found to be 0.74, and it can distinguish 81% of previously undetermined eating disorder cases.[16,17] The scale, which questions eating control, taking out what they eat and body dissatisfaction, consists of 5 questions. Individuals scoring 2 or more points on the scale where each item is given 1 point are considered at risk for an eating disorder.[18,19]

Intuitive Eating Scale - 2

The IES-2, developed by Tylka and Kroon Van Diest in 2013, was used to evaluate intuitive eating.[9] In the IES-2, a five-point Likert scale consisting of 23 items ranging from "strongly agree" to "strongly disagree" is used. Mean scores are evaluated for the total scale and each subscale, and higher scores indicate those who consume more intuitive eating. The sub-factors of the scale are unconditional permission to eat, eating for physical

reasons rather than emotional reasons, trusting hunger satiety signals, and body food selection compliance.[9] The Turkish validity and reliability of IES-2 have been reported by Bas et al.[13]

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) (version 22.0; SPSS Inc., 2016) was used for Statistical evaluation in this study. After analyzing the normality of the dispersion of the quantitative data by the Kolmogorov-Smirnov test, the Student t-test was employed in distanced groups to compare the means of scores that fulfill the parametric test hypothesis and a Mann-Whitney U test was employed in cases where not met. The chi-square test was employed in the assessment of categorical data. Pearson's test was employed for correlation analysis. Descriptive statistics were shown as a mean ± standard deviation (minimum-maximum), numbers and percentages, and $p < 0.05$ was accepted as the limit of significance.

Results

The mean age of the participants is 33.53 ± 8.24 (18 - 61) years. Of the 200 health workers in the study, 118 (59%) were women, and 82 (41%) were men. Forty-five (22.50%) participants were doctors, and 155 (77.50%) were nurses. One hundred twenty-one (60.50%) participants were married, and 79 (39.50%) were single. Sixty-six (33%) participants finished high school, 109 (54.50%) finished university and 25 (12.50%) finished postgraduates.

The participants' IES-2 mean score was 3.50 ± 0.59 (1.50 - 4.80). The mean scores of the IES-2 and its subscales are shown in Table 1. There was no significant difference in the IES-2 means score according to gender, marital status and educational status ($p = 0.653$, $p = 0.657$, $p = 0.349$, respectively).

Table 1. Intuitive Eating Scale-2 total and subscales scores of participants

Intuitive Eating Scale-2 total score and subscales	Mean ± SD
Intuitive Eating Scale-2 total score	3.50 ± 0.59
Eating for physical rather than emotional reasons	3.38 ± 0.74
Unconditional permission to eat	3.23 ± 0.55
Reliance on hunger and satiety cues	3.59 ± 0.80
Body-food choice congruence	3.91 ± 0.87

(SD: Standard Deviation)

There were 92 (46%) participants in Group 1 with an IES-2 median score below 3.60 and 108 (54%) participants in Group 2 with an IES-2 median score of 3.60 and above. The BDI mean score of those with an IES-2 score below 3.60 (Group 1) was 12.84 ± 8.42, while for those who were 3.60 and above (Group 2), it was 9.71 ± 6.52. The BDI mean score of the participants in Group 1 was significantly higher than that of those in Group 2 (p = 0.004). Moreover, the number of participants with normal mental well-being according to BDI score in Group 1 was lower than that of Group 2 (Table 2). The Pearson correlation analysis revealed that as the IES-2 scores of the participants increased, the BDI scores decreased (Table 3).

The mean SCOFF score of those with an IES-2 score below 3.60 (Group 1) was 1.25 ± 1.37, while for those who were 3.60 and above (Group 2), it was 0.59 ± 0.95. The mean SCOFF score of the participants in Group 1 was significantly higher than that of those in Group 2 (p<0.001). Moreover, the number of participants with risky eating behavior according to the SCOFF score (n=23, 25%) in Group 1 was significantly higher than that for Group 2 (Table 2). The Pearson correlation analysis revealed that as the IES-2 scores of the participants increased, the SCOFF scores decreased (Table 3).

Table 2. The mental well-being and eating disorder risk of participants according to IES-2

	Group 1 (n=92)	**Group 2 (n=108)**	***p***
BDI score, mean ± SD	12.84 ± 8.42	9.71 ± 6.52	**0.004***
Mental well-being according to BDI score, n (%)			
Normal (0-9)	33 (35.87)	57 (52.78)	
Mild depression (10-16)	38 (41.30)	35 (32.41)	**0.046†**
Moderate depression (17-29)	16 (17.39)	15 (13.89)	
Severe depression	5 (5.44)	1 (0.92)	
SCOFF score, mean ± SD	1.25 ± 1.37	0.59 ± 0.95	**<0.001***
Eating disorder risk according to SCOFF score, n (%)			
Risk free	69 (75)	100 (92.59)	**0.001†**
Risky	23 (25)	8 (7.41)	

(* Mann-Whitney U test, † Chi-square test, IES-2: Intuitive Eating Scale-2, BDI: Beck Depression Inventory, SD: Standard Deviation)

There was no statistically significant difference in the weight and BMI means scores according to IES-2 (p=0.411 and p=0.273, respectively). Nevertheless, the mean score of IES-2 (3.10 ± 0.79) in obese participants according to BMI was statistically lower than that of underweight, normal and overweight participants (3.77±0.58, 3.53±0.58, and 3.53±0.55, respectively) (p=0.044). Also, the Pearson correlation analysis showed that as the IES-2 scores of the participants increased, their weight decreased (Table 3).

Table 3. Correlation between the Intuitive Eating Scale scores and the Beck Depression Inventory and the SCOFF Questionnaire scores and weights in the health workers

	n	r	*p* *
IES-2 - BDI scores	200	-0.209	**0.003**
IES-2 - SCOFF Questionnaire scores	200	-0.306	**<0.001**
IES-2 – Weights of health workers	200	-0.146	**0.040**

(* Pearson's test, IES-2: Intuitive Eating Scale-2, BDI: Beck Depression Inventory, SCOFF: Sick, Control, One, Fat, Food)

Discussion

In our study, the health workers with high IES-2 scores had lower BDI and SCOFF scores. The number of health workers with normal well-being was higher in the group with a high IES-2 score. Also, it was determined that as the IES-2 scores of the participants increased, their weight decreased.

Intuitive eating is described as eating by hearing to and adapting to the physical hunger, satiety and satisfaction reactions given by the body naturally. It is basically the knowledge of the amount and type of nutrients the body needs, which is developed as evidence for the control of body weight without a special health problem. As long as the individual who eats intuitively has no chronic disease, the individual instinctively prefers foods in a way that ensures nutritional balance and thus increases the variety of food. Intuitive eating emphasizes a real harmony of body, food and mind in the individual.[1]

Higher intuitive eating was associated with better psychological and behavioral health, according to studies on intuitive eating.[3,5,10,11] Intuitive eating was inversely related to negative emotion and depressive symptomatology.[5] Shouse and Nilsson showed that higher intuitive eating levels were related to greater emotional awareness.[20] Schoenefeld and Webb discovered a positive relationship between intuitive eating and distress tolerance.[21] The results of Hazzard et al.'s study showed that intuitive eating predicts better psychological and behavioral health also in the long term.[12] The higher intuitive eating was related to less

depressive symptoms and a more positive mood in our study, consistent with these studies. Also, the correlation analysis showed that as the IES-2 scores of the participants increased, the BDI scores decreased. It may be thought that people who eat intuitively are better mentally because of their ability to differentiate between biological and emotional hunger and organize emotions with alternative strategies.

Studies had shown that intuitive eating was inversely associated with symptoms of eating disorders and was negatively related to food occupation and binge eating behaviors.[5,11,19,22] In addition, intuitive eating was found to be negatively associated with restricted eating patterns, such as little or no eating.[22] It was found that eating disorders decreased with increasing intuitive eating in a study.[5] In our study, it was found that participants with high intuitive eating scores had lower eating disorder scores, and the number of people with eating disorder risk was lower among these participants. Also, the correlation analysis revealed that as the IES-2 scores of the participants increased, the mental well-being scores decreased. These results show that people who eat intuitively have a more nutritious diet intake, healthier eating behaviors, and more positive eating patterns than those who do not intuitively eat.

Many cross-sectional studies have shown that intuitive eaters have lower BMI than those who do not intuitively eat.[3,6,7] This relationship could not be shown only in the studies of Augustus-Horvath and Tylka and Hawks et al.[23-24] In addition, it was found that the intuitive eating approach helped maintain weight in overweight and obese women.[3] There is evidence that an intuitive eating program can help maintain weight, although the traditional diet can initially lead to weight loss and then regain weight[3,25] and, completing an intuitive eating program can result in weight loss.[3] There was no significant difference in the weight and BMI means scores according to IES-2 in our study. Nevertheless, the mean score of IES-2 in obese participants according to BMI was statistically significantly lower than that of underweight, normal and overweight participants. Also, the correlation analysis showed that as the IES-2 scores of the participants increased, their weight decreased. These data showed that intuitive eating practices could help maintain weight, especially in the long term.

The superior aspect of our study is that it is the first study in Turkey to research the relationship between intuitive eating and mental well-being. Some limitations of this study are that we conducted a study of health workers from only one hospital. The number of health workers was low, which may have affected the results. External stressors, years of experience, and a previous history of anxiety/depression were not investigated. These limitations may have influenced the results of the study.

As a result, the health workers who ate intuitively had better mental well-being and fewer eating disorders. Also, the intuitive eating scores of the participants were negatively associated with their weight. In order to increase intuitive eating, intuitive eating programs should be arranged in schools and primary health care centers within the scope of preventive medicine.

Ethical considerations: Ethical approval was acquired from the Trakya University Ethics Committee (TÜTF-BAEK-2019/466).

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Research Article

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KNOWLEDGE, ATTITUDES AND BEHAVIOR OF PHYSICIANS TOWARDS INFLUENZA INFECTION AND VACCINATION DURING PREGNANCY

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Abstract

Objectives: In our study, we aimed to determine the knowledge, attitudes and behaviors of Family Physicians (FP) and obstetrics and gynecology (OB-GYN) physicians about influenza infection during pregnancy and the influenza vaccine administered during pregnancy, as well as the factors that influence them.

Materials and Methods: This descriptive cross-sectional study included 419 OB-GYN and FP practicing in Istanbul between November 15, 2017, and March 15, 2018. A 14-item survey was administered face-to-face to assess sociodemographic factors, influenza vaccination recommendation status, and knowledge level regarding influenza infection and vaccination.

Results: A total of 11.48% of the participants themselves had been vaccinated against influenza during pregnancy, and 48.68% of the participants recommended influenza vaccination during pregnancy. There was a statistically significant difference between vaccination recommendation status ($p=0.014$) and age ($p=0.014$), the institution of employment ($p=0.002$), specialty ($p=0.008$), having received the influenza vaccine during pregnancy ($p<0.001$), and find it beneficial to include pregnant women in the risk group for influenza vaccination ($p<0.001$). The independent variables of knowledge regarding influenza infection and vaccination during pregnancy (OR=2.60, $p=0.034$) and survey total score (OR=1.36, $p<0.001$) were found to be significantly associated based on the results of a multiple logistic regression analysis of influenza vaccine recommendation.

Conclusion: The knowledge and awareness of physicians should be improved to increase influenza vaccination rates during pregnancy. Furthermore, incorporating influenza vaccines into the standard maternal immunization schedule might help in increasing the vaccination rates.

Keywords: Influenza, pregnancy, vaccination.

Introduction

The influenza virus causes a highly contagious respiratory illness known as seasonal flu. The illness typically has a self-limiting clinical course, with symptoms improving within a week without medical treatment. However, it also has the potential to cause significant illness or death, particularly in those who are at high risk. Seasonal influenza epidemics are estimated to cause serious illness in 3–5 million people and the death of 290,000–650,000 people each year around the world.[1] Pregnant women are at higher risk than the general population for influenza-related complications, including higher rates of hospitalization and fetal and maternal death.[2] Hence, the vaccination of pregnant women against influenza has become a common practice in recent years to protect the mother and fetus from seasonal influenza infection.[3] The World Health Organization's Vaccine Experts Strategic Advisory Group reported in 2012 that pregnant women were given the highest priority for receiving seasonal influenza vaccination.[4] Similarly, various national and international organizations recommend a single dose of influenza vaccine in any trimester of pregnancy.[5-7] Influenza vaccination is recommended by the Turkish Ministry of Health for people aged ≥65 years, those with chronic diseases, healthcare workers and pregnant women in their second and third trimesters, with the expense of the vaccination reimbursed.[8] The advice of healthcare professionals is effective in vaccination acceptance during pregnancy.[9] In Turkey, physicians that are specialized in family medicine (FM) and specialized in obstetrics and gynecology (OB-GYN) perform pregnant women's follow-ups.[9]

Our study aimed to investigate FM and OB-GYN physicians' knowledge, attitudes and behaviors toward vaccination and influenza infection during pregnancy, as well as the factors that influence them.

Materials and Methods

This descriptive cross-sectional study was conducted between November 15, 2017, and March 15, 2018. The study population was determined as FM and OB-GYN physicians practicing in Istanbul (N = 13,575). The sample size was calculated as 378 at a 95% confidence interval (n = 378). A total of 419 physicians participated in the study voluntarily. Ethical approval was obtained from the local ethics committee.

Data collection tools

The survey used in the study was developed by a pediatrician and a family physician, considering the information in recent medical articles. The survey included multiple-choice, closed-ended and semi-closed-ended questions. The participants only gave one answer to the questions regarding their knowledge about influenza infection and vaccination, such as "I agree," "I disagree," or "I do not have an opinion." The

participants' responses to the survey questions were evaluated and coded as "1 = True" or "0 = False/I do not know." The total survey score was calculated by summing the scores obtained through this coding.

Statistical analysis

IBM SPSS 25.0 program (SPSS Inc. Chicago, Illinois, USA) was used for statistical analysis. Frequency tables and graphs were used to present descriptive statistics regarding the participants' sociodemographic information. In addition, the Cronbach's α reliability coefficient was determined using the participants' minimum, maximum, and average survey scores.

As there were more than 200 participants in the study, parametric tests were used in the analysis.[10] The independent samples test and one-way analysis of variance were used to determine whether there was a significant difference between the independent variables and the total survey score. When there was a significant difference between the groups, we performed a post-hoc test to determine the significance between the groups. Sidak post-hoc test was preferred as the variance was homogeneously distributed and the sample sizes were unequal.[11] Chi-square test was used to compare categorical variables, and a multivariate logistic regression analysis was performed to determine the factors predicting influenza vaccine recommendation during pregnancy. Statistical significance was defined as $p<0.05$.

Results

The study initially included 444 physicians; however, 25 of them were excluded due to incomplete surveys. Thus, the study was conducted with 419 physicians who completed the survey in its entirety, with 364 being FM (86.88%) and 55 being OB-GYN (13.12%) physicians. The sociodemographic characteristics of the participants are shown in Table 1.

The reliability analysis of the knowledge survey showed that the Cronbach's α internal consistency coefficient for the items was 0.76. A Cronbach α level above the 0.70 limit indicates that the internal consistency of the survey is adequate.[12] Therefore, the internal consistency of the survey in this study was considered to be adequate. The mean score obtained by the participants from the survey was 4.25±2.04 (min=0, max=7). Table 2 depicts the frequency distributions of the participants' "true" and "false/I do not know" responses to the survey items.

Table 1. Sociodemographic data of participants (n= 419)

Demographic variables	n or X_{mean} (min–max)	% or Mean ± SD
Age (Years)	39.00 (24.00–64.00)	39.55 ± 8.14
20–30 years	55	13.13
31–40 years	178	42.48
41–50 years	142	33.89
>50 years	44	10.50
Sex		
Male	117	27.93
Female	302	72.07
Marital Status		
Single	70	16,71
Married	349	83.29
Institution of Employment		
Primary healthcare	312	74.47
Secondary healthcare	46	10.97
Tertiary healthcare	61	14.56
Duration of practice (Years)	11.00 (1.00–38.00)	13.63 ± 8.61
Specialty		
Family medicine	364	86.88
Obstetrics and Gynecology	55	13.12
Have you or has your partner had the influenza vaccine during pregnancy?		
No	339	88.52
Yes	44	11.48
How many pregnant women do you follow on average per month?	20.00 (0.00–2000.000)	59.37 ± 192.57
Do you recommend influenza vaccination during pregnancy?		
No	215	51.32
Yes	204	48.68
If the answer is no, why not suggest it? (n = 298) *		
a) I do not recommend it because it is not in our standard maternal immunization schedule.	117	39.27
b) I do not have sufficient knowledge about influenza vaccination for pregnant women.	99	33.23
c) I do not recommend it due to its side effects.	32	10.73
d) I do not believe in its protective effects.	43	14.42
e) Pregnant women should not be vaccinated.	7	2.35
What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group? (n = 414)		
Beneficial	237	57.25
No opinion	77	18.60
I do not find it necessary	100	24.15
Did you receive information regarding influenza infection and vaccination during pregnancy?		
No	337	80.43
Yes	82	19.57
Where did you receive information about influenza infection and influenza vaccination during pregnancy? (n = 217)*		
Scientific conventions and meetings	69	31.80
Colleagues	47	21.66
Company meetings	11	5.07
Company representatives	10	4.61
Scientific articles	60	27.65
Other	20	9.21

(*n exceeds the sample size as the answers include multiple responses.)
(SD: Standard deviation)

Table 2. Frequency distribution of participants' responses to the items of the information survey (n = 419)

Survey items		n	%
1- Influenza vaccination during pregnancy protects the baby from influenza infection in the first six months after birth.	False/I do not know	279	66.58
	True	140	33.42
2- Compared to the general population, complications and mortality in influenza infection during pregnancy increased.	False/I do not know	131	31.26
	True	288	68.74
3- The efficacy of the influenza vaccine in pregnant women ranges from 54% to 89%.	False/I do not know	268	63.96
	True	151	36.04
4- The symptoms of influenza (fever, runny nose, myalgia, headache, and sore throat) are the same during pregnancy.	False/I do not know	84	20.04
	True	335	79.06
5- Nausea, vomiting, and hydration-resistant tachycardia are more common in influenza infection in pregnant women.	False/I do not know	148	35.32
	True	271	64.68
6- The hospitalization period is extended when influenza infection-related complications develop in pregnant women.	False/I do not know	80	19.09
	True	339	80.91
7- Influenza infection can be diagnosed in pregnant women based on clinical findings.	False/I do not know	159	37.94
	True	260	62.06

The results of the analysis comparing several variables with the vaccination recommendation status of the participants are shown in Table 3. The results showed that there was a statistically significant difference between vaccination recommendation and age (p=0.014), the institution of employment (p=0.002), specialty (p=0.008), the question “Have you had the influenza vaccine during your pregnancy?” (p<0.001), as well as the question “What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group?” (p<0.001).

We found a statistically significant difference between the total survey scores and the specialty of practice in the analysis we conducted on the comparison of the total survey scores with some variables. OB-GYN physicians had higher total survey scores than family physicians (t=−2.417, p=0.018). There was a significant difference between the total survey score and the item "Do you recommend influenza vaccination during pregnancy?". The total survey score was higher in those who recommended influenza vaccination during pregnancy compared with that in those who did not (t=−11.596, p<0.001)

The regression equation and the significance levels of the variables are illustrated in Table 4. Multiple logistic regression analysis results showed that not recommending influenza vaccination (dependent variable used in the model) was significantly associated with the following variables: practicing in a secondary healthcare (OR=6.01, p=0.001), finding it unnecessary to recommend influenza vaccination by placing pregnant women in the risk group (OR=76.79, p<0.001) or having no opinion on the issue (OR=30.90, p<0.001), having no knowledge about influenza infection and vaccination during pregnancy (OR=2.60, p=0.034), and the total survey score (OR=0.73, p<0.001).

Table 3. Comparison of Participants' Recommendations for Influenza Vaccination during Pregnancy and Several Variables

	Do you recommend influenza vaccination during pregnancy?			
Variables	**Group**	**No (n=204)**	**Yes (n=215)**	**p**
Age	20–30 years	21 (38,18)	34 (61.82)	**0.014**
	31–40 years	84 (47.19)	94 (52.81)	
	41–50 years	81 (57.04)	61 (23.96)	
	>50 years	29 (65.90)	15 (34.10)	
Institution of Employment	Primary healthcare	175 (56.08)	137 (43.92)	**0.002**
	Secondary healthcare	20 (43.47)	26 (56.53)	
	Tertiary healthcare	20 (32.78)	41 (67.22)	
Specialty	Family Medicine	196 (53.84)	168 (46.15)	**0.008**
	Obstetrics and Gynecology	19 (34.54)	36 (65.46)	
Have you or has your partner had the influenza vaccine during pregnancy?	No	192 (56.63)	147 (43.37)	**<0.001**
	Yes	8 (18.18)	36 (81.82)	
What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group?	Beneficial	46 (19.40)	191 (80.60)	**<0.001**
	No opinion	70 (90.90)	7 (9.10)	
	I do not find it necessary	95 (95.00)	5 (5.00)	

(Pearson Chi-Square Test, p<0.05)

Table 4. Multivariate logistic regression results on various variables and not recommending influenza vaccination

	Not recommending influenza vaccination	
Variables	**OR (95% CI)**	**p**
Institution of employment (Tertiary Healthcare)	-	**0.001**
Institution of employment (Secondary Healthcare)	6.01 (2.09–17.09)	**0.001**
Institution of employment (Primary Healthcare)	1.53 (0.36–6.42)	0.557
What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group? (Beneficial)	-	**<0.001**
What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group? (I do not find it necessary)	76.79 (25.30–233.09)	**<0.001**
What is your opinion about the Ministry of Health's recommendation for influenza vaccination by placing pregnant women in the risk group? (No opinion)	30.90 (11.84–80.62)	**<0.001**
Did you receive information about influenza infection vaccination during pregnancy? (No)	2.60 (1.07–6.31)	**0.034**
Survey Total Score	0.73 (0.61-0.86)	**<0.001**

(R^2 = 0.68, -2 loglikelihood = 278.65)

The results showed that the following variables in the model explained 68% of the factors contributing to the non-recommendation of the influenza vaccination (R2 = 0.68, -2 loglikelihood = 278,65) practicing in a secondary healthcare facility, believing that the Ministry of Health's recommendation to place pregnant women in the risk group for influenza vaccination is unnecessary or having no opinion on the matter, having no knowledge about influenza infection and vaccination during pregnancy, and low total survey scores. Results of multiple logistic regression analysis showed that the vaccination recommendation dependent variable was

significantly associated with the following dependent variables: practicing in a secondary healthcare (OR = 3,91, p = 0,020), practicing in a tertiary healthcare (OR = 6,01, p = 0,001), influenza infection during pregnancy, knowledge about influenza vaccination (OR = 2,60, p = 0,034), and survey total score (OR = 1,36, p < 0,001).

Discussion

Although the pregnant women were defined as a high-risk group for influenza infection, only half of the physicians in our study recommended influenza vaccination for pregnant women. We found that having knowledge regarding influenza infection and vaccination during pregnancy, being a gynecologist and obstetrician, and working in a secondary or tertiary healthcare facility were the primary factors affecting the rate of recommendation of influenza vaccination to pregnant women. Influenza vaccination rates during pregnancy are below the desired level in both developed and developing countries. This rate was 42,3% in the UK, whereas it was reported as 4% in Thailand.[8,13] The highest influenza vaccination rate during pregnancy in Turkey was during the 2009–2010 influenza pandemic (9.1%); however, it was substantially lower than that in the USA (45.7%).[14,15] The rate of influenza vaccination during pregnancy was low in our study.

Vaccination of pregnant women against influenza depends on factors related to both pregnant women and their healthcare providers. In a study conducted in Italy, only 9,7% of women had been vaccinated against the flu, and not receiving a vaccination recommendation was one of the main reasons for not getting vaccinated.[16] In a study conducted in Turkey, the rate of receiving vaccination advice by the healthcare provider during pregnancy was 10,6%, and the rate of influenza vaccination was 1,1%.[17] The same study reported that the most important determining factor in vaccine acceptance during pregnancy was "following the doctor's advice".

In our study, the rate of recommendation for influenza vaccination for pregnant women was low. Most of the physicians in our study believed that the complication and mortality rates due to influenza infection during pregnancy were higher than those in the general population. The fact that physicians were more knowledgeable about influenza infection and vaccination during pregnancy, especially in institutions such as universities and training and research hospitals, where education and research services in the field of health continue, increased the rate of vaccine recommendations, according to our findings. We also determined that being knowledgeable caused a difference in influenza vaccination recommendations during pregnancy. We found in our study that physicians who received knowledge regarding influenza vaccination and infection recommended vaccination at a higher rate. Similar to our results, studies report that the level of knowledge of healthcare professionals influences the vaccination recommendation rate.[18-21]

A study conducted with midwives in Paris reported that having a high level of knowledge about influenza vaccination is an important factor contributing to the recommendation of vaccination to pregnant women.[22] In

our study, we found that physicians who had received influenza vaccination during their own pregnancy recommended the vaccine at a higher rate. This result was consistent with the results of previous research. The "personal influenza vaccination status" of healthcare providers is the most important determinant of influenza vaccination recommendation during pregnancy, according to a recent study that examined 32 studies from 15 countries.[23] Different results have been reported in studies examining the effects of physicians' specialties on recommending influenza vaccination to pregnant women.

A Thai study reported that OB-GYN physicians were more likely to recommend vaccination of pregnant women.[24] According to a study conducted in Germany, the vast majority of women received influenza vaccination advice from their OB-GYN physicians.[25] A study conducted in Turkey showed that 50% of the pregnant women who were vaccinated for the flu had it recommended by an FM specialist, whereas 10% had it recommended by an OB-GYN specialist.[17] We also found that OB-GYN physicians recommended vaccination at a higher rate than FP's in our study. Physicians working in secondary and tertiary healthcare were more likely to recommend vaccinations. We believe that this is because insurance companies require a specialized physician's report to reimburse the vaccine. Although the influenza vaccine is administered to pregnant women in Turkey for free, it is not currently a part of the standard immunization schedule. A medical report states that the patient is in the second or third trimester of pregnancy, and a prescription for the vaccine is required for this vaccine to be provided free of charge.[26]

Family physicians do not meet these requirements for a pregnant woman who wants to receive the vaccine for free, especially in the primary healthcare setting that is not within their specialization. Furthermore, the fact that we found no differences in the answers given by family physicians to questions assessing their degree of knowledge in our study supports this opinion.

The first three reasons why physicians did not recommend the influenza vaccine to pregnant women in our study are as follows: the vaccine was not in the standard immunization schedule, the physicians did not have sufficient knowledge about influenza vaccination for pregnant women, and the physicians did not receive training regarding influenza vaccination. These findings are consistent with those of previous research. Fear of the side effects of the vaccine, misunderstandings about vaccine safety and efficacy, and insufficient knowledge about influenza infection all contribute to low rates of influenza vaccination, according to a study conducted with healthcare professionals.[27]

The reasons for not recommending the influenza vaccine include physicians' and pregnant women's lack of awareness of the severity of influenza during pregnancy, their concerns about the vaccine's safety and efficacy, and the difficulty in acquiring the vaccine, according to several studies.[28,29] In a study conducted in Germany, OB-GYN experts, stated that they did not recommend the vaccine because of concerns about the vaccine's

harmful effects on the fetus or baby, as well as concerns of potential side effects during pregnancy, a lack of time to discuss vaccination and a belief that the influenza vaccine was not necessary for pregnant women.[30]

There are few studies on influenza vaccination in pregnant women involving family physicians and OB-GYN specialists. The strength of our study is that it examines the knowledge and attitudes of physicians from both specialties about influenza vaccination during pregnancy. The small size of the study population is one of the limitations of this study. Our study limitations include the small number of participants and the fact that it is based on a survey

The fact that influenza infection is severe in pregnant women and that it increases mortality reveals the importance of vaccination. Physician advice is one of the most influential factors in vaccination. In our study, only half of the physicians recommended influenza vaccination during pregnancy. The factors affecting the rate of recommending influenza vaccination to pregnant women included having sufficient knowledge regarding influenza infection and vaccination during pregnancy, having received influenza vaccination during pregnancy, and being an OB-GYN physician.

The results of our study indicated that in order to increase influenza vaccination rates during pregnancy, the knowledge and awareness of physicians on this issue should be improved. We believe that organizing physician training programs would be advantageous in this regard. Furthermore, increased immunization rates can be accomplished by facilitating free vaccine access for pregnant women and, if possible, integrating the influenza vaccine into the standard maternal immunization schedule.

Ethical Considerations: The approval of the ethics committee was obtained from the Clinical Research Ethics Committee of Health Sciences University of Şişli Hamidiye Etfal Training and Research Hospital (approval no. 1761/dated 14.11.2017). The study was conducted in accordance with the principles of the Declaration of Helsinki. Participation in the study was on a voluntary basis, and informed consent was obtained from each participant. Confidentiality and anonymity of the respondents were also ensured.

Conflict of Interest: The authors declare no conflict of interest. There is no funding for this study.

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ANALYSIS OF INTRAMUSCULAR INJECTIONS ADMINISTERED IN A FAMILY HEALTH CENTER

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Abstract

Objectives: In this study, the objective was to evaluate the intramuscular injections administered to patients admitted to a family health center with sociodemographic characteristics.

Materials and Methods: This is a cross-sectional, analytical and, retrospective file review study. The sample population was formed by patients who had intramuscular injections administered between the dates of January 01, 2017 – and December 31, 2019. A total of 5648 injections for 2059 adults/children were evaluated. The data set was analyzed using SPSS 17.0 (IBM, USA) statistical package program.

Results: An average of 2.74 injections per person was administered. The rate of myorelaxant injection administration was higher among women ($p<0.001$), and the rate of antibiotic and analgesic + myorelaxant injection administrations was higher among men ($p<0.001$ for both). With the increase of age, the rate of analgesic injection administrations increases ($p<0.001$) and the rate of antibiotic injection administrations decreases ($p<0.001$). The highest analgesic + myorelaxant injection administration rate was during autumn, and the lowest was during winter ($p=0.001$).

Conclusion: The results showed that the frequency of analgesic, myorelaxant, antibiotic, steroid, vitamin/mineral, hormone and combination drugs used for intramuscular injection were affected by demographic variables such as age, gender and season. Identifying family health center dynamics may contribute to creating rational health policies.

Keywords: Intramuscular, injection, family medicine, demographic.

