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CHRONIC HEPATITIS C WITH CRYOGLOBULINEMIA: FEATURES AND MANIFESTATIONS

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Abstract

Demographic, clinical, laboratory, epidemiological aspects of cryoglobulinemia in chronic HCV infection in patients of the Crimean population remain insufficiently studied.

To study the incidence of cryoglobulinemia in chronic HCV infection and analyze the features of chronic HCV infection with the presence of cryoglobulinemia in the study population of patients.

***Materials and methods**. A study was conducted on 65 patients with chronic HCV infection (with chronic hepatitis C and liver cirrhosis). All patients underwent blood testing for the presence of cryoglobulins. Standard diagnostic tests included a biochemical blood test with determination of the levels of total bilirubin, ALT, AST, alkaline phosphatase, rheumatoid factor. The stage of liver fibrosis was determined using ultrasound elastography. The diagnosis of liver cirrhosis was established based on the results of a comprehensive clinical, laboratory and instrumental study; scores were calculated on the* Model for End-stage Liver Disease *scale and the Child-Pugh scale.*

***Results and its discussion.** Among 65 patients with chronic HCV infection, 37 % (n = 24) had a positive test for the presence of* cryoglobulins *in the blood. Groups with and without cryoglobulins were comparable in gender (p > 0.05), BMI (p > 0.05) and duration of infection (p > 0.05). In the group with cryoglobulinemia, patients with the syndromes of hepatomegaly (p < 0.001), splenomegaly (<0.05), ascites (p < 0.05), and there were also lower platelet counts (p < 0.001) and higher rates of AST (p < 0.01), γ-glutamyl transpeptidase (p < 0.01), bilirubin (p < 0.05), ESR (<0.01) and rheumatoid factor (p <0.001), which indicates the severity of the disease. A higher incidence of patients with already developed cirrhosis was found in the group of HCV infection with the presence of cryoglobulinemia (p < 0.001).*

***Conclusions.** The study revealed a high incidence of cryoglobulinemia (37 %) in the study population of patients and showed that chronic HCV infection with the presence of cryoglobulinemia is characterized by polymorphism and severity of clinical manifestations.*

***Keywords:** chronic hepatitis C, cryoglobulinemia, extrahepatic diseases*

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Introduction

Chronic infection caused by the hepatitis C virus (HCV infection) is widespread in the world, which, along with the progressive course of the disease and the variety of immune reactions, including mixed cryoglobulinemia, makes it one of the most important not only in hepatology, but also in internal medicine in general *[Asrani SK et al., 2018; Roubertou Y.,2022; Rajendran N, 2023*].

Historically HCV infection accounted for the majority of mixed cryoglobulinemia [*Miailhes P., 2018; Boleto G, 2020*].

It has been established that HCV can exhibit hepatotropic and lymphotropic properties with the predominant involvement of B-lymphocytes [*Kolopp-Sarda M et al., 2020*]. The resulting effect of the interaction of virus antigens with specific receptors on the surface of B-lymphocytes is poly-, oligo- or monoclonal proliferation of B-lymphocytes with the formation of immune complexes, including mixed cryoglobulins [*Codes-Méndez H., 2024, Vermeersch P, 2008*], which creates a substrate for immunopathological reactions [*Ignatova T et al., 2017*]. In some (8–10 %) patients, a prolonged period of B-lymphocyte activation with the accumulation of genetic mutations leads to the transformation of B-lymphocytic proliferation into malignant B-cell lymphoma [*Zhu X. et al., 2019*].

There is evidence of numerous extrahepatic manifestations and syndromes that are associated with HCV and cryoglobulinemia and involve one or more organs and systems [*Aydeniz A. et al., 2010; Karadag O, 2021; Kondili LA.,2022*].

It should be noted that despite the great interest in the problem of cryoglobulinemia in chronic HCV infection, some of its aspects, including demographic, clinical, laboratory, epidemiological, in patients of the Crimean population remain insufficiently studied. Thus, the frequency of occurrence of CHE in chronic HCV infection and the spectrum of extrahepatic diseases (EHDs) and syndromes are unclear.

Objective: to study the incidence of cryoglobulinemia in chronic HCV infection and analyze the features of chronic HCV infection with the presence of cryoglobulinemia in the study population of patients.

Materials and methods.

A study was conducted in a sample of 65 patients with chronic HCV infection.

The study included individuals who sought medical help of both sexes, aged 20 to 80 years, with chronic hepatitis C and liver cirrhosis, and whose blood contained antibodies to HCV (anti-HCV) and HCV RNA. All patients underwent blood testing for the presence ofcryoglobulins.

Criteria for non-inclusion in the study:

- ➢- viral hepatitis B
- ➢- autoimmune hepatitis
- ➢- concomitant HIV infection
- ➢- history of liver and kidney transplantation
- ➢- receiving antiviral treatment
- ➢- refusal to test for the presence of cryoglobulins.

All patients included in the study completed the full range of planned procedures. Demographic and epidemiological data were collected, including information on age, gender, estimated duration of the infectious process (more than 20 years or less than 20 years), and methods of infection. The duration of HCV infection was assessed only in individuals with an established epidemiological history, calculated from the date of blood transfusion or from the date of initial exposure to other potential parenteral sources.

Standard diagnostics tests included a biochemical blood test with determination of the levels of total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, rheumatoid factor, as well as a general blood and urine test. All patients underwent blood tests for cryoglobulins, chest radiography and ultrasound of the abdominal organs and kidneys.

The stage of liver fibrosis was determined using ultrasound elastography. The diagnosis of liver cirrhosis was established based on the results of a comprehensive clinical, laboratory and instrumental study, scores were calculated on the Model for End-stage Liver Disease scale and the Child-Pugh scale.

The presence of pathologies classified in the literature as extrahepatic [*Milovanova S. et al., 2014*] was determined by analysis of outpatient records. When extrahepatic manifestations were initially detected in patients, consultations and additional examinations were carried out with specialists in order to establish a final clinical diagnosis. These extrahepatic mani-

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festations were classified according to ICD-10 and were subsequently referred to as extrahepatic diseases [*Adinolfi LE.,2003*].

Descriptive statistics in the study population for quantitative variables were presented by median (Me), for qualitative variables – frequency and percentage. Group comparisons on quantitative variables were performed using the Mann-Whitney test. The results of the analysis were considered statistically significant at $p < 0.05$. Calculations were carried out in the statistical package R (version 3.6).

Results and its discussion.

Among 65 patients with chronic HCV infection, 37 % (n = 24) had a positive test for the presence of cryoglobulins in the blood, 63 % (n = 41) had a negative test.

Groups with and without cryoglobulins were comparable in gender ($p > 0.05$), BMI ($p > 0.05$) and duration of infection ($p > 0.05$). In the group with cryoglobulinemia, patients over 60 years of age were more common. The data is presented in Table 1.

The risk factors for infection presented in Table 2 showed that in the group with the presence of cryoglobulinemia, patients with a history of previous surgical interventions were identified statistically significantly more often ($p < 0.001$).

When systematizing clinical data in the group of HCV infection with the presence of cryoglobulinemia in comparison with the group without cryoglobulinemia, a higher incidence of patients with already developed cirrhosis was found ($p < 0.001$). This relationship may indirectly indicate an existing relationship between the presence of cryoglobulins in a pa-

Table 1

Demographic data and duration of infection of patients with chronic HCV infection (n = 65)

Indicators	Cryoglobulin detected		P
	No (n = 41)	Yes (n = 24)	
Age, years	55	58	0.021
Gender, n (%):			
women	24 (58.5 %)	13 (54 %)	0.105
men	17 (41.5 %)	11 (46 %)	
BMI, (kg/m^2)	27.7	27.9	0.913
Disease duration ≥ 20 years*, n (%)	18/26 (69 %)	13/18 (72 %)	0.479

Notes: * - in patients with established epidemiological history

Table 2

Possible risk factors for infection of patients with HCV infection according to the survey (n = 65)

Risk factors for infection	Cryoglobulin detected				P
	No (n = 41)		Yes (n = 24)		
	n	%	n	%	
Parenteral administration of psychotropic substances	1	2.4	1	4.1	0.556
Surgical interventions	22	53.6	15	62.5	<0.001
Blood transfusions	8	19.5	4	16.6	0.361
Dialysis	1	2.4	0	0	
Tattoos, piercings	4	9.7	0	0	
Visiting the dentist	3	7.3	2	8.3	0.981
Medical activities associated with the risk of occupational infection	3	7.3	1	4.1	0.977

Table 3

Clinical and laboratory data of patients with chronic HCV infection (N = 65)

Clinical and laboratory data	Cryoglobulin detected		P
	No (n = 41)	Yes (n = 24)	
Clinical diagnosis, n (%):			
- chronic hepatitis C	31 (74.6)	13 (54.2)	<0.001
- liver cirrhosis	10 (24.4)	11 (45.8)	
Hepatomegaly, n (%)	8 (19.5)	12 (50)	<0.001
Splenomegaly, n (%)	15 (36.6)	11(45.8)	<0.05
Ascites, n (%)	7 (16.7)	5 (23.8)	<0.05
Platelets, ($\times 10^9/l$),	160	132	<0.001
Leukocytes, ($\times 10^9/l$)	5.3	5.4	<0.05
ESR, (*mm/h*), Me	11	13	<0.01
Bilirubin, ($\mu mol/l$),	15.5	19.6	<0.05
Alanine aminotransferase, (*U/l*)	63.1	74.8	<0.05
Aspartate aminotransferase, (*U/l*)	55.6	78.0	<0.001
Alkaline phosphatase, (*U/l*)	99	109	<0.05
Gamma-glutamyl transferase, (*U/l*)	44	56.3	<0.01
Rheumatoid factor, (*IU/ml*)	31.3	68.2	<0.001

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tient and liver cirrhosis.

In addition, in the group with cholinesterase, patients with the syndromes of hepatomegaly ($p < 0.001$), splenomegaly (<0.05), ascites ($p < 0.05$), and there were also lower platelet counts ($p < 0.001$) and higher rates of AST ($p < 0.01$), GGTP ($p < 0.01$), bilirubin ($p < 0.05$), ESR (<0.01) and rheumatoid factor (p <0.001), which indicates the severity of the disease. The data is presented in Table 3.

An analysis of patient complaints upon admission to hospital or during outpatient consultations made it possible to combine them into three main syndromes (Table 4). More than 50 % of patients had complaints that were characteristic of asthenovegetative and dyspeptic syndromes, less than 50 % had manifestations characteristic of arthralgic syndrome. When studying the frequency of occurrence of syndromes in the study groups, it was found that in chronic HCV infection with the presence of cryoglobulinemia, each of them occurred statistically significantly more often ($p < 0.001$).

Along with the detected changes in clinical and laboratory parameters that characterize the involvement of the liver in the pathological process, in patients with HCV infection, attention is drawn to the presence of various immunopathological diseases, classified in the literature as extrahepatic manifestations of viral hepatitis C [*Milovanova S.Yu., et al., 2014; Bonacci M., 2017*]. An overview of the data is presented in Table 5.

Analysis of extrahepatic diseases showed that during HCV infection, diseases of the following groups were identified with varying frequencies: 1) diseases of the musculoskeletal system and connective

Table 4

Frequency of occurrence of the main clinical syndromes in patients with HCV infection (n = 65)

Clinical syndromes	Cryoglobulin detected				P
	No (n = 41)		Yes (n = 24)		
	n	%	n	%	
Asthenovegetative syndrome	30	73.2	19	79.2	<0.001
Arthralgic syndrome	11	26.8	10	41.7	<0.001
Dyspeptic syndrome	23	56.1	17	70.8	<0.001

Table 5

Incidence of extrahepatic diseases in patients with chronic HCV infection (n = 65)

Extrahepatic diseases and syndromes	Frequency of occurrence	
	n	%
Diseases of the musculoskeletal system and connective tissue, as well as certain disorders involving the immune mechanism		
Cryoglobulinemic vasculitis (D89.1)	13	20.0
Rheumatoid arthritis (M05, M06)	6	9.2
Ankylosing spondylitis (M45)	3	4.6
Systemic lupus erythematosus (M32)	1	1.5
Antiphospholipid syndrome (D68.6)	3	4.6
Dermatomyositis (M33.1)	1	1.5
Endocrine system diseases:autoimmune thyroiditis (E06.3)	11	16.9
Diseases of the skin and subcutaneous tissue, cutaneous sarcoidosis		
Psoriasis (L40)	6	9.2
Vitiligo (L80)	2	3.1
Vasculitis (L95)	4	6.1
Scleroderma (L94)	1	1.5
Erythema nodosum (L52)	2	3.1
Livedo mesh (L95)	3	4.6
Purple (D 69)	3	4.6
Lichen planus (L43)	2	3.1

Table 6

Frequency and the incidence of extrahepatic diseases in chronic HCV infection (n = 65)

Clinical syndromes	Cryoglobulin detected				P
	No (n = 41)		Yes (n = 24)		
	n	%	n	%	
No extrahepatic diseases	34	82.9	2	8.3	<0.001
Diseases of the musculoskeletal system and connective tissue, as well as certain disorders involving the immune mechanism	5	12.2	21	87.5	<0.001
Endocrine system diseases: autoimmune thyroiditis	3	7.3	8	33.3	<0.001
Diseases of the skin and subcutaneous tissue, cutaneous sarcoidosis	3	7.3	20	83.3	<0.001

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tissue, as well as certain disorders involving the immune mechanism; 2) diseases of the endocrine system (autoimmune thyroiditis); 3) diseases of the skin and subcutaneous tissue and cutaneous sarcoidosis. This aspect characterized the systematic manifestation of the disease.

In the course of the comparative analysis presented in Table 6, it was revealed that in case of HCV infection with the presence of cryoglobulinemia, the proportion of patients with extrahepatic diseases of the first, second and third groups was statistically significantly higher than in the absence of cryoglobulinemia ($p < 0.05$).

In development extrahepatic diseases in case of HCV infection, immune reactions that occur in response to virus replication in the liver and in tissues of lymphoid and non-lymphoid origin are of primary importance [*Rose K, 2024*]. Along with immune disorders, the presence of a direct cytopathic effect of HCV is important for the occurrence of systemic damage; in this case, EDs often serve as the only manifestation of the infectious process and can determine the prognosis and treatment tactics [*Baykova T. et al., 2013*].

The pathogenesis of chronic HCV infection is characterized by the development of extrahepatic manifestations of predominantly immunocomplex origin, including disruption of the intestinal microbiota, as described in hepatic steatosis [*Maksimova E.V., et al.,2022*], often caused by the formation of CHE. The frequency of detection of CH in individuals with chronic viral hepatitis C in different populations varies from 19 % to 66 % [*Baykova T. et al., 2013; Galossi A. et al., 2007*]. The incidence of extrahepatic diseases in mixed cryoglobulinemia ranges from 40 % to 74 % [*Ignatova T.M., et al., 2017*]. Differences in methodological features of studies and geographical differences in samples are discussed as the main reasons for this heterogeneity. Regarding the first factor, since the methods for detecting cryoglobulinemia are the same in almost all studies, it seems unlikely that methodological features could significantly affect the results obtained by the authors. Geographical differences are important and require additional targeted study in each specific country. Taking this fact into account, we carried out a statistical analysis of the incidence of cryoglobulinemia and extrahepatic diseases in chronic HCV infection in the Republic of Crimea.

One clinical study found that the main variables associated with the presence of extrahepatic diseases or cholinesterase in patients with HCV infection were female sex, older age, and advanced fibrosis [*Galossi A. et al., 2007*]. Studies by other authors have identified an association between CHE and female gender, low viral load and low platelet levels, duration of HCV infection, higher stage of fibrosis and liver cirrhosis [*Batsaikhan B. et al., 2018*].

CONCLUSIONS

The study revealed a high incidence of cryoglobulinemia (37 %) in the study population of patients and showed that chronic HCV infection with the presence of cryoglobulinemia is characterized by polymorphism and severity of clinical manifestations. The results obtained are consistent with the literature data.

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NUTRITIONAL AND HEALTH STATUS OF COLORECTAL CANCER PATIENTS - BASELINE STUDY

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Abstract

Colorectal cancer represents a significant global health burden, impacting millions of lives each year. Beyond the immediate challenges of diagnosis and treatment, colorectal cancer patients often face a cascade of complex issues affecting their quality of life, including impaired nutritional status and compromised health. The aim of this study was to examine initial nutritional and health status of patients with colorectal cancer. Basic anthropometric measurements were collected from 45 colorectal cancer patients at the initial nutritional counselling and prior to the start of treatment. At the same time, health-related quality of life was examined using a 36-Item Short Form Survey (SF-36). Results show weight loss amongst a substantial proportion of patients in the three months prior to the first nutrition counseling, 68.9% of patients lost weight, and 15.6% of patients gained weight. Patients with right-sided colorectal cancer have unfavorable anthropometric indices in comparison to left-sided colorectal cancer patients including lower muscle mass, higher waist to hip ratio, body fat, visceral fat index and higher waist circumference. In regard to health-related quality of life, the results show that right-sided and left-sided colorectal cancer patients significantly differ only in emotional functioning (p=0.036). Early nutritional assessment and implementation of nutritional support can contribute to the quality of life and maintenance of normal nutritional status in patients with colorectal cancer.

Keywords: Colorectal Cancer; Nutritional Status; Anthropometrics; Health-Related Quality of Life

Introduction

Colorectal cancer is a malignant neoplasm of the large bowel and/or rectum. It is the third most common cancer in the world, and it is one of the most common tumors in western countries [*WHO, 2023*] with the significant cause of morbidity and mortality worldwide [*Jayasinghe M et al., 2023*]. colorectal cancer is the 3rd most common cancer in men and the 2nd most common cancer in women [*WCRF, 2023*]. It predominantly affects older individuals, with the majority of cases occurring in people of 50 years or older [*WHO, 2023*].