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Introduction

Injection is the most commonly used form of pharmacological treatment. Intramuscular (IM) injections are one of the most prevalently used methods in injection applications.[1] IM injection is the method used to administer medication to large muscle masses with a sterile needle, and it is routinely performed in almost all medical disciplines.[2] Generally, it is used for increasing the speed of the medication's effect or when the oral form is irritating.[3] IM injection is used widely in inpatient treatment institutions and is administered to healthy/ill individuals during vaccination and outpatient treatments in primary health care services.[1] Worldwide, 12 billion treatments are administered annually by injection.

Moreover, 5% or less of these are vaccinations, and >95% are injections for treatment.[3] In the literature, there are many studies on which diagnosis, how often, type of health institutions, anatomical localizations injections are applied and what side effects it may cause.[4-6] Apart from routine, this study aimed to evaluate IM injections administered to patients who applied to a family health center (FHC) with sociodemographic characteristics.

Materials and Methods

Study design

This is a cross-sectional, analytical and, retrospective file review study. A total of 5648 injections of 2059 different individuals were evaluated.

Case selection

The study universe was formed by 17,000 individuals registered to the 'Asarcık Meydan FHC' identified as a primary healthcare institution providing service in the Asarcık district[7] located in a rural area connected to the city of Samsun on the coast of Turkey, 44 kilometers south of the city center with an area of 214 square kilometers. The sample population was formed by patients who had IM injections administered between January 01, 2017 – December 31, 2019.

Five doctors and eight assistant healthcare personnel worked in this FHC and all data were archived by them. Without the need for sample analysis, age, gender, injection date and content of the administered drug were recorded for all patients, except those who lacked information. Vaccine applications were also excluded, and name or identification numbers were not specified. The nurses got the injections of the patients who applied with various diagnoses.

Statistical analysis

Graph representations were used to reveal the change in the number of injections according to sex, age, season and months. Chi-square analyses were used to determine the significance and the degree of the relationship between two qualitative variables. The ratio test determined the differences between the categories of demographic variables. Moreover, to determine the variables that affect the distribution of the number of injections, multiple correspondence analysis was used to determine the relationship and correspondence between the categories by bringing the levels of all categorical variables together on the same plane. The acquired data set was analyzed using SPSS 17.0 (IBM, USA) statistical package program, and the significance level was accepted as $p<0.05$.

Results

In this study, an average of 2.74 injections (min:1 – max:6) per person was administered. Female gender, 43-65 years of age and use of analgesics were predominant (Table 1).

The rate of myorelaxant injection administration was higher among women ($p<0.001$), and the rate of antibiotic and analgesic + myorelaxant injection administrations was higher among men ($p<0.001$ and $p<0.001$, respectively) (Table 2).

With the increase of age, the rate of analgesic injection administrations increases ($p<0.001$) and the rate of antibiotic injection administrations decreases ($p<0.001$), contingency coefficient = 0.514 for both comparisons. Steroid injection administration rates were found to be the highest among the 19–42 age group and at lowest among the 0–18 age group ($p<0.001$). Vitamin/mineral injection administration rates were reported to be the highest among the 0–18 age group and the lowest among the 43–65 age group ($p<0.001$). The administration rate of injections containing hormones was highest among the 19–42 age group ($p<0.001$). Administration of analgesic + myorelaxant injection increased until the age of 65, but there was a decrease after the age of 65 ($p<0.001$) (Table 3).

As the summer season approached, the rate of analgesic injection and myorelaxant injection administrations decreased ($p<0.001$, $p = 0.006$, respectively). The rate of antibiotic injections, which showed an increase from autumn to spring, decreased in the summer ($p=0.004$). The highest rate of steroid injection administration was during summer and autumn; the lowest was during the winter season ($p = 0.003$). The highest rate of vitamin/mineral injection administration was during the summer; the lowest was during the spring ($p=0.002$). The highest administration rate of injections containing hormones was during autumn and winter, and the least amount of administrations was found to be during spring ($p = 0.001$). The highest rate of analgesic +

myorelaxant injection administration was during winter, and the lowest was during autumn (p = 0.001) (Table 4).

Table 1. Distribution of Injection Medications According to Variables

Variables	Categories	n	% *
Gender	Female	4152	73.51
	Male	1496	26.49
Age	0-18 Years	271	4.80
	19-42 Years	1470	26.03
	43-65 Years	2573	45.56
	+65 Years	1334	23.61
Season	Autumn	1465	25.94
	Winter	1491	26.41
	Spring	1476	26.13
	Summer	1216	21.52
Injection Medications	Analgesic	1395	24.70
	Myorelaxant	270	4.78
	Antibiotic	403	7.14
	Steroid	586	10.38
	Vitamin / Mineral	863	15.28
	Hormone	181	3.20
	Analgesic + Myorelaxant	1764	31.23
	Other	186	3.29
TOTAL		5648	100

* Percentage of the column is shown.

Table 2. Relationship between the Distribution of Number of Injections and Sex*

			Number of Injections		
			Female	**Male**	**Total**
Injection	Analgesic	n	1036_a	359_a	1395
		%	25.06%	24.04%	24.70%
	Myorelaxant	n	234_a	36_b	270
		%	5.63%	2.47%	4.87%
	Antibiotic	n	263_a	140_b	403
		%	6.31%	9.42%	7.13%
	Steroids	n	429_a	157_a	586
		%	10.32%	10.53%	10.42%
	Vitamin / Mineral	n	652_a	211_a	863
		%	15.78%	14.14%	15.39%
	Containing Hormone	n	181_a	0_b	181
		%	4.49%	0.0%	3.25%
	Analgesic + Myorelaxant	n	1236_a	528_b	1764
		%	29.81%	35.32%	31.22%
	Other	n	121_a	65_b	186
		%	2.90%	4.38%	3.32%
TOTAL		n	4152	1496	5648

* p=1.73e-23<0.05.
Each different index letter represents a subset of gender categories, of which the column ratios differ at a statistically significant level.
a-b: Shows from which group in the column related to each other.

While the season variable and gender variable had the same and low separation measures in both dimensions, it was observed that the age variable had more separation effectiveness than these two variables in both dimensions (Figure 1). The effect of season and gender variables, with coordinate values close to zero, on the injection variable was less, while the effect of the age variable on the injection variable was high (Table 5).

It is observed that women got more injections during the summer and spring seasons, and men got more injections during the winter and autumn seasons. The injections for the 0–18 age group were independent of season and sex. The correspondence graph showing all categories on the same coordinate plane is shown in Figure 2.

Table 3. Relationship between the Distribution of Number of Injections and Age *

			Number of Injections				Total
			0-18 Years	19-42 Years	43-65 Years	+65 Years	
Injection	Analgesic	n	8 $_{a}$	222 $_{b}$	741 $_{c}$	424 $_{c}$	1395
		%	3.02%	15.15%	28.84%	31.87%	24.70%
	Myorelaxant	n	5 $_{a}$	67 $_{a}$	139 $_{a}$	59 $_{a}$	270
		%	1.84%	4.63%	5.46%	4.40%	4.81%
	Antibiotic	n	167 $_{a}$	89 $_{b}$	78 $_{c}$	69 $_{b}$	403
		%	61.64%	6.11%	3.01%	5.23%	7.15%
	Steroid	n	12 $_{a}$	176 $_{b}$	256 $_{b}$	142 $_{b}$	586
		%	4.40%	12.02%	9.93%	10.69%	10.41%
	Vitamin / Mineral	n	63 $_{a}$	262 $_{a,b}$	345 $_{c}$	193 $_{b,c}$	863
		%	23.23%	17.84%	13.40%	14.51%	15.33%
	Containing Hormone	n	2 $_{a}$	179 $_{b}$	0 $_{c}$	0 $_{c}$	181
		%	0.72%	12.20%	0.00%	0.00%	3.22%
	Analgesic + Myorelaxant	n	3 $_{a}$	399 $_{b}$	940 $_{c}$	422 $_{b}$	1764
		%	1.11%	27.11%	36.51%	31.64%	31.22%
	Other	n	11 $_{a,b}$	76 $_{b}$	74 $_{a}$	25 $_{a}$	186
		%	4.14%	5.23%	2.95%	1.96%	3.36%
TOTAL		n	271	1470	2573	1334	5648

* p=6.32e-288 <0.05.
Each different index letter represents a subset of age categories, of which the column ratios differ at a statistically significant level.
a-c: Shows from which group in the column related to each other

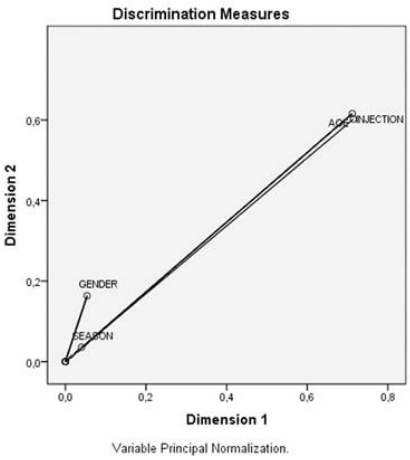

Figure 1. Separation Measures Chart for Variables

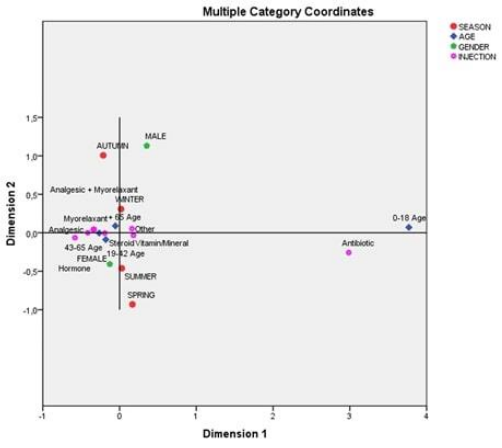

Figure 2. Multiple Category Coordinates Correspondence Chart

Table 4. Relationship between the Distribution of Injection Administrations and Seasons*

			Number of Injections				
			Autumn	**Winter**	**Spring**	**Summer**	**Total**
Injection	Analgesic	n	400 $_{a}$	356 $_{a, b}$	385 $_{a}$	254 $_{b}$	1395
		%	27.31%	23.90%	26.11%	20.95%	24,73%
	Myorelaxant	n	96 $_{a}$	76 $_{a, b}$	61 $_{b, c}$	37 $_{c}$	270
		%	6.62%	5.13%	4.12%	3.02%	4,81%
	Antibiotic	n	65 $_{a}$	117 $_{b, c}$	141 $_{c}$	80 $_{a, b}$	403
		%	4.44%	7.87%	9.62%	6.60%	7.12%
	Steroid	n	161 $_{a. b}$	123 $_{b}$	159 $_{a, b}$	143 $_{a}$	586
		%	11.03%	8.29%	10.85%	11.83%	10,43%
	Vitamin / Mineral	n	219 $_{a}$	223 $_{a}$	184 $_{a}$	237 $_{b}$	863
		%	14.90%	15.00%	12.57%	19.56%	15,30%
	Containing Hormone	n	79 $_{a}$	43 $_{b}$	25 $_{b}$	34 $_{b}$	181
		%	5.42%	2.91%	1.70%	2.80%	3.21%
	Analgesic + Myorelaxant	n	406 $_{a}$	500 $_{b}$	470 $_{a, b}$	388 $_{a, b}$	1764
		%	27.72%	33.53%	31.80%	31.91%	31,26%
	Other	n	39 $_{a}$	53 $_{a}$	51 $_{a}$	43 $_{a}$	186
		%	2.76%	3.67%	3.53%	3.53%	3.34%
TOTAL		n	1465	1491	1476	1216	5648

* p=6.62e-19 <0.05.
Each different index letter represents a subset of season categories, of which the column ratios differ at a statistically significant level.
a-c: Shows from which group in the column related to each other.

Table 5. Central Coordinates of Variables

	Dimension 1	**Dimension 2**
Season	0.040	0.036
Gender	0.054	0,163
Age	0.711	0,616
Injection	0.714	0,602

Discussion

In this primary care study, demographic factors were found to play a decisive role in the distribution of intramuscular injection. It shows the value of this study that it is not easy to reach a study analyzing intramuscular injection administrations in primary healthcare services in the literature. In this way, a statistical photograph of the rural area could be taken.

Primary health care institutions are the first step for all health problems.[8] Therefore, FHC admissions come with a wide range of symptoms and findings. Generally, musculoskeletal pain is at the forefront of IM injection needs.[4] Use of curative injection is common among healthcare professionals and patients. This is supported by studies conducted among patients in outpatient settings in developing countries. However, how much of these are needed is a separate subject of discussion. Antibiotics, vitamins or analgesics are often prescribed by

injection for upper respiratory tract infections, diarrhea, fever, or general fatigue.[9] In this study, the existence of many types of diagnosis for administering IM injection therapy and most of the injections being performed for analgesia support the literature. Skeletal diseases accompanying aging and pain related to obesity are frequently associated with IM analgesic use.[10] The results of this research showed the relationship between aging and this medication. Although the number of analgesic IM injections decreased during summer, the increase in the number of steroid injections may be explained by the increase in the number of pain cases caused by problems such as disc herniation and trauma triggered by physical activity rather than myalgia, which causes relatively milder pain.[11,12] As known, rural areas have their own dynamics different from the urban areas. Reasons, such as the concentration of agricultural activities at certain times of the year, may cause some medications to be used more frequently, whether necessarily or unnecessarily, compared to the patients in the city.[13] The increase in the number of antibiotic injections among children may be a result of the higher prevalence of respiratory tract infections.[14] The age and sex distribution in hormone injections appear to be related to the use of contraception or miscarriage prevention among pregnant women.

According to their study by Garcia et al., the IM administration method was used more among women, people over the age of 50 and those with a very low level of education. Most patients believe that this method is superior to enteric treatments.[15] According to the study by Talaat et al., when pediatric vaccines are excluded, the injection rate is higher among the elderly and women.[16] In a study conducted in Turkey, it was observed that the injection rate increases among patients with low levels of health literacy and among single patients.[17] According to this research, age, sex, and season were concepts that show effectiveness in IM injection. This was thought to be related to sociodemographic situations. In this rural district, men go to work in larger cities to earn money. People are interested in agriculture and animal husbandry. These facts may explain the difference in the parameters involved.

When technique rules are not followed in IM injection applications, many complication risks arise.[18] Unfortunately, home injections can also be found in Turkey. It is therefore important that the injections be made in the FHC. Because although the main task of FHCs is to provide preventive healthcare services, curative healthcare services constitute an important part of daily practice.[19] Vaccines were not included in this study, and analyses were conducted on the injections administered for treatment purposes.

A population-based study in Egypt reported that the average annual number of injections of 4.2 per person is higher compared to other low-income countries.[16] The average per person reported in this research is lower. The difference may be related to rational medication use or sociodemographic differences.

While certain clinicians choose to only perform a few types of musculoskeletal injections, others may inject to any anatomically possible target. Current evidence does not lead us to a definitive point for musculoskeletal

injections.[20] Unless there is an additional situation, the routine practice of FHC professionals is injections into the ventral/dorso-gluteal region. Therefore, statistical analysis was not needed.

In conclusion, one of the unique dynamics of primary health care institutions is that they cater to every segment of society. IM injection is an important curative intervention administered to this broad spectrum of the patient population. In this study, the objective was to roughly determine which patient groups were preferred to administer IM injection. The results showed that the frequency of analgesic, myorelaxant, antibiotic, steroid, vitamin/mineral, hormone and combination drugs used for intramuscular injection were affected by demographic variables such as age, gender and season. Identifying FHC dynamics can contribute to the creation of rational health policies.

Strengths and Limitations

The strengths of the study were the high number of analyzed administrations, study duration spreading over a long period of time and representing only primary healthcare.

Some patients received multiple injections, so the number of injections should not be confused with the number of patients. Also, the fact that the study was single-centered is a limitation in its generalization.

Ethical Considerations: Following the preliminary approval from the responsible physician of the FHC, a study protocol was signed with the provincial health directorate, and approval numbered GOKA/2021/18/9 was obtained from the Samsun Training and Research Hospital non-invasive ethics committee.

Conflict of Interest: The authors declare no conflict of interest. No financial disclosure was declared by the authors.

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Research Article

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RETROSPECTIVE ANALYSIS OF 32,749 PATIENTS: A PILOT ORTHOPEDIC TRAUMA REGISTRY STUDY IN ANKARA PROVINCE

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Abstract

Objectives: Musculoskeletal injury is a public health concern that substantially increases the workload of emergency healthcare providers and hospitals in developing countries. Successful management of the diagnosis and treatment processes reduces healthcare costs and shortens the duration of preventable disabilities in patients with musculoskeletal injuries. Here, we aimed to investigate the musculoskeletal trauma distribution within five years within the borders of Ankara province.

Materials and Methods: In this study, preliminary diagnoses of the patients with musculoskeletal injury made within five years (2014 – 2018) by emergency healthcare providers in Ankara were retrospectively screened through the Emergency Health Automation System – EHAS. The patients were classified according to age, gender and diagnosis.

Results: This study included data for the time period between 2014 and 2018 from 32,749 patients, i.e., 19,523 male and 13,226 female patients in Ankara province. The number of patients was recorded for each year, and it was found that there was an increase in the number of patients between years. Musculoskeletal trauma was most commonly seen in the 19-64 (adult) age group, and the highest number of cases was observed in April. Hips and thighs were the most common trauma regions in the body.

Conclusion: This study is the first to analyze data obtained from emergency healthcare providers in Turkey, and it can be considered a pilot study that can be utilized to eliminate the existing drawbacks and optimize registry systems by updating them.

Keywords: Musculoskeletal injury, national registry, trauma, emergency healthcare.

Introduction

Musculoskeletal injury is a public health concern that substantially increases the workload of emergency healthcare providers and hospitals in developing countries.[1,2] Such injuries have a greater impact, particularly in developing countries, due to resulting labor and economic losses. Therefore, it is important to investigate the demographic distribution of musculoskeletal injuries in developing countries and determine new treatment strategies and the necessary measures.[3,4]

Successful management of the diagnosis and treatment processes reduces healthcare costs and shortens the duration of preventable disabilities in patients with musculoskeletal injuries.[5,6] Therefore, updating existing infrastructure and service conditions after thoroughly evaluating emergency healthcare providers' orthopedics and traumatology databases would be highly beneficial.[7,8,9]

National registries are increasingly becoming important sources of data, and therefore all recorded data should be thoroughly analyzed in today's studies.[7,8] Proper registry of musculoskeletal injury data involves various challenges from both regional and national aspects.[9,10] It is particularly challenging to ensure that emergency healthcare providers in Turkey keep up-to-date and accurate records since multiple registry processes take place simultaneously for a high number of patients. On the other hand, more accurate data in Turkey has become available in the last five years because service provider registry systems have been revised and updated.

The aim of this study was to answer the following questions:

1. What is the number of trauma patients who presented to emergency healthcare providers within the borders of Ankara within the specified five-year period?

2. What is the distribution of trauma patients by month-year and affected body region?

3. How did the emergency response cases end up?

Materials and Methods

In this study, preliminary diagnoses of the patients with musculoskeletal injury made within five years (January 2014-December 2018) by emergency healthcare providers in Ankara were retrospectively screened. Data were obtained from the registry of the Ankara Provincial Directorate of Health (Emergency Health Automation System – EHAS). The ethical approval was obtained from the local ethics committee. According to the inclusion

criteria, all patients registered in the EHAS system with trauma complaints were included in the study. Patients with incomplete EHAS records and those who died were excluded. Patients were initially diagnosed by emergency healthcare personnel according to their complaints. Patient complaints were broadly classified as upper and lower extremity traumas and torso traumas. In addition, extremity traumas were also classified into subgroups. Data were analyzed by grouping the patients according to age, gender, and the time and month when the trauma occurred. To ensure standardization, the cases defined according to the ICD-10 codes were grouped under certain categories. Upper extremity traumas were classified as wrist and hand injuries, elbow and forearm injuries, and shoulder and upper arm injuries. Lower extremity traumas were classified as ankle and foot injuries, knee and calf injuries, and hip and thigh injuries. Injuries to the body parts were classified as multiple injuries.

Statistical Analysis

Statistical analysis was performed using SPSS (version 17.0, SPSS Inc., Chicago, IL, USA). The Wilcoxon test was used to make comparisons between average mean values. The reliability of the implementations was tested using a reliability test and correlation coefficients. $p < 0.05$ was considered statistically significant.

Results

This study included data from 32,749 patients, i.e. 19,523 male (59.61%) and 13,226 female (40.39%) patients (Table 1). The patients were evaluated according to age distribution, and the highest incidence of musculoskeletal trauma was observed in the 18-49 age group in both genders (Figure 1). Considering the total number of traumas observed within the specified five-year period, the highest number of patients admitted with trauma was observed in 2017 (8,951), and an increase of 37.90% was observed in the number of patients admitted with trauma between 2015 and 2016 (Figure 1). Moreover, considering the distribution of traumas by month, the highest number of patients with trauma was observed in the fourth and eighth months (April; 3,284 and August; 3,251), without any significant difference between the years (10%) (Figure 2).

The distribution of the number of patients by month was also analyzed separately for each year, and it was reported that 386 patients were admitted in the 8th month (August) of 2014, 577 patients in the 10th month (October) of 2015, 803 patients in the 5th month (May) of 2016, 1335 patients in the 8th month (August) of 2017 and 1412 patients in the 4th month (April) of 2018 (Figure 2). Accordingly, it was found that the highest incidence of musculoskeletal trauma was observed between the 4th and 10th months within the specified five-year period. Considering the distribution of the number of patients by time of trauma, the highest number of patients admitted with trauma was observed between 12:00-18:59, i.e., 14,596 cases, and there was no significant difference between genders ($p > 0.05$) (Figure 3). According to patient follow-up results obtained

from emergency healthcare providers, the most common incident was the transport of patients with musculoskeletal trauma to the hospitals (20,883 patients) and the least common incident was the death in 33 patients (0.10%) within five years (Table 2).

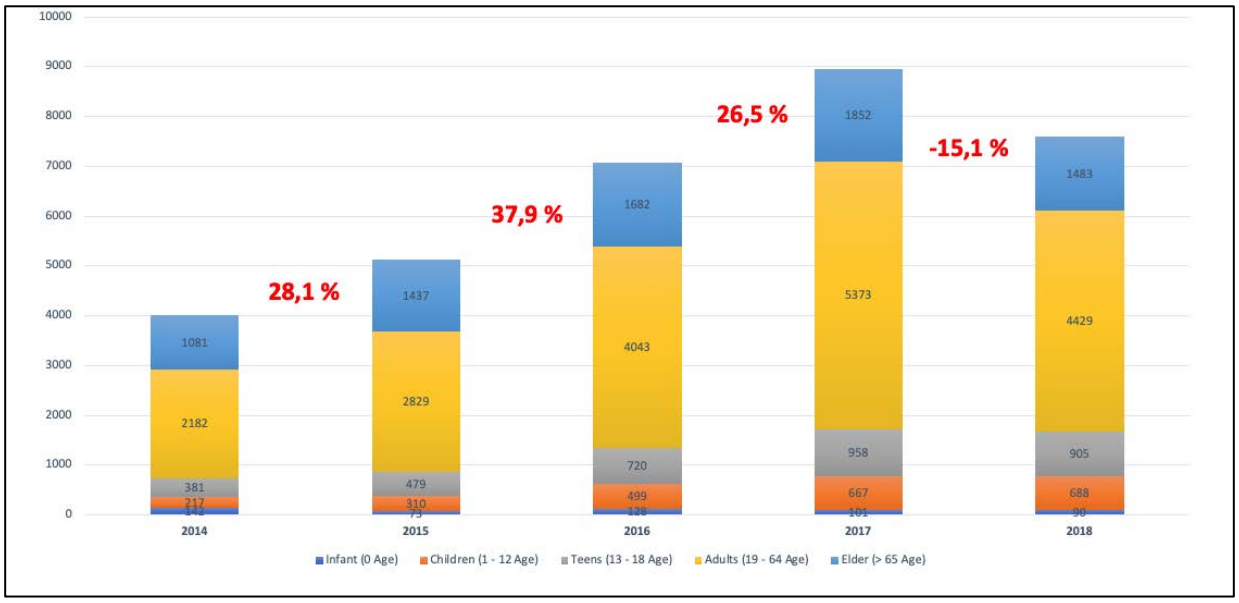

Figure 1. Total number of cases by age groups

Table 1. Total number of cases by gender

	2014 n (%)	**2015 n (%)**	**2016 n (%)**	**2017 n (%)**	**2018 n (%)**	**Total n (%)**
Male	2.286 (%57.10)	2.905 (%56.64)	4.298 (%60.77)	5.488 (%61.31)	4.546 (%59.85)	**19.523 (%59.61)**
Female	1.717 (%42.90)	2.223 (%43.46)	2.774 (%39.23)	3.463 (%38.68)	3.049 (%40.15)	**13.226 (%40.39)**
Total	**4.003 (%100)**	**5.128 (%100)**	**7.072 (%100)**	**8.951 (%100)**	**7.595 (%100)**	**32.749 (%100)**

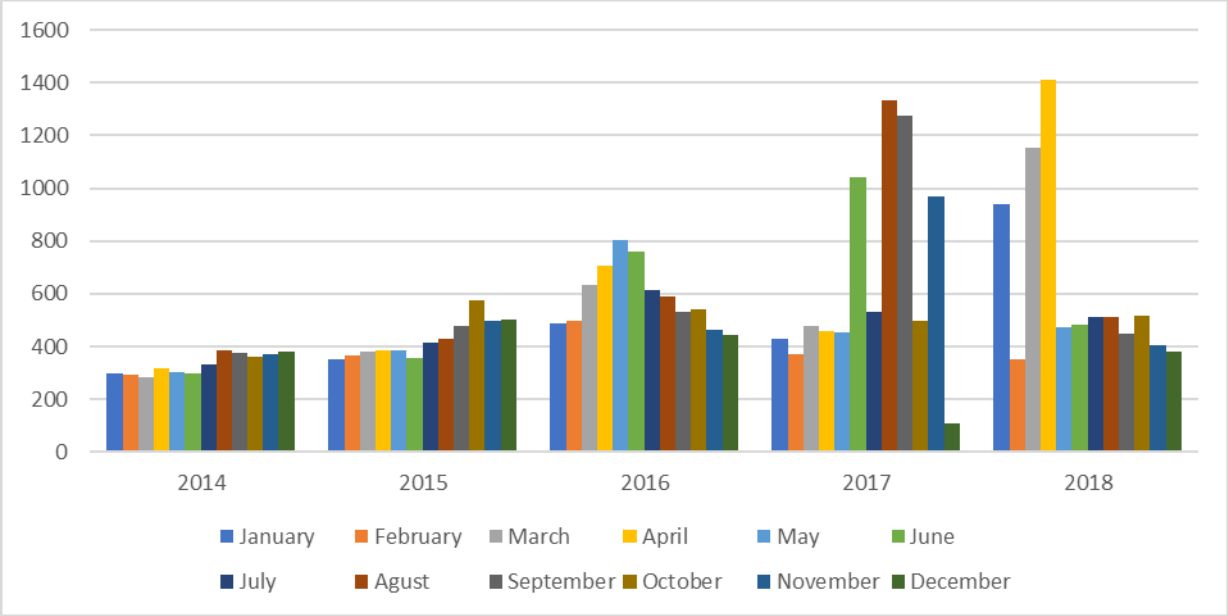

Figure 2. Total number of cases by months-year

Table 2. Total number of cases by results

	2014 n (%)	**2015 n (%)**	**2016 n (%)**	**2017 n (%)**	**2018 n (%)**	**Total n (%)**
Transfer to the Hospital	2.220 (%55.45)	2.968 (%57.87)	4.379 (%61.92)	6.262 (%69,95)	5.200 (%68.46)	**21.029 (%64.21)**
Transfer Between Hospitals	875 (%21.9)	929 (%18.11)	1.128 (%15.95)	1.180 (%13.18)	1.205 (%15.86)	**5.317 (%16.23)**
Transfer to the Home	560 (%13,98)	727 (%14.17)	851 (%12.03)	401 (%4.47)	360 (%4.73)	**2.899 (%8.85)**
Rejection of Transport	230 (%5.74)	367 (%7.15)	508 (%7.18)	775 (%8.65)	632 (%8.32)	**2.512 (%7.67)**
Others	40 (%0.99)	59 (%1.15)	109 (%1.54)	217 (%2.42)	116 (%1.52)	**541 (%1.65)**
On-Site Intervention	72 (%1.79)	73 (%1.42)	93 (%1.31)	110 (%1.22)	70 (%0.92)	**418 (%1.27)**
Exitus	6 (%0.14)	5 (%0.09)	4 (%0.05)	6 (%0.06)	12 (%0.15)	**33 (%0.10)**
Total	**4.003 (%100)**	**5.128 (%100)**	**7.072 (%100)**	**8.951 (%100)**	**7.595 (%100)**	**32.749 (%100)**

According to the case distribution by affected region within the specified five-year period, hip and thigh injuries had the highest incidence rate (23.34%), followed by ankle and foot injuries (18.58%), multiple injuries (16.48%) and elbow and forearm injuries (1986 cases, 6.09%), respectively (Figure 4).

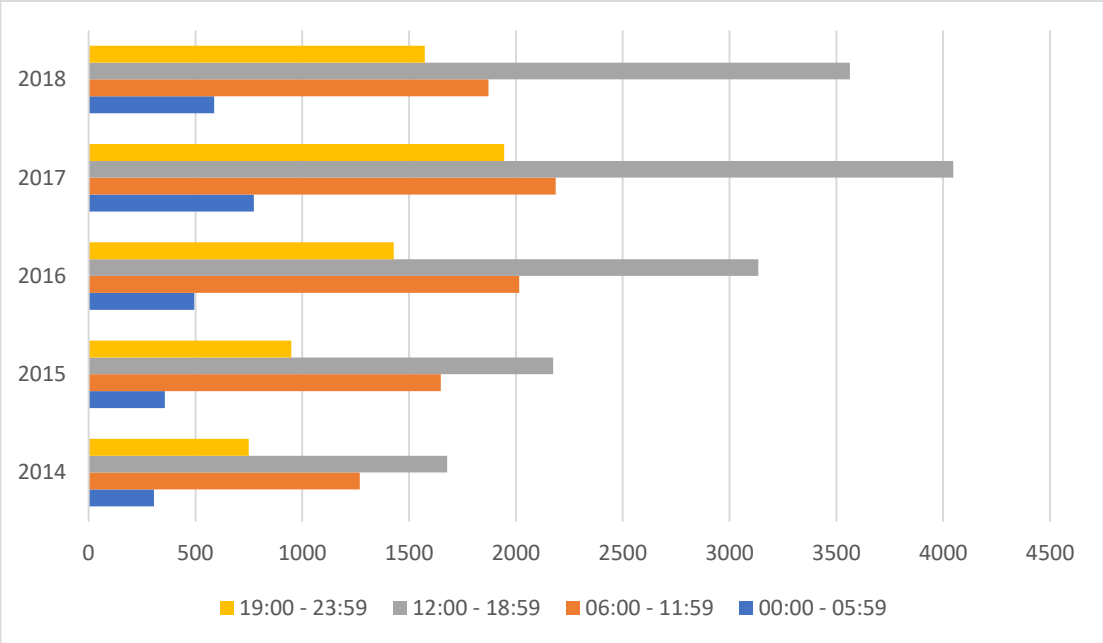

Figure 3. Total number of cases by the hour

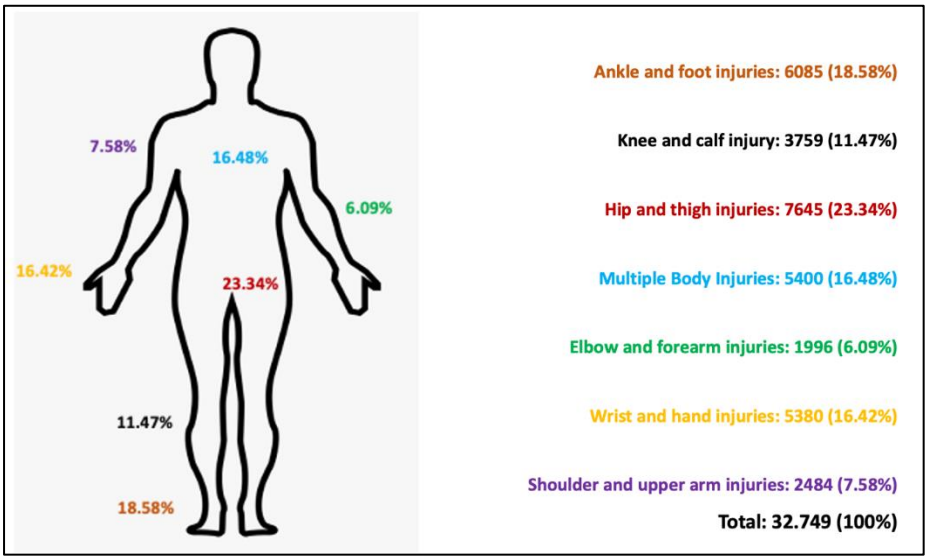

Figure 4. Total number of cases based on diagnosis

Discussion

The purpose of this pilot study was to obtain fundamental data for the effective and reliable design of a Turkish National Trauma Registry System. National Registry Systems provide researchers with reliable data and lead to the description and proper implementation of healthcare policies. The most important finding of this study

was that the majority of trauma patients were male and most of the traumas were found in the hip-thigh region. Considering the studies that investigate localized trauma in the literature: Laurila et al. conducted a study in 2019 to investigate the incidence of tibial fractures following isolated acute trauma between 1997 and 2014 in the Finland registry and found that the incidence of acute tibial fractures was 57.3% in males.[11] Moreover, in another study by Shah et al. conducted in 2017, the incidence of shoulder dislocations in England was investigated for the time period between 1995 and 2015, and it was stated that 72% of the dislocations occurring due to acute trauma were observed in males.[12] In this study, the incidence of trauma exposure was significantly higher in the male population as compared to females (59.6% in males, 40.4% in females), which was attributed to the fact that men work at jobs that require higher effort and therefore, have a higher risk of trauma exposure. Considering the publications relating to trauma registries in the literature, it was found that most publications had information about the regional distribution of trauma, increase in the number of cases according to years, gender distribution, and localization, whereas information on the distribution of trauma cases by month of the year was not available.[3, 8, 13] In this respect, our study will contribute to the literature.

There is also the possibility of generalizing on a population basis rather than on a limited group of patients.[14,15] Musculoskeletal traumas become more and more prevalent with the increasingly faster pace of life, and it is important to manage these traumas from various aspects.[16,17] The majority of centers providing healthcare services are highly populated by patients with musculoskeletal trauma.[18,19] Therefore, planning can be more appropriately conducted by management, and cost analyses can be evaluated according to trauma types through proper record keeping and archival of trauma records. There are studies concerning various data registries for health services in the literature.[3,7,8,20] Particularly, there are studies conducted in numerous countries on orthopedics and traumatology concerning trauma, arthroplasty, and arthroscopy.[21-23] These studies have shown that proper analysis of data enables conducting various cost analyses and important managerial planning processes. There is only one study previously conducted by Ceyhan et al. concerning the arthroplasty records in Turkey, even though registries are of utmost importance in healthcare.[24] This descriptive study is the first one to investigate the data concerning services provided by emergency healthcare providers, i.e., the first line of emergency health service delivery for patients with musculoskeletal trauma in Turkey.

The success of the initial intervention depends on the consistency between the initial diagnosis and the final diagnosis made at the center that will provide treatment for the patient.[25-27] Proper use of registry systems would pave the way for various in-service training and planning processes after the analysis of patient data. Therefore, it would be possible to provide training on the approach to the musculoskeletal system and planning processes for emergency service provider personnel. Moreover, the regional distribution of patient volume can be studied when planning emergency healthcare services in order to employ additional service providers during months and time intervals when patient volume is higher.

There is a considerably limited number of studies investigating trauma according to the localization in the literature, wherein research mainly focuses on isolated localized trauma.[7,8,11,28] In this sense, this study will also contribute to the literature in terms of identifying trauma exposure in all parts of the body in the adult population. Trauma to the hip joint generally manifests with hip fractures, especially in the older adult population.[29] The number of patients and age distributions reported in the literature were similar to the figures obtained in this study.[30]

Unlike previous studies on trauma registries in the literature, this study also investigated the time of the day when patients were exposed to trauma. The highest incidence of exposure to trauma was found to be between 12:00-18:59, i.e., within working hours when people become more tired and have less attention with more hours worked. In this respect, it can be recommended to also consider the working periods while taking precautions to reduce trauma exposure. This study also mentioned the outcomes of trauma, on which there is limited information in the literature. The study showed that 64% of trauma patients were transferred to the hospital and a very low proportion of patients, i.e., 0.1%, died at the trauma scene. Therefore, it is possible to say that almost all patients were transferred to a treatment center while they were still alive, regardless of the extent of trauma.

The weakness of our study was that the regional distribution of patients within Ankara and final diagnoses could not be evaluated. On the other hand, our study included a high number of patients, the data loss was low, and the distribution of the patients by month and time of the day could be observed, which were the strengths of the present study.

This study is the first one to analyze data obtained from emergency healthcare providers in Turkey, and it can be considered a pilot study that can be utilized to eliminate the existing drawbacks and optimize registry systems by updating them. Professional use of the existing registry infrastructure can enable further studies that could involve a more thorough investigation of case distributions and more proper data analysis.

Ethical Considerations: This study was organized with the permission of the Ankara Provincial Directorate of Health and was approved by the local Ethics Committee (Ankara Yildirim Beyazit University; approval number 2021 - 36).

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

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INFLUENCE OF COVID-19 PANDEMIC ON SEXUAL LIFE: WHAT IS THE SITUATION IN AN URBAN REGION OF TURKEY?

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Abstract

Objectives: It was aimed to evaluate the effect of the Covid-19 pandemic on couples' sexual life and to investigate whether any difficulties were faced in supplying the proper counseling in family planning.

Materials and Methods: Volunteers who attend to family medicine outpatient clinic were recruited in the study to complete a cross-sectional online survey. Participants were asked to sociodemographic characteristics, used family planning methods, difficulty in accessing the method, development of unplanned pregnancy, Covid infection of the spouses, and retrospectively report their sexual behavior frequency, desire, and relationship satisfaction during and before the pandemic. Then International Index of Erectile Function (IIEF), and the Female Sexual Function Index (FSFI) were administered.

Results: A total of 195 volunteers whose mean age was 40±7.94 participated in the study. Difficulty in accessing family planning methods was 3.59%, and the unintended pregnancy rate was 57.14%. Sexual intercourse frequency was once a month or less for 17.95% and once a week or more for 67.18%. A decrease in sexual intercourse was observed in 33.33%. Erectile dysfunction was detected in 68.92%. Sexual dysfunction was found in 52.07% of the women. The mean FSFI score was 23.77 ± 8.27, while the median IIEF score was 60. The frequency of sexual intercourse, and change in sexual desire were not influenced by Covid-19 pandemic.

Conclusion: Sexual life in both genders was not regressed, but access to methods and counseling about family planning was negatively affected by Covid-19 pandemic. Moreover, unintended pregnancies were observed at higher rates even in a highly educated population during the Covid-19 pandemic.

Keywords: Covid-19 pandemics, family planning, FSFI, IIEF, sexual dysfunctions.

Introduction

The Covid-19 pandemic has become one of the most crucial health problems worldwide. The virus is transmitted from droplets and contaminated surfaces through the hands, eyes, nasal mucosa, or mouth.[1] It is a significant cause of mortality and morbidity all over the world. It has made it compulsory to take drastic measures worldwide, including social isolation and quarantine. During the epidemic, individuals are expected to maintain social distance from other people as much as possible. It is thought that this process may cause some changes in the sexual behavior of individuals.[2,3]

In the Covid-19 pandemic, several factors can affect sexual behavior. Increased time spent together, less workload, and fewer social or family obligations may facilitate sexual intimacy. However, constant coexistence may increase the possibility of interpersonal conflict and stress, loss of income, and mental and physical weakness due to medical conditions that may negatively affect sexual life. The net result in an individual's sexual behavior is the cumulative effect of facilitating and limiting factors.[3]

Regarding sexual health, the current debate is whether there will be an increase in sexual activity with the pandemic; therefore, it is in the direction of whether it will be a new birth explosion or vice versa. More depression and less sexuality are predicted for acute anxiety and uncertainty. But it should not be forgotten that even in survival-mode societies, sexuality has a place because it is a fundamental expression of the human experience.[4]

Sexuality is an essential issue within the relationship dynamics of the partners. It should be a part of the health service to be offered to individuals with a holistic perspective. There are few studies conducted on different groups from Turkey in the early stages of the pandemic. However, no population-based studies have been found.[5-7] Available studies have focused only on sexuality, and there is no comprehensive data also, including family planning and reproductive health. Sexuality is not an easy subject for our country to discuss and study, and the fact that there are few studies on this subject has encouraged us.

In this study, we aimed to evaluate the changes in the sexual lives of our participants and to investigate whether people have faced difficulties in reaching the proper counseling in family planning one year later from the beginning of the pandemic.

Materials and Methods

This descriptive study was conducted on volunteers over the age of 18 who applied to a university hospital department of family medicine outpatient clinic in Turkey. Between 01-28 February 2021, 228 individuals applied to our outpatient clinic for various reasons. When detailed, a wide range of reasons for application, from a drug prescription to general preventive healthcare management, were observed. We informed them about the aims, stages, and confidentiality of the study, and 195 people volunteered to participate.

Only one of the spouses was included in the study. Those who are physically handicapped (visually disabled, deaf and speech handicapped persons), those whose mental faculties were not sufficient to answer the questions in the questionnaires, women in menopause, individuals who did not have active sexual activity in the last eight weeks, and incomplete questionnaires were excluded from the study.

In the research, the data form consisting of 40 questions for women and 35 questions for men was filled by the participants through online questionnaires. To reduce the risk of disease transmission and to acquire correct answers without being influenced by researchers, online questionnaire forms were directed to participants. Filling out the questionnaire took an average of 10-15 minutes.

In the first part of the data form, there were 20 questions prepared by the researchers, including 12 questions for sociodemographic characteristics, three questions that are thought to affect sexuality, and five questions to evaluate the impact of the Covid-19 pandemic. Additionally, vaginal dryness was asked to the women participants. With the personal information form, participants' age, education/income level, marital status, duration of the marriage, number of children, place of residence, occupation, smoking-alcohol use, existing chronic diseases, and medications were learned. Again, in this section, the family planning method used for the last year, the difficulty in accessing this method, the development of unplanned pregnancy after March 2020, the illness of themselves or one of the spouses during the Covid period, and whether there was a change in sexual desire and frequency of sexual intercourse during this period, and coexistence with different partners were questioned. In the second part, sexual functions were evaluated. For this purpose, the Female Sexual Function Index (FSFI) was used for female participants and the International Index of Erectile Function (EIIF) for males.

Female sexual function index (FSFI): It was developed by Rosen et al. in 2000 to evaluate the sexual functions of female participants. Its Turkish adaptation was performed by Öksüz et al.[8,9] A validation study determined the validity and reliability of the Turkish version of the questionnaire. It is a 19-item inventory of six subgroups: desire, arousal, lubrication, orgasm, satisfaction, and pain. Subscale scores and FSFI total scores are calculated according to a different scoring system by the researchers who developed the questionnaire. The scale reflects

sexual function in the last month according to sub-scores and total scores. The Cronbach's alpha value of the scale was 0.75-0.95, and the complete test-retest reliability was high for all domains (r=0.80-0.90) and the total score (r=0.92). Rosen et al. updated the cutoff value to 26 in 2005.[10]

International index of erectile function (IIEF): It was validated by the Society of Andrology in 2002 to assess the sexuality of male participants. It is a 15-item questionnaire consisting of five subgroups: erectile function, orgasmic function, sexual desire, sexual satisfaction, and overall satisfaction. Subgroup scores and total IIEF scores are obtained by scoring between 0 and 5 for each of the first ten questions and 1 to 5 points for the questions among 10-15. Bayraktar and Atun evaluated the reliability of the IIEF with Cronbach's alpha analysis and its correlation with the test-retest correlation coefficient in two consecutive IIEF queries, and they found a high degree of internal consistency and correlation for each of the five domains and the total scale (Cronbach's $\alpha > 0.91$, $r = 0.909$).[11-13]

Statistical Analysis

The data were uploaded to the SPSS (vers. 23.0) program. The normality of distribution was tested with the Kolmogorov-Smirnov test and Q-Q plots. Descriptive statistics (frequency, percentage, mean, median, minimum-maximum value, interquartile range) were used in the analysis of the categorical variables. Qualitative data were analyzed with the Chi-Square test, Fisher's Exact Test in 2x2, and multi-eyed layouts. The data obtained by measurement was evaluated with the Mann-Whitney U test. A value of $p<0.05$ was considered statistically significant.

Results

A total of 195 people (female/male: 121/74) were participated in the study. Sexual partners were not included in the study. The mean age was 40.00±7.94 (min-max:25-59); it was 40.54±7.41 (28-59) in men and 39.64±8.26 (25-59) in women. Among men participants, 86.49% (n=64) were at a university or higher educational level and 95.95% (n=71) were married. The rates were higher in women (93.39% (n=113), 99.17% (n=120), respectively). The mean duration of marriage was 13.95±9.54 (min-max:1-39) years. It was found out that 79.72% of men (n=59) and 95.04% of women (n=115) lived in the city center. Among women, level of income was low at 9.92% (n=12), medium at 31.40% (n=38), and high at 58.68% (n=71). For men, these rates were 17.56% (n=13), 25.68% (n=19), and 56.76% (n=42), respectively. Among participants, 70.27% of the men (n=52), 64.46% of the women (n=78) did not have any chronic disease and 83.78% of men (n=62), 75.21% of women (n=91) were not using any medication. When current chronic diseases were detailed, the women/men ratio was as follows: thyroid diseases n=16 (16/0), insulin resistance- type 2 diabetes n=12 (5/7), allergic diseases-urticaria-eczema n=11 (4/7), musculoskeletal and connective tissue diseases n=10 (6/4),

hypertension n=8 (5/3), ischemic heart diseases-rhythm disorder n=7 (2/5), rheumatological diseases n=6 (2/4), hyperlipidemia n=3 (3/0), attention-deficit/hyperactivity disorder n=3 (3/0), respiratory system diseases n=2 (2/0).

Sexual and reproductive health predictors of participants during the pandemic period were given in **Table 1**. Totally 14 (female; n=5 (4.13%), male; n= 9 (12.16%)) participants reported that they experienced pregnancy during the pandemic period. When detailed the unintended pregnancies, 2 (40.00%) of female participants and 6 (66.67%) of male participants were indicated. It was observed that 26.15% (n=51) of the individuals did not use any contraception method. While the rate of ineffective contraceptive methods (coitus interruptus method, calendar method) was 17.95% (n=35), the rate of those who used effective methods (condom, intrauterine device, oral contraceptive, etc.) was 55.90% (n=109). It was determined that 5 of those who experienced unintended pregnancy used condoms, while 3 used ineffective contraceptive methods.

Table 1. Sexual and Reproductive Health Predictors of Participants During the Pandemic Period

	Total n (%)	**Female n (%)**	**Male n (%)**
Family Planning Method (FPM) Used			
Condom	63 (33.32)	44 (36.36)	19 (25.68)
Intrauterine device	30 (15.39)	18 (14.88)	12 (16.22)
Oral contraceptive	5 (2.56)	2 (1.65)	3 (4.05)
Coitus interrupts	32 (16.41)	18 (14.88)	14 (18.92)
Calendar method	3 (1.54)	2 (1.65)	1 (1.35)
Not using	51 (26.16)	28 (23.14)	23 (31.08)
Tubal ligation/vasectomy	9 (4.62)	9 (7.44)	0
Those who have difficulties in accessing FPM	7 (3.59)	4 (3.31)	3 (4.05)
Unintended pregnancies (Pregnant women=14)	8 (57.14)	2 (1.65)	6 (8.10)
Frequency of sexual intercourse			
Once a month or less	35 (17.95)	22 (18.18)	13 (17.57)
Once every two weeks	29 (14.87)	15 (12.40)	14 (18.92)
Once a week	67 (34.36)	46 (38.02)	21 (28.38)
Twice a week or more	64 (32.82)	38 (31.40)	26 (35.13)
Change in frequency			
Increased	13 (6.67)	7 (5.79)	6 (8.11)
Constant	117 (60)	70 (57.85)	47 (63.51)
Decreased	65 (33.33)	44 (36.36)	21 (28.38)
Change in sexual desire			
Increased	7 (3.59)	3 (2.48)	4 (5.41)
Constant	129 (66.15)	76 (62.81)	53 (71.62)
Decreased	59 (30.26)	42 (34.71)	17 (22.97)
History of association with another partner	6 (3.08)	0	6 (8.11)

The individuals were examined in terms of sexual intercourse frequency, and among experienced a change (n=78), a decrease was observed in 83.33% (n=65). When read in terms of sexual desire, among participants who experienced differences (n=66), the rate of individuals with a decrease was 89.39% (n=59).

The effect of Covid-19 infection on sexual functions is given in Table 2. This effect was evaluated according to gender. While 24.32% (n=18) of men and 28.38% (n=21) of their sexual partners had a history of Covid-19 infection; these rates in women were 26.45% (n=32), and 24.79% (n=30), respectively. It was observed that being infected with Covid-19 and infection of sexual partners did not create a statistically significant difference in sexual functions.

When detailed according to gender, 60.00% (n=18) of the women whose partners had Covid-19 (n=30) did not change in their sexual desire (x^2=0.135, p=0.713) and 53.33% (n=16) of them did not change the frequency of sexual intercourse (x^2=0.334, p=0.563). It was observed that 85.71% (n=18) of male participants did not change their sexual desire (x^2=2.865, p=0.091), and 61.90% (n=13) did not change the frequency of sexual intercourse if their sexual partners had a history of the disease (n=21), (x^2=0.033, p=0.856).

Table 2. The Effect of Covid-19 Infection on Sexual Functions

	History of Covid19 infection, n=50, (%)	**The history of Covid-19 in the partner, n=51, (%)**
Sexual dysfunction		
No (n=81)	23 (46)	21 (41.18)
Yes (n=114)	27 (54)	30 (58.82)
p	0.458	0.951
Frequency of sexual intercourse		
Once a month or less (n=35)	8 (16)	7 (13.73)
Once a week or less (n=96)	27 (54)	26 (50.98)
Twice a week or more (n=64)	15 (30)	18 (35.29)
p	0.735	0.651
Change in Frequency of Sexual Intercourse		
Increased (n=13)	2 (4)	3 (5.88)
Constant (n=117)	29 (58)	29 (56.86)
Decreased (n=65)	19 (38)	19 (37.26)
p	0.548	0.780
Change in Sexual Desire		
Increased (n=7)	1 (2)	1 (1.96)
Constant (n=129)	30 (60)	36 (70.59)
Decreased (n=59)	19 (38)	14 (27.45)
p	0.334	0.639

Scale scores evaluating participants' sexual dysfunctions were summarized for both genders in Table 3. Among female participants, the effect of vaginal dryness on arousal, orgasm, satisfaction, and pain subscale scores and the total score was not found to be statistically significant (U1=1.314, p1=0.215, U2=1.372.5, p2=0.103, U3=1.276, p3=0.319, U4=1.262, p4=0.360, U5=1.386.5, p5=0.086 respectively). Sexual desire scores were significantly affected by vaginal dryness (U1=1.495, p1=0.013).

Table 3. Scale Scores Evaluating Participants' Sexual Dysfunction

Female (n=121)	**Mean ±SS (min-max, median)**
FSFI total score	23.77 ± 8.27 (2-34, 26.20)
Sexual desire	3.14 ± 1.01 (1-6, 3)
Sexual arousal	3.28 ± 1.38 (0-6, 3.30)
Lubrication	4.39 ± 1.67 (0-6, 4.80)
Orgasm	4.05 ± 1.88 (0-6, 4.80)
Sexual satisfaction	4.34 ± 1.63 (1-6, 4.80)
Pain	4.56 ± 1.85 (0-6, 5.20)
Sexual disfunction (FSFI total score <26.55), n (%)	63 (52.07)
Vaginal dryness, n (%)	23 (19.01)
Male (n=74)	**Median (IQR, Min-Max)**
IIEF Total score	60 (28.75, 5-70)
Erectile disfunction	24 (9, 1-27)
Orgasmic function	10 (6, 0-10)
Sexual desire	7 (3, 2-10)
Sexual satisfaction	11 (6, 0-15)
Overall satisfaction	8 (6, 2-10)
Erectile disfunction level	**n (%)**
Serious level	11 (14.86)
Medium level	5 (6.75)
Mild to moderate level	13 (17.57)
Mild level	22 (29.73)
None	23 (31.09)

FSFI: Female sexual function index, IIEF: International Index of Erectile function

The Covid-19 history of the women themselves or their sexual partners did not significantly affect the FSFI total score. The sexual desire scores of women who had Covid-19 were found to be significantly higher than those who did not have the infection (p=0.044). In terms of other subscale scores, there was no significant difference between women or their sexual partners having Covid-19. The total score, sexual desire, and lubrication subscale scores of the women who had no change in sexual desire were found to be significantly higher. The scale scores were also found to be high in women who had no change in the frequency of sexual intercourse, but it was not statistically significant (Table 4).

There was no statistically significant difference in male participants' or their sexual partners' history of Covid-19 in terms of total and subscale scores. The median total score, sexual satisfaction, and overall satisfaction subscale score of men who did not change in sexual intercourse frequency and in sexual desire were found to be significantly higher. In addition, the median erectile function, orgasmic function, and sexual desire score were higher in men who had no change in their sexual desire (Table 4).

Table 4. Comparison of Sexual Dysfunction Scale Scores in Both Genders According to Covid-19 Status and Change in the Sexual Function

	The participant's Covid-19 status			Partner's Covid-19 status			Change in the frequency of sexual intercourse			Change in sexual desire		
	Yes	No	p	Yes	No	p	Yes	No	p	Yes	No	p
Female (FSFI)												
Total score	26.75 (7.53)	25.90 (11.90)	0.362	26.25 (9.48)	26.20 (11.20)	0.829	24.60 (10.10)	27.20 (9.55)	0.237	23.90 (11.55)	27.25 (8.58)	**0.029**
Sexual desire	3.60 (1.20)	3 (1.20)	**0.044**	3.60 (1.20)	3 (1.20)	0.803	3 (1.20)	3.60 (1.20)	0.228	3 (1.20)	3.60 (1.20)	**0.026**
Sexual arousal	3.30 (1.73)	3.30 (2.10)	0.394	3 (1.80)	3.30 (2.10)	0.609	3 (2.10)	3.30 (2.10)	0.448	3 (2.10)	3.30 (1.80)	0.104
Lubrication	4.80 (0.60)	4.80 (2.10)	0.871	5.10 (1.13)	4.80 (2.10)	0.760	4.50 (1.80)	5.10 (1.58)	0.137	4.50 (2.10)	5.10 (1.73)	**0.016**
Orgasm	4.80 (1.80)	4.80 (3.20)	0.694	4.40 (2.50)	4.80 (2.40)	0.296	4.40 (3.20)	4.80 (2.50)	0.554	4 (3.40)	4.80 (2.40)	0.113
Sexual satisfaction	5 (1.90)	4.80 (2.60)	0.341	4.80 (2.90)	4.80 (2.40)	0.889	4.80 (2.40)	4.80 (2.50)	0.460	4.80 (3)	4.80 (2)	0.059
Pain	5.40 (1.40)	5.20 (2.40)	0.740	5.20 (1.30)	5.20 (2.40)	0.651	4.80 (2.40)	5.60 (2.10)	0.172	4.80 (2.40)	5.60 (1.90)	0.250
Male (IIEF)												
Total score	63 (31)	59 (28.25)	0.610	62 (11.50)	57 (34.50)	0.257	52 (32)	62 (14)	**0.041**	33 (33)	62 (13.50)	**0.002**
Erectile dysfunction	24 (9.25)	24 (9)	0.569	24 (5)	24 (12)	0.230	24 (9)	25 (7)	0.218	17 (15)	25 (5)	**0.005**
Orgasmic function	10 (2.75)	10 (6.75)	0.837	10 (1)	10 (7.50)	0.088	8 (6)	10 (2)	0.080	4 (8)	10 (1)	**<0.001**
Sexual desire	8 (3.50)	7 (3)	0.286	8 (2.50)	7 (3.50)	**0.019**	7 (3)	7 (2)	0.663	5 (3.50)	8 (1)	**0.005**
Sexual satisfaction	12 (6.50)	10.50 (6)	0.747	12 (4)	10 (7.50)	0.458	9 (7)	12 (4)	**0.005**	6 (6.50)	12 (5)	**0.001**
Overall satisfaction	8 (6)	8 (6)	0.698	8 (3)	8 (6)	0.392	6 (4)	9 (3)	**0.003**	4 (6)	8 (3.50)	**<0.001**

Mann- Whitney U test was used, and values were given as median (IQR). Statistically significant parameters were shown in bold characters.

Discussion

Pandemic deeply affects social life. Quarantines implemented in countries, the increase in the density of health centers, and the rapidly increasing number of cases can cause anxiety and depression in individuals. The Covid-19 pandemic has made it mandatory for people to maintain social distance from the people around them to reduce the risk of disease transmission. This situation is expected to affect not only interpersonal social communication but also the sexual lives of partners with each other. Sexual life can be affected by diseases, dysfunctions, and physical, emotional, and mental states. This may have resulted in sexual dysfunctions in individuals. While this study was being planned, it was investigated whether the pandemic had such an effect. The main findings of this study were that there was no regression in sexual life in both genders in most of the participants, and unintended pregnancies were observed even in the high-level educated population during the Covid-19 pandemic.

There are many studies in the literature to determine the variability in the frequency of sexual intercourse and sexual reluctance in the pandemic process. Conducted on 9000 patients in China during the pandemic process, Ossola A et al. found that 47% of people were negatively affected in their sex lives.[14] On the contrary, Arafat et al. reported an increase in sexual activity in Bangladesh, India, and Nepal.[3] In a study of 868 people in England, it was shown that sexual activity decreased in social isolation. [2] In another study conducted in China, a decrease in sexual desire was found in 25% of the cases. In addition, a reduction in sexual satisfaction was observed. Again, in the same study, a decrease was found in risky sexual behavior acts (another partner).[15] However, it has been determined that individuals have avoided being with different partners during the pandemic. Authors reported that this situation might be caused by legal restrictions, anxiety, fear, and psychological stress.[16] Differences between countries may be related to the level of development and culture.

In our study, sexual desire and the frequency of sexual intercourse were not influenced by most of the participants when compared to the pre-pandemic period. Erectile dysfunction was the most common sexual dysfunction in men and vaginal dryness and pain in women. When the sexual dysfunction scale scores of the women and men participating in the study were examined, it was seen that there was no significant change in terms of sexual intercourse, sexual desire, and sexual satisfaction compared to the pre-pandemic period. Among all the individuals participating in the study group, no changes were detected in the sexual life of those with a history of Covid-19 infection in themselves or in their partners. The rate of intercourse with different partners was calculated as 3.10%. Although individuals with a high level of education are in the majority in our study, the existence of this risky situation seems to be a separate discussion topic.

In addition, in our study, sexual desire decreases when female partners are infected. In the pre-pandemic period, sexual avoidance behaviors in females have generally been associated with different conditions, such

as psychiatric diseases (major depression, obsessive-compulsive disorder, etc.),[17,18] problems between the couples (lack of intimacy, attachment problems)[19,20] or some chronic diseases (hypertension, coronary artery disease, cancer, rheumatoid arthritis, epilepsy, migraine).[21,22]

In studies conducted in the early stages of the pandemic, it was observed that women were more affected by the pandemic process. Karagöz et al. reported that females had twofold higher sexual avoidance behaviors than males. They stated that the intimate life of couples might also be influenced by the living conditions (children staying at home due to closed school, tiny house, housework, etc.).[5] In our study, which was carried out one year later from the beginning of the pandemic, it was found that the situation remained the same. However, these conditions were not questioned in both studies. So, further studies are needed to examine this issue.