The statistics show that Bosnia and Herzegovi-

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na is not far behind. According to the latest available data of International Agency for Research on Cancer, in Bosnia and Herzegovina, colorectal cancer is the second most frequent cancer, after lung cancer [*Ferlay J et al.*, 2024].

Different parts of the world have varied rates of colorectal cancer, mostly because of genetic predisposition, but also due to environmental factors and lifestyle of the people in a given area [*Lewandowska A et al., 2022*]. The risk of colorectal cancer increases approximately linearly with increasing body mass index, from 23 kg/m^2 to 30 kg/m^2. This relation is stronger in men than in women. In addition, dietary patterns such as high fat and calorie intake and red meat consumption exceeding 100 g per day increase the risk of colorectal cancer [*Mohammad NMAB et al., 2022*].

The basic treatment of colorectal cancer is classical or laparoscopic resection of the tumor with the removal of the surrounding lymph nodes [*Lewandowska A et al., 2022*]. Chemotherapy or chemoradiation is advised for patients with unresectable tumors or who are medically unfit for surgery. Adjuvant chemotherapy is recommended for post-surgical patients with stage III or high-risk stage II disease. Pre-operative neoadjuvant therapy may be administered to patients with resectable high-stage colonic disease (T4 tumors) in order to downstage the tumor [*Szymańska K, 2018*].

The location of the primary tumor, in terms of right- or left-sided origin, is significant because the two sides have different characteristics in terms of symptoms, treatment approaches and prognosis [*Brule S et al., 2015; Pugh S et al., 2016; Baran B et al., 2018*]. The right-sided colorectal cancer include cecum, appendix, ascending colon, hepatic flexure and proximal two thirds of the transverse colon. The left-sided colorectal cancer includes the left-side of the colon, more precisely transverse colon, splenic flexure, descending colon, sigmoid colon and rectum [*Baran B et al., 2018*]. The incidence of left-sided colorectal cancer has been higher than right-sided colorectal cancer [*Lee GH et al., 2015*].

Nutrition status of patients is important at all stages of the treatment. Malnutrition, either underweight or with excess body weight [*Davis JN et al., 2020*], can impair treatment outcomes and tolerance, promote the development of early and late complications of treatment, and worsen quality of life. Malnutrition may result from the anticancer therapies, the tumor itself, or the patient's reaction to the tumor. On the other hand, one of the most common risk factors for colorectal cancer and other illnesses is obesity. Obesity is a risk factor both before and after a colorectal cancer diagnosis because obese or underweight colorectal cancer patients may have higher mortality rates than normal or overweight patients [*Negrichi S and Taleb S, 2020*].

Early detection of those who have a high risk of complications is essential for developing good nutritional and clinical standards, which will improve the quality of care for colorectal cancer patients [*Karin M. et al., 2020*] but also improve their quality of life. Health-related quality of life is generally recognized as a multidimensional evaluation of how illness and treatment impact a patient's perception of overall function and wellbeing, including physical, psychological, and social aspects of life [*Sitlinger A, Syed Yousuf Z, 2018*].

The aim of this study was to examine the initial nutritional and health status of patients with colorectal cancer.

Materials and Methods

The study was conducted between August 2021 and April 2023 at the University Clinical Hospital Mostar, Bosnia and Herzegovina. This study was approved by the Ethics Committee of the University Clinical Hospital Mostar, Bosnia and Herzegovina. All patients signed informed consent prior enrolling in the study.

A total of 45 individuals with colorectal cancer of all clinical stages were included, with 26 (57.8%) men and 19 (42.2%) women.

Data on sociodemographic characteristics (place of residence, number of children, number of household members, working status, education, average monthly income) were collected via direct interview. Data regarding the diagnosis of colorectal cancer (e.g. date of diagnosis, type of treatment) were collected from personal health charts.

Anthropometric measurements were collected at the initial nutritional counseling, prior to the start of treatment. Digital column scale Seca 769 (Hamburg, Germany) was used to measure body height and weight, and a non-elastic measuring tape to measure waist, hip and mid-upper arm circumference. The waist circumference cut-

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off points were 88 *cm* in females and 102 *cm* in males according to the World Health Organization (WHO) [*WHO*, 2008]. Tanita BC-545N was used for bioelectrical impedance analysis (BIA) in order to measure body fat (%), muscle mass (*kg*), and total body water. Patients were divided into four categories depending on their calculated Body Mass Index (BMI): underweight, normal weight, overweight and obese, based on WHO classification [*WHO*, 2008].

Health-related quality of life was assessed by using the 36-Item Short Form Survey (SF-36). The 36-Item Short Form Survey (SF-36) is a widely used and well-established health-related quality of life questionnaire. It was developed by the RAND Corporation and is designed to assess an individual's physical and mental health across various dimensions. The SF-36 questionnaire is used in both clinical research and healthcare settings to measure and monitor health outcomes and quality of life. It comprises 36 questions that cover eight different health domains, which are grouped into two main categories: physical health and mental health.

Physical functioning: assessing an individual's ability to perform physical activities and daily tasks.

Role-Physical: Evaluating the extent to which physical health issues interfere with an individual's work or daily activities.

- Bodily pain: Measuring the presence and intensity of pain and discomfort.
- General health: Assessing overall perceptions of health and well-being.
- Vitality: Gauging energy levels and fatigue.
- Social Functioning: Evaluating the impact of health on social interactions and relationships.
- Role-Emotional: Assessing how emotional health affects work or daily activities.
- Mental Health: Measuring psychological distress and well-being [*Mchorney CA et al., 1993*].
- Physical functioning scores ranged from 0 to 100, with higher scores indicating better condition.

Statistical Analysis: The obtained data were analyzed by using IBM SPSS for Windows, version 25 [*IBM Corp., Armonk, NY, USA*]. For categorical variables, results are expressed as percentages, mean and standard deviation.

The normality of the distribution of the investigated variables was tested using Shapiro-Wilk tests prior to providing answers to the predetermined research problems. An independent sample t-test was performed to examine differences between genders in health status as also for anthropometric measurements. For all analyses, p-value <0.05 was considered statistically significant.

Results

The average age of all respondents was 62.69 years (SD=7.81). Most of the patients were retired (60%), 24.4% were employed and 15.6% unemployed. 88.8% of participants were living in marriage and the highest percentage of respondents (60%) had a non-university degree.

Out of the total number of respondents, 8 patients were with right-sided and 37 with left-sided colorectal cancer.

Nutritional status: Anthropometric measurements are presented in Table 1. There was a statistically significant difference between gender in weight (*kg*), body fat (%), muscle mass (*kg*), total body water (%) and waist-to-hip ratio ($p<0.05$) (Table 2).

The percentage of underweight, overweight, and obese patients according to gender is shown in Figure 1. In the three months prior to the first nutrition counseling, 68.9% of patients lost weight, and 15.6% of patients gained weight. Although a large percentage of patients lost body weight three months prior to the first nutrition counseling, only one patient was in the underweight category, while 24.4% of patients were overweight, and 15.5% obese. For the majority of patients,

Table 1.

Anthropometric measurements of colorectal cancer patients

	Mean ± SD	Min	Max
Weight (*kg*)	76.23 ± 17.18	48.80	127.70
BMI (kg/m^2)	25.34 ± 5.48	17.00	41.70
Waist circumference (*cm*)	92.70 ± 13.22	71.00	125.00
Hip circumference (*cm*)	104.39 ± 10.31	87.00	137.00
Waist-to-hip ratio	0.88 ± 0.07	0.72	1.05
Mid-upper arm circumference (*cm*)	29.15 ± 4.28	22.00	42.00
Body fat (%)	27.99 ± 10.31	8.90	57.60
Muscle mass (*kg*)	51.95 ± 11.66	32.00	81.50
Total body water (%)	53.04 ± 9.22	39.10	96.40

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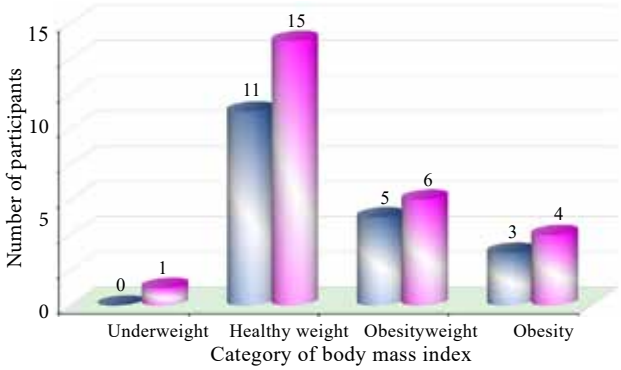

Figure 1. *Distribution of participants according to BMI category for males (like grey columns) and females (pink columns).*

57.8% BMI was within the range of reference values. The mean value for mid-upper arm circumference was 29.08 cm. According to waist circumference, abdominal obesity was present in 63.4% of patients, with mean value of waist-to-hip ratio (WHR) of 0.85 for women and 1.03 for men (Table 2). Statistically significant difference was observed between percent of body fat, muscle mass, and total body water between gender. Males had higher percent of muscle mass and total body water.

In Table 3, comparison of anthropometric measurements between right-sided and left-sided colorectal cancer patients are shown, and results clearly confirm that patients with right-sided colorectal cancer have unfavorable anthropometric indices. Particularly, right-sided patients have statistically significantly higher waist-to-hip ratio ($p<0.001$) and total body water ($p=0.003$). Additionally, right-sided colorectal cancer patients also have lower BMI, muscle mass and lower left-hand circumference, more body fat, higher visceral fat index, higher waist and lower hip circumference.

Health status: For 36-Item Short Form Survey (SF-36) mean scores were as follows: physical functioning 71.11, role functioning/physical 46.11, role functioning-emotional 73.48, energy/fatigue 49.67, emotional well-being 56.44, social functioning 62.61, pain 63.11, general health 45.40, health change 22.95. There was no statistically significant difference in health status with regard to gender ($p>0.05$) (Table 4). Of all participants, 12 females and 15 males were diagnosed with ad-

Table 2.

Anthropometric measurements between gender

	Mean ± SD		t	df	p
	F (n=19)	M (n=26)			
Weight (*kg*)	70.14 ± 16.59	80.68 ± 16.51	-2.111	43	0.041
BMI (kg/m^2)	26.01 ± 5.68	24.84 ± 5.39	0.702	43	0.487
Waist circumference (cm)	89.44 ± 14.88	96.24 ± 11.73	-1.633	39	0.110
Hip circumference (cm)	104.97 ± 12.21	100.25 ± 17.41	0.990	39	0.328
Waist-to-hip ratio	0.85 ± 0.07	1.03 ± 0.55	-1.471	39	0.165
Mid-upper arm circumference (*cm*)	29.16 ± 14.88	29.01 ± 11.73	0.109	37	0.914
Body fat (%)	34.83 ± 5.99	22.80 ± 9.93	4.667	42	0.000
Muscle mass (*kg*)	41.93 ± 6.46	56.94 ± 13.84	-4.369	42	0.000
Total body water (%)	47.12 ± 4.74	57.54 ± 9.31	-4.446	42	0.000
Visceral fat index	8.03 ± 2.89	1.88 ± 17.595	-1.676	42	0.099

Notes: M-males; F-females; SD-standard deviation, t-t-test value; df-degrees of freedom; p-statistical significance

Table 3.

Comparison of anthropometric measurements between right-sided and left-sided colorectal cancer patients

	Mean ± SD		t	df	p
	R (n=8)	L (n=37)			
Weight (*kg*)	78.20 ± 20.21	75.81 ± 16.74	0.353	43	0.155
BMI (kg/m^2)	24.81 ± 6.44	25.45 ± 5.35	-0.297	43	0.488
Waist circumference (*cm*)	97.64 ± 16.31	92.15 ± 13.00	0.975	39	0.257
Hip circumference (*cm*)	92.71 ± 29.48	104.44 ± 9.94	-1.916	39	0.014
Mid-upper arm circumference (cm)	27.75 ± 4.44	29.32 ± 4.31	-0.820	37	0.871
Waist-to-hip ratio	1.29 ± 0.97	0.88 ± 0.07	2.537	39	0.000
Body fat (%)	30.02 ± 5.11	27.54 ± 9.16	0.611	42	0.065
Muscle mass (*kg*)	43.98 ± 18.45	51.89 ± 11.94	-1.527	42	0.577
Total body water (%)	57.16 ± 17.78	52.12 ± 6.04	1.413	42	0.003
Visceral Fat index	22.06 ± 30.20	9.67 ± 4.46	2.442	42	0.000

Notes: *R-right sided colorectal cancer; L-left sided colorectal cancer; SD-standard deviation, t-t-test value; df-degrees of freedom; p-statistical significance*

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ditional health conditions, mostly hypertension, hyperlipidemia and benign prostatic hyperplasia in men. No statistically significant difference was found in any subscale in the health status of SF-36 with regard to the presence of other health conditions ($p<0.05$).

Right-sided and left-sided colorectal cancer patients significantly differ only in emotional functioning ($p=0.036$) (Table 5). However, every aspect, except social functioning was worse in right-sided in comparison to left-sided colorectal cancer patients.

Discussion

Nutritional status: Research results on the number of patients with right-sided colorectal cancer (n=8) and left-sided colorectal cancer (n=37), are consistent with the literature, where two thirds of colorectal cancer occur in the left colon and one-third in the right colon [*Szymańska K, 2018*].

In previous studies, it was observed that patients with gastrointestinal neoplasms who had poor nutritional status as well as delayed and insufficient nutritional support after surgery had worse surgical treatment outcomes [*Zietarska*

Table 4.

Comparison between the measures of health, based on the SF-36 questionnaire between gender

Variable	Mean ± SD		t	df	p
	F (n=19)	M (n=26)			
Physical functioning	65.00 ± 20.06	75.58 ± 20.31	-1.73	43	0.090
Role functioning/physical	38.16 ± 45.92	51.92 ± 46.32	-0.98	43	0.329
Role functioning/emotional	64.91 ± 46.44	80.00 ± 40.82	-1.14	42	0.259
Energy/fatigue	45.79 ± 16.68	52.50 ± 19.98	-1.31	43	0.194
Emotional well-being	57.89 ± 17.90	55.38 ± 15.25	0.50	43	0.615
Social functioning	52.89 ± 32.76	69.71 ± 31.69	-1.73	43	0.090
Pain	52.63 ± 41.91	69.71 ± 37.67	0.143	43	0.136
General health	47.84 ± 24.36	43.62 ± 20.93	0.428	43	0.536
Health change	18.42 ± 33.37	25.38 ± 31.90	0.614	43	0.482

Notes: *M-males; F-females; SD-standard deviation, t-t-test value; df-degrees of freedom; p statistical significance*

Table 5.

Comparison between the measures of health, based on the SF-36 questionnaire between right-sided and left-sided colorectal cancer patients

Variable	Mean ± SD		t	df	p
	R (n=8)	L (n=37)			
Physical functioning	68.75 ± 25.60	71.62 ± 19,82	-0.353	43	0.379
Role functioning/physical	50.00 ± 53.45	45.27 ± 45.19	0.260	43	0.159
Role functioning/emotional	50.00 ± 53.45	78.70 ± 39.96	-1.728	42	0.036
Energy/fatigue	51.25 ± 24.89	49.32 ± 15.23	0.287	43	0.076
Emotional well-being	53.50 ± 22.31	57.08 ± 14.98	-0.560	43	0.154
Social functioning	64.06 ± 33.03	62.30 ± 33.28	0.136	43	0.972
Pain	66.25 ± 41.29	62.43 ± 40.37	0.242	43	0.722
General health	52.63 ± 22.30	43.84 ± 22.26	1.012	43	0.770
Health change	32.50 ± 40.97	20.27 ± 30.41	-0.709	43	0.086

Notes: *R-right sided colorectal cancer; L-left sided colorectal cancer; SD-standard deviation, t-t-test value; df-degrees of freedom; p-statistical significance*

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M et al., 2018]. Although only one patient was in the underweight category and the majority of patients had BMI within the reference range, 68.9% of patients reported weight loss prior to the examination and nutrition counseling. The findings support the preexisting issue of nutritional status disruption in colorectal cancer patients. The research results are consistent with the results of [*Zietarska M et al. 2018*] who conclude how severe malnutrition or actual cancer cachexia do not often occur in colorectal cancer patients. Prevalence of malnutrition is generally lower in patients with colorectal and breast cancers with some exceptions [*Bossi P et al., 2021*]. Also, if pre-cachexia is observed in patients, it is reversible by appropriate nutritional support [*Zietarska M et al., 2018*].

In this study, the mean BMI was 25.34 ± 5.48 kg/m^2. According to a review [*Moghaddam AA et al., 2007*] for every 2 *kg/m*^{2} increase in BMI, the risk for developing colorectal cancer increases by 7%. BMI at the time of diagnosis is an independent prognostic factor among patients with early-stage disease whose primary tumors were resected and who received adjuvant chemotherapy with curative intent [*Renfro LA et al., 2016*]. In analysis by [*Sinicrope FA et al. 2013*] based on 25,291 patients from the Adjuvant Colon Cancer End Points (ACCENT) database, during a median follow-up of 7.8 years, obese and underweight patients with stage II or III disease had significantly poorer survival compared with overweight and normal-weight patients. For metastatic colorectal cancer, BMI was prognostic factor for the overall survival and progression-free survival, with an L-shaped pattern [*Renfro LA et al., 2016*]. Risk of progression and/or death was greatest for low BMI, risk decreased as BMI increased to approximately 28 *kg/m*^{2}, and then it plateaued [*Renfro LA et al., 2016*].