Like India, most countries worldwide have noticed that it has to take drastic compulsory measures against the infection as a national emergency.[23] Due to the coordination of their strategies regarding early detection, treatment, isolation of sick people, and crucial healthcare problems, some preventive healthcare practices could not be maintained as their priority. As shown in the past pandemics, counseling and using of contraception, which is an indispensable part of sexual health, might also be neglected. It had been stated that during the West African Ebola epidemic, the practice of contraception declined by 65% in Liberia and 23% in Sierra Leone.[24]

The latest estimates of the influence of Covid-19 on contraception have been announced by UNFPA (United Nations Population Fund) and Avenir Health with an analysis of 115 low- and middle-income countries. An estimated 12 (4-23) million women may have been unable to access family planning services. As a result of these disruptions, as many as 1.4 million (500 000-2.7 million) unintended pregnancies may have occurred before women could resume the use of family planning services. Compared with the pre-Covid-19 period, 41% of countries reported that services had been interrupted.[25]

World Health Organization reported that some 59% of countries (n=102) had 'partial', and 9% had 'severe' disruptions in family planning and contraception services.[26] In a study from Bangladesh, the prevalence of family planning use was 36.03% suggesting a 23% (approximately) decrease compared to pre-pandemic data. Besides, it was shown that 24.42% of the participants were using oral contraceptive pills; versus 61.7% before the pandemic period.[27] The decline in the prevalence of contraceptive use due to Covid-19 disruptions among women of reproductive age (15–49 years) has been estimated to be 10% for each modern method and 20% for female and male sterilization.[28]

However, countries have needed to coordinate their strategies to hinder unintended pregnancies. Ensuring that people have access to contraceptive services will reduce avoidable pressures on the health system to manage the consequences of unintended pregnancy and future detrimental consequences of population

growth. To prevent unintended pregnancy, couples who are waiting for the provision of contraception should be reminded to use an effective, reversible method of contraception at this time as the pandemic has limited health care accessibility.[29] Existing low use of health services for permanent contraception compounded with compromised access to family planning services may lead to an increased number of unintended pregnancies and unsafe abortions, thus adversely affecting maternal and neonatal health.[30]

We asked whether there was a problem in supplying the used family planning method compared to the pre-pandemic period. Unfortunately, 3.59% of the participants stated that they had difficulty accessing family planning methods, and the unintended pregnancy rate was found to be 57.14%. Most of the volunteers who participated in our study had a high level of education. On the other hand, considering the negative consequences of unintended pregnancies, we think these rates may be higher for individuals with lower education levels.

Our study had some limitations due to the lack of evidence-based data on the participants before the pandemic. It was designed this way because the pandemic has been an unpredictable disaster. The evaluation was made based on the individuals' self-reports with an online survey of the patients who were admitted to the hospital. Moreover, it was carried out in the family medicine outpatient clinic, which may be thought to point the society. However, descriptive study findings could not be naturally generalized to the whole population. The study was conducted at the end of the first year following the first case of the pandemic in our country may have turned into an old routine harmony over time. In addition, a gender comparison could not be made in terms of dysfunction rates due to the different measurement tools. Unfortunately, in low-income and the low-educated population was not volunteer to participate in the study, and the number of participants was small. It may have caused the study topic because sexuality is still an issue that is being prohibited in terms of social and religious customs in our city.

However, our study had some superior aspects. First of all, this study was conducted on both genders, and secondly, the research was designed to evaluate full of sexual life. Also, people filled out the online surveys themselves, and it may have caused them to answer more frankly. Finally, it is thought to be beneficial for professionals working in this field to obtain data on reproductive health, such as unintended pregnancies and different partners. Although it is a single-center study, it is thought that this study provides essential data on reproductive health and maybe a guide for completing the deficiencies in terms of public health.

Conclusion

When all these findings were evaluated, it was concluded in our study that if individuals had sexual dysfunctions before the pandemic, this situation continued during the pandemic period but did not increase. Similarly, there was no change in the pandemic process in individuals who did not experience sexual problems.

Most of the participants in our study were high-income and well-educated individuals living in the city center. Concerning this, the majority may not have had difficulty supplying and using family planning methods. But unfortunately, the unintended pregnancy rate was high. It was not ignored that if unintended pregnancy occurred such this population and at such a higher rate, how the low-income and low-educated population should be investigated thoroughly. Moreover, this study was done in the first year of the pandemic, and the conclusions may have come out like this. When the pandemic is over, prospective studies with broad participation will enable us to make more precise inferences on the subject.

Ethical Considerations: The necessary ethics committee approval was obtained for the study from the Non-Invasive Clinical Research Ethics Committee with the date 21.10.2020 and decision number 2020-10/28. All participants included in the study were informed about the study and signed informed consent forms.

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

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CAN ISCHEMIA MODIFIED ALBUMIN LEVEL BE REGARDED AS AN INDICATIVE MARKER OF ULCERATIVE COLITIS AND ITS ACTIVITY?

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Abstract

Objectives: We studied the effectivity of serum ischemia-modified albumin (IMA) levels in the diagnosis and clinical activity of patients suffering from Ulcerative Colitis (UC).

Materials and Methods: Eighty-eight clinically and pathologically confirmed UC patients and 48 age- and sex-matched healthy volunteers were included in the study. The patients were classified according to the Rachmilewitz Score [Endoscopy activity index (EAI)], and those with a score below five were considered in the remission group, and those above five were considered as active disease group. The IMA levels were calculated by the colorimetric method.

Results: When UC patients were compared to the control group, higher IMA levels were observed in the patient's serum (0.48±0.25 g/L *vs.* 0.28±0.08 g/L), and the difference was statistically significant ($p<0.001$). Among UC patients, higher IMA levels were found in the active group (n:36) compared to the remission group (n:52) (0.72±0.20 g/L vs. 0.32±0.12 g/L, and $p<0.001$). Positive and statistically significant correlations between serum IMA levels and EAI scores ($r:0.81$, $p<0.001$) were detected.

Conclusion: Serum IMA level may be a suitable biomarker for the diagnosis and the clinical and endoscopic activity of UC disease. It may have diagnostic and prognostic features in UC disease.

Keywords: Endoscopic activity index, ischemia-modified albumin, serum marker, ulcerative colitis.

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Introduction

Ulcerative colitis (UC) is an inflammatory disease of the colon causing diffuse fragility and superficial erosions of the colonic membrane associated with blood loss of unknown origin. It is the most common type of inflammatory bowel disease involving the inflammation limited to the colonic mucosa and submucosa. Typically, the disease starts in the rectum and spreads to the proximal colon without interruption.[1, 2] Although the etiology of UC is unknown, environmental and genetic factors, immune system diseases and oxidative stress may play a role in the etiopathogenesis. The increase in reactive oxygen/nitrogen species (ROS/RNS) is believed to take part in the oxidative stress in the UC. As a product of standard cellular metabolism, the excessive production of ROS/RNS causes oxidative stress, ischemia and tissue hypoxia. ROS/RNS corresponding to hydroxyl radicals were generated almost in every ischemic disease and adjusted the N-terminus of serum albumin leading to ischemia-modified albumin (IMA) formation. It is known that IMA formed in this way is considered an indirect marker of oxidative stress.[3-5] Since it has been shown that IMA was associated with oxidative stress and tissue perfusion, we designed this study considering that IMA may play a role in the pathogenesis of UC.

We aimed to assess serum IMA level as a diagnostic marker in UC patients and determine whether it is efficient in assessing the disease severity or not.

Materials and Methods

Study population

This study was conducted in our hospital between August 2017 and January 2020. Eighty-eight patients with UC and 48 healthy volunteers were enrolled in the study. UC and the control group were older than 18 years. The control group consisted of volunteers without any disease who applied to our outpatient clinic for control purposes and were similar to UC patients in terms of age and gender. Patients with any chronic systemic disease, ischemic disease or a history of ischemic diseases, such as myocardial infarction and pulmonary embolism, patients with hepatic and renal failure or infectious disease, were excluded from the study because IMA levels may be affected.

Endoscopic evaluation

To evaluate the degree of the disease, the history of the patients at the time of admission and all blood values, including inflammatory markers, were investigated. All procedures were performed by the same experienced endoscopist under the same conditions to calculate the endoscopic activity index (EAI) of Rachmilewitz scoring

(MC). Colonoscopy evaluation was performed by the same clinician using a high-definition white-light colonoscope (Olympus Medical Systems, Tokyo, Japan), each lasting approximately 15-20 minutes, by evaluating the colonic mucosa and calculating the EAI.

Clinical and endoscopic values were used for the staging of the disease. Endoscopic activity index values were calculated for UC activity using the Rachmilewitz scoring system, and values of 5 and above were accepted as active disease.[6]

Ischemia-modified albumin evaluation

For the measurement of IMA levels, 5 ml of blood was taken from all patients and controls between 08.00 and 10.00 a.m. after at least 12 hours of fasting, using the antecubital vein. Then the blood was centrifuged at 4000 rpm for 10 minutes, and the serum samples were placed in Eppendorf tubes and stored in -80-degree cabinets until the day of the procedure. All the IMA measurements were done by the same operator throughout the study period via Shimadzu UVmini-1240 spectrophotometry, including cobalt chloride, dithiothreitol, and sodium chloride 0.9%, by the spectrophotometric method reported by Bar-Or et al.[7]

Biochemical parameters evaluation

Complete blood count, alanine aminotransferase (ALT), (aspartate aminotransferase (AST), total protein, albumin, C-reactive protein (CRP) levels and erythrocyte sedimentation rates (ESR) were evaluated in the morning of the colonoscopy procedure and then recorded as data for analysis. ALT, AST, total protein, and albumin were calculated by using Roche Cobas Integra 800 (Roche Diagnostic Corp., Indianapolis, Indiana, USA) autoanalyzer. CRP was assessed by using a Hitachi Modular P800 analyzer. Also, ESR was evaluated by Alifax Test 1 THL (Alifax s.p.a Comp. Polvera, Italy) machine.

Statistical analysis

All statistical analysis was performed using SPSS (Statistical Program for Social Sciences) version 26 software. To assess the normality of data Kolmogorov-Smirnov/Shapiro–Wilk tests were used. Quantitative factors with normal distribution were indicated as mean ± standard deviation (SD), while those with non-normally distributed were indicated as median (IQR). Categorical factors were presented by numbers and percentages. In two group comparisons of normally distributed quantitative variables, the Student t-test was used, while for comparison of non-normally distributed quantitative variables, the Mann–Whitney U test was used. Comparison of categorical data was made by chi-square/ Fisher's exact tests where appropriate. The correlation analysis was done using Pearson/Spearman correlation analysis tests among quantitative variables. A $p<0.050$ was considered statistically significant.

Results

There were 88 patients at UC and 48 volunteers in the control group. No statistically significant difference was found when the control group and the UC group were compared in terms of age, sex and body mass index (BMI). There was no significant difference between the groups regarding hemoglobin, platelet, white blood cells (WBC), glucose, total protein, albumin and ESR. CRP levels were significantly higher in the UC group compared with the control group (1.5 (6.3) mg/L *vs.* 0.43 (0.2) mg/L) respectively, and p<0.001. When compared in terms of IMA levels, it was determined that there were higher levels in the UC group than in the control group, and the difference was statistically significant (0.48 ± 0.25 g/L *vs.* 0.28 ±0.08 g/L, respectively) and p<0.001. Comparisons of demographic characteristics and laboratory findings of control and UC patients are represented in Table1. The comparison of IMA levels between the control and UC group is shown in Figure 1.

Table 1. Comparison of Demographic and Clinical Factors of Ulcerative Colitis and Control Groups.

Factors	Control group (n:48)	UC group (n:88)	p
Gender, F (%)	16 (33.33%)	33 (37.50%)	0.629
Age, years	43 (21-77)	43 (18-77)	0.726
BMI (kg/m^2)	24.9 (3.48)	25.78 (3.59)	0.841
Hemoglobin (g/dl)	13.8 (1.8)	14.05 (1.2)	0.350
Platelet (x10^3 µL)	271.0 (68)	264 (96)	0.784
WBC (x10^3 µL)	7640 (3100)	7095 (2500)	0.071
Glucose (mg/dl)	94.62 ± 17.2	97.10 ± 16.3	0.752
T protein (g/L)	7.40 ± 0.43	7.31 ± 0.44	0.831
Albumin (g/L)	4.64 ± 0.36	4.36 ± 0.29	0.324
ESR (mm/h)	12 (9)	13 (20)	0.494
CRP (mg/L)	0.43 (0.2)	1.5 (6.3)	<0.001
IMA (g/L)	0.28 ± 0.08	0.48 ± 0.25	<0.001

Factors were expressed as mean ± SD (Standard deviation) for normally distributed and median (Interquartile range) for non-normally distributed factors. Categorical factors were defined as numbers (%).
(BMI; body mass index, CRP; C-reactive protein, EAI; endoscopic activity index, ESR, erythrocyte sedimentation rate, F; female, IMA, ischemia modified albumin; NS, non-significant; UC, ulcerative colitis; WBC, white blood cell)

Patients with a diagnosis of UC were classified as having an EAI < 5 in remission (n:52) and those with a score of greater than five as an active disease (n:36). There was a good positive correlation between IMA levels and EAI scores (r:0.81 and p<0.001) and CRP levels (r:0.37 and p<0.001). There was no statistically significant difference regarding age, gender, BMI, hemoglobin, platelet, WBC, ALT, AST, total protein, Albumin and ESR between the remission and active groups. There was a significant difference among remission and active groups regarding CRP levels (1.46 ± 1.04 mg/dL *vs.* 10.34 ± 9.6 mg/dL, respectively) and p<0.001. When the remission and active UC groups were compared in terms of IMA levels, a statistically significant difference was calculated (0.32 ± 0.12 g/L *vs.* 0.72 ± 0.20 g/L, respectively) and p<0.001. A comparison of demographic and

clinical factors among remission and active UC group are demonstrated in Table 2. Figure 2 represents the comparison of IMA levels between remission and active UC groups.

Table 2. Comparison of Demographic and Clinical Factors of Remission and Active Ulcerative Colitis Groups.

Factors	Remission group (n:52)	Active group (n:36)	P
Gender F (%)	17 (32.69%)	16 (44.44%)	0.263
Age, years	55 (32-68)	47 (20-66)	0.365
BMI (kg/m^2)	24.43 ± 3.51	26.68 ± 3.30	0.203
Hemoglobin (g/dl)	12.86 ± 3.35	13.8 ± 0.63	0.519
Platelet (x10^3 µL)	323 ± 256	267 ± 70.4	0.783
WBC (x10^3 µL)	5720 ± 3900	6620 ± 1290	0.368
ALT (IU/L)	10 ± 3.6	17 ± 9.2	0.663
AST (IU/L)	16.67 ± 3.21	16.29 ± 3.1	0.974
T protein (g/L)	7.40 ± 1.10	6.98 ± 0.36	0.191
Albumin (g/L)	4.16 ± 0.90	4.3 ± 0.29	0.691
ESR (mm/h)	15 ± 15.7	10.43 ± 7.77	0.212
CRP (mg/L)	1.46 ± 1.04	10.34 ± 9.6	<0.001
IMA (g/L)	0.32 ± 0.12	0.72 ± 0.20	<0.001

Factors were expressed as mean ± SD (Standard deviation) for normally distributed and median (Interquartile range) for non-normally distributed factors. Categorical factors were defined as numbers (%).
(ALT; alanine aminotransferase, AST; aspartate aminotransferase, BMI; body mass index, CRP; C-reactive protein, EAI; endoscopic activity index, ESR, erythrocyte sedimentation rate, F; female, IMA, ischemia modified albumin; NS, non-significant; UC, ulcerative colitis; WBC, white blood cell.)

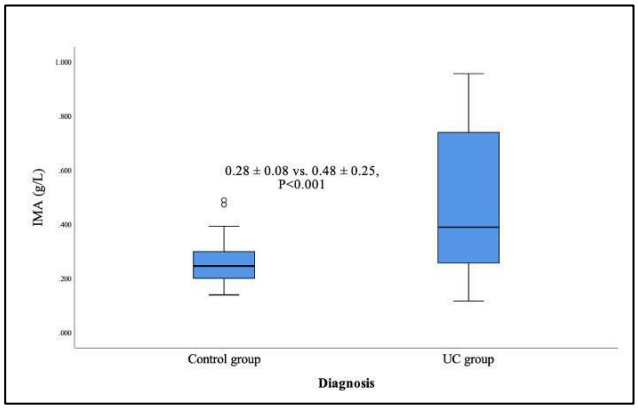

Figure 1. The Comparison of IMA Levels Between the Control and the UC Groups

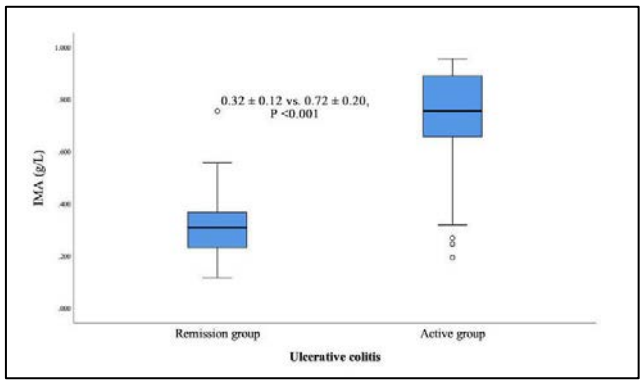

Figure 2. The Comparison of IMA Levels Between Remission and Active UC Groups.

Discussion

According to the results of our present work, when IMA levels were compared between the control group and UC group, statistically significantly higher levels were found in the UC group. A good positive correlation was found between IMA levels, CRP and EAI scores among patients in the UC group. The reason for the increased level of IMA may be due to tissue hypoxia developing after intestinal microvascular ischemia in the classical UC pathology. Therefore, a significant difference was observed between UC and healthy controls, and as the endoscopic activity of the disease increased, the detection of increased IMA levels revealed its relationship with tissue hypoxia.[8-10]

The IMA level, which has a valuable role in both diagnosis and disease severity in UC patients, gains value in the diagnosis and follow-up of the UC as a non-invasive serum biomarker. It has been reported that IMA levels have a significant role in some diseases, such as sepsis and cholestatic jaundice, both at the diagnostic and prognostic level. [11] Our results also confirm these studies and support the usability of IMA in the diagnosis and prognostic evaluation of UC.

Inflammatory bowel diseases are chronic inflammatory disorders and may also have the gastrointestinal system and extraintestinal involvement, which are not clarified in terms of etiology. [12] Although the onset of the deterioration of the immune system response and how the damage to the colonic mucosa occurs has not been clarified; it has been proven that reactive oxygen radicals, redox modules, nitrogen particles (ROS/RNS) and subsequent oxidative stress molecules may take part in its physiopathology. [9, 13] Recently, it has been reported that colonic oxidative stress may stimulate the formation of colitis.[9] It has been shown that the IMA level was an indirect marker of oxidative stress.[14-16] In our study, the presence of higher serum IMA levels was demonstrated in patients with UC compared to controls. Furthermore, higher levels were found in UC patients with active disease compared to those in remission, which is important in terms of showing that this hypothesis was proven in our study.

Several studies have shown that the proinflammatory cascade that causes the creation of reactive oxygen species (ROS) is induced by ischemia.[17, 18] In addition, the role of IMA in inflammation-related diseases has been shown recently.[14, 19] As a result of our study, we indirectly showed that serum IMA level could be obtained both diagnostically and prognostically in UC disease which has chronic inflammatory pathophysiology.

Although IMA levels were associated with acute coronary syndrome, liver ischemia, and ischemic conditions in organs such as the brain, kidney and intestine in adults, the association between IMA levels and UC disease and activity has not been demonstrated.[14-16] In the previous studies, the relationship between IMA levels and inflammatory bowel diseases was investigated, but different results were found.[15, 20] In our study, the

relationship between serum IMA levels and UC was important because it was significant both in diagnosis and prognosis. With this study, we obtained results that support the studies showing that there is a relationship between the level of ima and the UC.

Normally, IBD patients have more oxidative stress than healthy people. Due to ongoing chronic oxidative stress, antioxidants that increase in the inflamed tissue decrease over time, and this causes an increase in oxidative stress in the tissue due to the decrease in antioxidants.[21] It is acknowledged that oxidative DNA damage is very effective in the pathophysiology of UC and in the carcinogenesis that may develop in the later stages of the disease.[22] Therefore, patients with UC are at risk of developing colon carcinoma.[23] This is thought to be due to the increase in unmet oxidative stress. Consequently, we thought that high IMA levels in active UC patients were due to oxidative stress.

Since some serum biomarkers are involved in severe inflammation as acute phase reactants, they have been accepted for use in inflammatory diseases such as UC. Therefore, the good positive correlation between IMA levels and CRP reinforces the usability of this marker in the diagnosis and prognosis of UC.[24, 25]

In conclusion, serum IMA level may be a suitable biomarker for the diagnosis and the clinical and endoscopic activity of UC disease. It may have diagnostic and prognostic features in UC disease.

Ethical considerations: The work was designed according to the Helsinki declaration, and the local ethics committee confirmed with the number 10022015.03.12. All applicants signed the consent form before being recruited into the study.

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

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NORMOCALCEMIC PRIMARY HYPERPARATHYROIDISM IS NOT INNOCENT AS IT SOUNDS

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Abstract

Objectives: Primary hyperparathyroidism (PHPT) is the most common cause of hypercalcemia. A group of patients who were admitted with PHPT and had normal calcium levels with high parathyroid hormone (PTH) levels was defined as normocalcemic PHPT (NPHPT). The data of PHPT operated patients were retrospectively analyzed, and biochemical and clinical characteristics of hypercalcemic and normocalcemic patients were compared.

Materials and Methods: The data of patients diagnosed with PHPT between January 2012 and January 2019 were retrospectively evaluated. A total of 318 patients were divided into two subgroups, hypercalcemic and normocalcemic, according to their calcium level. The two groups were compared regarding clinical and biochemical properties.

Results: Female gender was dominant in both groups ($P = 0.072$). The mean age was similar in both groups ($P = 0.362$). As expected, serum corrected calcium (Ca), PTH levels, and urinary Ca excretion were higher in the hypercalcemia group ($P < 0.001$). There was no difference between the two groups in alkaline phosphatase, creatinine, and vitamin D levels. The percentage of localization with preoperative was similar. Also, there was no difference in adenoma features (echogenicity, cystic appearance) and localization on ultrasonography (US). The positive result obtained on neck MRI and MIBI scanning was similar. There was no difference between the two groups in terms of kidney stone and osteoporosis prevalence.

Conclusion: In our cohort, the NHPT phenotype was found to be like the hypercalcemic group. These findings suggest that the frequency of surgical indications is similar.

Keywords: Primary hyperparathyroidism, normocalcemic primary hyperparathyroidism, complications.

Introduction

Primary hyperparathyroidism (PHPT) is characterized by autonomous secretion of parathyroid hormone (PTH) from one or multiple parathyroid glands and involves a wide spectrum of clinical presentations, including symptomatic and sometimes life-threatening hypercalcemia on one side and mild asymptomatic hypercalcemia on the other side. [1]PHPT is one of the most common endocrine diseases after diabetes mellitus (DM) and thyroid disorders and the most common cause of hypercalcemia in outpatient clinics. [2]

Normocalcemic hyperparathyroidism (NPHPT) was first defined in 2009 at the Third International Workshop on Asymptomatic PHPT. It was defined as consistently elevated PTH with normal calcium (Ca) levels which should be confirmed with at least two consecutive measurements. [3] It is a diagnosis of exclusion and other possible etiologies that may increase PTH secretion, such as drugs (lithium), vitamin D deficiency, renal insufficiency, renal calcium loss, and malabsorptive bowel diseases (celiac disease, inflammatory bowel disease or previous bariatric surgery) should be excluded in the differential diagnosis. Prevalence of NPHPT in the literature varies in a wide range between 0.1- 8.9 %. [4]

It is not well known if NPHPT is a milder form of PHPT or if it has distinctive features, different courses, and natural history. Some studies suggest that it will progress into the hypercalcemic state eventually, whereas others oppose that.[4,5] The data on the disease course, biochemical progress and complications are scarce and difficult to draw any direct conclusion. Regarding the proceedings of the Fourth International Workshop on Asymptomatic Primary Hyperparathyroidism, NPHPT is not fully described, and its epidemiology, natural history, and management were not well defined.[6] We still do not clearly know how to diagnose, how many measurements we are supposed to make, how to manage, when to advise operation and how frequently we should see the patient during follow-up.

In this study, we compared the biochemical and clinical profiles of the patients with asymptomatic PHPT and NPHPT. All patients were previously operated and had histopathologically proven parathyroid adenoma. The operation criteria were determined according to the last version of the Endocrine Society guideline. Our primary aim was to evaluate the complications and prevalence of end-organ damage in NPHT. Secondary outcomes were to detect differences regarding biochemical features, preoperative imaging results and persistent/recurrent disease prevalence after the operation.

Materials and Methods

The patients who underwent parathyroid surgery for PHPT between January 2012 and January 2019 were retrospectively evaluated. Approval from the local ethics committee was provided before the data collection. A

total of 318 patients were enrolled. The patients were subdivided into two groups (NPHPT) and hypercalcemic PHPT. The diagnosis of NPHPT was made on persistently high serum PTH levels with normal serum total and ionized calcium levels. Ca was measured at least three times consecutively in a period of 3 to 6 months, and secondary causes that may lead to elevated serum PTH have been excluded. All patients who underwent operation had at least one indication for surgery were enrolled. Indications were determined according to the 'The Fourth International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism in 2014', which were as follows: Serum calcium exceeding 1 mg/dL above the normal range, a reduction in bone mineral density (BMD) at dual-energy x-ray absorptiometry (DEXA) that is significantly decreased over the baseline measurement and a T-score < -2.5 at that site, presence of a fragility fracture, the presence of nephrolithiasis, reduced GFR< 60 mL/min. [6] The decision of surgery was made by a multidisciplinary team including surgeons, endocrinologists, and nuclear medicine specialists. All patients had preoperative imaging tests, including Tc99m sestamibi scintigraphy and neck ultrasonography (US) with/without neck computed tomography (CT)/magnetic resonance imaging (MRI) or parathyroid hormone washout from the lesion.

The normal range of calcium, according to our hospital's assay was (8.8–10.2 mg/dL) (Roche Diagnostics, Manheim, Germany). Plasma intact PTH was measured using the Allegro immunoradiometric assay (Roche Diagnostics, Manheim, Germany). The detection limit of the assay was 1 pg/mL (normal range, 10–65 pg/mL), and the intra- and interassay coefficients of variation were 2% and 10%, respectively. In all individuals we calculated albumin-adjusted Ca by using the following equation (Ca+(4-serum albumin) x0.8). Vitamin D was measured by liquid chromatography coupled with tandem mass spectrometry (Schimadzu-API LC-MSMS API 3200, Canada). The lower and upper detection limits were 4 and 150 μg/L, respectively

Statistical analysis

All statistical analyses were performed with the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA). Descriptive analyses were presented using mean ± standard deviation (SD) for normally distributed variables, median and range (min-max) for non-normally distributed variables and as number of cases and (%) for nominal variables. The Chi-square test was used to investigate the difference between the groups regarding the categorical variables. The comparisons between groups were performed by the student's t-test for parametric variables and the Mann-Whitney U test for non-parametric variables to determine the best predictor(s). A p-value less than 0.05 was accepted as statistically significant.

Results

A total of 318 patients with PHPT who underwent surgery and histopathological proven parathyroid adenoma were enrolled in this study. Of all, 101(31.76%) had NPHPT, whereas 217 had hypercalcemic PHPT (68.24%). All patients were asymptomatic or had nonspecific symptoms that cannot be attributed to hypercalcemia alone. In the NPHPT group number and percentage of female/male patients were 93(92.07%)/8(7.93%), whereas it was 184(84.79%)/33(15.21%) in the hypercalcemic group. There wasn't any significant difference between the two groups. The mean age of the NPHPT and hypercalcemic PHPT groups were similar between the two groups. Mean serum Ca, median PTH and daily excretion of Ca in 24 hours urine collection were significantly higher in the NPHT group, whereas serum phosphorus (P) was significantly lower in the NPHT patients (Table 1). There was no significant difference between the two groups regarding serum 25 OH Vitamin D and alkaline phosphatase (ALP) levels (Table 1).

Table 1. Biochemical Parameters in Normocalcemic and Hypercalcemic PHPT Groups

Number of patients (n)	NPHPT 101	Hypercalcemic PHPT 217	p
Female/Male (number/percentage)	93 (92.07%)/8(7.93%)	184 (84.79%)/33(15.21)	0.072
Age (years)	54.08±10.37	55.41±12.82	0.362
Serum corrected Ca (mg/dl)	10.1± 0.33	11.4±0.92	<0.001
Serum P(mg/dl)	2.80±0.53	2.50±0.54	<0.001
PTH pg/ml (median; min-max)	123(90-260)	214 (70-1883)	<0.001
25 OH Vitamin D (µg/L)	25.9±6.32	27.4± 4.71	0.285
GFR (ml/min)	67.50± 6.53	72.5± 4.41	0.106
Creatine mg/dl	0.69±0.14	0.77±0.57	0.153
ALP (U/L)	99.8 ±6.50	115.75±12.70	0.061
24 hours urinary Ca (mg/day)	354 (80-770)	459(90-1216)	0.005
Mean Largest diameter of PA (mm)	14.73 ±7.82	17.75± 8.84	0.006
Volume of PAs (ml) (median)	0.87 (0.04-10.32)	1.9(0.03-31.63)	0.008

(GFR: Glomerular Filtration Rate, ALP: Alkaline Phosphatase, PA: Parathyroid adenoma)

When the preoperative imaging test results were evaluated, the rate of lesion detection with US was similar between the two groups. (Table 2). The rate of lesion detection with MIBI scintigraphy was also similar between the two groups (Table 2). Among 101 patients in the normocalcemic group, neck MRI was performed only in 11 patients, and it correctly localized the lesion in 4 patients. In the hypercalcemic group, MRI was performed in 58 patients, and a lesion was detected in 24 (Table 2). In the NPHPT group, 70 of the lesions that were suspicious for parathyroid adenoma were hypoechoic, whereas the rest had mixed echogenicity. In the hypercalcemic group, 141 of the lesions were hypoechoic, and the rest had mixed echogenicity (Table 2). The largest diameter of the adenoma and volume were measured significantly higher in the hypercalcemic group (Table 2).

The presence of nephrolithiasis was evaluated with US in 95 of 101 patients in the NPHPT group, and 22 had kidney stones. Renal US was performed in 212 of 217 hypercalcemic PHPT patients, and 58 were detected to have kidney stones. Prevalence of nephrolithiasis and estimated glomerular filtration rate (GFR) were similar between the two groups (Table 1 and 3).

BMD was evaluated with DEXA in all patients with PHPT. In the NPHPT group, 40 had osteoporosis (T score <-2.5 in one of three areas), 32 had osteopenia (T score between -1 and -2.5), and the rest had normal scores. In the hypercalcemic group, 102 patients had osteoporosis, 73 patients had osteopenia, and the rest had normal scores. There was not any significant difference between the two groups in the prevalence of osteoporosis (Table 3).

Table 2. Rate of Lesion Detection with US, MIBI Scintigraphy and MRI

	Total	Positive	Negative
NPHPT			
US*	101	92	9
MIBI**	98	56	32
MRI***	11	4	7
Hypercalcemic PHPT			
US*	217	208	9
MIBI**	210	129	81
MRI***	58	24	34

* p-value for the correct lesion localization of the lesion with US was 0.11
** p-value for the correct lesion localization of the lesion with MIBI was 0.570
*** p-value for the correct lesion localization of the lesion with MRI 0.062

Table 3. Rate of Complications of Hyperparathyroidism in Normocalcemic and Hypercalcemic Patients

	NPHPT n: 101(100%)	Hypercalcemic n: 217(100%)
DEXAs	101 (100%)	217(100%)
patients with a normal T score	29 (28.71%)	42 (19.35%)
patients with osteoporosis*	40 (39.60%)	102 (47.01%)
patients with osteopenia	32 (31.68%)	73 (33.64%)
Renal US	95(94.05%)	212(97.69%)
patients with kidney Stones**	22 (21.78%)	58 (26.72%)
patients without kidney stones	73 (72.27%)	154(70.96%)

* NPHPT vs hypercalcemic PHPT;p=0.082
** NPHPT vs hypercalcemic PHPT;p=0.721

Discussion

PHPT may present in different clinical subtypes, including obviously symptomatic type, which has been seen less frequently in the last decade, as well as the one with mild or nonspecific symptoms and asymptomatic subtypes.[7-9]The recently defined normocalcemic variant became a focus of interest among endocrinologists and surgeons with expertise in parathyroid diseases and surgery.

NPHPT is diagnosed by elevated serum PTH levels together with normal total corrected calcium and ionized serum Ca, after possible etiologies that may increase PTH are excluded.[9]Previous studies reported that NPHPT is a rare disorder. However, the prevalence is variable, probably due to different selection criteria used to define NPHPT patients. [2] The serum Ca level can fluctuate with time and may exceed the upper limit of the normal range and eventually become classical PHPT with hypercalcemia. Some authors recommend that the least significant change (LSC) of albumin-corrected calcium should be used to define and follow NPHPT. LSC shows if the quantitative change in the measured parameter is significant or not during the follow-up. [5,10]

The faith of the disease is unknown, and there is scarce data about the progression. In one study, it was suggested that the disease has a biphasic course and eventually will evolve to hypercalcemia.[11] In another study, 151 patients (81%) remained normocalcemic, and 36 (19%) became hypercalcemic; 24 (67%) had increased Ca to high levels within two years, 10 (28%) within 2 to 4 years, and 2 (5%) after more than four years.[12]

In our study, the clinical and biochemical profiles of 318 PHPT patients who underwent operation were evaluated retrospectively. In our cohort, almost one-third of the patients who had an indication for surgery were normocalcemic at the time of diagnosis. The localization of the parathyroid adenoma was not more difficult in normocalcemic patients despite the smaller lesion size and the volume measured with ultrasonography. Urinary calcium excretion was lower in the NPHPT group compared to hypercalcemic PHPT patients. In contrast to our study, in the reports by Díaz-Soto et al. and Kiriakopoulos et al., no significant difference in urinary calcium excretion between NHPT and PHPT was detected. [13,14]

NPHPT is characterized by several medical complications.[15] The first and major one is osteoporosis and consequent bone fractures. There is no study evaluating the incidence of fractures distinctively in patients with NPHPT. In our cohort, there weren't any reported femur or radius fractures. Since vertebral fracture assessment (VFA) was not routinely performed during DEXA and X-ray or CT/MRI was not ordered unless the patient had overt symptoms, we could not detect the exact prevalence of vertebral fractures. Among operated patients, the prevalence of osteopenia and osteoporosis was similar between NPHPT and PHPT groups in our study. In the previous reports from tertiary reference centers, NPHPT patients had decreased BMD compared

to controls and were classified either as osteopenia or osteoporosis.[16] As a result, all patients in those studies had decreased BMD. In our cohort, the prevalence of osteopenia and osteoporosis in the NPHPT group was like the hypercalcemic group. In the light of those findings, it may be suggested that increased serum PTH results in bone loss even if the serum calcium is normal and there is a lack of any other etiology explaining the elevated hormone levels. In addition to that, NPHPT patients with decreased bone mineral density may experience a further decrease in their BMD with time. The most well-organized and detailed study of NHPT was reported by Palermo et al., which was cross-sectional and prospective. [17] In that study, three groups were compared with each other which were PHPT (41 patients), NPHPT (47 patients) and age and gender-matched healthy controls (39 patients). BMD values at the lumbar spine, femur and distal radius in the NPHPT patients were detected to be between those in the PHPT and healthy controls. There wasn't any statistically significant difference between NPHPT and control groups, whereas it was lower at all sites in PHPT patients compared to controls. No statistical differences were found between BMD in NHPT and PHPT, except for a lower BMD value at the one-third distal radius in PHPT. Vertebral fractures detected by VFA were significantly more common in patients with PHPT (60%) compared to NPHPT (28%) or controls (23%). Bone turnover markers (serum carboxy-terminal [CTx]-telopeptide of type 1 collagen and procollagen type 1 N-terminal propeptide [P1NP]) were significantly higher than controls in both patient groups and significantly higher in PHPT than NPHPT. The authors concluded that NPHPT might be considered a milder form of classical PHPT and controls regarding biochemical parameters, but not associated with increased bone complications, reduced BMD, vertebral fractures, or increased bone turnover markers.

In the study of Palermo et al. that was prospective and cross-sectional, the presence of nephrolithiasis was evaluated from the medical database of medical records and patient notes.[17]They found that the prevalence of nephrolithiasis was 13% in NPHPT patients while it was 10% in patients with PHPT, and there wasn't a significant difference between the two groups. Controls had a kidney stone prevalence of 3%, although that might have been underestimated since subjects were not screened in detail with US or CT. Those findings suggest that nephrolithiasis prevalence is similar between NPHPT and PHPT; however, they couldn't demonstrate the pathophysiology of kidney stones in hyperparathyroidism. Our study confirms those findings. A study from Brazil reported that 20% of patients with NPHPT undergoing abdominal ultrasound imaging had kidney stones.[18]This prevalence is higher than that reported in the general population of approximately 10.6% in the United States but might be affected by selection bias, for example, caused by previous kidney stones.[19]

Our study has limitations. The most important one is including patients who underwent surgery alone. That might cause a selection bias since operated patients have more severe diseases. In addition to that, we can't discuss and compare the rate of indications for surgery in NPHPT and PHPT groups. The other limitations were retrospective nature, lack of follow-up data and bone fracture prevalence.

In conclusion rate of the patients with NPHPT was one-third of all hyperparathyroidism patients, which means being normocalcemic does not indicate the lack of need for surgery as a permanent treatment. End organ damage, defined as osteoporosis and kidney stones, is as prevalent as hypercalcemic PHPT in NPHPT patients. It should not be accepted as a milder form of the disease and should be followed like PHPT patients as advised in the guidelines for asymptomatic disease.

Ethical Considerations: Ethical approval was obtained from the clinical research ethics committee of Yıldırım Beyazıt University (date: 17.03.2021, decision number: 32).

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

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Research Article

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WHAT ARE THE PARAMETERS THAT PREDICT THE DEVELOPMENT OF NEPHROLITHIASIS AND OSTEOPOROSIS IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM?

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Abstract

Objectives: Primary hyperparathyroidism (PHPT) is associated with an increased risk of nephrolithiasis and osteoporosis, and predicting the development of these diseases will reduce PHP-related morbidities.

Materials and Methods: A total of 311 patients with PHPT due to parathyroid adenoma were evaluated retrospectively. The patients were divided into groups, as patients with and without nephrolithiasis and those with and without osteoporosis. Demographic and biochemical variables that could predict the development of nephrolithiasis and osteoporosis in these groups were examined.

Results: Nephrolithiasis was observed in 24.44% of 311 PHPT patients. Serum creatinine (Cr), serum calcium (Ca), adjusted Ca (adj Ca), albumin and 24-hour urinary phosphorus (24h uP) levels were higher, and serum P-value was significantly lower in patients with nephrolithiasis than those without nephrolithiasis. In the Receiver Operating Characteristic (ROC) analysis, serum Cr ≥0.66 mg/dl, adj Ca ≥10.72 mg/dl, serum P ≤2.71 mg/dl and 24h uP ≥635 mg/day cut-off values were found to have high sensitivity and low specificity values on the risk of developing nephrolithiasis. Of all patients, 43.09% had osteoporosis, and it was determined that only ≥50.50 years (sensitivity 81.34%, specificity 49.51%) and intact parathyroid hormone ≥ 201.50 pg/mL (sensitivity 75.14%, specificity 41.04%) values could predict osteoporosis (Area Under the ROC curve ranged from 0.57 to 0.67).

Conclusion: While biochemical parameters are useful in predicting nephrolithiasis in patients with PHPT, the development of osteoporosis seems to be less related to biochemical parameters.

Keywords: Primary hyperparathyroidism, nephrolithiasis, osteoporosis.

Introduction

Primary hyperparathyroidism (PHPT) is one of the common endocrine diseases, and it was diagnosed with various clinical findings such as significant hypercalcemia, fractures, nephrolithiasis and pancreatitis in the past, but today it is diagnosed with minimal or no symptoms owing to the routine measurement of serum calcium (Ca) levels.[1,2]

Even asymptomatic, PHPT often causes bone loss, and patients with PHPT have an increased risk of silent fractures in the cortical and trabecular regions.[3-5] Therefore, bone mineral density (BMD) measurement and vertebral fracture analysis are recommended for patients with PHPT at the time of diagnosis and periodically thereafter.[2]

The risk of nephrolithiasis is increased in PHPT, and the prevalence of silent kidney stones in asymptomatic PHPT(aPHPT) patients is up to 35%.[6,7] Renal imaging and 24-hour urinary Ca (24-h uCa) measurement are recommended for all patients, and it is stated that the biochemical urinary stone risk profile should be evaluated when 24-h uCa excretion exceeds 400 mg.[8,9]

The aim of this study was to determine the factors that predict the development of osteoporosis and nephrolithiasis in patients with PHPT, and thus predict the development of nephrolithiasis or osteoporosis in these patients.

Materials and Methods

Patients

The records of 390 patients followed for PHPT were reviewed, and 311 patients with PHPT due to parathyroid adenoma were included in the study. Patients with parathyroid carcinoma, parathyroid hyperplasia, normocalcemic HPT, hypercalcemia due to malignancy or bone metastases, and those using thiazide diuretics or lithium were not included in the study. In addition, patients with PHPT who also had gastrointestinal, hematological, rheumatological or other endocrinological diseases that could cause osteoporosis were not included in the study. The diagnosis of PHPT was made in the presence of hypercalcemia with the presence of elevated or normal intact PTH (iPTH).

Patients' demographic characteristics, a history of nephrolithiasis and fractures, laboratory [serum Ca, albumin, serum phosphorus (P), iPTH, alkaline phosphatase (ALP), 25-hydroxyvitamin D, creatinine (Cr) and 24-h uCa and 24-h urinary P (uP)], renal imaging and BMD results were recorded. The patients were divided

into groups as patients with and without nephrolithiasis, and those with and without osteoporosis, and demographic characteristics and laboratory findings were compared among the patients.

Biochemistry and Imaging

Serum Ca, albumin, serum P, Mg, ALP, Cr, 24-h uCa and 24-h uP were measured by standard laboratory methods. Adjusted Ca (Adj Ca) levels were calculated [Adj Ca= Serum Ca + [0.8 x (normal albumin – patient albumin)]]. Allegro IRMA (Roche Diagnostics) was used to detect plasma iPTH (normal range, 15–60 pg/mL). The detection limit was set at 1 pg/mL, with intra-assay and inter-assay coefficients of variation of 2% and 10%, respectively. Liquid chromatography with tandem mass spectrometry was used to determine 25-hydroxyvitamin D levels (Shimadzu-API LC-MS-MS API 3200, Canada), and the normal range was 20–80 µg/L.

Renal ultrasonography (US) or abdominal computed tomography (CT) was used to evaluate nephrolithiasis. Patients with a history of renal stone disease and patients with renal stones/calcifications on imaging were included in the nephrolithiasis group. BMD was evaluated with dual-energy X-ray absorptiometry (DEXA) (QDR-4500, Hologic Inc, Waltham, MA). DEXA measurements were made from the lumbar vertebra, femur and forearm (distal 1/3 radius). BMD was expressed as T-score or Z-score. In the diagnosis of osteoporosis, the region with the lowest T score in the lumbar spine, femoral neck and forearm was considered. The results were classified as normal, osteopenia and osteoporosis according to the criteria defined by WHO.

Statistical analysis

All statistical analyses were performed on IBM SPSS Statistics Version 21.0 for Windows (IBM Corp. Released 2012. Armonk, NY), and the statistical significance value was accepted at <0.05. The continuous variables were summarized as median (Quartile 1-Quartile 3) after examining the normality. The sex was given as frequency and percentage. The Yates Chi-Square or Fisher's exact test was used to analyze categorical variables. The comparisons between patients with versus without the condition (nephrolithiasis, osteoporosis) were analyzed by the Mann-Whitney U test. The Odds ratio was calculated from the univariate logistic regression model to obtain significant differences. When a significant difference was found between groups, the Area Under the Receiver Operating Characteristic (ROC) was calculated to decide whether to determine the cut-off value(s) to discriminate between patients with and without the condition. The Area Under the ROC curve (AUC) takes values between 0 and 1. To interpret the AUC values, we use the following rule of thumb given by Hosmer et. al: 0.50: no discrimination, 0.51-0.70: Poor, 0.71-0.80: Acceptable, 0.81-0.90: Excellent, >0.91: outstanding discrimination.[10]

Results

Of the 311 patients, 34 (10.93%) were male, 277 (89.07%) were female, and the median age was 54 years.

Nephrolithiasis

Nephrolithiasis was found in 24.44% (n=76) of the patients with PHPT. Gender and age distribution were similar in patients with and without nephrolithiasis. Nephrolithiasis was observed in 32.35% of male and 23.47% of female patients. Serum Cr, serum Ca, adj Ca, albumin, and 24-h uP levels were higher, and serum P-value was significantly lower in patients with nephrolithiasis than those without nephrolithiasis. There was no significant difference between the groups in terms of ALP, iPTH, 25 (OH)D and 24-h uCa level (Table 1).

Table 1. The Comparison of Patients with Nephrolithiasis and Those without Nephrolithiasis

Variables	Without Nephrolithiasis	With Nephrolithiasis	p*	OR (95% CI lower - upper)	AUC (95% CI lower-upper)
Sex (Male/Female)	23 (67.65) / 212 (76.53)	11 (32.35) / 65 (23.47)	0.354		
Age (years)	54 (47-61)	53.50 (46-59)	0.414		
Serum Cr (mg/dL)	0.67 (0.59-0.80)	0.74 (0.64-0.89)	**0.006**	16.336 (2.856 – 93.451)	0.636 (0.543 – 0.728)
Serum Ca (mg/dL)	11.19 (10.80-11.60)	11.53 (10.90-12.10)	**<0.001**	1.698 (1.280 – 2.252)	0.639 (0.565 – 0.713)
Adj Ca (mg/dL)	10.80 (10.41-11.28)	11.19 (10.65-11.76)	**0.002**	1.522 (1.161 – 1.996)	0.619 (0.543 – 0.694)
Serum P (mg/dL)	2.60 (2.30-2.94)	2.42 (2.10-2.82)	**0.012**	1.838 (1.105 – 3.059)	0.596 (0.523 – 0.668)
Albumin (g/L)	4.43 (4.20-4.61)	4.55 (4.23-4.80)	**0.027**	2.251 (1.085 – 4.670)	0.584 (0.506 – 0.662)
ALP (U/L)	96 (78-132)	94.50 (78.25-128.75)	0.745		
iPTH (pg/mL)	151 (102-214)	177.50 (112.25-287.75)	0.069		
25 (OH) D (µg/L)	16.10 (10.10-25.55)	15.38 (9.40-24.35)	0.388		
24-h uCa (mg/day)	357.20 (249.50-491)	397.50 (306.25-519.50)	0.106		
24-h uP (mg/day)	720 (532-973)	820 (680-1030)	**0.017**	1.001 (1.000-1.001) p>0.05	0.592 (0.522-0.662)

Abbreviations: Cr: Creatinine, Ca: Calcium, Adj Ca: Adjusted calcium, P: Phosphorus, ALP: Alkaline phosphatase, iPTH: intact parathyroid hormone, CI: confidence Interval, AUC: Area Under the Curve, OR: Odds ratio obtained univariate logistic regression.
Data were summarized as median (quartile 1-quartile 3) or frequency (percentage) according to variable type.
**Continuity Correction Chi_Square test for sex, Mann Whitney U test results for quantitative variables.*
The limits for interpretation of the AUC: 0.50:no discrimination, 0.51-0.70:Poor, 0.71-0.80:Acceptable, 0.81-0.90:Excellent, >0.91: outstanding discrimination.

As a result of the ROC analysis performed to determine the cut-off point on the risk of nephrolithiasis, it was determined that AUC values caused poor discrimination. Serum Cr ≥0.66 mg/dl, adj Ca ≥10.72 mg/dl, serum P ≤2.71 mg/dl and 24h uP ≥635 mg/day cut-off values were found to have high sensitivity and low specificity values on the risk of developing nephrolithiasis (Table 2).

Osteoporosis

Osteoporosis was found in 43.09% (n=134), osteopenia in 33.76% (n=105) and normal BMD in 23.15% (n=72) of the patients with PHPT. In addition, forearm BMD values were compatible with osteoporosis in 40.7% of the patients and osteopenia in 33.3%. The median age of patients with osteoporosis was significantly higher than those without osteoporosis (p<0.001). While the median iPTH was 159.50 (Q1:110.50-Q3:277.43) pg/mL in patients with osteoporosis, the median iPTH was 152 (Q1:100.85-Q3:201.50) pg/mL in patients without osteoporosis, and a borderline significance was determined between the two groups (p=0.049). There was no significant difference between the groups with and without osteoporosis in terms of serum Cr, serum Ca, adj Ca, serum P, albumin, ALP, 25 (OH) D, 24-h uCa and 24-h uP levels (Table 3).

As a result of the ROC analysis performed to determine the cut-off point on the risk of osteoporosis, it was observed that the risk of osteoporosis increased at ≥ 50.50 years (sensitivity 81.34%, specificity 49.51%) and iPTH ≥ 201.50 pg/mL (sensitivity 75.14%, specificity 41.04%) (Table 2).

Table 2. The Cut-Off Values for Variables That Show a Statistically Significant Difference Between Groups

Variables	AUC (95% CI lower-upper)	Cut off value	Sensitivity (%)	Specificity (%)
For Nephrolithiasis				
Serum Cr (mg/dL)	0.636 (0.543 – 0.728)	≥0.66	73.91	45.52
Serum Ca (mg/dL)	0.639 (0.565 – 0.713)	≥11.09	71.50	44.68
Adj Ca (mg/dL)	0.619 (0.543 – 0.694)	≥10.72	72.37	45.30
Serum P (mg/dL)	0.596 (0.523 – 0.668)	≤2.71	71.50	40.43
Albumin (g/L)	0.584 (0.506 – 0.662)	≥4.40	69.74	43.16
24-h uP (mg/day)	0.592 (0.522-0.662)	≥635	80.01	40.61
For Osteoporosis				
Age (years)	0.672 (0.611 – 0.733)	≥50.50	81.34	49.51
iPTH (pg/mL)	0.565 (0.500-0.631)	≥201.50	75.14	41.04

Abbreviations: Cr: Creatinine, Ca: Calcium, Adj Ca: Adjusted calcium, P: Phosphorus, iPTH: intact parathyroid hormone, CI: confidence Interval, AUC: Area Under the Curve. The limits for interpretation of the AUC: 0.50:no discrimination, 0.51-0.70:Poor, 0.71-0.80:Acceptable, 0.81-0.90:Excellent, >0.91: outstanding discrimination.

Table 3. The Comparison of Patients with Osteoporosis and Those without Osteoporosis

Variables	Without Osteoporosis	With Osteoporosis	p*	OR (95% CI lower-upper)	AUC (95% CI lower-upper)
Sex (Male/Female)	22 (64.71) / 155 (55.95)	12 (35.29) / 122 (44.05)	0.430		
Age (years)	51 (44-57)	56 (52-64)	**<0.001**	1.051 (1.027-1.076)	0.672 (0.611 – 0.733)
Serum Cr (mg/dL)	0.70 (0.60-0.86)	0.67 (0.59-0.79)	0.203		
Serum Ca (mg/dL)	11.20 (10.85-11.79)	11.28 (10.89-11.90)	0.595		
Adj Ca (mg/dL)	10.79 (10.42-11.42)	10.92 (10.46-11.49)	0.638		
Serum P (mg/dL)	2.60 (2.20-3.05)	2.50 (2.28-2.84)	0.330		
Albumin (g/L)	4.48 (4.18-4.70)	4.43 (4.20-4.67)	0.991		
ALP (U/L)	87 (76-128)	99 (81.75-133.25)	0.080		
iPTH (pg/mL)	152 (100.85-201.50)	159.50 (110.50-277.43)	**0.049**	1.002 (1.001 – 1.004)	0.565 (0.500-0.631)
25 (OH) D (µg/L)	15.60 (10-24.70)	16.71 (10.09-25.83)	0.585		
24-h uCa (mg/day)	361.50 (247.85-479.75)	385 (264-517.50)	0.392		
24-h uP (mg/day)	755 (560-980)	725 (531-980)	0.566		

Abbreviations: Cr: Creatinine, Ca: Calcium, Adj Ca: Adjusted calcium, P: Phosphorus, ALP: Alkaline phosphatase, iPTH: intact parathyroid hormone, CI: confidence Interval, AUC: Area Under the Curve, OR: Odds ratio obtained univariate logistic regression.
Data were summarized as median (quartile 1-quartile 3) or frequency (percentage) according to variable type.
**Continuity Correction Chi_Square test for sex, Mann Whitney U test results for quantitative variables.*
The limits for interpretation of the AUC: 0.50:no discrimination, 0.51-0.70:Poor, 0.71-0.80:Acceptable, 0.81-0.90:Excellent, >0.91: outstanding discrimination.

Discussion

In this study, the factors that may predict the development of nephrolithiasis and osteoporosis in patients with PHPT were evaluated. Serum Cr ≥0.66 mg/dl, adj Ca ≥10.72 mg/dl, serum P ≤2.71 mg/dl and 24-h uP ≥635 mg/day values were found to predict the development of nephrolithiasis; and age ≥50.50 years and iPTH ≥201.50 pg/ml values to predict the development of osteoporosis with high sensitivity, low specificity and weak strength.

Today, the main renal manifestations of PHPT are hypercalciuria and nephrolithiasis.[11] Symptomatic nephrolithiasis is present in approximately 10-20% of patients.[11,12] The prevalence actually appears to be much higher when asymptomatic patients are screened for nephrolithiasis.[7,8,11] While younger age and male gender have been shown as risk factors for nephrolithiasis, less consistent relationships have been observed between

the degree of hypercalcemia and hypercalciuria, PTH levels, and other urinary factors and nephrolithiasis.[9,11] In a study by Reid et al., nephrolithiasis was found in 13.9% of 611 patients with PHPT, and only younger age and male sex were found to be independently associated with nephrolithiasis. The authors attributed this to the hypothesis that younger individuals are at higher risk of nephrolithiasis due to greater renal activation of 1,25-dihydroxy vitamin D, greater intestinal Ca absorption and, consequently, greater calciuria.[13,14]

Saponaro et al. detected nephrolithiasis in 21.6% of 176 aPHPT patients, and hypercalciuria was reported to be a predictor for nephrolithiasis.[15] In a case-control study of 617 PHP patients, 23% of the patients had renal calcification (12% nephrolithiasis, 12% nephrocalcinosis, both 1%), with most being mild.[16] Cipriani et al. reported the prevalence of nephrolithiasis as 35.5% in 76 patients with aPHPT.[7] In our study, the prevalence of nephrolithiasis was found as 24.44%. Differences in the prevalence of nephrolithiasis in patients with aPHPT may be due to the retrospective, prospective, or observational study design, the use of different imaging modalities, or patient selection. We included only patients with parathyroid adenoma in our study. We did not include patients with parathyroid carcinoma with higher serum Ca levels and more complications. The prevalence of nephrolithiasis may be higher in studies including these patients.

Current guidelines recommend a value of 24-h uCa >400 mg/day to define hypercalciuria. However, it has been stated in the literature that this cut-off value for hypercalciuria is found in approximately one-third of patients with aPHPT, indicating a very low positive predictive value.[15,17] Saponaro et al. reported that the cut-off values of 250 mg/day for women and 300 mg/day for men showed higher sensitivity as a predictor of nephrolithiasis, but the specificity was lower than >400 mg/day. In the same study, it was reported that the sensitivity of the cut-off value of 231 mg/day obtained after ROC analysis was high, but the specificity was low.[15] Tay et al. reported that a threshold of >211 mg/day in patients with aPHPT had a sensitivity of 84.2% and a specificity of 55.3% for nephrolithiasis.[17] In another study evaluating the relationship between nephrolithiasis and hypercalcemia, it was shown that the risk increases at the upper limit of normal even if the 24-h uCa level is within the normal range.[18] In our study, although 24-h uCa was higher in patients with nephrolithiasis than in those without nephrolithiasis, this difference was not statistically significant, and therefore, the 24-h uCa value that could predict nephrolithiasis could not be calculated. This finding suggests that other urinary factors associated or not associated with hypercalciuria may also play a role in the development of nephrolithiasis. There are limited and conflicting results regarding the potential role of high 24-h urinary oxalate and low citrate in patients with PHPT.[9,11] However, these variables were not evaluated in our study. Changes in serum albumin levels affect total serum Ca levels. Therefore, it is important to use the adj Ca level in the analysis. In our study, serum albumin levels and accordingly adj Ca levels were found to be higher in patients with nephrolithiasis, and this is consistent with an increased risk of nephrolithiasis. On the other hand, there are studies in the literature in which serum albumin levels are similar in patients with and without nephrolithiasis.[19]

On DEXA performed in PHPT patients, BMD loss is greater in cortical regions such as the distal one-third of the forearm than cancellous sites such as the lumbar spine, reflecting the catabolic or anabolic effects of PTH on different skeletal parts.[20] However, epidemiological data suggest an increased risk of both vertebral and peripheral fractures in PHPT.[4] New technologies for noninvasively imaging skeletal microarchitectures, such as high-resolution peripheral quantitative CT (HRpQCT) and trabecular bone score (TBS), demonstrate that trabecular deterioration occurs both in the spine and in the radius and tibia.[21,22] Using these technologies, studies have shown that the risk of vertebral fracture (VF) is increased in PHPT compared to age- and sex-matched controls and many PHPT patients have silent Vfs.[3,23]

The prevalence of osteoporosis in PHPT differs among studies (39-62.9%).[7,24,25] Mean T scores are in the osteopenic range in most studies.[24,25] In a study by Reid et al., osteoporosis was observed in 48.4% of the cases, osteopenia in 39.9% and normal BMD was in 11.7% with DEXA, and greater age, lower body mass index, and lower Cr level were reported to be independently associated with osteoporosis.[13] Liu et al., investigated the risk factors associated with VF in PHPT, and found the prevalence of osteoporosis at 54.7% and the prevalence of VF at 12.8%. The authors stated that VF was associated with age, osteoporosis at the hip, prior fractures, osteoporosis treatment, and poorer renal function, but these risk factors were similar to those in patients without PHPT, and there was no association between VF and TBS, spine BMD, or biochemical severity of PHPT.[5] Unlike the study of Liu et al., different studies have shown that TBS was associated with common VFs in PHPT.[26,27] In our study, 33.76% of our patients had osteopenia, 43.09% had osteoporosis, and 23.15% had normal BMD. Age and serum iPTH levels were found to be associated with the development of osteoporosis. However, since our study was retrospective, HRpQCT and TBS measurements were not available in our study population, and the risk of VF could not be evaluated.

This study has some limitations. First, only patients who were operated for PHPT and were found to have parathyroid adenoma were included in the study. Patients who did not meet the surgical indications and were followed up and patients with parathyroid carcinoma who had a higher risk of complications such as severe hypercalcemia, nephrolithiasis and osteoporosis were not included in the study. Second, urinary stone risk factors other than 24-h uCa could not be evaluated to assess the risk of nephrolithiasis. Finally, VF risk assessment could not be performed in our study group.

In conclusion; the factors that may predict the development of nephrolithiasis and osteoporosis in patients with PHPT were evaluated, and serum Cr, adj Ca, serum P, and 24-h uP values were found to predict the development of nephrolithiasis; and age and iPTH values to predict the development of osteoporosis with high sensitivity and low specificity. However, the low AUC values indicate that the predictive power of these parameters is weak. The exclusion of cases with parathyroid carcinoma in the study may have reduced the

power of the cut-off point for these variables. In addition, increasing the number of patients included in further studies might change these rates.

Ethical Considerations: Local ethical committee approval was obtained according to the ethical standards of the Helsinki declaration (Approval date and number: 2021/21-2080).

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

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Research Article

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THE ASSOCIATION BETWEEN PLATELET TO LYMPHOCYTE RATIO AND LEFT ATRIAL APPENDAGE THROMBOGENIC MILIEU IN PATIENTS WITH NON-VALVULAR ATRIAL FIBRILLATION

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Abstract

Objectives: To assess the diagnostic value of platelet to lymphocyte ratio (PLR) with respect to the risk of left atrial appendage thrombogenic milieu (LAA TM) in patients with nonvalvular atrial fibrillation (AF), which has not been studied before.

Materials and Methods: This is a retrospective study that included consecutive patients with non-valvular AF who underwent transesophageal echocardiography (TEE) prior to electrical cardioversion or prior to AF catheter ablation. The potential association between PLR and LAA TM, which was defined as the presence of a thrombus, sludge and spontaneous echo contrast in LAA, was analyzed using multivariate logistic regression analysis.

Results: A total of 120 patients (59 females, mean age: 66.15 ± 10.2 years) with nonvalvular AF were included in the study. The thrombogenic milieu was determined in 37 (30.83%) patients on TEE examination. Patients with LAA TM were found to have a higher mean CHA2DS2-VASc score (3.00 vs. 2.00, p=0.009), decreased LAA velocity (23.60 vs. 36.20 m/s, p=0.002) and left ventricular ejection fraction (49.70 vs. 56.90 %, p=0.010), greater left atrial diameter (4.70 vs. 4.30 cm, p= 0.001) and higher PLR value (157.91 vs. 126.13, p=0.023) compared to those without thrombogenic milieu. Only LAA velocity (OR=0.854; p=0.001) and PLR (OR=1.024; p=0.012) were found to be independently associated with LAA TM.