The minimum value for mid-upper arm circumference was 22 *cm* and the highest 42*cm*, which also indicates a reduced risk of malnutrition in this study population.

Bioelectrical impedance analysis (BIA) was also conducted to support basic anthropometric measurements. It is an accessible and cheap method to measure fat-free mass [*Ræder H et al., 2018*]. The results for BMI (*kg/m*^{2}), muscle mass (*kg*), and percent of fat mass were comparable with findings of [*Zietarska M et al., 2018*]. Excess abdominal fat is an important, independent risk factor for disease, especially for cardiovascular disease and diabetes. In the study [*Popovici D et al. 2023*] obesity was found to be a significant predictor of rectal cancer in the context of BMI, meaning that patients who were obese were more likely to develop rectal cancer than those who were normal weight or overweight.

The evaluation of waist circumference to assess the risks associated with obesity or overweight is supported by research [*WHO, 2011*]. A 2-cm increase in waist circumference, a measure of central obesity, was associated with a 4% greater risk of colorectal cancer [*Moghaddam AA et al., 2007*]. Waist circumference and waist-to-hip ratio could also be predictors of mortality and morbidity after colorectal surgery, and according to *[Kartheuser AH et al., 2013*] even better than BMI or body surface area. Thirty-nine percent of men and 24.39% of women in our study, were classified as abdominally obese (≥88 *cm* for women and ≥102 *cm* for men). Obesity is an important factor in predicting the recurrence of colorectal cancer [*Choi Y et al., 2016*] and according to study [*Choi MH et al., 2018*] visceral obesity tended to shorten disease-free survival (time from surgery to the time of recurrence) in rectal cancer patients.

Visceral obesity, the accumulation of visceral adipose tissue, as a more reliable indicator of obesity than BMI, had a negative impact on the outcomes of patients with cancer. That includes longer operative time, greater intraoperative blood loss, longer hospital stays, higher postoperative complications after elective colorectal surgery and even higher mortality rate [*Zhou CJ et al., 2023*]. Viscerally obese rectal cancer patients after neoadjuvant chemotherapy and resection showed shorter disease-free survival than non-obese patients [*Cederholm et al., 2019*]. WHO has highlighted "a double burden of malnutrition" - characterized by the coexistence of undernutrition and being overweight or obese or having diet-related non-communicable diseases, as a real and growing global health challenge [*Zhou CJ et al., 2023*].

Study of [*Zhou CJ et al., 2023*] showed that the combination of visceral obesity and malnutrition resulted in higher postoperative complications and mortality rates and was a good indicator of poor

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prognosis in patients with rectal cancer. Also, some studies have proposed that the progress of cachexia may be directly linked to an imbalance between the catabolic and anabolic processes occurring in peripheral tissues, particularly adipose tissue [*Batista Jr ML et al., 2012*].

Higher waist-to-hip ratio , body fat, visceral fat index and waist circumference in right-sided colorectal cancer patients (Table 3) indicate increased abdominal obesity. The results clearly confirm that patients with right-sided colorectal cancer have unfavorable anthropometric indices, which can affect both their treatment and survival success. Beside higher waist circumference, right-sided colorectal cancer had also lower muscle mass than left-sided colorectal cancer, and according to the previous studies, reduced muscle mass and increased visceral fat mass are considered negative prognostic factors for colon cancer patients [*Choi MH et al., 2018*]. The findings of the previously mentioned study indicate the importance of patients' muscle mass at initial diagnosis as an important factor in oncologic outcome.

Most of the studies have shown that the prognosis of right-sided colorectal cancer is worse than that of left-sided colorectal cancer [*Lee G et al., 2015; Baran B et al., 2018*]. Patients with right-sided colorectal cancer present with more advanced tumor stages compared with patients with left-sided colorectal cancer. One of the possible reasons could be that symptoms in right-sided colorectal cancer are often manifesting with subtle signs including microcytic anemia and weight loss, rather than the more obvious symptoms in left-sided colorectal cancer like rectal bleeding and altered bowel habits [*Lee GH et al., 2015*]. In the systematic review and meta-analysis by [*Petrelli F et al. 2016*], which included 66 studies with more than 1.4 million patients, a significant prognostic impact of tumor site in the overall survival was found with a 20% reduced risk of death for cancers arising on the left side.

Health status: The SF-36 is widely used as a generic short-form measure of functional health and wellbeing of different population groups [*Jureša V et al., 2000*].

Although, there was no statistically significant difference in health status with regard to gender ($p>0.05$) (Table 4), mean value for role functioning/physical were lower in females than in men, as well for role functioning/emotional. The lower scores of role functioning/physical means problems with work or other daily activities as a result of physical health, and lower scores of functioning/emotional means certain problems with work or other daily activities as a result of emotional problems. In the study by [*Domati F et al., 2014*] about quality of life in colon cancer patients during chemotherapy, physical role (perception of physical capacities) was reduced in both sexes (though not significantly) when compared to the reference Italian population.

Results from our study show that right-sided and left-sided colorectal cancer patients significantly differ only in emotional functioning ($p=0.036$). However, every aspect, except social functioning, was worse in right-sided in comparison to left-sided colorectal cancer patients. These results were expected given the more severe manifestation of the right-sided colorectal cancer and their unfavorable anthropometric indices, which have an impact on health status. Also, the biochemical effects of adjuvant therapy for colorectal cancer and the unavoidable morphological modification of intestinal anatomy resulting from surgical resections may cause changes in the physical and functional aspects of health-related quality of life [*Theodoropoulos GE et al., 2012*]. The study of [*Bosma E et al., 2015*] revealed that patients with severe complication after colorectal surgery have a larger postoperative decrease in health status compared to patients with none or minor complications. The most notable decrease was 6 weeks postoperatively and most notably in the domains of limitations in physical activities and social activities, the general mental health domain, vitality and general health perception [*Bosma E et al., 2015*].

The increasing importance given to the quality of life makes a significant impact on how cancer patients are treated. It is becoming increasingly recognized that fatigue, a non-specific, multifaceted syndrome with psychological, social, and physiological components, is the most prevalent and frequent side effect that colon cancer patients experience during their treatment (surgery, radiation and/or chemotherapy) [*Domati F et al., 2014*].

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Conclusion

Considering that the anthropometric measurement was done at the very beginning, before the therapy, it is possible that the loss of body mass is one of the disease's signs and symptoms or is connected to the surgery that some of the patients have already undergone.

Obese patients can be malnourished, and as we show, there are significant difference in nutritional status between right-sided and left-sided colorectal cancer patients. Increased visceral fat (associated with inflammation and sarcopenia risk) within right-sided colorectal cancer patients, could be the reason for a more severe clinical presentation as compared to left-sided colorectal cancer. This warrants further evaluation and monitoring of nutritional status, physical activity and quality of life in right and left-sided colorectal cancer throughout the course of treatment. Recognition of deterioration in the nutritional status early in the course of the treatment can be successfully treated with appropriate nutritional support.

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CHARACTERISTICS OF MORPHOLOGICAL ELEMENTS OF LESIONS OF THE ORAL MUCOSA IN PATIENTS WITH HIV INFECTION

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Abstract

The human immunodeficiency virus remains a global public health problem, having claimed more than 35 million lives to date. Oral lesions in people infected with HIV are among the first symptoms of the disease, which are characterized by great diversity. WHO suggests using oral mucosal diseases in HIV infection as an important diagnostic criterion. Signs of HIV in the oral cavity are inflammatory-dystrophic changes in the mucous membrane. In essence, HIV suppresses the proliferation of CD4+ T-lymphocytes, thereby significantly reducing the production of these important lymphocytes. The aim of this study was to identify the characters of the most typical morphological changes in the oral mucosa in patients with HIV.

The study included 190 patients (group I HIV n= 90, group II control group n=100) with lesions of the oral mucosa in the age range 29 – 64 years. The control group involved 45 subjects without HIV with lesions of the oral mucosa, their age: 25 to 67. The content of cytokines IL-2, IL-4, IL-10 and γ-IFN in the oral fluid was determined by ELISA. Biopsies taken from the buccal mucosa and gums were subjected to histological examination. Immunohistochemical study of mucous membrane biopsies was carried out using monoclonal mouse antibodies to CD3 + and CD20+.

The morphological signs may be conditionally subdivided into indicators of severity and activity of inflammation. The healing of oral mucosa injures occurred due to the higher density of newly formed blood vessels and the appearance of collagen fibers. Significant morphological changes developed in the microvasculature have dual influence: it makes worse the tissue trophism and accelerate the healing with differentiation into coarse-fibrous connective tissue. The immunohistochemical findings indicate the decrease in tissue local immune response. An increase in pro-inflammatory IL2 and a decrease in anti-inflammatory IL4 were detected in comparison with the control group.

Keywords: Morphology, oral mucosa, cytokines, immunohistochemistry, HIV.

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Introduction

Human immunodeficiency virus (HIV) remains a global public health problem, having claimed more than 35 million lives to date. In 2023, HIV-related causes will kill approximately 1.5 million people worldwide. At the end of 2023, there were an estimated 37.8 million people living with HIV worldwide, and 2.7 million people acquired HIV- infection in 2021 [*WHO, 2022*]. In Armenia from 1988 to October 31, 2023 4583 cases of HI-infection were registered [*Azatyan VYu et al., 2022; NCID, 2024*].

Oral lesions in people infected with HIV are among the first symptoms of the disease, and are characterized by great diversity. A stomatologyst may be the first specialist an HIV-infected patient sees [*Castillejos-García I et al., 2018*]. World Health Organization (WHO) proposes to use oral mucosal diseases in HIV-infection as an important diagnostic criterion [*WHO, 2022*]. Oral mucosa lesions serve as clinical markers of HIV viremia and immune suppression as HIV- infection progresses [*Kolisa Y et al., 2019; Johnson N et al., 2020*.

The stomatologyst faces great difficulties in diagnosing oral lesions in patients with HIV-infection and AIDS due to their diversity and nonspecificity[*Jambeiro de Souza A et al., 2018; Weinberg A et al., 2020*]. Therefore, diagnosis and treatment of oral lesions in this group of patients should be carried out in close contact with infectious disease specialists, immunologists, oncologists and other specialists [*Xia HS et al., 2021*].

The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity is due to the development of mechanisms of cellular damage and modification of tissue structures that are common to the entire body, with the acquisition of autoantigenic properties. The leading role in the occurrence of these changes is played by failures and dysfunctions of cytokine regulation of immunobiological processes [*Bostanci N et al., 2018; Maney P et al., 2015*]. The development of inflammatory diseases is determined by the state of cytokine regulation [*Pan W et al., 2019; Sun X et al., 2021*]. Most of both pro- and anti-inflammatory cytokines (such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNFα, ɤ-INF) are present not only in peripheral blood but also in mixed saliva or oral fluid [*Sehgal HS et al., 2019; Sheshukova OV et al., 2021*]. Compelling evidence has accumulated in favor of the cytokine concept of pathogenesis of chronic inflammatory diseases oral mucosa [*Taylor J, 2014; Groeger S et al., 2019*].

Signs of HIV-infection in the oral cavity are inflammatory-dystrophic changes in the oral mucosa: hyperemia, dryness, edema and desquamation of the epithelium. Essentially, HIV-infection inhibits CD4+ T lymphocyte proliferation [*MacParland SA et al., 2015*; *Skardasi G et al., 2018; Tavares TS et al., 2021*] thus reducing significantly the production of these important lymphocytes. This impairs virus-specific T-cell response by altering cooperation among various components of the immune system [*Huang Y et al., 2015; Yang Z et al., 2021*]. Moreover, CD3 lymphocytes in HIV-infection patients are less effective in blocking HIV replication as compared to healthy individuals [*Kokordelis P et al., 2015; Warnakulasuriya S et al., 2021*].

The impaired immune system also leads to the development of oral cavity diseases among HIV-infection patients [*Cekici A et al., 2014; Weinberg A. et al., 2020; Yura Y et al., 2022*]. The diagnostic methods available to the dentist include immunological [*Stadler AF et al., 2016*] and morphological studies, forming a group of morphological diagnostic methods [*Noguera-Julian M et al., 2017; Sehgal HS et al., 2019*; *Sun X et al., 2021*]. In case of discrepancy between the preliminary clinical diagnosis and the results of histopathological examination (the gold standard of oral mucosa lesions diagnostics) [*Mendez M et al., 2016*], along with classical histological research methods [*Yu CH et al., 2014; Azevedo AS et al., 2016*], modern methods of immunohistochemistry should be used [*Sanchez-Quevedo MC et al., 2007*].

The **aim** of this study was to identify the characters of the most typical morphological changes in the oral mucosa in patients with HIV-infection.

Material and methods

The study included 190 patients who were divided into groups (group I: patients of the main group with HIV-infection n= 90, group II: individuals of the control group n=100). In addition to the underlying disease, patients with HIV-infection had lesions of the oral mucosa. The age of patients ranged from 29 to 64 years, hospitalized in the period 2021-2023 in the infectious diseases clinic of the ‘’Mikaelyan Institute of Surgery’’, Yerevan

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State Medical University (Yerevan, Armenia). The control group included 100 individuals without HIV-infection with oral mucosa lesions who applied to the Stomatology Scientific and Educational Clinical Center No. 1 of Yerevan State Medical University during the same period. Their ages ranged from 25 to 67 years. The viral nature of HIV-infection was verified by detecting HIV virus RNA in the blood serum of examined individuals using the reaction WESTERN- BLOT.

A clinical examination was done to assess the oral cavity status. This included an external examination of the: lips, corners of the mouth, an assessment of various parts of the oral mucous membrane (color, relief, presence of hemorrhages and telangiectasia), well as the condition of tongue (color, coating and foci of epithelial desquamation).

Study of cytokines in oral fluid: Cytokines of the oral fluid were tested among 24 patients with HIV-infection and 30 patients without HIV-infection, who agreed to pass this test. The test material was unstimulated mixed saliva – oral fluid, obtained without stimulation and collected with a sterile syringe into sterile Eppendorf tubes. Samples were frozen and stored at –20 °C. The samples after were thawed at room temperature, centrifuged at 5000 rpm in the cold. Mucin was precipitated using 6 units of Lydase per 1.0 *ml* of oral fluid by our patented method (*Patent RA No. 3295 A dated at May 16, 2019*). The concentrations of cytokines IL-2, IL-4, IL-10 and γ-INF was determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA) using the Vector-Best test systems (Vector-Best JSC, Novosibirsk, Russia) and was registrated on a Statfax 303 Plus photometer (Awareness Technology, Inc. Palm City, FL 34990, USA)

Morphological study: The material for morphological studies served samples of biopsy tissues excised from mucous membrane in the area of immediate localization of the pathological process in all patients with HIV-infection. According to the standard histological scheme, the pieces of tissue were fixed in 10% neutral formalin, dehydrated and embedded in paraffin. A series of sections of 4 μm thickness were stained with hematoxylin - eosin and picrofuchsin by Van Gieson for a general assessment of the condition of the examined tissues [*Prento P, 1999*]. Histological micro preparations were studied with a ZEISS Primo Star trinocular microscope (ZEISS Microscopy, Germany) under 100 and 400 times magnification. Microphotographs were taken with a ZEISS Axiocam ERc 5s (Carl ZEISS Microscopy, Germany). All features were examined in accordance with the international standards, WHO recommendations and recognized research methods [*WHO, 2005*].

Immunohistochemical study: Immunohistochemical study was carried out with reagents produced by Zytomed (Germany), i.e. a manual polymer detection system and positive control. Immunohistochemical study of mucous membrane biopsies was carried out using monoclonal mouse antibodies to CD3 + (clone SP7 for the determination of T-lymphocytes), CD20 + (clone L26 for the determination of B-lymphocytes). The listed immunohistochemical markers were chosen after the control researches, which allowed to reveal the most informative indicators to evaluate the activity of T- and B-lymphocytes. It has helped to evaluate more preciously character of oral mucosa inflammation.

Statistical analysis: Descriptive analysis (Mean ± SD for continuous and frequencies/proportion for categorical variables) were calculated for all variables of interest. Differences between two groups were evaluated using "chi-square" or "Fisher's exact" tests for categorical variables and "Wilcoxon signed rank test" for continuous variables. Spearmen correlation was performed for determination of relationships between continuous variables. P-value was considered significant at <0.05 and <0.001 for highly significant results. Analyses were conducted using Excel 2013 and R software software and program Vassar Stats to calculate Odds Ratio and 95% Confidence Intervals (CI).

Results

The study included 90 patients with HIV-infection, 81 men (90.0%) and 9 women (10.0%). The control group involved 100 subjects without HIV-infection with lesions of oral mucosa: 62 men (62.0%) and 38 women (38.0%). The average age in HIV-infection patients group was 45.2 ± 8.34, and in the control group 37.99 ± 16.66. Patient complaints and data from the clinical examination of the oral cavity were taken into account when examining the dental status, including: external examination of the lips and corners of the mouth, assessment of the state of various parts of the oral mucous (Table 1).

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Table 1.

Clinical examination data of the oral mucosa in patients with HIV-infection and in the control groups

Sign	Control group n=100		HIV-infection n=90		p-value*
	n	%	nr	%	
Erosion on the lips					
Absent	99	99	46	51.1	
Present	1	1	44	48.9	<0.001
Cracks in the corners of the mouth					
Absent	99	99	24	26.7	
Present	1	1	66	73.3	<0.001
Disorders in the mucous membrane relief					
Absent	97	97	22	24.4	
Present	3	3	68	75.6	<0.001
Hemorrhages on the buccal mucosa and the hard palate					
Absent	100	100	83	92.2	
Present	0	0	7	7.8	<0.00471
Telangiectasia on the buccal mucosa					
Absent	100	100	84	93.3	
Present	0	0	6	6.7	<0.0103
Coated tongue					
Absent	100	100	0	0	
Present	0	0	90	100	<0.001
Foci of epithelial desquamation on the surface of the tongue					
Absent	100	100	41	45.6	
Present	0	0	49	54.4	<0.001

Notes: ** p-value test result from the comparison between HIV-infection and control group*

The objective examination of the lips in HIV-infection patients revealed 44 cases (48.9%) erosion and 66 cases (73.3%) cracks in the corners of the mouth. There were only 1 (1.0%) patients/cases of erosions and cracks in mouth corners in the control group. Disorders in the mucous membrane relief in the HIV-infection patients were detected in 75.6%, in the control group – in 3.0%. Some manifestations inherent in HIV-infection were absent in the control group. Namely, hemorrhages on the buccal mucosa and on hard palate were observed in 7 (7.8%) of the examined patients. A similar picture was observed in terms of telangiectasias on the buccal mucosa, absent in the control group, while in the HIV-infection group it was detected in 6.7% patients. The examination of the tongue of patients in HIV-infection group also revealed symptoms that were absent in the control group. The presence of coated tongue was detected in 100.0% and foci of tongue surface epithelial desquamation in 54.4% examined HIV-infection patients.

Pro-inflammatory and Anti-inflammatory Cytokines: Within the scope of the research we studied the content of pro-inflammatory cytokines - IL2, ɤ-INF and anti-inflammatory cytokines - IL4 and IL10 in the oral fluid (Table 2).

The comparison of the results of oral fluid cytokines in HIV-infection and in the control group shows that the amount of pro-inflammatory IL2 increases with a high significant difference 24.57 ± 21.58 (8.7 times, $p<0.001$). The level of anti-inflammatory IL4 decreases with the same significant difference ($p<0.001$) 0.21 ± 0.48 (68.05 times). The amount of IL10 also significantly increases 3.29 ± 6.55 (3.5 times, $p<0.05$). The increase of ɤ-INF in HIV-infection patients is statistically insignificant in comparison with the control group ($p>0.561$).

Pathohistological and immunohistochemical study: Pathological processes in the oral cavity were mainly localized in buccal mucosa (by 55.0% of patients) and in gums (by 45.0% of patients). Five groups of major pathomorphological changes were identified in the mucous membrane in HIV-infection, such as: inflammatory infiltration (lymphoplasmocytic or plasmocytic infiltration with admixture of neutrophils), circulatory disorders, mucosal ulceration with fibrinous film, mucosal fibrosis and dystrophic changes in squamous epithelium. The inflammatory reaction was observed in all morphologically examined patients with HIV-infection and detected in the form of lymphoplasmocytic infiltration in 91.3%. The inflammation predominantly had proliferative properties and was localized mostly in the upper parts of the mucous membrane. Lymphoid infiltration was also

Table 2.

Oral fluid cytokines levels in the control group and in patients with HIV-infection (mean ± SD)

Cytokines	Control group (n=30)	HIV-infection (n=24)	Odds Ratio	95% CI	p value*
IL2	2.83±5.67	24.57±21.58	-21.75	[-31.06; -12.44]	<0.001
IL10	0.94±1.33	3.29 ±6.55	-2.35	[-5.15; 0.45]	<0.05
IL4	14.29±26.11	0.21 ±0.48	14.08	[4.33; 23.84]	<0.001
ɤ-INF	0.72±3.04	0.34 ±1.4	0.38	[-0.88; 1.64]	0.561

Notes: ** p-value test result from the comparison between control and HIV-infection groups*

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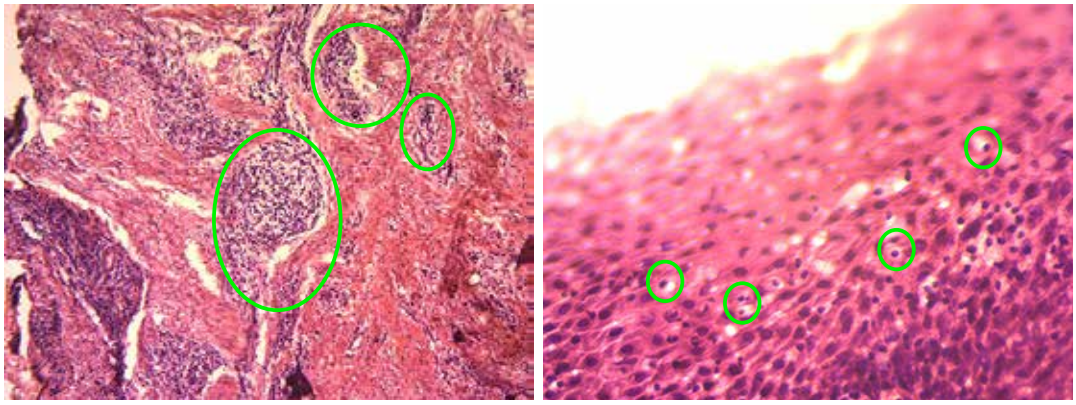

Figure 1. *Lymphoplasmocytic infiltration in the oral mucosa of the patient with HIV-infection. Rounds – inflammatory cell infiltrate, rounds – sclerosing connective tissue of the oral mucosa (stained with hematoxylin - eosin, x100) (a); Migration of the cells of the inflammatory infiltrate into the thickness of the epithelial layer of the oral mucosa of the patient with HIV-infection (rounds; stained with hematoxylin - eosin, x 400) (b).*

found around unevenly congested small blood vessels, at the epithelium border with the underlying tissue (Fig.1a), in some cases with the migration of inflammatory infiltrate cells into the thickness of the epithelial layer (Fig. 1b). Circulatory disorders were revealed in 100% of the examined patients in the form of edema, hemorrhages of various sizes due to destruction of the blood vessel walls, stasis in capillaries, marginal standing of blood cells corpuscles in venules and capillaries, hyperemia and angiomatosis. Obliteration of the vascular lumina, fibrinoid necrosis and fibrinoid swelling of the vessel walls were observed. Hyperplastic, metaplastic and dystrophic changes in squamous epithelium in form of acanthosis, parakeratosis and thickening were revealed in 99.7% of the examined HIV-infection patients. Fragments of necrotic bone tissue, most likely due to sequestration of the jawbone, were found in few patients 7.9% with HIV-infection. Damage of the epithelial cells was revealed as cytoplasmic vacuoles up to balloon dystrophy, death and desquamation of the epithelium with formation of microerosions. The comparative analyses of these five pathomorphological changes showed that mucous membrane fibrosis was reliably detected in 97.8% of HIV-infection patients with a high significant difference ($p<0.001$). Changes caused by the development mucous membrane sclerosis were determined in 100% of cases.

Immunohistochemical research of biopsies mucous oral membrane taken of from patients with HIV-infection us to evaluate the quantative composition of infiltrate to T-lymphocytes (CD3+) and B-lymphocytes (CD20+). Diffuse lymphocytes in its plate mucous oral membrane are represented mainly with T-cells, though T-lymphocytes were singly localized in the thick epithelial stratum. B-lymphocytes were diffuse in scanty quantity. Single plasmocytes were also scattered in the infiltrate mainly were in the surface part of the mucous membrane under the epithelium (Fig. 2a and 2b).

Thus, in the patients with HIV-infection we saw single diffuse CD20+ - positive lymphocytes, which is evident of local weakly expressed humoral immune response.

Discussion

For the first time, a comprehensive clinical, immunological, pathomorphological and immunohistochemical study of the oral mucosa was conducted in patients with HIV-infection and control group individuals who did not have HIV-infection but had lesions of the oral mucosa.

There is practically no pathology that would not affect the state of the oral mucosa. At the same time, the similarity of clinical manifestations in the oral cavity of diseases of different etiology and pathogenesis contributes to difficulties in making a final diagnosis [*Takai S et al., 2015*]. Lesions of the oral mucosa and periodontium aggravate the course of diseases and serve as an important addition to the characterization of the general clinical picture of hepatitis and HIV infection [*Andrusiów S et al., 2020*]. The experience of a dentist working in infectious disease

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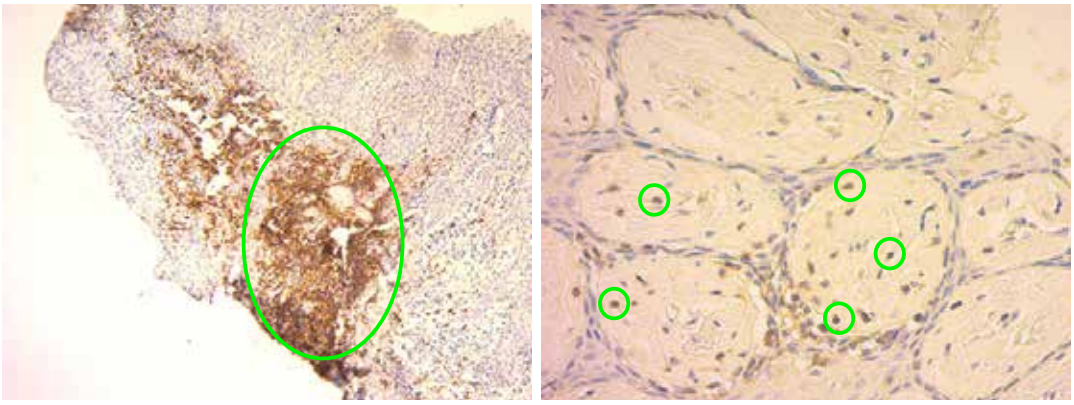

Figure 2. *Diffuse positive reaction to CD3 + in the cells of the inflammatory infiltrate (rounds) in the oral mucosa of the patients with HIV-infection (x 100) (a). Focal positive reaction to CD20+ in scattered single cells of the inflammatory infiltrate (rounds) of the oral mucosa of the patient with HIV-infection (x 400) (b).*

departments has shown that the effectiveness of diagnosis and treatment of oral mucosa lesions depends on the earliest possible examination of the admitted patient [*Bagewadi SB et al., 2015*].

Analyzing the frequency of occurrence of symptoms of oral mucosa lesions, it was found that in HIV-infection, the leading positions with a high degree of reliability are occupied by two leading pathognomonic symptoms (disorders in the mucous membrane relief, coated tongue) with fluctuations from 75.6% to 100.0% of cases. On the contrary, cracks in the corners of the mouth and especially erosions on the lips are more characteristic of HIV-infection, which cannot be said about the presence of hemorrhages on the buccal muca and the hard palate, which were observed in 7.8% of cases.

There are few studies in the available literature that have studied and systematized the symptoms of oral mucosa damage in HIV-infection, especially the early manifestations of the disease. The reliability of the frequency of occurrence of one or another symptom of the lesion has not been studied either. Some authors even point to the contradictory nature of the data indicating the connection between oral mucosa lesions and HIV-infection. In our opinion, these conclusions are related to the incorrect conduct of the research. It should be noted that the literature contains reviews on this problem, which describe in detail the epidemiological data and pathophysiological mechanisms of extrahepatic lesions in HIV-infection [*El Howati A et al., 2018; Meer S, 2019*]. However, there are few original studies in this area. We will try to discuss and compare those individual works that were found in the available literature.

Stomatological aspects of HIV-infection are diverse and not fully understood. HIV-associated stomatological diseases may be the first symptoms of infection, are characterized by high frequency, clinical polymorphism and manifestation of the course, significantly aggravate the course of the underlying disease and reduce the patient's quality of life. Jambeiro de Souza A et al. *(2018)* studied the prevalence and structure of periodontal diseases in HIV-infected patients, but the authors did not aim to study individual symptoms of the lesion and limited themselves to an index assessment [*Jambeiro de Souza A et al., 2018*].

Pakfetrat A. et al. *(2015)* examined 110 HIV-positive patients to study the prevalence of oral lesions. The authors identified severe periodontitis in 27.3% of patients [*Pakfetrat A et al., 2015*]. As indicated above, in our work, periodontitis and oral mucosa damage are detected more often in HIV-infection. In addition, we studied individual symptoms of oral mucosa lesions, which (almost all) had a high frequency of occurrence. Candidly lesions of the oral cavity were found in 42.7% of cases (*Pseudomembranous Candidiasis* - 23.6%; *Erythematous Candidiasis* - 19.1%) [*Zeng BS et al., 2021*]. Our results are somewhat at odds with the data of the above-mentioned authors. Acute candidiasis is indicated by the presence of plaque on the surface of the tongue, which was detected in 100% of our patients with HIV-infection. The discrepancies also concern angular cheilitis, which they identified in 9.1% of cases; according to our

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results, cracks in the corners of the mouth occurred in 73.3% of patients.

The basis of the inflammatory process of any etiology is the launch of the cytokine cascade, which includes, on the one hand, proinflammatory cytokines, and on the other, anti-inflammatory mediators. The balance between the two opposing groups largely determines the nature of the course and outcome of the disease [*Zatoloca PA et al., 2013; Ketlinsky SS et al., 2018*]. The main problem is the lack of available laboratory diagnostic methods that would sufficiently clearly reflect the shift in the cytokine balance towards inflammatory or anti-inflammatory/immunosuppressive reactions.

In connection with the above, we consider it appropriate to discuss some methodological aspects of our work in studying cytokines. The latter can come from the blood serum as a result of their transudation, but the content of cytokines in saliva does not correlate with their level in the blood, which indirectly indicates the predominance of their local synthesis [*Ketlinsky SS et al., 2018; Zhang H et al., 2021*]. Taking into account the recommendations of the literature, we studied the content of both pro-inflammatory - interleukin 2 (IL-2), ɤ-interferon (ɤ-INF), and anti-inflammatory cytokines - interleukin 4 (IL-4) and interleukin 10 (IL-10) specifically in the oral fluid, which is more accessible and non-invasive.

There are a sufficient number of works in the literature devoted to the study of the content and ratio of pro- and anti-inflammatory cytokines in oral fluid in various pathologies [*Grimm S et al., 2020; Esmaeilzadeh A et al., 2021*]. However, it should be noted that the available data are highly contradictory. This also applies to the data from control groups, which creates certain difficulties in terms of a clear understanding of normal cytokine levels in oral fluid. There are a few studies in the literature devoted to studying this issue in HIV-infection, but there are no studies on the simultaneous study of proinflammatory (IL-2, ɤ-INF) and anti-inflammatory cytokines (IL-10, IL-4) in the same group of patients.

Given the high variability of the normal cytokine levels in oral fluid, we found it interesting to analyze the available literature data regarding the cytokines identified in our work. According to various authors, the content of IL-4 in the stomach fluid of practically healthy people ranges from 2.3 (1; 8.5) to 15.2±1.5 *pg/ml*, IL-10 – from 4.83 ± 0.40 to 22.59 *pg/ml* (11.04-43.74), IL-2 – from 0.1 ± 0.02 *pg/ml* to 10.0 (8.5; 28.5), ɤ-INF from 18.35 ± 0.47 to 23.8±1.5 pg/ml [*Chibichyan EKh et al., 2017; Shafeev IR et al., 2016*]. Our data on IL-4 and IL-2 levels in the control group coincide with literature data, but there are discrepancies in IL-10 and ɤ-INF levels, which once again proves the high variability of normal cytokine levels in oral fluid.

Based on the above, it can be assumed that the level of cytokines in oral fluid and their ratio depend on various factors: the duration, prevalence and severity of the process, the rate of progression and development of complications of the disease, as well as the individual characteristics of the body. In this regard, we are more inclined to agree with the opinion of the following authors, who believe that the diagnostic significance of assessing the level of cytokines lies in the statement of the very fact of its increase or decrease in a given patient with a specific disease, and, in order to assess the severity and predict the course of the disease, it is advisable to determine the concentration of both pro- and anti-inflammatory cytokines in the dynamics of the development of pathology [*Pan W et al., 2019; Surlin P et al., 2020*]. It is certainly advisable to evaluate the dynamics of cytokine profile indicators in order to determine the effectiveness of the treatment and reduce the risk of therapeutic failure.

The results of our research has shown circulatory disorders and inflammation in the oral mucosa which were revealed in 100% of the examined patients. In cases of HIV-infection, significant morphological changes have developed in microvascular channel had dual influence. On the one hand, impaired blood circulation affects the tissue trophism, on the other hand, the high density of blood vessels in the regenerating granulation tissue ensured acceleration of metabolic processes, which promoted healing and differentiation into coarse-fibrous connective tissue. Azatyan V. et al. *(2021)* performed a morphological study of oral mucosal biopsies in patients with HBV, HCV, HIV-infection and also revealed general morphological changes in the oral mucosa [*Azatyan V et al., 2021*].

An immunohistochemical study revealed a significant decrease in CD3+ and CD20+ lymphocytes

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in patients with HIV-infection, which indicates a decrease in local immune responses. Available studies have been dedicated to the study of minor salivary glands in patients with HCV+HIV-infection suffering from Sjögren's syndrome with the use of monoclonal antibodies CD3, CD8, CD20, HLA-DR [*Coll J et al., 1997*].

The problem of HIV-infection remains extremely relevant due to its widespread prevalence. The global coverage of territories and the high epidemic potential of this group of diseases maintain their social and economic significance. Pathologies caused by HIV are most often found in young, working-age individuals and lead to disability and fairly high mortality. Thus, the study of the state of the oral mucosa in HIV-infection, the features of the cytokine profile of the oral fluid, morphological and immunohistochemical lesions of the mucous membrane is very relevant, which is why our work was carried out.