Conclusion: PLR may be an independent risk factor for LAA TM in nonvalvular AF patients; however, beyond research purposes, the rather low sensitivity and specificity values must be interpreted with caution in the routine clinical setting.

Keywords: Atrial fibrillation, platelet to lymphocyte ratio, left atrial appendage thrombogenic milieu.

Introduction

Atrial fibrillation (AF) is the most common arrhythmic disorder with an important association with cardio-embolic stroke.[1] Formation of thrombus, sludge and spontaneous echo contrast (SEC) in the left atrium or its appendix is the precursor mechanism of cardiac thromboembolism, and these formations are firmly related to the presence of AF. Current literature adopted the novel terminology of left atrial appendage thrombogenic milieu (LAA TM), an umbrella term consisting of thrombus, sludge and SEC.[2] Although the mechanisms behind thrombogenesis in patients with non-valvular AF are multifactorial, including left atrial stasis, inflammation and oxidative stress, identifying predictors of LAA TM is crucial because early treatment can help prevent thromboembolic complications. Several studies have sought to determine predictors of LAA TM.[3-5] These studies showed that predictors for LAA TM in patients with AF include higher natriuretic peptide levels, increased left atrium diameter, decreased left ventricular ejection fraction, longer AF duration, and persistent or permanent AF.[3-5] Recently, Fu and colleagues revealed that blood group A is an independent risk factor for left atrial and/or left atrial appendage thrombogenic milieu in patients with non-valvular AF.[6] Platelets have a pivotal role in thrombogenesis and inflammation, and platelet to lymphocyte ratio (PLR) has emerged as a new inflammatory marker.[7]

Although the association between inflammatory status and thrombosis risk in patients with non-valvular AF has been shown in previous studies, the relationship between the presence of LAA TM and PLR has not been studied yet. Therefore, we aimed to investigate the predictive value of PLR for LAA TM in patients with non-valvular AF.

Materials and Methods

We retrospectively analyzed the data of patients with non-valvular AF who had undergone TEE examination prior to electrical cardioversion (2) or prior to AF catheter ablation between January 2016 and June 2018 at our hospital. All patients included in the study had provided written informed consent for the scheduled procedure(s) and the possible use of their data for scientific purposes. Ethical approval was obtained prior to the study.

Study group and variables

Patients with mitral stenosis or a mechanical prosthetic heart valve, patients with concomitant infection and incomplete medical records on blood count were excluded. Patients with signs of infection that may affect PLR levels were not included in our study. The patients did not have a history of antibiotic use. Apart from infection and valvular disease, there were also several other clinical conditions that were excluded because they might

have affected PLR values. Thus, patients with systemic diseases, hematological disorders, metabolic diseases, congenital abnormalities, malignancies, those receiving chemotherapy treatment, individuals with a history of previous percutaneous coronary intervention or coronary artery bypass grafting, and patients with chronic renal, respiratory or hepatic disease were excluded. The CHA2DS2–VASc score (congestive heart failure or left ventricular dysfunction, hypertension, age ≥75 or 65–74 years, diabetes, thromboembolism or a history of stroke, vascular disease, and sex) was obtained for assessing stroke risk in AF.

The complete blood counts, which were obtained immediately before TEE, included total white blood cell, neutrophil, lymphocyte and platelet count analyses which were measured via automated blood counters, the Sysmex XS1000i and XE2100 devices (Sysmex Corporation, Kobe, Japan). Platelet-to-lymphocyte ratio was calculated as the ratio of the platelets to lymphocytes obtained from the blood samples.

The TEE Procedure

Echocardiographic examinations were performed with System V and a Vivid T8 from GE Ultrasound (G E Medical Systems, Wisconsin, USA) with a 1.7/3.4 MHz harmonic transducer and a multiplane 6.7 MHz transoesophageal probe. Subcutaneous heparin was administered prior to TEE. Assessment of the LAA function was done by the recording of LAA velocity by placing a pulsed wave Doppler sample volume just inside the base of the appendage. TEE images were reviewed for the presence of LAA thrombus/sludge and spontaneous echo contrast by experienced observers. The patients were classified as having an LAA TM if any of the following were present: dense spontaneous echo contrast, sludge or thrombus in LAA, and LAA flow velocity ≤20 m/s.

Statistical analysis

All statistical analyses were performed using the SPSS 20 (SPSS INC, Chicago, Illinois, USA). For the normality check, the Shapiro-Wilk test was used. Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to the normality of distribution and frequency (percentage) for categorical variables. Normally distributed variables were analyzed with the independent samples t-test, and non-normally distributed variables were analyzed with the Mann-Whitney U test. Chi-square tests were used to compare the distribution of categorical variables between the groups. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal PLR cut-off value for predicting LAA TM. A calculated difference of $p<0.05$ was considered to be statistically significant.

Results

A total of 120 patients (59 female, mean age: 66.15±10.2 years) with nonvalvular AF were included in the study. The comparison of baseline characteristics of the study population according to the presence of LAA TM is summarized in Table 1.

Table 1: Baseline Characteristics of the Groups

	LAA TM+ (n=37)	**LAA TM - (n=83)**	**p**
Age, y	67.39±10.32	64.89±10.01	0.528
Female / Male (n)	20(54.05%) / 17(45.94%)	39(46.98%) /44(53.01%)	0.555
Persistan AF(n, %)	22 (59.00%)	37 (44.50%)	0.054
AF duration, month	7.50 (2.00-24.00)	10.00(2.50-36.00)	0.480
Coronary artery disease (n%)	18 (48.60%)	32 (38.60%)	0.322
Hypertension (n, %)	28 (75.70%)	49 (59.00%)	0.100
Diabetes mellitus (n, %)	11 (29.70%)	17 (20.50%)	0.350
Hyperlipidemia (n, %)	10 (27.00%)	15 (18.10%)	0.331
Stroke (n, %)	3 (8.10%)	2 (2.40%)	0.170
CHA_2DS_2-VASc score	3.00 (2.00-5.00)	2.00 (1.00-4.00)	0.008
Left atrium, cm	4.70±0.45	4.30±0.62	0.001
Left ventricular ejection fraction, %	49.70(38.00-60.00)	56.90 (55.00-60.00)	0.010
Left atrial appendage velocity	23.60±15.92	36.20±16.44	0.002
Hemoglobin, g/dL	13.41±1.74	13.54±1.77	0.804
Platelet	254.83±74.26	241.21±77.7	0.037
Lymphocyte	2.09±0.77	2.20±0.84	0.194
Platelet to lymphocyte ratio	157.91±73.41	126.13±57.20	0.023
Glucose, mg/dL	115.12 (103.06-131.03)	114.01 (100.06-121.64)	0.285
Glomerular filtration rate	71.54 (61.02-85.04)	72.04 (65.04-89.03)	0.200
Antiplatelets (n, %)	12 (32.40%)	22 (26.50%)	0.518
Oral anticoagulants (n, %)	31 (83.80%)	70 (84.30%)	0.939
NOAC (n, %)	15 (40.50%)	58 (69.90%)	0.002
VKA (n, %)	16 (43.21%)	12 (14.54%)	0.001

Abbreviations: LAA TM: Left atrial appendage thrombogenic milieu; AF: atrial fibrillation; CHA_2DS_2-VASc: congestive heart failure or left ventricular dysfunction, hypertension, age ≥75 or 65–74 years, diabetes, thromboembolism or a history of stroke, vascular disease, and sex, NOAC: non–vitamin K antagonist oral anticoagulant; VKA: vitamin K antagonist.
Data are given as mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables

A univariate logistic regression analysis identified persistant AF (odds ratio [OR] = 2.511; p=0.045), CHA_2DS_2-VASc score (OR= 1.416; p=0.006), left atrial diameter (OR= 3.358; p=0.003), left ventricular ejection fraction (OR= 0.952; p=0.006), LAA velocity (OR= 0.943; p=0.003) and PLR (OR= 1.008; p=0.018) as predictors of LAA TM. However, in multivariate logistic regression analysis, only LAA velocity (OR=0.854; p=0.001) and PLR

(OR=1.024; p=0.012) were found to be independently associated with LAA TM in patients with non-valvular AF (Table 2). ROC curve analysis showed that the optimal PLR cut-off value for predicting LAA was 124.50 with a sensitivity of 62.20% and specifity of 60.20% (AUC= 0.641, 95% CI: 0.534-0.748, p=0.014) (Figure 1).

Table 2. Univariate and Multivariate Regression Analysis for Predicting Left Atrial Appendage Thrombogenic Milieu

Variables	Univariate OR (95 % CI)	p	Multivariate OR (95 % CI)	p
Age, year	1.025 (0.985-1.067)	0.218		
Female	1.327 (0.610-2.887)	0.475		
Persistan AF	2.511 (1.023-6.162)	0.045	1.267 (0.170-9.462)	0.817
AF duration, month	0.987 (0.964-1.011)	0.285		
Coronary artery disease	1.510 (0.691-3.299)	0.302		
Hypertension	2.159 (0.905-5.148)	0.083		
Diabetes Mellitus	1.643 (0.679-3.975)	0.271		
Hyperlipidemia	1.679 (0.672-4.196)	0.268		
Stroke/TIA	3.574 (0.571-22.354)	0.173		
CHA_2DS_2-VASc score	1.416 (1.102-1.818)	0.006	1.156 (0.740-1.805)	0.524
Left atrium, cm	3.358 (1.528-7.381)	0.003	1.575 (0.368-6.745)	0.540
LV ejection fraction, %	0.952 (0.920-0.986)	0.006	1.004 (0.943-1.070)	0.899
LA appendage velocity, cm/sn	0.943 (0.907-0.981)	0.003	0.854 (0.778-0.938)	0.001
Hemoglobin, g/dL	0.972 (0.780-1.212)	0.803		
Platelet to lymphocyte ratio	1.008 (1.001-1.014)	0.018	1.024 (1.005-1.042)	0.012
Glucose, mg/dL	1.007 (0.996-1.018)	0.209		
Glomerular filtration rate	0.984 (0.960-1.008)	0.191		
Antiplatelets	1.331 (0.573-3.093)	0.507		
Oral anticoagulants	1.189 (0.391-3.617)	0.761		

Abbreviations: OR: odds ratio; CI: Confidence interval; TIA: transient ischemic attack; LV: left ventricle; LA; left atrium. Other abbreviations are as in Table 1.

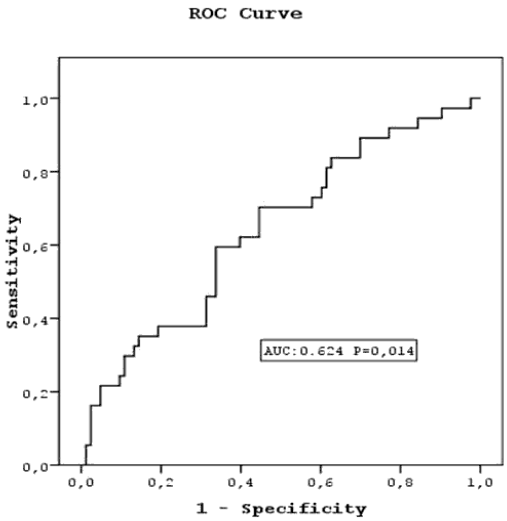

Figure 1: ROC Curve Analysis for PLR on Predicting LAA TM (AUC: 0,641, 95%ci:0.534-0.748, p=0.014).

Discussion

In the present study, we observed a significant association between PLR and increased risk of LAA TM in patients with nonvalvular AF. Although previous studies documented the potential use of TEE parameters as markers of LAA TM, to the best of our knowledge, this is the first study that reports an association between PLR and LAA TM in AF patients. However, taking into account the low sensitivity and specificity values, it is evident that PLR values cannot be used for diagnostic purposes in this context.

Previous studies have established that the left atrium, particularly LAA, is the most frequent location of cardiac thromboembolism. Therefore, prediction of LAA TM is important.[8-11] Transesophageal echocardiography has been the recommended procedure for this purpose.[12, 13] Because of its semi-invasive nature, there has been an interest in the prediction of LAA TM by the routine, non-invasive parameters such as clinical, transthoracic echocardiographic and biochemical markers. However, current literature indicates inconclusive findings on this topic. Yarmohammadi et al. found a positive relationship between CHADS2 score and left atrial thrombosis in patients with low left ventricular ejection fraction (≤20%) (2). Contrary to this, Jaroch and colleagues' retrospective study consisting of 202 patients with persistent AF failed to demonstrate the predictive value of CHA_2DS_2-VASc score for LA TM.[3] They found that duration of AF (exceeding one year), left atrial diameter exceeding 51 mm, left ventricular end-diastolic dimension (exceeding 52 mm), and radiographic evidence of aortic plaques were independent predictors of LAA TM. In a recent study, Kizawa et al. found that patients with TM had a lower incidence of paroxysmal AF and higher CHA_2DS_2-VASc scores compared to those without TM.[14] Presence of TM was associated with greater left atrial volume index, lower left ventricular ejection fraction, lower glomerular filtration rate and higher prevalence of left ventricular hypertrophy. In addition to the controversy on these prediction rules, the inconclusive findings still persist in the laboratory parameters. In patients with nonvalvular AF, Habara et al. revealed that plasma D-dimer was the most powerful predictor of LAA thrombus.[15] Similarly, Ochiumi et al. showed that brain natriuretic peptide levels higher than 251.2 pg/mL may predict LAA thrombus.[16] In contrast, Pant and colleagues showed that brain natriuretic peptide predicts spontaneous echo contrast, but its predictive ability does not include LAA thrombus.[5] In addition to this, Bejinariu and colleagues revealed that neither D-dimer nor BNP values were predictors of LAA TM.[17] Cianfrocca et al. Showed that C-reactive protein had an additive effect on left atrial appendage velocity on the prediction of LAA TM. [18] Our study also showed that LAA velocity was independently associated with LAA TM in patients with nonvalvular AF.

High platelet counts may increase thrombocyte activation and aggravate the release of inflammatory mediators. PLR reflects both inflammation and thrombosis and is more valuable than either platelet or lymphocyte counts alone. Recent data have shown that PLR, a novel systemic inflammatory response marker, is an important prognostic factor in numerous diseases such as acute coronary syndromes.[19]

In addition, many studies have shown that PLR is an important parameter in predicting the occurrence and recurrence of AF. In a study examining patients after CABG surgery, Gungor et al. showed that in addition to age and PLR values higher than 119.3 predicted AF recurrence with a sensitivity of 64% and a specificity of 56%.[20] In another study, Dereli et al. showed that PLR values higher than 147 predicted AF recurrence with a sensitivity of 83.3% and a specificity of 84.5% in nonvalvular AF patients undergoing electrical cardioversion.[21] However, the predictive value of PLR in LAA TM has not been investigated up to date. In our study, patients with LAA TM had a higher mean CHA_2DS_2-VASc score, decreased LAA velocity and left ventricular ejection fraction, increased left atrial diameter and higher PLR than patients without LAA TM. Of these parameters, only LAA velocity and PLR were found to be independently associated with LAA TM in patients with non-valvular AF.

Limitations

As this was a retrospective, single-center study, which included relatively small sample size, the potential cause-effect relationship could not be determined. All subjects in the current study were scheduled to receive cardioversion or ablation treatment, and selection bias must be considered when applying the results to the entire population of AF patients. The absence of measurement of other established inflammatory markers such as CRP, interleukin-6, tumor necrosis factor or BNP may also be considered a potential limitation.

Although there was no difference between the groups, the use of antiplatelet may have affected the results. In addition, the statistically significant difference between NOAC and vitamin K usage rates between the groups is a limitation of our study that may affect the results.

Our data also showed that CHA_2DS_2-VASc score could not predict LAA TM. This finding may raise concerns about the statistical power of the study; however, similar results have been reported previously. Comprehensive studies examining more cases may yield different results in this regard.

Conclusion

To our knowledge, this is the first study that combines echocardiographic, clinical and laboratory parameters for the prediction of the LAA TM presence. Although TEE is the gold-standard technique for the detection of LAA TM, identifying these novel predictors, such as PLR, may provide clinical insight and also may guide clinicians to conduct further research. This study also showed that PLR predicts LAA TM regardless of clinical prediction rules in patients with nonvalvular AF; however, as mentioned, caution must be taken to implement these findings in bedside practice as the current cut-off value of 124.5 had rather low sensitivity and specificity values and therefore can not reliably useful in the routine clinical setting.

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Ethical Considerations: The study protocol was approved by the TOBB Economics and Technology University local ethic committee (No: 118028, Date: 16/01/2019).

Conflict of Interest: The authors declare no conflict of interest

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Research Article

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EVALUATION OF INFECTION AGENT AND ANTIBIOTIC RESISTANCE DISTRIBUTION IN PALLIATIVE CARE PATIENTS WITH PRESSURE ULCERS

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Abstract

Objectives: In our study, it was aimed to examine the distribution of infectious microorganisms, and antibiotic resistance status in palliative care patients with pressure ulcers followed in Ankara Polatlı Duatepe State Hospital Palliative Care Service in 2019- 2020.

Materials and Methods: The sex, age, and detected diseases of a total of 178 palliative care patients included in our study were analyzed retrospectively. For determining the causative agents of pressure ulcer infections in these patients, Gram staining was performed on the bacterial cultures that developed in the wound samples, and the Vitek-2 (bioMérieux, France) automatic test device was used to identify these cultures and determine their antibiotic susceptibility.

Results: It was observed that the single-agent microorganism grew in 26 of the cultures. When the 26 active microorganisms we detected in the wound culture growths were examined; it was observed that *Escherichia coli* (n=9, 34.62%) and *Proteus mirabilis* (n=3, 11.53%) grew more frequently in enteric bacteria and *Pseudomonas aeruginosa* (n=3, 11.53%) in non-fermentative bacteria. In our study, the absence of antibiotic resistance in *Pseudomonas aeruginosa* isolates was considered remarkable. In our study, 100% resistance was found to antibiotics such as Ampicillin, Cefepime, Ceftriaxone, Ciprofloxacin, Amoxicillin-clavulanate, and Gentamicin in gram (+) bacteria, while 100% resistance was found against antibiotics such as Ceftriaxone, Ciprofloxacin, and Trimethoprim/sulfamethoxazole in gram (-) bacteria.

Conclusion: In the treatment of infection pressure ulcers, starting antibiotic therapy at the appropriate time and choosing the right antibiotic is one of the most important factors that determine the success of treatment.

Keywords: Pressure ulcer, palliative care, bacteria, antibiotic resistance.

Introduction

Pressure ulcers are defined as localized tissue damage to the skin and/or subcutaneous tissue, often by pressure on bony prominences or by friction with pressure.[1] Among the body regions where pressure ulcers are most common are sacrum, hip, heel, leg, rib, and scalp.[2-4] Pressure ulcers are an important health problem that increases morbidity and mortality, prolongs hospitalization, and increases the cost of treatment, especially in bedridden patients with limited mobility and in the elderly. 70% of pressure ulcers are seen in people over 65 years of age, who have long periods of inactivity and have neurological or vascular diseases.[5]

Pressure ulcer infections are usually polymicrobial. These infections can cause more serious infections such as cellulitis, osteomyelitis, and sepsis. Although many agents are blamed as causative agents in pressure ulcer infections, the most commonly isolated aerobic bacteria are; staphylococci, enterococci, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, anaerobic bacteria; *Peptosterptococci*, *Bacteriodes fragilis,* and *Clostridium spp*.[6, 7]

In the treatment of pressure ulcer infections, "knowledge of the causative agent" is decisive in the selection of antibiotics and the duration of treatment. According to the results of the culture antibiogram, starting the treatment by considering the antibiotic resistance increases the success of the treatment.

In this study, it was aimed to examine the distribution of causative microorganisms and antibiotic resistance in pressure ulcer infections developed in palliative care patients followed in the Palliative Care Service of our hospital in 2019-2020.

Materials and Methods

Our study included 178 patients who were hospitalized with various diagnoses in Ankara Polatlı Duatepe State Hospital Palliative Care Service from 1st January 2019 to 31th December 2020 and had clinical signs of pressure ulcer infection during the hospitalization. In the staging of pressure ulcers, the classification specified in the guideline of the European Pressure Ulcer Advisory Panel (EPUAP), which provides recommendations for the prevention and treatment of pressure sores, was used.[8] According to EPUAP staging; in stage 2, there was partial depth tissue loss affecting the epidermis and/or the upper layer of the dermis. Clinically, peeling and blistering of the skin were observed, and the wound was superficial. In stage 3, there was full-depth tissue loss or necrosis, including all tissue from the epidermis to the upper fascia. In stage 4, there was full-depth tissue loss as in stage 3. Tissue loss and necrosis had progressed below the fascia, into bone tissue, and supporting structures such as tendons and joint capsules. In patients who developed pressure ulcers, findings such as tenderness, temperature increase, discharge, and redness, which are signs of local infection, and swab cultures

were evaluated together, and the diagnosis of active infection or colonization was made. The distribution of 11 causative microorganisms isolated from 26 of these patients with growth in their wound swab cultures and their antibiotic resistance status were examined. Results considered as colonization and/or contamination were excluded from the study.

All pressure ulcer infections included in the study were clinically evaluated by the same physician. Topical antibiotics were used 3-5 days before sample collection was discontinued. After the swab sticks were moistened with sterile saline, the samples were taken by sufficiently pressing and rotating 360 degrees in 1 cm^2 area of the wound bed and placed in a Carry-Blair transport medium. Wound culture samples in transport medium were inoculated on 5% sheep blood agar and eosin methylene blue (EMB) agar medium. Preparations of the samples were stained with gram stain. Identification of growing bacteria and antimicrobial susceptibility tests were performed using the Vitek-2 (bioMérieux, France) automated system.

The leukocyte-white blood cell (WBC) (10^3/mL), C-reactive protein (CRP) (mg/dL), and erythrocyte sedimentation rate (ESR) (mm/h) measurements in the blood samples were taken from these patients were made in an automatic blood count device.

Before starting the study, local ethics committee approval was obtained. Data obtained from the hospital automation system and patient files (The demographic characteristics of the patients, underlying diseases, hospitalization history, etc.) were evaluated retrospectively.

Statistical Analysis

All statistical analyses were performed by using the statistical package SPSS for Windows, version 22.0 (SPSS, Chicago, Illinois, USA). Descriptive statistics such as frequency, percentage, and ratio were used to evaluate the data, and Student's t-test, and chi-square test were used for comparisons. The value of $p<0.05$ was considered statistically significant.

Results

Of 178 patients sampled from pressure ulcer infections, 72 (40.40%) were male, 106 (59.60%) were female, and the mean age was 76.79±12.04 (40-101) years. While there was no growth in 152 (85.40%) of the patients, growth was detected in the culture in 26 (14.60%), and a total of 11 bacteria grew. While no microbial growth was observed in 59 (81.90%) of male patients' wound cultures, growth was observed in 13 cultures. There was no microbial growth in 93 (87.70%) of the female patients, while growth was observed in 13 (12.30%) of the female patients. The most common diagnoses were Alzheimer (37.64%, n=67), Malignancy (20.22%, n=36),

Diabetes mellitus (DM) (11.80%, n=21), and Cerebrovascular disease (CVO) (11.24%, n=20). Among the evaluated pressure ulcers. Eight of them (4.50%) were stage 4; 84 (47.19%) were stage 3, and 86 (48.31%) were stage 2.

It was observed that the single-agent microorganism grew in 26 of the cultures. When the 26 active microorganisms we detected in the wound culture growths were examined; it was observed that *Escherichia coli* (n=9, 34.62%) and *Proteus mirabilis* (n=3, 11.53%) grew more frequently in enteric bacteria and *Pseudomonas aeruginosa* (n=3, 11.53%) in non-fermentative bacteria.

The distribution of antibiotic resistance rates of an enteric, non-fermentative, gram (+), and gram (-) bacteria in which growth was detected in the culture are shown in Tables 1, 2, and 3; respectively.

In our study, the most effective agents against enteric bacteria were found to be Tobramycin, Meropenem, Imipenem, Tigecycline, Colistin, Amikacin, Cefazolin, Gentamicin, Ceftriaxone, Cefoxitin, Netilmicin, Levofloxacin, Cefuroxime, Cefixime, and Cefuroxime axetil, while in *Proteus mirabilis*, Tobramycin, Meropenem, Imipenem, Tigecycline, Colistin, Ertapenem, Cefuroxime, Cefixime, Cefuroxime axetil, Levofloxacin, Piperacillin/tazobactam, Trimethoprim/sulfamethoxazole, Aztreonam, Cefoxitin, and Cefepime. It was observed that no antibiotic group came to the fore in terms of resistance level.

In our study, the absence of antibiotic resistance in *Pseudomonas aeruginosa* isolates was considered remarkable.

In our study, 100% resistance was found to antibiotics such as Ampicillin, Cefepime, Ceftriaxone, Ciprofloxacin, Amoxicillin-clavulanate, and Gentamicin in gram (+) bacteria, while 100% resistance was found against antibiotics such as Ceftriaxone, Ciprofloxacin, and Trimethoprim/sulfamethoxazole in gram (-) bacteria.

In our study, the WBC (11.29±6.38) (10^3/mL), CRP (13.15±6.50) (mg/dL), and ESR (92.38±20.64) (mm/h) were found to be higher than the reference ranges ((4-10), (0-0.50), and (0-30); respectively) in patients with growth in wound culture (n=26).

Table 1. Resistance rates to various antibiotics in enteric bacteria isolated from wound cultures

Antibiotic	Escherichia coli n (%) 7 (26.92%)	Escherichia coli (ESBL+) n (%) 2 (7.69%)	Proteus mirabilis n (%) 3 (11.54%)	Klebsiella pneumoniae ssp. n (%) 2 (7.69%)	Serratia marcescens n (%) 1 (3.85%)
Amikacin	1 (14.29)	0 (0)	2 (66.67)	1 (50)	0 (0)
Ampicillin	4 (57.14)	1 (50)	2 (66.67)	1 (50)	1 (100)
Cefazolin	0 (0)	1 (50)	2 (66.67)	1 (50)	0 (0)
Cefepime	4 (57.14)	1 (50)	1 (33.33)	1 (50)	0 (0)
Ceftriaxone	1 (14.29)	1 (50)	2 (66.67)	1 (50)	0 (0)
Ciprofloxacin	4 (57.14)	1 (50)	2 (66.67)	1 (50)	0 (0)
Cefoxitin	1 (14.29)	0 (0)	0 (0)	1 (50)	1 (100)
Netilmicin	1 (14.29)	1 (50)	2 (66.67)	1 (50)	0 (0)
Amoxicillin-clavulanate	2 (28.57)	1 (50)	2 (66.67)	1 (50)	0 (0)
Aztreonam	4 (57.14)	0 (0)	1 (33.33)	1 (50)	0 (0)
Levofloxacin	1 (14.29)	0 (0)	0 (0)	0 (0)	0 (0)
Ceftazidime	4 (57.14)	1 (50)	2 (66.67)	1 (50)	0 (0)
Cefuroxime	1 (14.29)	1 (50)	0 (0)	1 (50)	1 (100)
Cefixime	1 (14.29)	0 (0)	0 (0)	0 (0)	0 (0)
Cefuroxime axetil	1 (14.29)	1 (50)	0 (0)	1 (50)	1 (100)
Piperacillin/tazobactam	3 (42.86)	1 (50)	1 (33.33)	1 (50)	0 (0)
Piperacillin	2 (28.57)	1 (50)	1 (33.33)	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole	7 (100)	0 (0)	1 (33.33)	1 (50)	0 (0)
Gentamicin	1 (14.29)	1 (50)	2 (66.67)	1 (50)	0 (0)
Ertapenem	2 (28.57)	1 (50)	0 (0)	1 (50)	0 (0)
Imipenem	1 (14.29)	0 (0)	1 (33.33)	0 (0)	0 (0)
Meropenem	1 (14.29)	0 (0)	0 (0)	1 (50)	0 (0)
Tigecycline	0 (0)	0 (0)	1 (33.33)	1 (50)	0 (0)
Tobramycin	1 (14.29)	0 (0)	0 (0)	0 (0)	0 (0)
Colistin	0 (0)	0 (0)	1 (33.33)	0 (0)	0 (0)

Table 2. Resistance rates to various antibiotics in gram (+) bacteria isolated from wound cultures

Antibiotic	Enterococcus faecalis n (%) 2 (7.69%)	Staphylococcus aureus n (%) 2 (7.69%)	Coagulase-negative Staphylococcus spp. n (%) 2 (7.69%)
Amikacin	0 (0)	N	N
Ampicillin	2 (100)	1 (50)	2 (100)
Cefepime	2 (100)	N	N
Ceftriaxone	2 (100)	N	N
Ciprofloxacin	2 (100)	1 (50)	2 (100)
Netilmicin	1 (50)	N	N
Amoxicillin-clavulanate	2 (100)	N	N
Aztreonam	1 (50)	N	N
Ceftazidime	1 (50)	N	N
Piperacillin/tazobactam	1 (50)	N	N
Penicillin G	0 (0)	1 (50)	N
Gentamicin	1 (50)	1 (50)	2 (100)
Ertapenem	0 (0)	N	N
Imipenem	0 (0)	N	N
Meropenem	0 (0)	N	N
Tigecycline	1 (50)	0 (0)	0 (0)

(N: Not tested.)