One of the limitations of the study was that despite the fact that the HIV-infection group had 90 participants and the control group – 100, only 24 of them from the HIV-infection group and 30 patients from the control group agreed to pass the test of cytokines of the oral fluid.

Conclusion

Thus, HIV-infection contributes to the damage of the oral mucosa. Pathomorphological examination revealed circulatory disorders and inflammation of the oral mucosa. Immunohistochemical study revealed a decrease in CD20 + lymphocytes in biopsies of patients with HIV-infection, which also indicates a decrease in local humoral immune responses. An increase in pro-inflammatory IL2 and a decrease in anti-inflammatory IL4 were detected in comparison with the control group. Further studies are needed to better understand IL-s levels in patients with HIV-infection and their relationship with oral mucosal lesions.

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THE EFFECT OF THE MEDICINAL COMPOSITION "EFLORNITHINE-ARMENICUM" ON THE PROGRESSION OF THE INFLAMMATORY PROCESS IN AN EXPERIMENTALLY INDUCED AEROBIC WOUND

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Abstract

Wound infection remains one of the most serious current challenges in modern medicine. Significant challenges in the symptomatic and pathogenetic therapy of wound infections arise due to the known symbiosis between pathogenic and opportunistic bacteria and certain pathogenic and opportunistic fungi. The mixed bacterial-fungal microflora persisting during a wound infection is often described as a biofilm. Notably, the addition of a fungal infection significantly worsens the wound healing process: on one hand, fungi that persist in the host's wound are inherently toxic; on the other, their association with bacteria often enhances the pathogenic potential of the bacteria.

The therapeutic efficacy of the medicinal composition Eflornithine-Armenicum was studied using an experimentally induced aerobic wound model. This medicinal composition was developed at the Research Center of the Yerevan State Medical University in collaboration with Arpimed LLC.

A wide range of morphological, morphometric, cytological, bacteriostatic, and immunomorphological studies were conducted. It was found that three applications of the composition to the wound surface on the skin of experimental rats led to an early activation of reparative and proliferative processes, ultimately resulting in complete restoration of the integrity of the damaged wound tissues through substitution.

The therapeutic effectiveness of the tested medicinal composition is, on one hand, due to the pronounced antibacterial activity of Eflornithine, which facilitated the early self-cleansing of the wound from opportunistic and pathogenic microorganisms persisting in situ. On the other hand, the effectiveness is attributed to the strong anti-inflammatory activity of Armenicum paste, thanks to the presence of ionized iodine in its composition.

Based on our studies, we believe there are broad prospects for further preclinical and clinical research on Eflornithine-Armenicum as an effective therapeutic agent for the pathogenetic treatment of wound inflammation.

Based on our comprehensive studies, we conclude that the medicinal composition Eflornithine-Armenicum, which we developed, should be considered an effective therapeutic agent in the treatment of aerobic wounds. This is particularly important, as both components of the composition have long been approved by prestigious pharmaceutical regulatory bodies as medicinal products with confirmed effectiveness and safety.

Keywords: *aerobic wound, wound infections, medicinal composition "Eflornithine-Armenicum, treatment.*

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Introduction

Wound infections remain a significant and serious problem in modern medicine. The situation is further complicated by the changing nature of pathological processes and the resistance of opportunistic and pathogenic microorganisms in wounds to many antibacterial drugs, especially antibiotics [*Pfaller M, 2012, Kapoor G et al., 2017; Reygaert W, 2018; Berman J, Krysan D, 2020*].

Significant challenges in symptomatic and pathogenetic therapy during wound infection arise from the known symbiosis of pathogenic and opportunistic bacteria, as well as certain fungi. This mixed bacterial-fungal microflora in wound infections is often described as a biofilm [*James G et al., 2008, Dowd S et al., 2011; Clinton A, Carter T, 2015; Percival S et al., 2015, Michael AJ, 2018, Rocha R, Wilson R, 2018*]. It is particularly noteworthy that the addition of a fungal infection significantly worsens the progression of the wound healing process. On one hand, the fungi persisting in the host wound are inherently toxic; on the other, when in association with bacteria, they often enhance the pathogenic potential of the bacterial presence. Unfortunately, current antifungal therapies are not always effective [*Becker W, 1991, Pruskowski K et al., 2021*].

The current situation regarding daily diet choices is concerning, as various supplements, including hormonal ones, are commonly used to increase the meat mass of poultry, fish, cattle and small ruminants [*Fritsche S, Steinhart H, 1999, Saha S, Pathak N, 2021*].

As a result of the factors mentioned above, the search for effective agents, and particularly the development of medicinal compositions with a broad, multipotent spectrum of action, represents a promising and relevant scientific and practical approach in modern medicine.

At the research center of Yerevan State Medical University named after M. Herats and Arpimed, LLC (Abovyan, Armenia), we have developed and successfully completed preclinical testing of the medicinal composition "Eflornithine (DFMO)-Armenicum" [*Ghazaryan H, Hovhannisyan A, 2022*].

The selection of agents for the medicinal composition was determined by the following consideration. It is well established that DFMO exhibits a strong inhibitory effect, suppressing the synthesis of aliphatic polyamines at the earliest stages of their enzymatic transformation, specifically, the conversion of ornithine to putrescine [*Meyskens F, Gerner E, 1999; Gerner E, Meyskens F, 2009*].

It is also worth noting that in recent years, highly informative data has emerged indicating that the vital activity and persistence of many resident pathogenic and opportunistic bacteria, viruses, and fungi within the host body are largely maintained by mechanisms that are specifically polyamine-dependent [*Wallace H, Fraser A, 2004; Shah P, Swiatlo E, 2008; Wallace H, 2009; Valdés-Santiago L et al., 2012; Valdés-Santiago L, Ruiz-Herrera J, 2014; Bae D et al., 2018; Berman J, Krysan D, 2020*]. In this context, there have been rare attempts to use DFMO to inhibit the synthesis of aliphatic polyamines not only in somatic cells but also to suppress their synthesis within microbial cells [*Wallace H, Fraser A, 2004; Wallace H, 2009; Bae D et al., 2018; Berman J, Krysan D, 2020*].

The second component of the medicinal composition, *Armenicum* paste, has a notably pronounced anti-inflammatory and partially bacteriostatic spectrum of action [*Zilfyan A et al., 2016*].

Material and methods

The investigation involved 180 male Wistar rats, in which an aerobic wound model was induced according to the method proposed by Hovhannisyan S.S. et al., for which a patent has been granted [*Hovhannisyan S et al., 1987*].

The animals in both the experimental and control groups were divided into three subgroups, which were removed from the experiment on the third, fifth, and ninth days of the investigation. The control group received only *Armenicum* paste, applied to the wound surface three times at 4-hour intervals, at a dose of 5.1 *mg/kg*. In addition to *Armenicum* paste, the experimental group also received the medicinal composition Eflornithine (DFMO)-*Armenicum*, applied to the wound surface three times at the same intervals. A single dose of *Armenicum* paste was 5.1 *mg/kg*, while a single dose of Eflornithine was 460 *mg/kg*.

The study employed conventional morphological methods, including staining with azure-II eosin and hematoxylin-eosin. Bacteriological methods were also used, incorporating staining with azure-II eosin and fluorochromizing with acridine orange.

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An immunomorphological method was utilized to detect fibronectin in tissues, specifically the indirect Coons method with rabbit anti-fibronectin serum (Sigma, USA) and FITC-labeled anti-rabbit IgG serum (Sigma, USA). The preparations were examined under a trinocular light microscope (Micros, Austria) and a trinocular fluorescence microscope (Boeco, Germany).

Statistical analysis was performed using the SPSS program, 13 ANOVA version, using Student's t-criteria.

Results and discussion

The study results indicated that local treatment of skin wounds in the experimental rats led to a marked activation of local reparative-proliferative processes. In contrast to the control group, which received only *Armenicum* paste, the experimental group showed activation of reparative-proliferative processes as early as the third day of the investigation. In the control group, where only *Armenicum* paste was applied to the wound surface, processes aimed at restoring defect integrity were observed only on the fifth and ninth days of the regional inflammatory process.

By the third day of the experiment, reparative processes were evident through the focal development of granulation tissue, which by the fifth day had become more widespread and showed increased differentiation (Fig. 1 a, b, c, d).

A clear trend toward differentiation of granu-

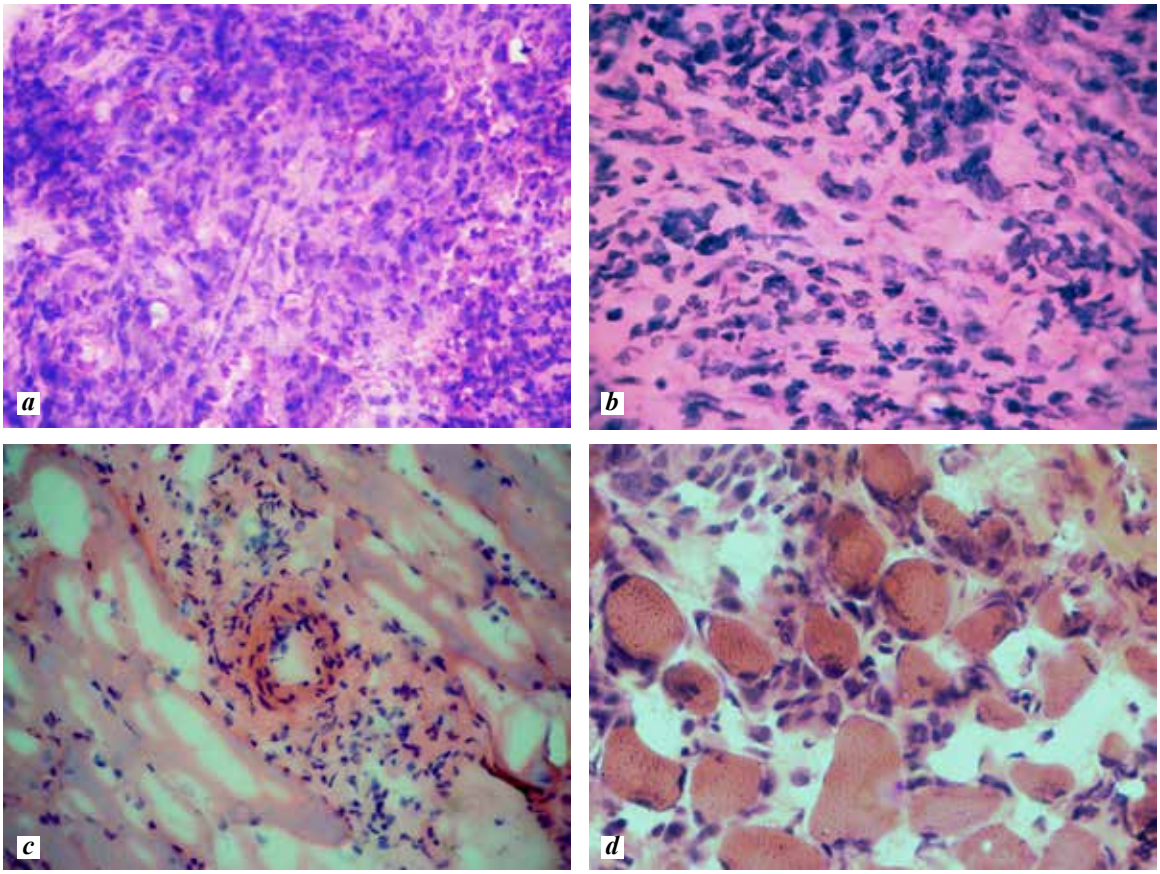

Figure 1. *Structural changes in the soft tissues of the wound in animals from the experimental group on the 5th day of investigation.*

(a). Further differentiation of granulation tissue transitioning to loose connective tissue in the superficial areas of the wound. Hematoxylin-eosin staining. Oc. 15, Ob. 60.

(b). Against a background of moderate edema, signs of granulation tissue growth with a tendency toward an organized structure of newly formed collagen fibers are observed. Stained with azure II-eosin. Oc. 15, Ob. 60.

(c). Productive subacute vasculitis with perivascular myocytolysis in the underlying muscle tissue. Stained with hematoxylin - eosin. Oc. 10, Ob. 10.

(d). Edema and cellular infiltration of the intermuscular tissue, with dystrophic changes observed in a distinct group of myocytes. Stained with hematoxylin and eosin. Oc 10, Ob. 60.

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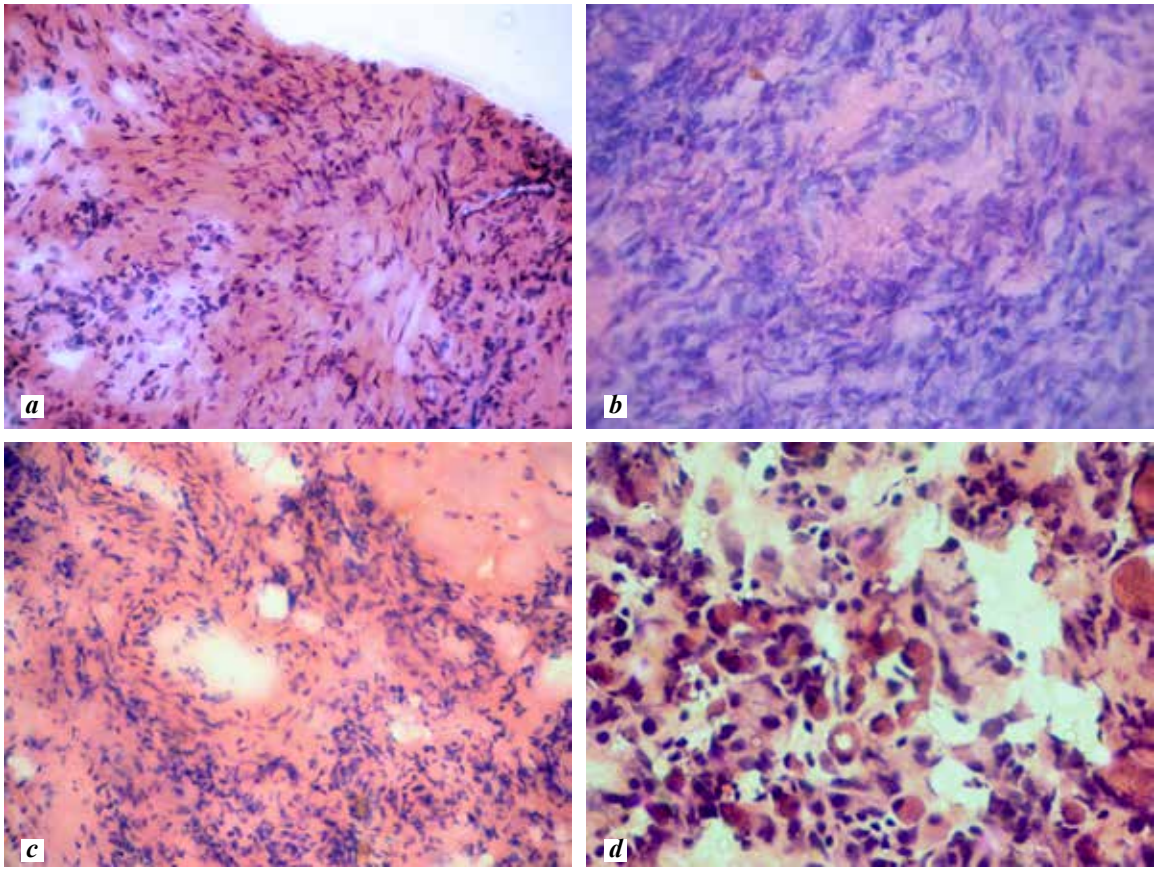

Figure 2. *Structural changes in the soft tissues of the wound in control group animals. Stained with hematoxylin - eosin. 5th day of investigation.*

(a). Dystrophic changes in inflammatory and connective tissue cells in the superficial areas of the wound, with early focal signs of granulation tissue revitalization in these regions. Oc. 15, Ob. 20.

(b). Poorly differentiated strands of granulation tissue with randomly oriented collagen fibers. Oc. 15, Ob. 20.

(c). Moderate perivascular infiltration of inflammatory cells, with focal activation of fibroblastic cells. Oc. 15, Ob. 10.

(d). Single muscle cells are visible in cross-section. The intermuscular spaces are compressed and infiltrated with inflammatory cells. Oc. 15, Ob. 60.

lation tissue was observed. In the control group animals, granulation tissue remained detectable even at the later stages of the investigation. Additionally, catabolic processes were observed more frequently in the control group, marked by areas of necrobiosis and death of connective elements, including fibroblastic and angiomatous cells, as well as myocytes (Fig. 2 a, b, c, d).

At relatively late stages of the regional inflammatory process (the 9th day of investigation), the wound induced in rats healed through substitution, meaning the integrity of the soft tissue covering the wound defect was restored by secondary intention and connective tissue growth (Fig. 3 a, b).

What are the possible mechanisms underlying the beneficial effect of the medicinal composition "Eflornithine-Armenicum" on the recovery process in an induced aerobic wound?

Primarily, the positive effect of local application of this medicinal composition can be attributed to the direct action of its components on the bacterial landscape of the wound. Notably, the composition exhibited a pronounced bactericidal effect, simultaneously targeting both pathogenic and opportunistic microflora persisting in the wound. This observation is supported by our bacterioscopic analysis of the wound exudate microflora. As mentioned earlier, many opportunistic and pathogenic microorganisms, including bacteria residing in the wound exudate and soft tissues, particularly in areas of tissue destruction, require aliphatic polyamines to support their reproduction and vital functions.

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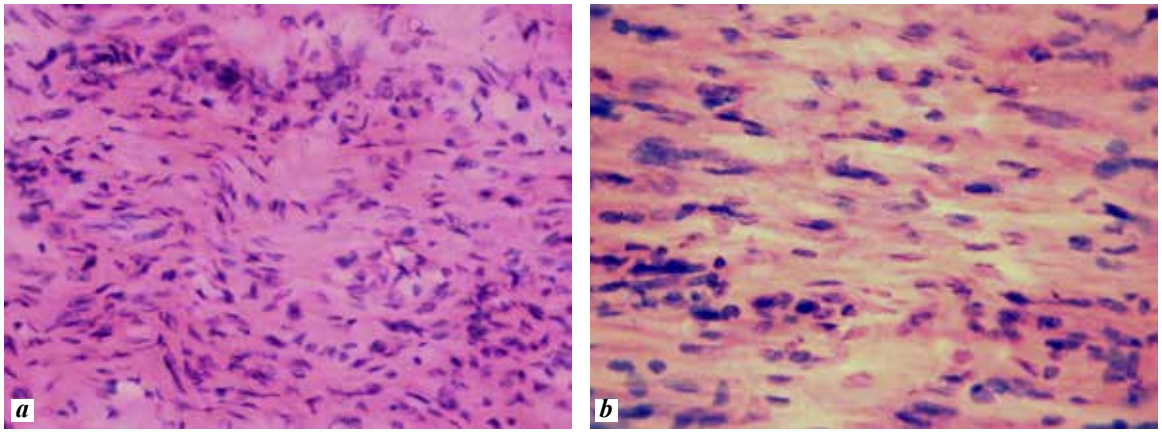

Figure 3. *Structural changes in the soft tissues of the wound in experimental group animals. 9th day of investigation.*
(a). Differentiated connective tissue is present in the superficial parts of the wound. Collagen fibers acquire a linear, ordered orientation, with mature fibrocytes beginning to predominate among the fibroblastic cells. Stained with hematoxylin - eosin. Oc 15, Ob. 20.
(b). Further differentiation of granulation tissue in the deep parts of the wound. Stained with hematoxylin and eosin. Oc. 15, Ob. 10.

It is particularly noteworthy that the pronounced bactericidal effect observed was primarily due to DFMO in the medicinal composition, which facilitated rapid cleansing of the wound from persisting microorganisms. This effect was confirmed by our cytological and bacterioscopic studies, using azure-II eosin and acridine orange staining (Fig. 4 a, b).

We also observed that, with the cleansing of the wound from microorganisms, the structural and functional characteristics of wound exudate cells, macrophages, leukocytes, and lymphocytes, became significantly normalized. Concurrently, complete phagocytosis was activated in structurally intact macrophages within the wound exudate. This facilitated early activation of reparative and proliferative processes in the soft tissue surrounding the wound, ultimately leading to full healing by substitution, wherein the entire length of the damaged tissues was replaced by loose connective tissue.

Additionally, our immunomorphological studies revealed that the presence of eflornithine in the medicinal composition promoted pronounced syn-

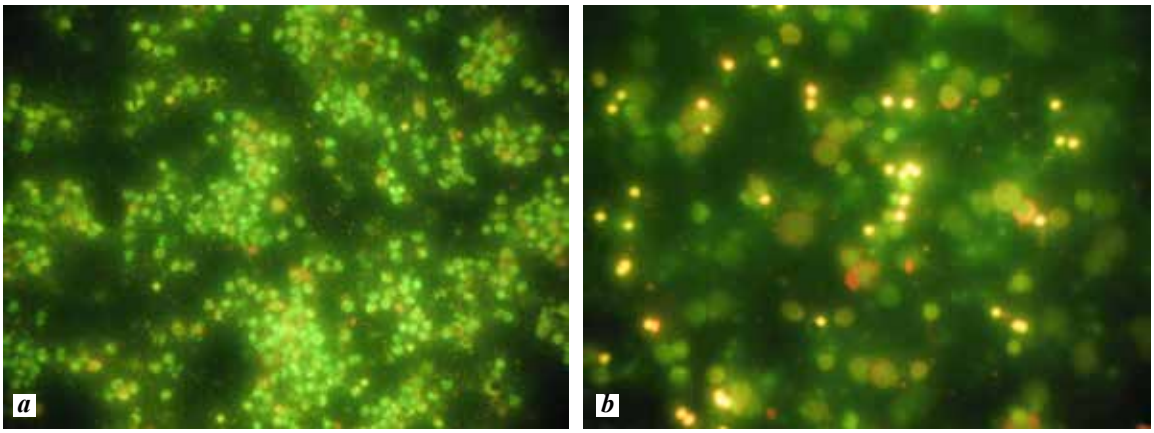

Figure 4. *Structural changes in immunocompetent cells of wound exudate in experimental group animals. Stained with acridine orange. Oc. 15, Ob. 20*
(a). Single macrophages and a moderate number of lympho-leukocyte cells are present, with changes in their tinctorial properties: green fluorescence shifts to orange-red. Extracellularly oriented single green and orange-red granules are also observed. 3rd day of investigation.
(b). Structurally preserved immunocompetent cells with green fluorescence dominate in the exudate. 5th day of investigation.

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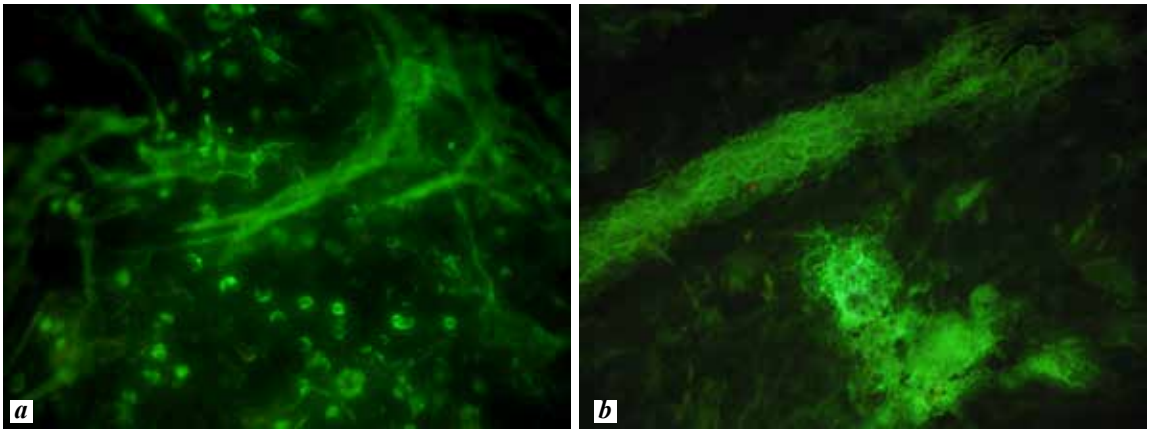

Figure 5. *Presence of fibronectin in the soft tissues of the wound in experimental group animals. Luminescent microscopy, 3rd day of the experiment.*
(a). Specific luminescence in the cytoplasm of fibroblast cells, indicating the presence of fibronectin. Oc. 15, Ob. 40.
(b). Fibronectin deposits in the superficial and deep layers of the wound's soft tissues. Oc. 10, Ob. 10.

thesis of fibronectin by fibroblasts in the wound's soft tissues, even at the early stages of the inflammatory process (Fig. 5 a, b).

Fibronectin is a well-known potent activator of fibroblast cell proliferation, which, in turn, initiates the production of collagen protein structures during the wound inflammatory process [*Grinnell F et al., 1981; Lenselink E, 2015; Gimeno-LLuch I et al., 2022*]

CONCLUSION

Based on our comprehensive studies, we conclude that the medicinal composition "*Eflornithine-Armenicum"* should be considered an effective therapeutic agent for treating aerobic wounds. This is particularly significant, as both components have been approved by prestigious pharmaceutical regulatory bodies, affirming their effectiveness and safety as medicinal products.

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EVALUATION OF KNOWLEDGE AND ATTITUDE REGARDING MORBID OBESITY AND BARIATRIC SURGERY PRACTICE: AN OBSERVATIONAL ANALYTICAL STUDY IN A NATIONALLY REPRESENTATIVE SAMPLE OF ARMENIAN POPULATION

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Abstract

The study evaluated the level of knowledge regarding morbid obesity (including its risk factors, signs, and symptoms as well as associated comorbidity) and bariatric surgery. The study has targeted also the attitude of participants toward bariatric surgery and barriers to surgical treatment of morbid obesity in cohorts with BMI higher than 40 kg/m^2.

A total of 600 questionnaires were distributed. The final number of participants who met the inclusion criteria was 570 patients.

The adopted questionnaire consisted of three parts. The first part was to verify the social-demographic and clinical characteristics of the cohort. The second part was consisted of questions revealing the level of awareness to morbid obesity. The third part was directed to revealing the barriers to bariatric surgery (psychological, physician related, financial issue related as well as the barriers related to the lack of awareness of bariatric surgery, its safety, availability, etc.)

The study demonstrated that the overwhelming majority of the survey respondents demonstrated moderate to good awareness about the inquired topics concerning morbid obesity. The poorest knowledge (with incorrect answer or answer "Don't know") regarding morbid obesity was observed in rural area residents as well as in secondary school and Secondary Vocational Education level having respondents. The level of awareness regarding morbid obesity was strongly associated with data categories of BMI, comorbidity burden index, history of another operation and positive family history of morbid obesity. Strong relationship was revealed in data categories regarding history of operation and smoking with barrier types as well as BMI and comorbidity data categories. The data obtained are also discovering the dominant association of BMI higher than 55 kg/m^2 with doctors-related issues, association of Comorbidity Burden Index 21-30 with the financial issue related barriers.

The association of psychological barriers was dominantly revealed with rural residence and with university diploma while the urban residents mainly stated physician related barriers. The knowledge related barriers were mostly demonstrated by respondents with positive family history of morbid obesity and positive history of another operation

Keywords: *morbid obesity, bariatric surgery, barrier, knowledge, comorbidity*

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Introduction.

Obesity is a multi-factorial pathology, associated with a combination of genetic, environmental, and metabolic factors [*Qasim, M et al., 2017, Flores-Dorantes MT et al., 2020*] From a public health perspective, obesity is a major risk factor for multiple comorbidity conditions and complications, increasing the cost of medical care and deteriorating the quality of life of patients. Being overweight and obese are among the risk factors for disability and death.

Morbid obesity accounts for 44% of diabetes, 23% of coronary heart disease, and 7 to 41% of cancer, and is also associated with hypertension, cardiovascular disease, sleep apnoea, and liver failure. Approximately 80-90% of alcoholic fatty disease occurs in obese people, the high degree of steatosis, reflecting the degree of fat accumulation in the liver. All this, in turn, results in reduction of life expectancy [*Kitahara C et al., 2014; Bhandari M et al., 2019*].

The obesity-directed measures (diet regulation, radical lifestyle revision, drug intervention), unfortunately, do not have proper efficiency and do not lead to reliable improvement of the condition [*Kitahara CM et al., 2014*]. With the use of traditional therapy, no more than 10% of patients with morbid obesity can achieve the desired treatment result. The results of long-term observation of large groups of patients showed that despite the use of various weight loss programs, including diet therapy, drug therapy, and physical exercises, they not only did not lead to a decrease in body weight over a 10-year period, but also led to increase of BMI and comorbidity. Currently, the widely used surgical approach, bariatric surgery, is the only one that has been proven effective in reducing body weight in patients for more than 10 years [*Qasim, M et al., 2017*].

Due to its increasing popularity, laparoscopic sleeve gastrectomy (LSG) is currently the most common bariatric treatment performed globally [*Varela JE, Nguyen NT, 2015; Mocian F et al., 2021*].

LSG was first used as a first-stage approach for superobese patients, but because of its safety and effectiveness, comparative technical simplicity, short learning curve, shorter duration of procedure and recovery period, feasibility even for extremely obese patients, and likelihood of revision and conversion to malabsorptive surgery, it is now widely accepted as a stand-alone procedure [*Angrisani L et al., 2015; Hirpara D et al., 2016; Gentileschi P et al., 2020*].

It has been demonstrated that bariatric surgery is more successful than medicinal therapy in maintaining weight loss and lowering mortality. It is the most successful therapeutic approach for treating obesity and its concomitant conditions. In spite of the fact, is still a glaringly underutilized intervention. Less than 1% of candidates undergo surgery despite these well-established benefits for a variety of reasons, including monetary burden, lack of resources, patient-physician relationship, and perceptions and attitudes of both parties.

Bariatric surgery may be hampered by a number of variables, including attitudes and views held by patients and doctors, interactions between patients and doctors, resource scarcity, and financial problems. Furthermore, long-term weight control is linked to high-risk failure and weight return, even in individuals who do undergo bariatric surgery and/or alternative weight loss therapies. Long-term patient support and supervision after the weight reduction period may be even more crucial for promoting long-term weight loss maintenance than medication. It was shown that monthly patient interaction combined with longitudinal follow-up enhanced long-term weight loss.

This observational correlative analytical study conducted in a representative sample of population aimed to evaluate the level of knowledge regarding morbid obesity (including its risk factors, signs and symptoms as well as associated comorbidity) and bariatric surgery. The study has targeted also the attitude of participants toward the bariatric surgery and barriers to surgical treatment of morbid obesity in a cohort with BMI higher than 40 kg/m^2.

Materials and methods

This observational correlative analytical study was carried out between April and November of 2023 in a representative sample of 20-60 years old Armenian patients, admitted to Shengavit MC with endocrine, vascular, metabolic or another disorder, associated with morbid obesity (BMI was higher than 40 kg/m^2). The sample of patients included in the study consisted of Armenia residents living in the country for 18 years or more. Random sampling was used to select participants. A total of 600 questionnaires were distributed. The response rate

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was 97.0% (582 participants returned the questionnaire). Twelve participants were excluded because of being representative of other nationality. The final number of participants who met the inclusion criteria was 570 patients. The determined representative sample included 570 patients.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the approval by human research committee. The protocol was approved by the Ethics Committee of Named after M.Heratsi Yerevan State Medical University. All participants gave written informed consent to participate in the trial and to use their data.

Data were generated by using structured interview with self-administered and newly designed knowledge and attitude questionnaire. Content validity of the questionnaire was assessed. The purpose was to indicate whether the questionnaire instrument appears logical to a group of experts. A panel of bariatric surgeons (n = 2), endocrinologists (n = 2), two experienced researchers with an academic degree, and two bariatric patients evaluated the questionnaire for content validity. The panel members have used the questionnaire in a pilot survey with 18 patients and provided feedback on how well each questionnaire point measures the construct in a question. The evaluation was addressing several key points: the goals of measurement, the target population, analysis of concepts (important aspects) targeted by the measurement, selection of questions, as well as concision or relevancy. The time to complete the questionnaire was indicated approximately 25 to 30 *min*.

The designed questionnaire consisted of domains with total 50 questions (Tables 1a 1b). The first part was to verify the social-demographic and clinical characteristics of respondents (eleven criteria, including age, gender, education level, area of residency, employment status, marital status and presence in family at least one child, health insurance coverage, estimated monthly income level, health status, smoking, a family history of obesity). Total amount of questions was 15.

The second domain was aimed to reveal the participant's knowledge of morbid obesity (including its risk factors, signs and symptoms as well as associated comorbidity) and bariatric surgery. Total amount of questions - 10.

The third domain of the questionnaire examined the attitude of participants toward the bariatric surgery and barriers to surgical treatment of morbid obesity as well as their attitude towards the efficacy of these measures in Armenia. This domain was divided into 4 subsets with 4 different barrier types, determined as the most popular and included in the survey. The subsets A, B, C and D contained the questions regarding attitude and barriers to bariatric surgery. The subset A was composed of questions assessing knowledge of bariatric surgery, its essence, indications, availability in Armenia etc. The subset B was composed of questions revealing the attitude to doctors, Health care facilities and Health care system generally. The questions included in the subsets CD revealed psychological barriers and financial issue-related barriers respectively. Total amount of questions in all four subsets of this domain was 25 questions. The questions of the second (regarding morbid obesity related knowledge) and third (regarding barriers to bariatric surgery) domains were designed to be answered with "Yes", "No" or "Don't know" (tables 1a, 1b).

Data Processing and Statistical Analysis: Data were analyzed by the Statistical Package for Social Sciences (SPSS) software version 23.0 (SPSS®): Inc., Chicago, IL, USA). The frequencies and percentages were calculated. Questions related to knowledge measure were calculated by adding the correct answers, then dividing them by the overall number of questions related to the parameter of interest to be measured then multiplying the number by 100%.

A self-developed scale was used to report the results as poor knowledge (0%–32.99%), intermediate knowledge (33%–67%), good to excellent knowledge (higher than 67%). Comparisons between social-demographic and clinical data and knowledge about morbid obesity as well as social-demographic and clinical data and types of barriers to bariatric surgery were measured using the Chi-square test. Under the null hypothesis, this sum has approximately a chi-squared distribution whose number of degrees of freedom is (number of rows-1)* (number of columns-1). The chosen level of significance was at ($P \leq 0.05$). A barrier with a maximum percentage of positive answers was considered dominant for the patient.

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Table 1a.

Questionnaire, domain 1 Obesity and comorbidity regarding awareness revealing questions

N	Questions	Do you know	
		Yes	No
1.	Morbid obesity resolving is possible ?		
2.	Morbid obesity is very common in the world and in Armenia?		
3.	Beside from lack of physical activity, unhealthy diet behaviors the risk factors of obesity also include insufficient sleep, stressful life, genetics and medicines ?		
4.	Diabetes Mellitus, Hypertension, Sleep apnoea etc. are associated with advanced stages of Morbid Obesity ?		
5.	Metabolic changes, induced by Morbid obesity can induce Depression		
6.	metabolic changes, induced by Morbid obesity can be life threatening ?		
7.	Conservative treatment of obesity (regular activity, modified diet, time restricted eating, cognitive behavioral therapy etc.) usually does not provide desirable and stable result ?		
8.	Bio-active supplements are not reliably contributing to excessive weight loss ?		
9.	Weight reduction improves the chance of curability and survival?		
10.	Early management of obesity complications improves the chance of curability and survival?		