Table 3. Resistance rates to various antibiotics in gram (-) bacteria isolated from wound cultures

Antibiotic	Burkholderia cepacia n (%) 1 (3.85%)	Citrobacter freundii n (%) 1 (3.85%)
Amikacin	0 (0)	0 (0)
Ampicillin	0 (0)	0 (0)
Cefepime	0 (0)	0 (0)
Ceftriaxone	0 (0)	1 (100)
Ciprofloxacin	0 (0)	1 (100)
Netilmicin	0 (0)	0 (0)
Amoxicillin-clavulanate	0 (0)	0 (0)
Aztreonam	0 (0)	0 (0)
Ceftazidime	0 (0)	0 (0)
Piperacillin/tazobactam	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole	1 (100)	0 (0)
Gentamicin	0 (0)	0 (0)
Ertapenem	0 (0)	0 (0)
Imipenem	0 (0)	0 (0)
Meropenem	0 (0)	0 (0)
Tigecycline	0 (0)	0 (0)

Discussion

Diagnosis of pressure ulcer infections is complex and should be evaluated together with clinical symptoms, condition of scar tissue and surrounding, markers of inflammation, microbiological examination of targeted specimens, and tissue biopsies. Changes in pain quality, crepitation, increased exudate, pus, serous exudate with inflammation, increased erythema, bad smell, edema, and local temperature increase in surrounding tissues suggest infection. Tissue biopsy culture is the gold-standard method, but it is an invasive method. It requires intensive work and experience, it is not applied due to difficulties in clinical use, cost, and the need for experienced personnel. Instead, local wound swab cultures, which are a more non-invasive method, are preferred as a more usable method when evaluated together with local signs of infection. Swab cultures may also be insufficient to distinguish between occasional colonization and active infection. Therefore, evaluation of the infection together with clinical findings such as tenderness, erythema, temperature increase, and discharge, which are local findings of infection, gives more accurate results.[7]

In the literature, it has been reported that gram (-) bacteria take the first place among bacteria isolated from pressure ulcer infections, and these species are mostly isolated from pressure ulcer infections in patients with spinal cord injury. This supports the view that the infection occurs due to colonization of the skin with the urogenital and digestive flora.[9]

Ozturk et al. determined that enteric bacteria reproduced most frequently (43.40%) in pressure ulcer infections, *Escherichia coli* in enteric bacteria, and *Pseudomonas aeruginosa* in non-fermentative bacteria grew more frequent.[2] These results are in agreement with the results of the present study.

In our study, when the 26 causative microorganisms we detected in the wound culture; it was observed that *Escherichia coli* (n=9, 34.62%) and *Proteus mirabilis* (n=3, 11.53%) grew more frequently in enteric bacteria and *Pseudomonas aeruginosa* (n=3, 11.53%) in non-fermentative bacteria.

Dundar et al. in the swab samples taken from the pressure sores of 68 patients who received home care service, determined, that 48% of the bacteria reproduced as non-fermentative, 38% as enteric, and 14% as gram-positive bacteria. The most common bacteria are *Pseudomonas aeruginosa* (n=23), *Proteus spp*. (n=20) and *Acinetobacter baumannii* (n=18).[10]

In a study conducted on 55 patients with spinal cord lesions, *Staphylococcus aureus* and *Escherichia coli* were found to be the most common pathogens.[7] Heym et al. in their study, isolated *Enterobacter* 29%, *Staphylococcus spp*. 28% and *Enterococcus faecalis* 16% in the deep tissue biopsy cultures of 101 patients with spinal cord injury and decubitus ulcer infection.[11]

Kilic et al. found pressure ulcer infection was in 13.80% of 2893 patients hospitalized in the rehabilitation center. They isolated *Staphylococcus aureus, Acinetobacter spp., Escherichia coli, and Pseudomonas spp*. as causative agents in order of frequency.[12]

Altan et al., in their study found that the five most commonly isolated microorganisms in wound cultures were *Acinetobacter spp*. (28%), *Pseudomonas spp*. (16.60%), *Candida spp*. (11.40%), *Escherichia coli* (9.30%), and *Enterococcus spp*.[13]

In many clinical studies, the most frequently isolated agents from wound cultures have been reported as *Coagulase-negative staphylococci*, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterococcus spp*.[1, 6] Again, it has been reported that *Coagulase-negative staphylococci* are the most common contaminating bacterium in wound cultures and they show a growth of over 20% in wound cultures.[7] In our study, *Escherichia coli*, 34.62%, *Pseudomonas aeruginosa* 11.53%, *Staphylococcus aureus*, and *Coagulase-negative Staphylococcus spp*. 7.70% were isolated, respectively.

In studies, the effectiveness of the Colistin agent in the treatment of non-fermentative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* has been accepted.[2, 14] Ozturk et al. did not detect Colistin resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumann*ii isolates.[2] Similarly, Colistin resistance was not detected in *Pseudomonas aeruginosa* isolates in our study.

Durmaz et al. in the study in which they evaluated a total of 137 *Pseudomonas aeruginosa* strains, the resistance rates in isolates were found against antibiotics; Amikacin 43%, Gentamicin 38%, Ceftazidime 42%, Cefepime 40%, Cefoperazone-sulbactam 44%, Ciprofloxacin 47%, Levofloxacin 47%, Piperacillin/tazobactam 71%, Imipenem 37%, Meropenem 37%, Colistin 7%, Netilmicin 28%, and Colistin has been reported as the most effective antimicrobial agent against *Pseudomonas aeruginosa strains*.[15]

Aktepe et al. reported that the most sensitive antibiotics in *Pseudomonas aeruginosa* strains were Amikacin and Tobramycin, and resistance was detected at a rate of 4.90%. Meropenem and Imipenem resistance were found to be 26% and 26.80%, respectively. While Ciprofloxacin resistance was determined as 33.30%, this rate was 70% and above for Cephalosporins. It has been stated that the resistance rates in Intensive Care Unit-derived strains increased to 46.40% for Carbapenems and 47.50% for Ciprofloxacin.[16] These results aren't in agreement with the results of the present study. In our study, no resistance was found to any of the antibiotics we used in *Pseudomonas aeruginosa* isolates. This may be since the patient population served by our hospital is not very large and it shows the importance of each hospital determining its resistance profiles.

Parlak et al. reported that they detected ESBL (extended-spectrum beta-lactamases) positivity at a rate of 48% in *Escherichia coli* strains and 67% in *Klebsiella pneumoniae* strains. It was stated that the most effective

antibiotics against isolated strains were the Carbapenem group, followed by Amikacin, Nitrofurantoin, and Cefoxitin in *Escherichia coli* strains, and Amikacin, Cefoxitin, and Ciprofloxacin in *Klebsiella pneumoniae* strains.[17] In our study, ESBL positivity was found 22.20% in *Escherichia coli* strains (2 of 9 strains), ESBL positivity was not found in *Klebsiella* strains. Especially *Escherichia coli* and *Klebsiella* strains showed 50% resistance to Ampicillin, Cefazolin, Cefepime, Ceftriaxone, and Ciprofloxacin.

Scivoletto et al. and Gürçay et al. found high WBC, CRP, and ESR in patients with pressure ulcer infection. [18, 19] In our study, we found that WBC, CRP, and ESR increased in patients with reproductive decubitus ulcer infection, which was consistent with the studies in the literature.

Pressure ulcer infections continue to be a health problem that reduces the quality of life in long-term care patients, despite the development of prevention and treatment methods.[20] It is natural to have superficial bacterial contamination in pressure ulcer infections. Patients with pressure ulcer infection often have an accompanying urinary system or respiratory tract infection, and if not treated, serious problems such as bacteremia and sepsis may occur, which can be fatal.[21]

When local swab cultures are evaluated together with the clinical findings of the infection in the diagnosis of pressure ulcer infection, it is still the most commonly used method. In pressure ulcer infections, aseptic conditions must be followed to prevent infection by colonized bacteria. When an infection develops, a culture should be taken, and antibiotic susceptibility testing should be performed.

In the treatment of infection, starting antibiotic therapy at the appropriate time and choosing the right antibiotic is one of the most important factors that determine the success of treatment. The distribution of infectious agents and the distribution of antibiotic resistance vary periodically and from clinic to clinic.

In our study, following the literature; in the diagnosis of pressure ulcer infection, local wound swab cultures should be evaluated together with the clinical findings of the infection, and we found that initiation of antibiotic therapy at the appropriate time and choosing the right antibiotic in the treatment of infection is one of the most important factors that determine the success of treatment.

Conclusions

As a result, determining the agent distributions and antibiotic resistance in patients with pressure ulcer infection, which we followed in our clinic, with the findings we obtained from our study, guided us in the treatment of our patients and the rational use of antibiotics. Since our study is single-centered, it provides limited information. It is aimed that our future studies will be carried out in a multi-center manner with clinics that periodically perform their surveillance work.

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Ethical Considerations: Ethics committee approval was obtained from Siirt University Non-Interventional Clinical Research Ethics Committee under the Declaration of Helsinki (Date: 06.12.2021, No: 1779, Decision No: 2021/11.01.04)

Conflict of Interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Review

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A SYSTEMATIC REVIEW OF EFFECTIVE BIOAGENT IN CHRONIC WOUNDS: THE MAGGOT BIOTHERAPY PYRAMID

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Abstract

Wound assessment is important in monitoring the effectiveness of treatment in chronic wounds. Therefore, a holistic approach is needed when evaluating effective wound treatment. Most chronic wounds require complementary treatment approaches and conventional treatments in modern medicine. This research was carried out by compiling studies on the past, present, and future of maggot (medical larvae) that cure nonhealing/hard-to-heal wounds; therapeutic larva types, therapy method, healing mechanism, wound healing effect with clinical studies, different usage areas and biological activities of the larvae and the metabolite components in the secretions that provide these activities. In conclusion, medical larvae applied with traditional and complementary medicine techniques to treat nonhealing, difficult-to-heal wounds have a wound-healing effect. However, more research is needed to identify the metabolite components in their secretions that provide their mechanism of action and biological activities.

Keywords: Maggot, Lucilia sericata, chronic wound, wound debridement treatment, bioactive.

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Introduction

The skin is a defense barrier of the organism, protecting the organism against external effects such as environmental microorganisms, chemicals, radiation, and allergens. Some physical and biomechanical factors may adversely affect skin integrity. The inability to prevent passage and accommodation of pathogenic microorganisms biofilm development disrupts the anatomical, functional tissue integrity, which is the main reason for wound formation and wound chronicity. Chronic wounds are those whose healing is delayed due to some underlying problems and do not show normal healing stages. If a wound does not heal within 4-6 weeks despite treatment, it is a chronic wound type. Infections related to chronic wounds are difficult to control. The most important of these difficulties that may be encountered is a risk of disease by tissue damage and tissue death and containing a high amount of bacteria. As a result of the inability to remove excess exudate, wound healing is delayed, so it is necessary to prepare the wound bed and clean death tissue in the wound to ensure wound management. This important stage is possible with the debridement technique, which reduces the risk of infection and speeds up wound healing to remove the dead tissue.[1] Maggot Debridement Therapy (MDT) is the most effective and natural way of debridement performed in necrotic tissue by mechanical and biochemical means. In this method, Maggot therapy applications, ulcerative lesions, burns, various types of malignant tumors, abscesses and osteomyelitis, and traumatic wounds also result in difficult wounds to heal. Low cost, fast, effective, easy, and practical application; It makes the larval treatment more preferred.[2]

Such names as 'Maggot Therapy,' 'Larval Therapy,' 'Therapeutic Myiasis,' 'Biosurgical Debridement,' and 'Biosurgery' are also given to Maggot Debridement Therapy (MDT). Is known that in the 1500s, Maya tribal natives wrapped the infected wound by drying the blood of cattle in the sun, and with this practice, the larvae entered the dead tissues and treated them by feeding on these necrotized tissues, ensuring that the wound was healed within a few days. There is information that a native tribe in Australia cleaned wounds with larvae and continued to relay for generations.[2]According to the first written documents on the treatment of larvae, the French physician Ambroise Pare is cited pioneer in battlefield medicine, especially in the treatment of wounds.[3] In the 1800s, during the civil war, he served as an officer of the surgeon Baron D.J.Larrey used the larvae in wound treatments of Napoleon's used soldiers. They stayed on the battlefield and observed that the larvae attacked only necrotic tissue and accelerated the healing of an infected wound.[4] Dr. W.S Baer conducted the first scientific studies on the clinical application of larvae.[4] After treating two soldiers in the First World War, Baer began working with extensive research on flies. Baer reported that during the 1920s and 1930s, more than 90 patients suffering from osteomyelitis and chronic leg ulcers were treated with the use of larvae. In the 1940s, it declined with the widespread use of antibiotics such as penicillin and sulfamide. At the end of the 1980s, it began to receive attention again due to a rise in antimicrobial resistance rates.[5] Currently, "Maggot Treatment" is accepted worldwide and approved by the national health authorities, and the practice methods are developing daily. At the beginning of the 1990s, more than 8,000 treatments took place in 600 centers in

five years in England alone. Again in the 1990s in the United States and around the world since 1995, Canada, Australia, England, Germany, Switzerland, Sweden, Finland, France, Austria, Denmark, Ukraine, Netherlands, Egypt, Israel, Thailand, and nonrecyclable stubborn larval therapy in the treatment of wounds or injured patients by applying positive results have been achieved.[6] Maggot Therapy in Turkey has been used at the Gulhane Military Medical Academy since 2002.[7] Since 2008; it has been involved in Istanbul University Cerrahpasa Faculty of Medicine, Department of Medical Microbiology.[8] T.R. With the efforts of the Ministry of Health, the 'Regulation on Traditional and Complementary Medicine Practices' was published in the Official Gazette dated 27.10.2014 and numbered 29158, and standards were established for Maggot (Larvae) practices.. MDT is defined as the use of sterilized larvae of the *Lucilia (Phoenicia) sericata* fly, species of fly belonging to the Insecta class, the Diptera order, the Cyclorrhapha suborder, the family Calliphoridae, the Lucilia lineage, in the treatment of infected open wounds. These larvae have the property of not damaging the dermis and subcutaneous tissue of the skin. The elimination products of *Lucilia sericata* larvae are a source of antimicrobial, antibacterial, antibiofilm, and other biological activity in the wound. The purpose of our study; To recognize wound healing maggots, develop maggot therapy, present research on the forms and methods of treatment used today, and emphasize their bioactivity with a multidisciplinary approach.

Materials and Methods

This study is a systematic review. The conditions that should be included in the writing of the Systematic Review research report for the research and the preparation of the study report are based on the relevant checklist protocol (PRISMA).[9] Firstly, Maggot Therapy is used to comprehensively analyze the literature and evidence on the potential activities of Maggot secretion and secretions;

- (‘maggot (OR) larvae’) (AND)
- (‘maggot (OR) larvae’) (AND) (’chronic wounds OR ‘wound debridement’)
- (‘*Lucilia sericata’*) (AND) (‘secretion’)
- (‘*Lucilia sericata* secretion’) (AND) (‘bioactivities)

A four-fold search algorithm was created using Turkish and English terms. PubMed, Science Direct, Scopus, Web of Science, and Cochrane databases were systematically interrogated. Four-layer search algorithms were determined as:

1. MDT development,
2. Therapeutic maggot species used in chronic wounds and chronic wounds,
3. MDT controlled clinical trials and MDT application,
4. According to the need for analysis, active metabolite components and bioactivity in larval secretion.

To better analyze and raise the development of treatment practices with unknowns about Maggot in the process from the history of Maggot therapy to the present, information sources works of literature that have reached twenty years ago to the present day were systematically evaluated. As summarised in Figure 1, priority in the relevant literature; titles and abstracts were then read in full text. A thorough evaluation was carried out by extracting copies of the same articles obtained from unrelated literature and databases.[10] The scale score was determined as 1-9 in full-text comparative randomized control articles. The methodological quality of the research included in this systematic review was evaluated using checklists published by CASP, Critical Appraisal Skills Programme. Accordingly, the quality assessment of randomized controlled trials was carried out with 9-item[11] checklists. Each item included in these lists is evaluated as "yes, no, indefinite, and not applicable." The status determined for each study is given in Table 2. The reference list of included studies was reviewed to obtain related studies that would contribute.

Selection Criteria and Selection of Research

The studies that are suitable for this systematic review have been selected according to the following inclusion criteria;

1. **Studying group:** Chronic wound treatment, biological activity in wound healing
2. **Intervention:** Maggot (larval) treatment using the larvae of *Lucilia sericata*
3. **Comparison:** Not using *Lucilia sericata* larvae, hydrogel treatment, traditional treatment
4. **Results:** Wound healing by achieving complete debridement in chronic wounds with maggot therapy with controlled clinical trials, identification of larval secretory metabolite components effective in wound healing
5. **Study design:** Descriptive research, qualitative research, randomized controlled or comparative clinical studies

The exclusion criteria in the study are the method of non-specific, full-text inaccessible, repeated studies of non-experimental studies published in different languages is the work of Turkish and English languages. This literature analysis describes the many functional features of the larvae used in MDT, which emerge from different disciplines, sources of information, and research findings and reveals their qualified nature.

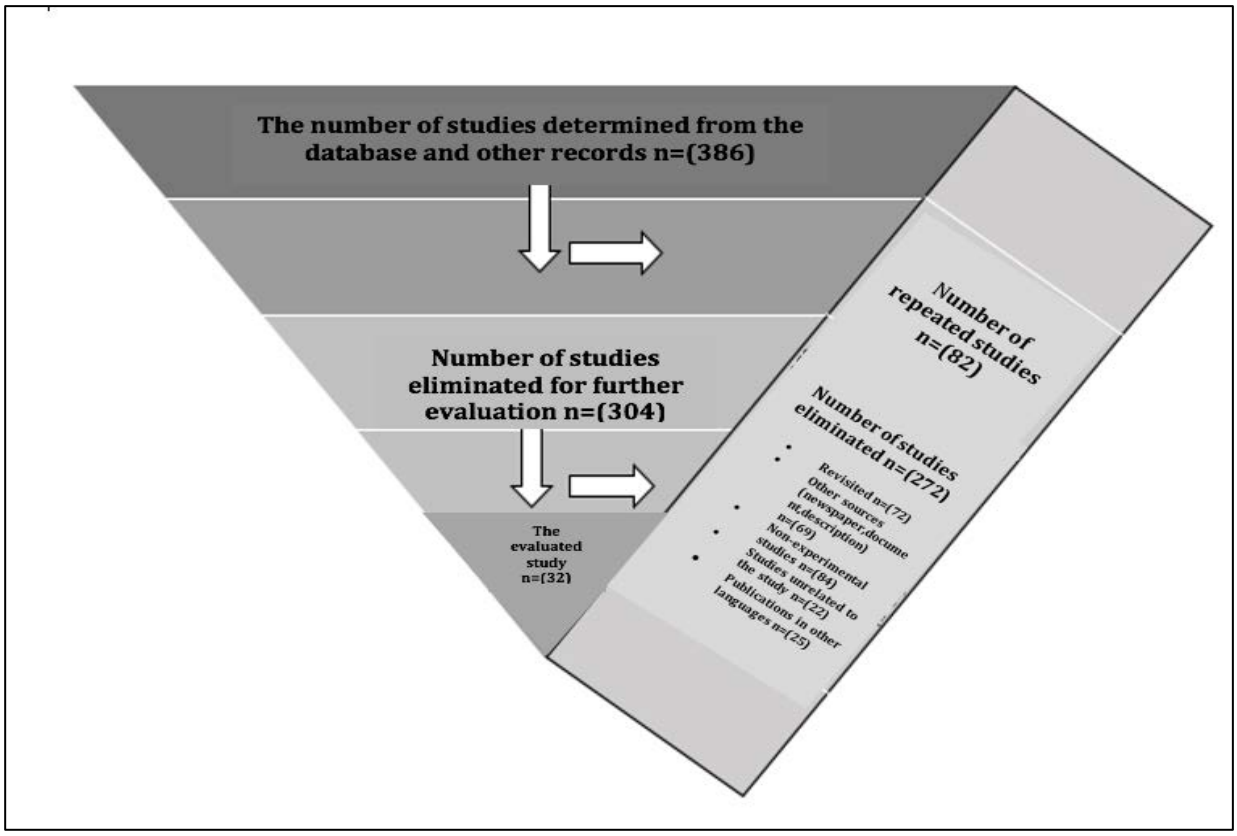

Figure 1: Selected Working Procedure Pyramid

Discussion

Larvae Used in Maggot Therapy

The fly larvae used for Maggot Therapy are usually found in the family Calliphoridae. The main thing in selecting larvae is the ability to feed only from dead tissues without damaging living tissues.[12] It has been noted that wound treatment can also be used for nonhealing skin infections, including eight species of obligate non-parasites from the family Calliphoridae as a worldwide treatment.[13] (Table-1). However, the other six species were not preferred in medical practice except *Lucilia sericata* (widespread preference) and *Lucilia cuprina* (limited choice). It has been observed that different types of larvae can change the effectiveness and reliability of treatment.[13]

Table 1. Calliphoridae species used in larval wound treatment

FAMILY	SPECIES
Calliphoridae	*Calliphora vicina*
	Chrysomya rufifacies
	Lucilia caesar
	Lucilia cuprina
	Lucilia illustris
	Lucilia sericata
	Phormium regina
	Protophormia terraenovae

Lucilia (Fly: Calliphoridae) members are in the group of organisms that are not parasitic in the adult period but cause infection (myiasis) by settling in human and animal tissues during the larval period.[14] In medical entomology, they are included in the group called myiasis flies.[15] Myiasis flies are examined in two groups obligate parasites and non-obligate parasites. The species *Lucilia sericata* is included in the mandatory non-parasitic group.[15] *Lucilia sericata*, first described by Meigen in 1826, was named the greenfly because of its metallic green color. *L.sericata* is preferred because it feeds on superficial necrophage in living tissues. It has been observed that *L.sericata* larvae starve to death when released into living healthy granulation tissue. It has been reported that *L. sericata* is a suitable species for Maggot Therapy due to their necrophage feeding.[16]

Members of this family are remarkable for exhibiting community behavior during reproduction. When the first female begins lay eggs, other females who see this also tend to ovulate, and different species of this family lay eggs on each other's eggs. They are holometabolous and undergo a complete metamorphosis with one egg, three larvae, one prepupa, one pupa, and an adult stage. Figure 2 gives an overview of a related simplified larval developmental biology.[17] Accordingly, the life cycle of *L.sericata* lasts about 16 days at 25ºC. The larvae of Calliphoridae do not resemble the adult at all. They differ in their structure, biology, and ecology.[18]

The maximum amount of nutrients that the larvae need is the third period. Due to the unbelievable feeding speed of the larvae, their growth is also proportionally quite fast. They change skin twice during development; the elasticity of the upper layer of the skin allows them to grow faster.[19] Towards the end of the third period, the larvae enter the navigation phase (post-feeding). The larva finishes feeding and moves away from the nutrient source, looking for a suitable place to pupate. Larva completely empties the digestive tract to pupate, and contractions of longitudinal muscles are observed. There is a rapid water loss from the upper skin until the pupation contraction is over. The adult emerges from the pupal sheath upwards with the help of its feet; when it first appears, it appears as a pupa, but in about one day, it regains its normal appearance and begins to fly.[19]

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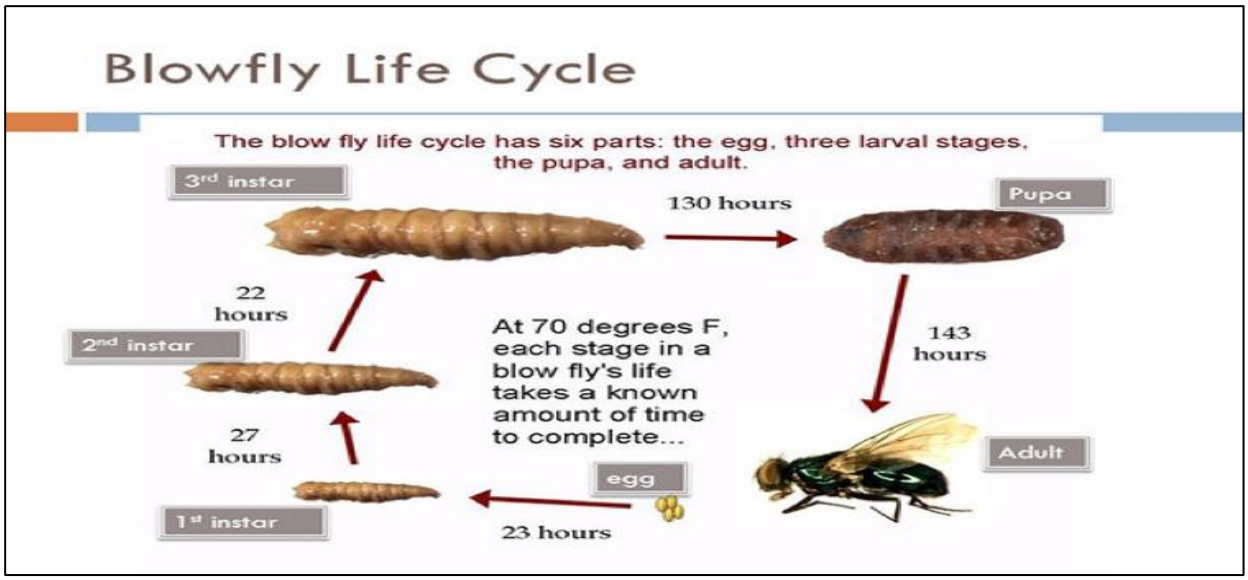

Figure 2. *"Lucilia sericata"* Blowfly Lifecycle
(https://www.nlm.nih.gov/ visibleproofs/galleries/technologies/blowfly.html)

The Mechanism of Action of Maggot Therapy

In deep wounds, blood flow slows down due to insufficient tissue oxygenation. It becomes difficult for antibiotics to enter the area and suppressive immune mediators to work; recovery is delayed. MDT method is preferred when there are situations where a healthy physical, biochemical macro, and microenvironment can not build with medical treatment methods, and progressive tissue loss can not be prevented.[20] The most powerful aspect of Maggot Treatment is the debridement of the wound. Others are the formation of tissue by defecation and granulation. Debridement is removing necrotic tissue and cellular tissue from the wound bed.[21] Full larval debridement requires an average of 2-3 larval cycles lasting 3-5 days.[15] Debridement occurs through two mechanisms. First is mechanical; the larvae themselves break down substrates into small particles with the help of hooks located in the mouth, then they liquefy and lubricate necrotic tissues in the wound with salivary enzyme secretions.[22] Second mechanism is more detailed and complex. In this complex mechanism, various chemical enzymes and substances are secreted. The pH values of larval secretions in the range of 8.6-8.7 provide an available environment for proteolytic enzyme activity such as trypsin and chymotrypsin. In disinfection, larvae secrete antibacterial substances from the intestinal, hemolymph, and salivary glands on the wound and ensure the destruction of bacteria. Another factor that plays an important role in the breakdown of bacteria is the changed pH value of the digestive tract of larvae. Bacteria can also be destroyed in the wound since antibacterial substances effective in the intestines and proteolytic enzymes such as protease lipase collagenase in the digestive system are released during nutrition.[23] With ammonia and calcium carbonate

metabolite components in secretions, it is removed from the acidic environment of the wound pH, and wound alkalinization is carried out. However, therapeutic substances such as allantoin and urease of larvae are supporters that will ensure the integrity of the disinfection stage.[23] The larvae act as a natural mechanical stimulant as they constantly move on the wound. Cytokines such as interferon-γ and interleukin-10 with secretions such as ammonium, ammonium bicarbonate, urea, and allantoin accelerate granulation tissue formation. They stimulate wound healing by secreting growth-promoting factors.[24]

Application of Maggot Therapy

Clinical applications of Maggot Therapy (MT) are usually performed by cage dressing or free larval application. (Figure-3a)[25] The larvae used in MT are disinfected and sterilized green bottle fly *Lucilia sericata* feed on necrotized cells rather than healthy tissues. Sterile larvae left on the wound can circulate freely on the wound bed. For this purpose, before applying the larvae, the wound should be thoroughly washed, and all possible tissue residues that can be removed from the residues of wound care products should be removed. The wound and its surroundings should be carefully cleaned. To prevent the escape of larvae from the wound and protect against secretory digestive enzymes., the upper part of the wound is covered with hydrocolloid dressings surrounding the wound or a sterile piece of thin nylon tulle that acts as a net fixed with non-allergic transparent adhesives. The wound surface is kept in the dressing at a density of 5-10 larvae/cm2 for 1-3 days during the treatment. In recent years, maggot Therapy practices have also been carried out using "Biobag." (Figure-3b)[25] In this method, the maggots are placed in pouches with foam particles inside, consisting of two pieces of tulle made of a special material (polyvinyl alcohol-hydro-sponge) with a thickness of 0.5 mm, similar to tea bags. The mouth of the bag is attached. Gauze or bandage keeps larvae in pouches fixed in the wound.[25,4]

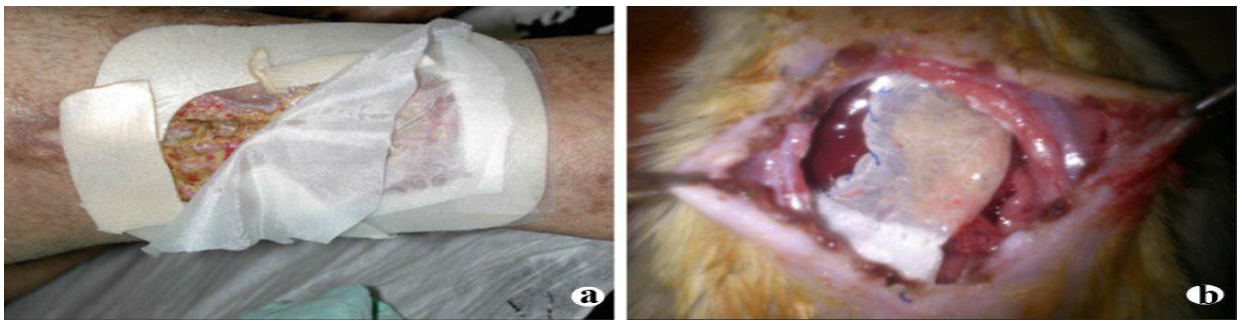

Figure 3. Method of Use of Larvae in MDT a) Cage-Shaped Dressing b) Biobag

In the bio-bag method, mechanical irritation is prevented so larvae cannot act directly on the wound. Accordingly, the decrease of pain sensation, inability of larvae to escape through the sac, and provision of a hygienic environment on the wound bed are beneficial aspects. On the contrary, the disadvantage of this method is that due to the restriction of movement of larvae on the wound, cleaning off dead tissues can not be done adequately. One of the factors that should be considered when applying Maggot Therapy is the number of larvae to be applied to the wound and how long it should be applied.[26] The number of larvae used in the treatment varies according to necrotic tissue, wound width, depth, and type of infecting bacteria. In the medical sector, commercial companies usually sell tubes containing about 300 larvae that have been sterilized. Less than 200 larvae should be used during treatment in low wound depth, while up to 1000 larvae can be applied to wounds with huge depth.[4] Control management is important in treatment. After each application, a sterile physiologic solution should be squeezed out, and larvae should be collected from the wound with pliers and removed. The larvae develop most rapidly between the sixteenth and fortieth hours after decanting from the egg stage. The nutritional needs during this period are about 20-25 mg. Applying 16-hour larvae kept in their substrate to the wound is recommended for effective treatment. (Figure-4) [4] In the application of wound care, it is practical to leave young larvae on the wound for 2-3 days; in application with large maggots, both faster and more effective treatment is performed.