Table 1 b.

Questionnaire, domain 2

N	Barriers revealing questions	Do you know	
		Yes	No
Questions revealing barriers related knowledge of bariatric surgery, its essence, indications,availability in armenia etc.			
1.	that the procedure of bariatric surgery is low traumatic?		
2.	that the length of stay at hospital after bariatric surgery is usually 3 days?		
3.	that the age frame of bariatric surgery focus includes 18 -65 years?		
4.	that bariatric surgery is indicated for the people whose BMI>40 without complications and for people > 35 with somatic complications?		
5.	that bariatric surgery is the most reliable method of Morbid Obesity management?		
6.	the most spread type of bariatric surgery?		
7.	that bariatric surgery can be performed in Armenia with high efficacy ?		
Psychological barriers revealing questions			
1.	Do you care about your health ?		
2.	Do you generally care about your external appearance?		
3.	Do you fear of diminishing social image?		
4.	Do you fear you can not cope with restrictions?		
5.	Are you afraid of postoperative period being painful?		
6.	Are you afraid of facility and medical instruments?		
7.	Do you consider operation is complicated enough and subconsciously avoid it?		
8.	Do you think that bariatric surgery is good way to solve morbid obesity's complications?		
9.	Do you think that post-operative period is very complicated and many efforts are required to follow the rules?		
10.	Do you believe that your health problem is caused by your overweight?		
Doctors related barriers revealing questions			
1.	Do you trust in terms of saving information?		
2.	Do you trust in terms of preoperative preparation methodology?		
3.	Do you trust Health care system and medical staff in our country in terms of performance accuracy of the procedure?		
4.	Do you trust in terms of sterility and safety of the procedure?		
5.	Do you trust in terms of proper management of postoperative period?		
Financial issue related barriers revealing questions			
1.	Would you apply to the bariatric surgery if it was free?		
2.	Would you like bariatric surgery to be covered by insurance?		
3.	Would you apply to the bariatric surgery with a postoperative follow-up treatment program, which is covered by the health insurance?		

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Results

Knowledge and beliefs regarding bariatric surgery: The data concerning relationship between knowledge regarding bariatric surgery and clinical and social - demographic characteristics of the investigated cohort are represented in the table 2 and 3.

***Relationship between knowledge regarding bariatric surgery and clinical characteristics*:** The strong association was revealed in BMI ($\chi2$ =15.37, *p=0.*0039), comorbidity burden index ($\chi2$ =15.37, *p=0.*0039) and history of any operational intervention ($\chi2$ =15.37, *p=0.*0039) with knowledge regarding bariatric surgery. There was no any association revealed between knowledge about morbid obesity and smoking ($\chi2$ =0.0041, p=0.9979). The data obtained also allows us to conclude about the dominant association of good knowledge with BMI higher than 55 (61 vs the expected 46), with comorbidity burden index [11- 20] (112 vs the expected 96) as well as with positive family history of Obesity (166 vs the expected 147). The excellent knowledge was associated with comorbidity burden index [21-30] (32 vs the expected 22).

Relationship between knowledge regarding bariatric surgery and social-demographic characteristics: As anticipated, there was no association between the age, gender and data categories of knowledge about morbid obesity and bariatric surgery ($\chi2$ =0.0351, *p=0.*0015 and ($\chi2$=0.0048, *p=0.*998 correspondingly for age and gender categories).

There is no enough association between the marital status data and awareness regarding morbid obesity ($\chi2$ =0.0468, p=0.999). No significant association was observed between the children (at least one child per family)_data and scores of knowledge about morbid obesity and bariatric surgery ($\chi2$ =0.029, p=0.986) and employment status ($\chi2$=0.0122, p=0.0999).

The strong difference was revealed in educational level category and knowledge concerning morbid obesity ($\chi2$ =16.32, p=0.1213) residence area ($\chi2$ =139.81, p<0,00001), as well as in insurance status ($\chi2$ =79.10, p<0,00001), level of income ($\chi2$ =24.80, p=3.72E-4), health status (by self-estimation) ($\chi2$ =37.781, p<0,00001) and positive family history of morbid obesity ($\chi2$ =3.72, 4, p=0.4458). There was enough evidence of these factors' high influence on the knowledge regarding morbid obesity.

Table 2.

Relationship of knowledge regarding morbid obesity with the clinical characteristics.

Variable	Total N (%)	Bariatric Surgery Outcomes			$\chi2$ test	P-value
		Poor N=190 (33.33%)	Intermediate to Good N=281(49.3%)	Excellent N=99 (17.37%)		
BMI, (kg/m^2),						
35--44.9	182 (31.9%)	71 (39.01%)	81 (44.50%)	30 (16.48%)	15.375	0.004
45-54.9	294 (51.6%)	103 (53.16%)	139 (47.27%)	52 (17.69%)		
higher than 55	94 (16.5%)	16 (17.02%)	61 (64.89%)	17 (18.09%)		
Comorbidity burden index,						
1-10	246 (43.2%)	93 (37.80%)	120 (48.78%)	33 (13.41%)	17.632	0.0014
11-20	196 (34.4%)	50 (25.51%)	112 (57.14%)	34 (17.35%)		
21-30	128 (22.5%)	47 (36.72%)	49 (38.28%)	32 (25%)		
History of another operation						
Positive	299 (52.5%)	81 (27.09%)	166 (55.52%)	52 (17.39%)	12.289	0.002
Negative	271 (47.5%)	109 (40.22%)	115 (42.44%)	47 (17.34%)		
Health Status (by self-estimation),						
Excellent	61 (10.7%)	28 (45.90%)	26 (42.62%)	7 (11.48%)	37.781	<.00001
Good	303 (53.2%)	124 (40.92%)	140 (46.20%)	39 (12.87%)		
Poor	206 (36.1%)	38 (18.45%)	115 (55.83%)	53 (25.73%)		
Family history of Morbid obesity,						
Yes	274 (48.1%)	81/91.33	153	40/47.58	11.876	0.0183
No	117(20.5%)	44/39	45/57.67	28/20.32		
Don't know	179 (31.4%)	65/59.66	83/88.24	31/31.08		

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Table 3.

Relationship of knowledge regarding morbid obesity with the social - demographic characteristics.

Variable	Total number of patients N (%)	Bariatric Surgery Outcomes			χ2 test	P-value
		Poor N=190 (33.33%)	Intermediate to Good N=281(49.3%)	Excellent N=99 (17.37%)		
Age (years),						
20-39	214 (37.5%)	71 (33.18%)	106 (49.53%)	37 (17.29%)	0.035	0.999
40-59	280 (49.1)	93 (33.21%)	138 (49.29%)	49 (17.5%)		
≥ 60	76 (13.3%)	26 (34.21%)	37 (48.68%)	13 (17.11%)		
Gender,						
M	271 (47.5%)	90 (33.21%)	134 (49.45%)	47 (17.34%)	0.005	0.998
F	299 (52.5%)	100 (33.44%)	147 (49.16%)	52 (17.39%)		
Smoking,						
Yes	179 (31.40%)	60 (33.52%)	88 (49.16%)	31 (17.32%)	0.004	0.998
No	391 (68.6%)	130 (33.24%)	193 (49.36%)	68 (17.39%)		
Educational level,						
Secondary school	31 (5.4%)	12 (38.71%)	12 (38.71%)	7 (22.58%)	16.321	0.012
Secondary Special/ Vocational diploma	241 (42.3%)	92 (38.17%)	103 (42.74%)	46 (19.09%)		
University diploma	268 (47.0%)	79 (29.48%)	151 (56.34%)	38 (14.18%)		
PhD	30 (5.26%)	7 (23.33%)	15 (50%)	8 (26.67%)		
Residence area,						
Urban	269 (47.2%)	25 (9.69%)	192 (71.38%)	52 (19.33%)	139.809	< 0.00001
Rural	301 (52.8%)	165 (54.82%)	89 (29.57%)	47 (15.61%)		
Marital Status						
Married or living together	282 (49.5%)	94 (33.33%)	139 (49.29%)	49 (17.38%)	0.0468	0.999
Single	116 (20.4%)	39 (33.62%)	57 (49.14%)	20 (17.24%)		
Divorced/separated	112(19.7%)	3 (2.68%)	55 (49.11%)	20 (17.86%)		
Widowed	60 (10.5%)	20 (33.33%)	30 (50%)	10 (16.67%)		
Presence of Children in family,						
Yes	458 (80.4%)	152 (33.19%)	226 (49.34%)	80 (17.47%)	0.029	0.986
No	112 (19.7%)	38 (33.93%)	55 (49.11%)	19 (16.96%)		
Employment Status,						
Working	318(55.8%)	106 (33.33%)	157 (49.37%)	55 (17.3%)	0.012	0.999
Non-working at the moment	210 (36.8%)	70 (33.33%)	104 (49.52%)	36 (17.14%)		
Student	42 (7.3%)	14 (33.33%)	21 (50%)	7 (16.67%)		
Insurance,						
Yes	204 (35.8%)	23 (11.27%)	144 (70.59%)	4 (1.96%)	79.097	< 0.00001
No	366(64.2%)	167 (45.63%)	137 (37.43%)	52 (14.21%)		
Income,						
<120,000 AMD	102 (17.9%)	36 (35.29%)	52 (50.98%)	14 (13.73%)	24.797	0.001
120,000–320,000 AMD	291 (51.1%)	101 (34.71%)	143 (49.14%)	47 (16.15%)		
320–600,000 AMD	161 (28.3%)	52 (32.3%)	81 (50.31%)	28 (17.39%)		
>600,000 AMD	16 (2.8%)	1 (6.25%)	5 (31.25%)	10 (62.5%)		

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The data obtained also allowed us to conclude about the dominant association of poor knowledge with rural residence (165 vs the expected 100), as well as good knowledge with being insured (144 vs the expected 100) and with poor health status (by self-estimation) (115 vs the expected 101).

Barriers to bariatric surgery as a method of Morbid Obesity management

The data regarding relationship of clinical and social - demographic variables with kind of barriers to bariatric surgery are represented in Table 4 and Table 5.

Relationship between type of barriers to bariatric surgery and clinical characteristics.

Comparison of clinical characteristics of participants (BMI, comorbidity, history of any operative intervention and smoking) and kinds of barriers to bariatric surgery rejected the null hypothesis with a very high probability. The comparative analysis demonstrated strong evidence of dependence between data categories concerning BMI and comorbidity and kinds of barriers to bariatric surgery (χ^2 =15.73, p=0.01524 and χ2=14.0217, p=0.0294 correspondingly for BMI and comorbidity. Another strong relationship was discovered in data categories regarding history of operation and smoking with kind of barrier (χ2 =8.7457, p=0.0329 and χ2 =15.7577, p=0.0013 correspondingly for history of operation and smoking).

The data obtained are also discovering the dominant association of BMI higher than 55 with the physician related issues (31 respondents vs expected 20) as well as association of Comorbidity Burden Index 21-30 with the financial issue related barriers (41 respondents vs the expected 29). The barriers, related to lack of the knowledge regarding obesity and bariatric surgery was dominantly revealed in the respondents with positive history of another operation (114 respondents vs the expected 97) and smoking respondents (70 respondents vs the expected 58).

Relationship between type of barriers to bariatric surgery and social-demographic characteristics. As anticipated, there was no association between the age and gender data categories with kind of barriers to bariatric surgery (χ2=0.0566, p=0.9996 and χ2=0.0192, p=0.9992 correspondingly for age and gender).

Comparison of clinical and some demographic characteristics of participants (education, residence area, employment status income level, family history of obesity and health status by self-estimation) and kinds of barriers to bariatric surgery rejected the

Table 4.

Relationship of clinical variables with kind of barriers to bariatric surgery.

Variable	Total number of patients N (%)	Barriers to bariatric surgery					
		Psychological N = 127	Knowledge - related N = 186	Doctors - related N =125	Financial issue related N = 132		
BMI, (kg/m^2),							
35-44.9	182 (31.9%)	50 (27.5%)	55 (30.2%)	39 (21.4%)	38 (20.9%)	15.736	0.01524
45-54.9	294 (51.6%)	67 (22.8%)	100 (34.0%)	55 (18.7%)	72 (24.5%)		
>55	94 (16.5%)	10 (10.6%)	31 (33%)	31 (33%)	22 (23.4%)		
Comorbidity,							
1-10	246 (43.2%)	39 (15.9%)	89 (36.2%)	63 (25.6%)	56 (22.8%)	14.022	0.029
11-20	196 (34.4%)	44 (22.5%)	64 (32.7%)	43 (21.9%)	45 (23.0%)		
21-30	128 (22.5%)	28 (21.9%)	41 (32.0%)	18 (14.1%)	41 (32.0%)		
History of another operation,							
Positive	299 (52.5%)	60 (20.1%)	114 (38.1%)	60 (20.1%)	65 (21.7%)	8.746	0.033
Negative	271 (47.5%)	67 (24.7%)	72 (26.6%)	65 (24.0%)	67 (24.7%)		
Health Status (by self-estimation),							
Excellent	61 (%)	15 (24.6%)	21 (34.4%)	15 (24.6%)	10 (16.4%)	16.916	0.0096
Good	301 (%)	72 (23.9%)	104 (34.6%)	71 (23.6%)	54 (17.9%)		
Poor	208 (%)	40 (19.2%)	61 (29.3%)	39 (18.8%)	68 (32.7%)		
Family history of Morbid obesity,							
Yes	274 (48.7%)	50 (18.3%)	101 (36.9%)	60 (21.9%)	63 (23.0%)	12.891	0.045
No	117(20.5%)	38 (32.5%)	26 (22.2%)	26 (22.2%)	27 (23.1%)		
Don't know	179 (31.4%)	39 (21.8%)	59 (33.0%)	39 (21.8%)	42 (23.5%)		

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Table 5.

Relationship of social - demographic variables with kind of barriers to bariatric surgery

Variable	Total number of patients N (%)	Barriers to bariatric surgery				χ2 test	P -value
		Psychological N = 127	Knowledge - related N = 186	Doctors - related N =125	Financial issue related N = 132		
Age (years),							
20-39	214(37.5%)	48 (22.4%)	70 (32.7%)	47 (22.0%)	49 (22.9%)	0.056	0.999
40-59 prev	280(49.1)	62 (22.1%)	91 (32.5%)	62 (22.1%)	65 (23.2%)		
≥ 60	76(13.3%)	17 (22.4%)	25 (32.9%)	16 (21.1%)	18 (23.7%)		
Gender,							
M	271 (47.5%)	60 (22.1%)	89 (32.8%)	59 (21.8%)	63 (23.3%)	0.019	0.999
F	299 (52.5%)	67 (22.4%)	97 (32.4%)	66 (22.1%)	69 (23.1%)		
Smoking,							
Yes	179 (31.40%)	38 (21.2%)	70 (39.1%)	30 (16.8%)	41 (22.9%)	15.7577	0.0127
No	391 (68.6%)	89 (22.8%)	116 (29.7%)	95 (24.3%)	91 (23.2%)		
Educational level,							
Secondary school	31 (5.4%)	7 (22.6%)	10 (32.3%)	8 (25.8%)	6 (19.4%)	18.348	0.031
Secondary Special/ Vocational	241 (42.3%)	37 (15.4%)	86 (35.7%)	58 (24.1%)	60 (24.9%)		
University diploma	268 (47.0%)	79(29.5%)	82 (30.6%)	51 (19.0%)	56 (20.9%)		
PhD	30 (5.26%)	4 (13.3%)	8 (26.7%)	8 (26.7%)	10 (33.3%)		
Residence area,							
Urban	269 (47.2%)	48 (17.8%)	88 (32.7%)	68 (25.3%)	67 (24.9%)	7.847	0.049
Rural	301 (52.8%)	79 (26.3%)	98 (32.6%)	60 (19.9%)	64 (21.3%)		
Marital Status,							
Married or living together	282 (49.5%)	61 (21.6%)	91 (32.3%)	68 (24.1%)	62 (22.0%)	9.873	0.361
Single	116 (20.4%)	28 (24.1%)	47 (40.5%)	20 (17.2%)	21 (18.1%)		
Divorced/separated	112(19.7%)	25 (22.3%)	29 (25.9%)	24 (21.4%)	34 (30.4%)		
Widowed	60 (10.5%)	13 (21.7%)	19 (31.7%)	13 (21.7%)	15 (25%)		
Presence of Children in family,							
Yes	458 (80.4%)	102 (22.3%)	149 (32.5%)	100 (21.8%)	107 (23.4%)	0.0589	0.996
No	112 (19.7%)	25 (22.3%)	37 (33.0%)	25 (22.3%)	25 (22.3%)		
Employment Status,							
Working	318 (55.8%)	90 (28.3%)	96 (30.2%)	62 (19.5%)	70 (22.0%)	17.912	0.006
Non-working at the moment	210 (36.8%)	34 (16.2%)	76 (36.2%)	51 (24.3%)	49 (23.3%)		
Student	42 (7.3%)	3 (7.14%)	14 (33.3%)	12 (28.6%)	13 (30.1%)		
Insurace,							
Yes	204 (35.8%)	45 (22.1%)	66 (32.4%)	44 (21.6%)	49 (24.0%)	0.987	0.135
No	366(64.2%)	82 (22.4%)	120 (32.8%)	81 (22.1%)	83 (22.7%)		
Income,							
<120 000 AMD	102 (17.9%)	25 (24.5%)	35 (34.3%)	20 (19.6%)	22 (21.6%)	18.944	0.026
120–320,000	291 (51.1%)	57 (19.6%)	80 (27.5%)	74 (25.4%)	80 (27.5%)		
320–600,000 AMD	161 (28.3%)	42 (26.1%)	62 (38.5%)	28 (17.4%)	29 (18.0%)		
>600,000 AMD	16 (2.8%)	3 (18.8%)	9 (56.3%)	3 (18.8%)	1 (6.3%)		

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null hypothesis with a very high probability.