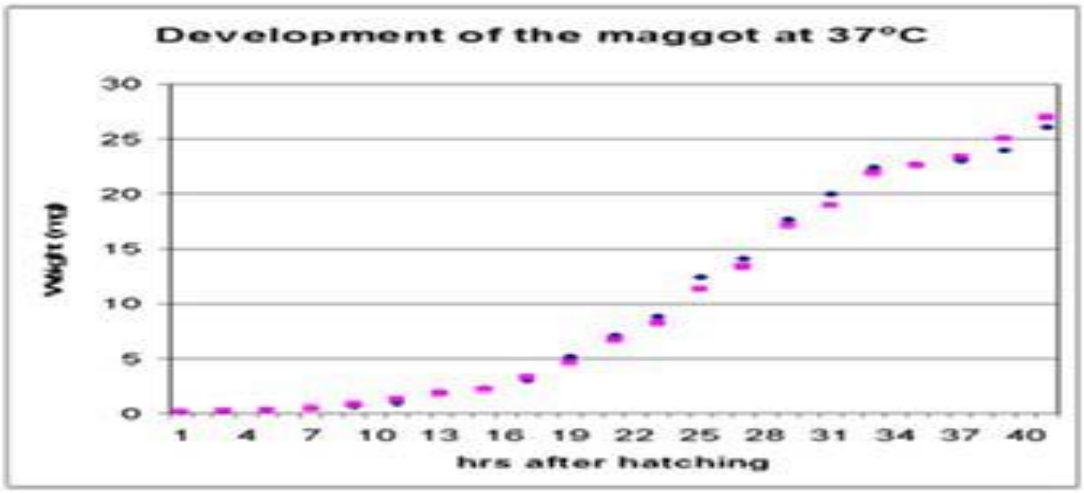

Figure 4. The change in the average body weight of maggots after their first hatching at 37 °C

The most effective function of Maggot Therapy in wound healing is debridement. This function is performed by maggots entering every place of necrotic tissue and separating living tissue from tissue destruction sites (Figure-5).[27] Wound monitoring is important for rapid progression of treatment; during control, the condition

of the wound, necrosis, drainage, inflammatory discharge, bad smell, and the bleeding should be monitored and recorded if the patient has pain.[4]

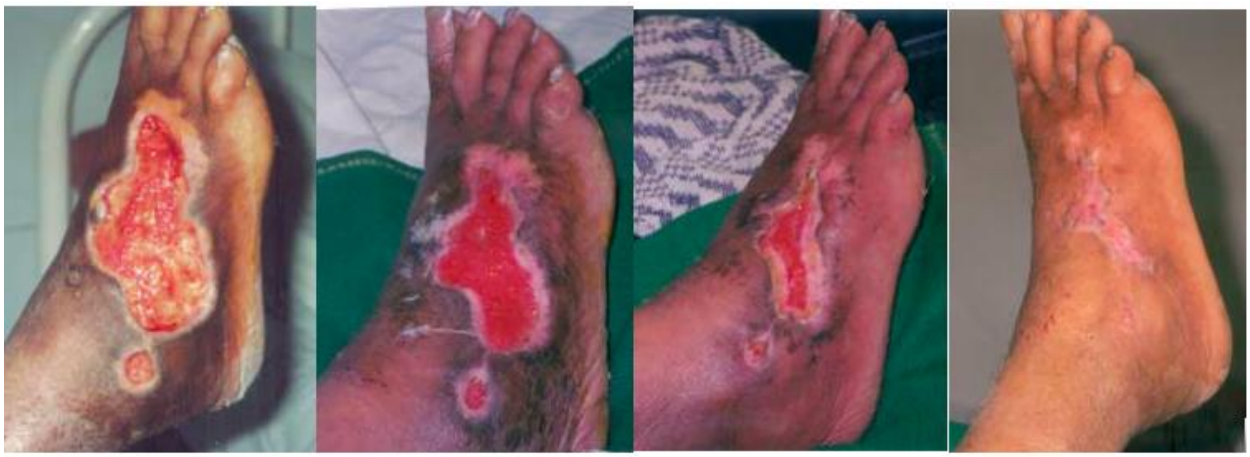

Figure 5. Two diabetic foot ulcers: one before MDT (a) and two after (b), four (c) and eight (d) weeks of MDT

Maggot Therapy can be used to treat ulcers due to conditions such as wounds that do not heal due to trauma, burns, bone marrow inflammation, mastoiditis, Burger's disease, necrotic tumors, and crusted or not completely healed wounds.[28] Psychological and aesthetic anxiety in patients is more prominent than possible surgical side effects. Pain is the most common complaint reported by 6-40% of patients during treatment. In Maggot Therapy, pain is related to the length of stay of the dressing on the wound. As this period increases, pain increases in sensation. The pain, which is mild when applied for the first time, increases gradually due to proteolytic enzymes secreted by larvae stimulating the nerve endings within 24 hours, growth, hardening of the skin, and larvae trying to escape from the wound within 48-72 hours.[29] It raises the patient's body temperature; if ammonium salts produced by larvae are not sufficiently absorbed by dressing, it increases its body temperature. The danger of septicemia can also arise if non-sterile larvae are used in therapy.[30] Systemic antibiotics are applied to prevent septicemia. Nevertheless, this can lead to blockage of the gill slits, through which the larvae supply oxygen, so antibiotics should not be used in ointment form.[28]

Clinical Studies

The first prospective randomized controlled trial was presented by Ronald Sherman, a key figure in the Maggot Therapy revival. In this study, the effect of Maggot Therapy on the treatment of pressure ulcers in patients with spinal cord injuries was investigated, and wound bed surface area and the healing speed and effect rates were observed. The result of treatment is that the larvae used do not cause any side effects in most necrotic wounds; they are debrided faster than all other medical treatment methods.[31] In a different controlled study conducted by Sherman's group, it is the treatment of chronic bilateral plantar foot ulcers that do not respond to

conventional treatments applied to all types but have positive results after using Maggot Therapy.[32] In Maggot treatment applied to 43 patients in pressure ulcer treatment in 2002; it was observed that wounds were completely healed with full debridement compared to patients who received conventional medicine; in another randomized study conducted in 2003 in which 14 patients with venous leg ulcers were treated with Maggot Therapy or hydrogel were followed up; it was stated that after only one application, larvae completely cleared ulcer of necrotic cells and provided effective debridement in all patients.[33-34]

Markevich and his team conducted another study of 140 patients with chronic wounds, diabetic foot neuropathy, medical treatment, and worm. Treatment had the wound and was followed ten days after treatment with maggot wound closure is found to be twice as effective.[35] In a randomized controlled study by Dumville and friends, which examined the comparison of hydrogel therapy and Maggot application, although MDT provided faster debridement than hydrogel therapy in neurotic leg ulcer cases, no difference was observed in bacterial load and healing rates between the two patient groups.[36] In different prospective controlled studies on foot ulcer disease in 2012, 723 patients who underwent Maggot Therapy were treated as outpatients. In 357 (82.1%) hospitalized patients, maggot treatment was performed with frequent controls, and complete debridement was achieved. In addition to supporting and creating evidence, these studies; reported that complex diabetic wounds were treated with Maggot Therapy in 17 of 23 patients, including last-stage renal disease, diabetes, heart disease, and identical conditions.[37] In Table 2, clinical studies in which Maggot Therapy was effective in the debridement, cleaning, and removal of infection of various wounds that did not heal with conventional treatments are discussed.

Opletalová and friends[38] statistically significant faster debridement of exfoliation with MT treatment but reported that this was only in the first week of MT. Mudge and friends [39] demonstrated that MDT debrides the wound more quickly with the difference in the number of wounds completely debrided in the MT group than Hydrogel treatment. In another study organized by Wang and friends, a significant difference between the MDT and hydrogel group was not found in rates of MRSA decimation capacity and the bacterial load reduction in traditional treatment and MT treatment applied to diabetic foot and leg ulcer patients for 60 days.[40]

Different Uses of Larvae

In medicine, Maggot Therapy is mostly used to treat necrotic, suppurative, watery, gangrenous wounds that are difficult to heal. Chronically infected wounds such as ulcers, burns, and osteoarthritis, as seen in Figure-6.[41] In treatment, larvae are classically applied as a last resort, usually after long-term systemic antibiotic administration and if successful recovery has not been achieved despite medical intervention.

Table 2. The summary of the included clinical trials

Authors/Year	Type of wound	Number of participants	Intervention and Control	Follow	Result	Quality score
Sherman (2002)	Pressure ulcers	MDT: 43 Control :49	MDT and traditional treatment	17-19 weeks	Debridement, Granulation tissue, Complete recovery of surface area,	Yes 5/9 No 2/9
					Adverse impact	Indefinite 2/9
Sherman (2003)	Diabetic foot and leg ulcers	MDT: 14 Control: 14	MDT and traditional treatment	Eight weeks	Debridement, Granulation tissue, Complete recovery of surface area,	Yes 5/9 No 2/9
					Adverse impact	Indefinite 2/9
Dumville et al. (2009)	Venous or mixed ulcers	MDT:180 Control:87	MDT and hydrogel therapy	6-12 weeks	Complete debridement, The recovery period, Bacterial growth, Adverse events	Yes 7/9 No 2/9
Mudge et al. (2014)	Venous or complex leg ulcers	MDT:46 Control:42	MDT and hydrogel therapy	28-35 days	Debridement, Wound surface area Bacterial growth, Adverse events	Yes 8/9 No 1/9
Markevich et al. (2000)	Diabetic neuropathic foot	MDT:70 Control:70	MDT and traditional therapy	Ten days	Complete recovery and debridement	Yes 8/9 No 1/9
Opletalova et al. (2012)	Venous leg ulcers	MDT: 51 Control: 54	MDT and traditional therapy	30 days	Complete debridement, Bacterial growth, Recovery period, Adverse events	Yes 8/9 No 1/9
Wang et al. (2010)	Diabetic foot and leg ulcers	MDT: 53 Control:53	MDT and traditional therapy	60 days	Debridement of granulation tissue, Bacterial growth, Adverse events	Yes 7/9 No 2/9

CASP, Critical Appraisal Skills Programme. 1 Did the trial address a clearly focused issue? 2 Was the assignment of patients to treatments randomized? 3. Were all of the patients who entered the trial properly accounted for at its conclusion? 4 Were patients, health workers and study personnel 'blind' to treatment? 5 Were the groups similar at the start of the trial? 6 Aside from the experimental intervention, were the groups treated equally? 7 Can the results be applied to the local population or in your context? 8 Were all clinically important outcomes considered? 9 Are the benefits worth the harms and costs?

If we talk about the use of larval therapy in veterinary, it is not as widespread as its use in medicine, but it is used, albeit limited. Infected wounds of 2 dogs, four cats, one rabbit, and 13 horses, seven lames and six dying, were started to be treated with Maggot Therapy by American veterinarians; animals survived amputation and death except for only one horse. There were no complications other than pain during the treatment process. After the study, it was understood that Maggot Therapy is also effective and safe for some serious hoof and leg wounds in horses.[41]

The interesting aspect of therapeutic agent larvae is that it has an important place in forensic entomology. The science of "Forensic Entomology" or "Biocriminal Entomology" can be defined as information about insects' biology, behavior, adaptation abilities, and ecology in forensic research using their life cycles. Carrion-feeding

(necrophage) flies are mediators that play an important role in the decay phase of corpses. This connection between insects and carcasses and their use in criminal investigations is important in forensic entomology. Insects are living species that detect and reach corpses as soon as possible. They lay eggs or larvae on the corpse in openings such as the face, inside the mouth, between lips and teeth, eye cavity, ear hole, nostrils, and wound surface area. They can be fed more comfortably and protected easily.[42] *L. sericata* is typically known by studies as a fly with a bad smell that is released when a corpse decays and explodes after death. Hence, it is important species from a Forensic Entomological point of view. The time of death was estimated according to the larvae or eggs arriving at the corpse in a certain order, the order of arrival of insects left on the corpse and the lengths and numbers of larvae emerging from them, and the number of respiratory slits in the stigma by Post Mortem Interval (PMI) calculation. In the last years, Entomotoxicological analysis studies using larvae have had an important place in providing information about the time after death, especially larval stages of insects found at the crime scene, and providing important evidence about poisoning or drug consumption by the victim.[43]

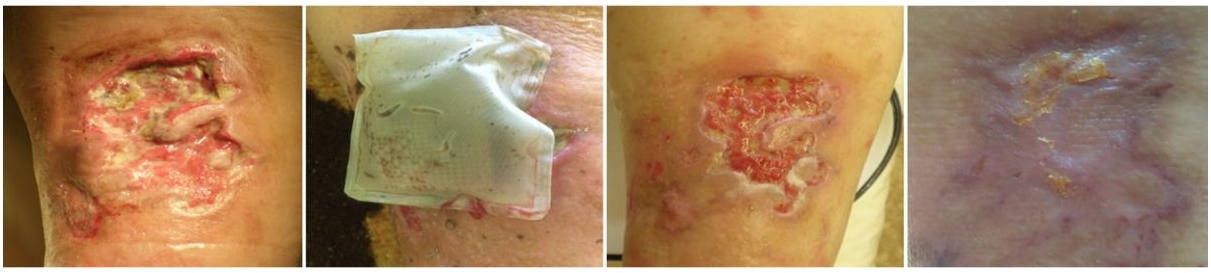

Figure 6. The successful outcome of the patient after treatment with *Lucilia sericata* larvae

Biological Activity of Larval Secretions

The secretion of *Lucilia sericata* larvae contains allantoin, cysteine, sulfhydryl radicals, glutathione, ammonia, calcium carbonate, and growth-stimulating factor. Additionally, they have many digestive enzymes (Table-3) while feeding on the wound.[44] With the latest in vitro studies, it has been determined that secretion (secretions) of larvae contain at least two substances with antibacterial properties. They are hydrophobic substances with a molecular weight of 3-10 kDa and hydrophilic substances of <1 kDa with peptide-like structures.[45]

Since the discovery of larvae, scientific studies in the process and today's larval development and the available function of larvae in the application of Maggot Therapy in wounds have been discussed in the simplified pyramid. The stage in each layer, specific to the potential impact of the larvae, is the hierarchy of the sublayers (Table-7). Regarding the activation step in effect, the fascinating power in the secretions obtained from the whole body fluid of the larvae is an antibacterial effect, which was first described in the 1930s. The mechanism of action is the ingestion of bacteria by larvae, the direct killing of bacteria in the digestive tract, and wound alkalinization. In addition, its debridement activities have also been reported to reduce bacterial load on the

wound with its numerous antibacterial effects. It has been noted that some substances in secretions of sterile *Lucilia sericata* larvae have a significant antimicrobial effect. Simmons was the first to perform an antibacterial analysis of larvae against various microorganisms (*Staphylococcus, Streptococcus, Proteus, Clostridium*). It was found that the secretory extract can kill bacteria in 5-10 minutes, and the bactericidal effect of the larvae was revealed.

Table 3. The secretory enzyme content and activities of *Lucilia sericata* larvae.

Enzymes	Ingredients	Specific activity (µ mol Min^{-1} Mg^{-1})	Km (MM)	Vmax (µ mol Min^{-1})	Molecular weight
General proteases	Kazin	0.688	-	-	-
Collagenase	Collagen	0	-	-	-
Trypsin	BApNA	0.010	0.2	0.5	26±2,9
Leucine aminopeptidase	LNA	0.043	0.1	2.7	280±37
Carboxypeptidase A	HPA	0.034	0.02	2.7	40±9.4
Carboxypeptidase B	HA	0.015	0.08	1.5	42±5.9
Elastase	Elastin Congo red	0.002	-	-	-
Carboxypeptidase B	Chymotrypsin	GPA	0	-	-

On the other hand, a study analyzed that 104 bacteriae were completely broken down; it has been attended the ability of larval secretions alone to accelerate wound healing. In addition to indicating the potential for lytic activity against *Pseudomonas and Candida*, it has also been reported that larvae completely degrade MRSA found in infected wounds and ulcers.[46] Since then, many findings in many studies have led to the need to investigate further antibacterial activity in larval secretions against both Grams (+) and Gram (-) bacteria.

When examined in terms of bioactivity of medical larvae, the main factors and mechanisms were systematized as in Table-5.[47] On the other hand, there is a strong activity of larval elimination products and body fluids against *the P.aeruginosa.* They showed various peptides and genetic rearrangements of *L.sericata*, especially against this species, and noted the synergistic effects of these peptides. It is highly effective against *Staphylococcus aureus* and *Escherichia coli* by applying various antibacterial activity analyses for the secretions obtained from sterile larvae.

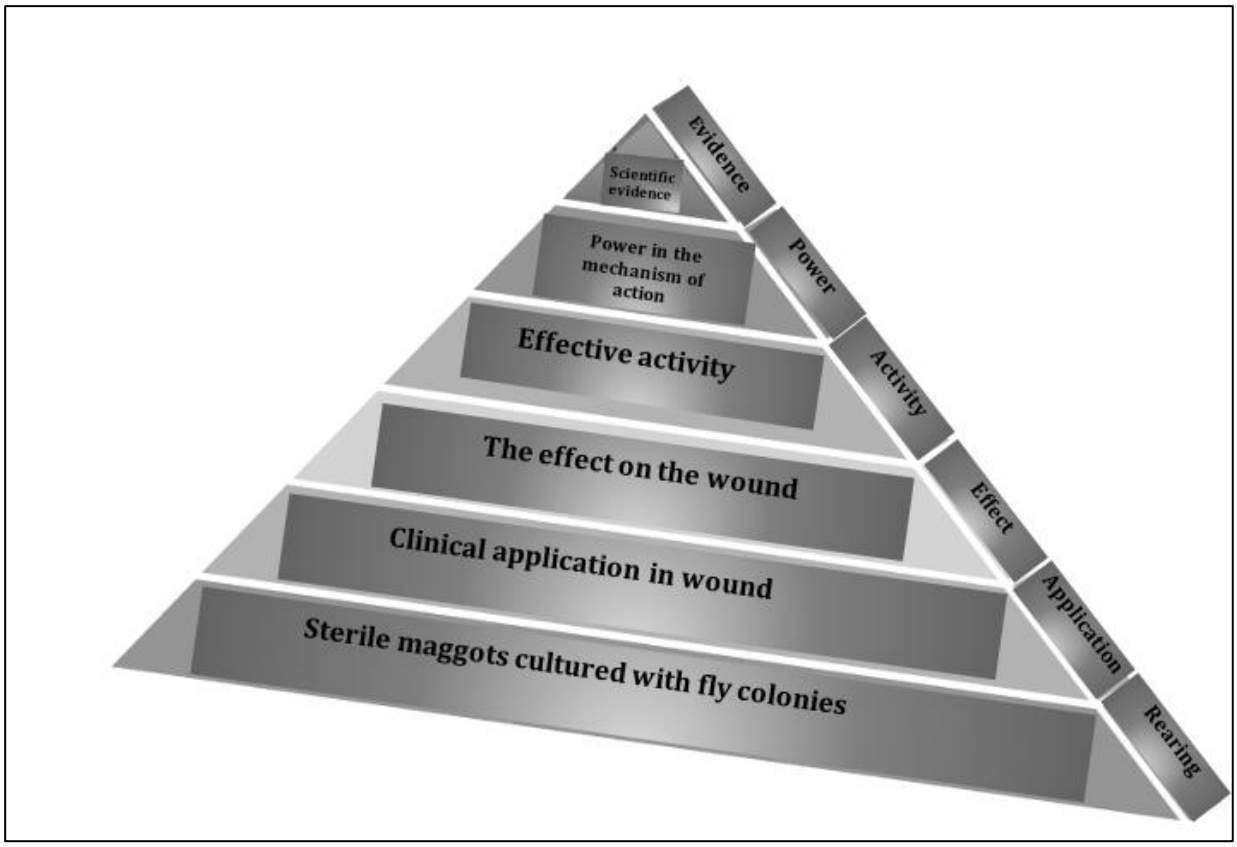

Figure 7. The Simplified Pyramid for Maggot Therapy

In contrast, its antibacterial effect is weak against *P. aeruginosa* and *S.aureus*.[48] Larval secretions are mediated by at least two different molecules, chymotrypsin, and DNAase, which can prevent bacterial biofilm formation and degrade resident biofilms. It has been revealed that it is necessary to study the antimicrobial molecules in larval secretions in more detail. By isolating several antimicrobial molecules from the *L. sericata*, secretory components' structural characterization and antimicrobial activity were revealed. Of these molecules, Lucifensin 1, Lucifensin 2, MAMP, and Seraticin 1 have been identified as potential antimicrobial molecules.[49] The molecular structure of Seraticin 1 is investigated so that it can respond to chemical synthesis. The mode of action, the minimum inhibitory concentrations, and molecular targets' determination are also being analyzed. (Nigam Y. and others, unpublished) Therefore, the presence of antibacterial molecules contained in *L.sericata* secretions has now become universally accepted.[50]

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Table 5. Overview of the bioactivity of maggots

Bioactivity	Component	Molecular weight	Case	Source	Mechanism
Antibacterial	Ammonia, calcium carbonate	0.5–10 kDa	Undefined	Whole-body extract	Increasing the pH value
	Ammonium carbonate	0.5–10 kDa		Whole-body extract	Increasing the pH value
	Allantoin	0.5–3 kDa		Whole-body extract	Stimulation of granulation
	Lucifensin I	4,11 Da	Strains of *Staphylococcus Streptococcus*	Intestinal salivary glands, body fat, hemolymph, ES	Regulating ion channels or transmembrane pores
	Lucifensin II	4,127Da	*Staphylococcus aureus, Pseudomonas aeruginosa*	Whole-body extract	----
	Lucilin	Undefined	Multidrug resistance Gram-negative bacterium	Excretion-Secretion (ES)	----
	MAP	45.1 kDa	Standard and antibiotic-resistant strains of *Staphylococcus aureus*	Excretion-Secretion (ES)	Increasing membrane permeability
	Alfa-metoksifenil	Undefined	Undefined	ES	
	C10H16N6O9 -Seraticin	<500 Da	MRSA, *Staphylococcus spp., Bacillus spp., E. coli, Pseudomonas spp., Proteus spp., Enterococcus spp., Serratia spp., Candida spp.,*	Whole-body extract	Melting of the bacterial membrane
	Alloferon I & II	Undefined	*Enterobacter spp.*		
	pHydroxyphenylacetic acid	152 Da	*Micrococcus luteus, Pseudomonas aeruginosa*	Whole-body essence	Changing the membrane potential of bacteria by the flow of K + in the cell membrane
	p-Hydroxybenzoic acid	138 Da		Whole-body essence	
	octahydro-dipyrrolo[1,2-a;1',2'-d] pyrazine-5,10-dione	194 Da Undefined	*M. luteus, P. aeruginosa*		
	Proline diketopiperazine	Undefined	*Micrococcus luteus, Pseudomonas aeruginosa*	Whole-body essence	
Antibiofilm	Chymotrypsin 1, MEP	25 kDa, 45kDa	*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruainosa*	Excretion-Secretion (ES)	Biofilm formation in protein
	DNAase				
Antifungal	Lucimysin	8.2 kDa	Ascomycota, Basidiomycota, Zygomycota, Candida albicans, Phytophthora parasitica	Excretion-Secretion (ES)	Formation of a metal complex at a particular receptor
Anti-inflammatory	Excretion-Secretion (ES)	56 kDa	Macrophage		MIP-1β, RANTES, PDGF-BB reduction
Immunomodulator	BLIP	Undefined	T lymphocytes	Excretion-Secretion (ES)	IFN-γ, IL-4, IL-10, IL-13, and CD25 reduction, TNF-α and TGF-β increasing editing
Antileishmania	Excretion-Secretion (ES) and Hemolymph	<10 Da	*Leishmania tropica* Promastigote and intracellular amastigotes	ES and Hemolymph	The effect of cytotoxicity on promastigote and intracellular amastigotes
Proangiogenesis	Unsaturated fatty acid	Undefined	Microvascular endothelial cell	Whole-body essence	Increase migration, activating the AKT1 signaling pathway
	Histidine	Undefined	Human umbilical vein endothelial cell		Increasing immigration
Procoagulant	Jonah-like protein	Undefined	Human plasma		Reduce clotting time
Antitumor	ω-6 PUFA	Undefined	A-549 lung cancer cells		Activating the p38MAPK signaling pathway
Antiatherosclerosis	Excretion-Secretion (ES)	Undefined	Murine serum		Increase HDL
Antiviral	Excretion-Secretion (ES)	Undefined	Cytotoxicity of Vero cells	Petroleum ether extract	Herpes Simplex Virus (HSV-1) potential

By isolating larval medical proteins, a new antifungal peptide called lusimycin was identified from larval secretions, and its functional characterization with its clones was determined. However, in vivo studies have shown that it prevents the cutaneous development of secretions. Its antiparasitic effect has been reported.[51] Platelets and neutrophils, and monocytes/macrophages are among the first cells to work, contributing to an unending inflammatory phase that can interfere with wound healing beyond their benefits to the young wound and prevent the progression of inflammatory reactions in the wound. Biological activity of maggot secretions in different studies; physical effects on the wound and secrete bioactive molecules with immunomodulatory function, proangiogenic activity, antitumor activity and antiatherosclerosis effect, antiviral effect with scientific information are described.[52]

Maggot therapy is known for its long history, from tribal medicine to today's conventional medicine, and there is solid clinical and biomedical evidence that it is effective.[53] Ideas and studies on larval rearing and supply alternatives in production are insufficient. However, comparatively controlled studies in academia will prove the effectiveness of larval treatment, information gaps in treatment management, potential biases in the treatment process, and psychological and aesthetic concerns.

The development of biological therapy and *Lucilia sericata* larvae are gaining new supporters among clinicians in medicine every year. Still, although the availability of the method is rising, the unit price is increasing due to the difficulties of growing larvae production.[54] In terms of the supply of larvae production and traditional academic studies, widening the website networks of medical larval producer companies, doing the current production, product, service, and distribution practices in functioning health services also provide industrial information.

L.sericata larvae are effective in treating larvae, and their use is widespread. The limited use of *L.cuprina* larvae shows as many positive effects as *L.sericata* larvae. However, it has been reported that only *L. sericata* species larvae were included in the case-cohort studies compiled for MDT treatment. In contrast, *L. cuprina* species that reported positive results in treatment were not included in the evaluation.[55,57] Therefore, it is useful in academic studies to present evidence on the effectiveness of treatment of *L. cuprina* larva species used for MDT. Its overall integrity and safety applicability are similar to that of the *L. sericata* species. For this reason, wider literature on Calliphoridae flies and developmental biology and physiology will contribute more.

In the production of medical larvae, the focus should be on changing growing methods and optimum standardization development parameters with minimum development times to achieve uniformity of larval culture continuity between laboratory colonies. The development of sterilization methodologies for larvae is important. There is no scientific literature on the conventional growing and genetic development of *L. sericata* or *L. cuprina* fly strains and laboratory populations to improve medical performance. The last study

investigates whether genetic modification can enhance the therapeutic gains of medical larvae.[56] It hopes to use Recombinant DNA techniques for these fly strains used in larval treatment to create cDNA libraries. On the other hand, human growth factors have been shown to encourage wound healing. Much research continues in which genetic engineering techniques are used to produce transgenic larvae capable of secreting human growth factors such as human platelet-derived growth factor (PDGF-BB).[56]

In the evaluation of MDT with systematic reviews, its successful role in antibiofilm effect against Gram-positive and Gram-negative bacterial strains, including *S. aureus, P. aeruginosa,* methicillin-resistant *S. aureus,* and other drug-resistant pathogens, have been highlighted. In some studies, it has been reported that MDT does not have a direct antimicrobial effect. In contrast, further clinical studies have confirmed the decrease in bacterial load after larval use;[57,58] the mechanisms of action in these activities are similar to different types of larvae used in Maggot Treatment, or is there a difference in degree? These questions are intriguing. More large-scale short-term studies are needed for specific results.

Currently, treatment methods used for maggot therapy may be replaced by patented drugs obtained due to secretion isolation of larvae in the future. To further determine the advantages of maggot therapy and to reveal the medical utility of species other than *L. sericata*, a large number and large-scale research and clinical studies are needed.[28] Scientists agree that interdisciplinary practices will contribute "synergically and positively to the treatment process" in chronic wound treatment. The biochemical mechanisms underlying curative properties of larval therapy have been studied since larval treatment was considered a medical treatment option. The findings on the molecular and cellular mechanisms of the curative effect of maggots are still unclear. Molecules from the secretions of larvae are valuable substances responsible for stimulating the remedial process of chronic wounds.[59] It is a matter of wonder whether any potential for bioactivity identified in the larvae is due to a symbiotic bacterium present in the maggot's body or due to the maggots themselves.[60] Using recombinant DNA techniques seems to be a more promising strategy. Data describing the molecules that provide bioactivity accelerate scientific discovery and help identify source proteases and antibacterial peptides to positive effects of larval therapy with the possibility of using different techniques. Additionally, it will be possible to prepare such peptides and proteins using recombinant techniques to test activity by in vitro experiments. This attitude will enable us to understand better how the larvae can remove necrotic tissue from the wound, effectively eliminate various pathogens and improve the curative process. Active recombinant peptides and proteins developed from larvae can be used as new biotherapeutic agents in the future treatment process of larvae transitioning to biosurgery.

CONCLUSIONS

MDT was an effective treatment when indigenous tribes first discovered it centuries ago. With the rise of drug-resistant pathogens and the incidence of atherosclerosis with the diabetes epidemic, MDT has re-emerged as a significantly useful therapy. MDT is a fast, easy-to-manage, safe, and cost-effective tool for wound care; It emerges as an effective method of multidisciplinary approaches in treatment. MDT is a dynamic concept, and the Maggot Therapy Pyramid provides a structured approach to therapy promotion and an answer to the curiosities in assessing and managing chronic wounds. The fascinating powers of *Lucilia sericata* and other medically useful species larvae used for debridement treatment support MDT in a generally private demonstrable effect on every step of the therapy pyramid from floor to ceiling. Interventions for treating chronic wounds are debridement, antimicrobial, antibacterial action, and degradation of the biofilm. The existence of antimicrobial, antibacterial, and other effects presented in our study is clear. It is thought that the metabolite components in the larval body fluids play a role in these effects. These metabolite components may also have a role in interventions that need further studies. In order to reveal the true mechanism of action of MDT, advanced functional component definitions should be made and supported by controlled studies.

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