The comparative analysis demonstrated strong evidence of dependence between these variables and kinds of barriers to bariatric surgery ($\chi2$ =18.3483, p=0.3134 for education, $\chi2$ =7.8472, p=0.0493 - for residence area, $\chi2$ =17.9124, p=0.0064 - for employment status, $\chi2$ =18.9444, p=0.0257 - for income level, $\chi2$ =12.8907, p=0.0448 - for family history of obesity and $\chi2$ =16.916, p=0.0096 - for health status by self-estimation). No association between the marital status ($\chi2$ =9.8730, p=0.3608), data categories with kind of barriers to bariatric surgery was revealed. No significant association was also observed between presence in family at least one child ($\chi2$ =0.0589, p=0.9963) and being or not being insured ($\chi2$ =0.1354, p=0.9873) variables with kind of barriers to bariatric surgery.

The data obtained also allows us to conclude about the dominant association of psychological barriers with rural residence (79 respondents vs the expected 67) and with university diploma (79 vs the expected 59 respondents) while the urban residents mainly stated doctors related barriers (68 respondents vs the expected 59). The knowledge related barriers were mostly revealed by respondents with positive family history of morbid obesity (101 respondents vs the expected 89), and positive history of another operation (114 vs the expected 97). The differences were statistically significant (p=0.0492, p=0.0313, p=0.0448 respectively for residence area, education level and family history of morbid obesity), causing the high likelihood of refusing bariatric surgery due to revealed barriers.

Discussion

Bariatric surgery is the most effective treatment for weight loss and improvement of obesity-related comorbidity with long-term efficacy. The aim of this study was to assess patients' awareness about morbid obesity and perception of bariatric surgery, a 50 item survey questionnaire was developed and distributed to patients who had BMI higher than 40 and applied to our Medical Center with a somatic problem for conservative treatment. The questionnaire consisted of four parts. The first part was to verify the social-demographic and clinical characteristics of cohort. The second part have been consisted of morbid obesity's awareness level revealing questions. The third part was directed to revealing the barriers to bariatric surgery (psychological barriers, physician related, financial issue related as well as the barriers related to the lack of awareness of bariatric surgery, its safety, availability etc.).

Overwhelming majority of the survey respondents demonstrated moderate to good awareness about the inquired topics concerning morbid obesity. The poorest knowledge (with incorrect answer or answer "Don't know") regarding morbid obesity was observed in rural area residents (total 54.2% given incorrect answer or answer "Don't know") as well as in Secondary school and Secondary Vocational Education level having respondents (respectively 38.71% and 38.17% given incorrect answer or answer "Don't know").

The answer "Don't know" was with the highest response rate in 28% of answers. Comparison of clinical and demographic characteristics of participants and their awareness regarding morbid obesity and bariatric operation, rejected the null hypothesis with a very high probability. Taking into consideration the middle level of awareness regarding morbid obesity, associated comorbidity with the consequent jeopardy it becomes obvious necessity to adopt a multidisciplinary approach for informing and team-implemented management of this category of patients.

Our results are complying with the conclusions of systematic review, highlighting the significance of understanding these challenges and the requirement for a multidisciplinary strategy to be used in the care of these complicated individuals. The authors considered as well that to assess the effectiveness of a structured interdisciplinary longitudinal strategy, further prospective studies are required [*Kallies KJ, Borgert AJ, Kothari SN. (2019)*, *Funk LM, Jolles SA, Greenberg CC, Schwarze ML, Safdar N, McVay]*.

The purpose of one another study was to collect data on individual patients' specific health knowledge before and after weight loss surgery and to investigate the association with weight loss, incidence of postoperative complications, health literacy, depression, and anxiety [*Köhler H et al., 2020*]. The study demonstrated "acceptable" particular knowledge of surgical candidates as well as strong information retention even years after performed training. "Good" specific knowledge and health literacy do not take the place of lifetime actual implementation of food

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guidelines and physical activity, and they were not linked to improved weight loss or problems following surgery. Authors came to the conclusion that to examine the knowledge in the same patients across time, more longitudinal studies involving knowledge assessments at different times (before to training commencement, prior to and following surgery) are required. We particularly percept the results of this study as a serious predisposition for informative training organizing for morbid obesity patients. Not less alarming was the distribution of the types of barriers to bariatric surgery. The proportion of the patients answered in positive way the question "Do you think that bariatric surgery is good way to solve morbid obesity's complication?" was about 18%, which the direct evidence and direct consequence of bariatric surgery related information insufficiency. From this point of view the primary care physicians referrals for surgical treatment of obese patients are crucial because they raise the surgical acceptability rate. According to a Canadian study, 42% of obese individuals received treatment recommendations from primary care physicians for a variety of reasons. Negative attitudes about the illness and its treatment, workload, ignorance, inadequate infrastructure, and a lack of motivation have all been identified as contributing factors [*Hirpara DH et al., 2016*].

Additional studies aimed to disclose patients' and primary care physicians' degree of awareness and attitude toward bariatric surgery. Patients' perceptions of physicians' attitudes on bariatric surgery were also looked into. The results of the study showed that, despite their willingness to participate in the treatment and follow-up of these patients and their basic understanding of obesity treatment, the physicians were unable to devote enough time to this problem because of the workload and the need for a multidisciplinary approach to the disease [*Kallies KJ et al., 2019*].

In a study of Rubio-Almanza M. et al (*2018*) only 67% and 81% of the respondents primary care physicians felt confident to suggest medication for obesity or bariatric surgery, respectively. Yet, bariatric surgery has shown positive impacts on morbidity and mortality in individuals with severe and complex obesity with significant and sustained weight loss [*Rubio-Almanza M et al., 2018*]. Carrasco, D., et al. (2022) considered it to be cost effective for society and the healthcare system due to decreased costs related to treatment of obesity comorbidity. The most important finding of that study is the positive association between physicians' knowledge and better adherence to obesity guidelines and feeling more confident to suggest obesity treatment. This study also shows that physicians had an ambivalent attitude towards obesity [*Carrasco D et al., 2022*].

In multiple studies, the authors found a significant positive association between primary care physicians' knowledge and positive attitudes about obesity and willingness to refer patients to bariatric surgery [*Turner M et al., 2016; Memarian E et al., 2021; Douglass B et al., 2023*]

Conclusion

The study demonstrated that the overwhelming majority of the survey respondents demonstrated moderate to good awareness about the inquired topics concerning morbid obesity. The poorest knowledge (with incorrect answer or answer "Don't know") regarding morbid obesity was observed in rural area residents as well as in Secondary school and Secondary Vocational Education level having respondents. The level of awareness regarding morbid obesity was strongly associated with data categories of BMI, Comorbidity burden index, history of another operation and positive family history of morbid obesity.

Strong relationship was discovered in data categories regarding history of operation and smoking with kind of barrier as well as BMI and comorbidity data categories.

The data obtained are also discovering the dominant association of BMI higher than 55 kg/m^2 with physician-related issues, association of Comorbidity Burden Index 21-30 with the financial issue related barriers.

The dominant association of barriers, related to lack of the knowledge regarding bariatric surgery was revealed in the respondents with positive history of another operation and smoking respondents. The association of psychological barriers was dominantly revealed with rural residence and with university diploma while the urban residents mainly stated doctors related barriers. The knowledge related barriers were mostly demonstrated by respondents with positive family history of morbid obesity and positive history of another operation.

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THE ROLE OF RESIDENT BACTERIAL-FUNGAL INTERACTIONS IN BIOFILM FORMATION DURING WOUND INFECTIONS: DOES BIOFILM FORMATION IN ECOLOGICAL NICHES CONTRIBUTE TO NORMAL FUNCTIONING IN VERTEBRATE MAMMALS?

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Abstract

Recent discussions have focused on the distribution of opportunistic-pathogenic and pathogenic microorganisms that migrate from their biological ecological niches to internal organs and tissues under pathological conditions. Resident gram-negative and gram-positive microorganisms colonize soft tissues, providing optimal conditions for their persistence. Apparently, a similar migration process occurs with resident pathogenic and opportunistic-pathogenic fungi. Resident microorganisms persisting in the soft tissues of wounds begin to form new structures, so called biofilms, which foster new functional interactions between bacteria and fungi. Simultaneously, the role and relative proportion of biofilm-forming microorganisms in the wound inflammatory process have not yet been fully established. Fungi within the biofilm, through their mycelia and pseudohyphae, provide an optimal surface for the adhesion of resident bacteria.

Such an interaction between bacteria and fungi, through biofilm formation, can be realized in three ways under pathological conditions: 1) By simultaneously activating the pathogenic potentials of both fungi and bacteria, 2) By activating only one type of microorganism, while preserving the principle of commensalism with others. 3) By activating only one type of resident microorganisms while inhibiting the activity of another.

Apparently, the formation of biofilms enhances the pathogenic potentials of both resident bacteria and fungi simultaneously, which has a negative effect on the nature and progression of the wound process, especially when it becomes chronic.

Our previous studies have shown that many resident-pathogenic bacteria, including E. coli, play an active role in maintaining immune homeostasis, cardiovascular activity and gastrointestinal function under normal conditions by modulating the production of biologically active factors with immunomodulatory, endocrine-stimulating and cardiostimulating effects.

We believe that the activity of resident opportunistic-pathogenic bacteria in a healthy organism follows the only evolutionarily justified mechanism: the maintenance of their existence by utilizing the energy resources of the macroorganism, while simultaneously participating in the activity of integrative systems at all levels of structural organization. In this regard, we pose the following question: Does bacterial-fungal biofilm formation occur in their ecological niches under normal functioning of vertebrate mammalian organisms?

Keywords: *wound, bacteria, fungi, biofilm, bacterial-fungal interactions in normal and pathological conditions.*

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Introduction

The interaction between microorganisms, involving both bacteria and fungi, leads to the formation of unique microstructures referred to as microbiomes. These microbiomes create optimal conditions for the joint activity of microorganisms within the host macroorganism, particularly in case of local or diffuse infectious and inflammatory processes.

Currently, there is no clear differentiation between the structural formations referred to as microbiomes (biofilm). In particular, this concept often refers to bacterial biofilms, which are presented by mono- or polymicrobial formations.

Such bacterial biofilms form aggregates within the macroorganism, embedded in a protective matrix composed of extracellular structures such as proteins and polysaccharides [*Vestby L et al., 2020; Nesse L et al., 2023*]. One aspect of this issue should be clarified. This review focuses on the role of specific intracorporeal microorganisms – bacteria and fungi, that participate in the formation of bacterial-fungal interactions, often referred to as "biofilm". Pathogenic and opportunistic-pathogenic fungi, when proliferating within the macroorganism, create a mesh-like film structure to which pathogenic and opportunistic-pathogenic bacteria adhere. Bacterial adhesion occurs via fungal hyphae that form the mycelium. These bacterial-fungal interactions are collectively known as "biofilms" [*James G et al., 2008; Clinton A, Carter T, 2015; Percival S et al., 2015*].

What is the biological role of bacterial-fungal biofilms in the onset and progression of a wide range of infectious diseases, including wound infections? In the case of the latter, biofilm structures can contribute to the infection process, which may be triggered by various factors: thermal (e.g., burns, frostbite and gunshot wounds), chemical (e.g., exposure to acids, alkalis, toxic products, including poisonous household and military substances) and traumatic (e.g., household and workplace injuries, knife wounds, crush syndrome), and others.

As we have previously noted, mixed bacterial-fungal microflora often persists in wound lesions during the infectious process [*Groll A, Walsh T, 2001; Gnat S et al., 2021; de Hoog S et al., 2024*].

Such regional inflammatory process is characterized by a severe clinical course and is difficult to treat with all known anti-inflammatory, antibacterial and antifungal drugs [*Becker W, 1991; Dowd S et al., 2011; Pfaller M, 2012; Kapoor G et al., 2017; Reygaert WC, 2018; Berman J, Krysan D, 2020; Pruskowski K, 2021*].

Over the past three decades, researchers have focused on identifying the structural and functional relationship between resident bacteria and fungi persisting in wounds.

A significant emphasis has been placed on this issue in relation to the phenomenon of "bacterial translocation" in the diseased organism [*Deitch E, 1994; Sousa L et al., 1996; Adawi D et al., 2001; Demetriades D et al., 1999; Pfalzgzaff A et al., 2018; Zegadlo K et al., 2023*], where opportunistic-pathogenic and pathogenic resident microorganisms leave their ecological niches (e.g., oral cavity, gastrointestinal tract) and begin to colonize new organs and tissues, initiating pathological processes [*Steffen E, Berg R, 1983; Gautreaux M et al., 1994; Sousa C et al., 1996; Wang X et al., 1996; Nikitenko V et al., 2001; Sahakyan K, 2005; James G et al., 2008; Clinton A, Carter T, 2015; Percival S et al., 2015; Zilfyan A, 2016*]. In this context, the wound inflammatory process is not an exception, particularly when not only bacterial microflora but also mixed bacterial-fungal microflora persist in the wound. The presence of a fungal infection significantly exacerbates the wound process, as fungi not only contribute toxicity but, in association with bacteria, often enhance the pathogenic potential of the bacteria.

In our opinion, the concept of the "microbiome", which includes not only bacteria but also fungi, should be considered from a qualitatively new perspective. Under pathological conditions, newly formed bacterial-fungal communities begin to function, establishing a characteristic network, commonly referred to as a "biofilm".

But what is this network? A complex fungal network provides a surface for the adhesion of a wide variety of pathogenic and opportunistic-pathogenic bacteria [*Dowd S et al., 2011; Short B et al., 2023*]. In fact, the biofilm is an optimal structural microobject, where associative relationships between bacteria and fungi are formed, explaining their long-term persistence in pathologically altered lesions.

Unfortunately, to date, aspects related to the

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potential functioning of such bacterial-fungal interactions under the physiological conditions of vertebrate mammals are not fully understood.

It is possible that associations formed by resident specific bacteria, fungi and archaea have a beneficial stimulatory effect on various integrative functions, primarily through the targeted synthesis and exocytosis of biologically active substances, particularly those with adaptogenic properties. In our opinion, this aspect of the associative activity of resident microorganisms needs further investigation, as it offers significant perspectives for understanding integrative processes within the interaction of micro- and macroorganisms.

The role of archaea in the vertebrate mammalian organisms remains insufficiently studied. However, even the limited available data suggest that archaea play a significant role in the integrative functions of humans. Thus, it has been established that archaea are involved in methane production during anaerobic respiration. Methanogenic archaea (methanogens) are found in the oral cavity, skin, lungs and gastrointestinal tract. Archaea are present in microbiomes, where they form biofilms with bacteria. In these biofilms, archaea can promote the growth of pathogenic bacteria [*Kuznetsova A, Dukat A, 2022*].

Microbiologists, bacteriologists and infectious disease specialists face considerable challenges in interpreting the nature of biofilms. It remains unclear whether biofilms should be viewed solely as a factor-substrate of the pathogenicity of resident microflora, or if they represent an adaptive process that has evolved in vertebrate mammals, especially humans, enabling microorganisms to adapt to the physiological conditions of the macroorganism.

It is widely believed that biofilm formation occurs primarily in organs and tissues under pathological conditions, particularly in a wide range of somatic diseases, and primarily, infectious bacterial and viral diseases.

From our point of view, the biological purpose of the microbiome should be considered from three perspectives:

From the perspective of biofilm formation, aimed at maintaining favorable conditions for microorganisms during their activity and persistence within the macroorganism, i.e., from a perspective that is beneficial only to the microorganisms.

From the perspective of the production of endogenously active substances by resident microorganisms, which have a modulatory effect. These substances support the activity of various integrative functions, particularly under pathological conditions, facilitating the development of protective and adaptive reactions.

From a mutually beneficial perspective, through which the macroorganism begins to function on a qualitatively new basis – on the principle of mutual interdependence with resident microorganisms.

A few words about the concept of "biofilm" that is frequently used by microbiologists and infectious disease specialists in their scientific studies. The term "biofilm" can also be referred to as "biofilm".

It is not clear what type of biofilm is being referred to. Is it a biostructure within the body that forms films? Are these artificially created biofilms, treated with various medicinal compositions for the treatment of various diseases? Moreover, the term "biofilm" lacks a principle that would define it as a specific community of microorganisms interacting within the macroorganism in the form of a pellicle.

That is why, we believe that the term "biofilm" by itself is not sufficiently informative, especially when interpreting the processes of persistence of opportunistic-pathogenic and pathogenic microorganisms within the ecological niches of the macroorganism.

In modern microbiology and bacteriology, the concept of the "'tree of life of microorganisms" is well-established, encompassing bacteria, eukaryotes and archaea. These domains form highly complex interactions within the macroorganism, the overall activity of which remains insufficiently studied to this day.

In our view, the mentioned microbial domains should be defined not as a biofilm, but rather as "bacterial-fungal" and "bacterial-viral interactions".

The term "interactions" refers to the structural and functional connections between specific microorganisms, which are realized through two mutually exclusive mechanisms.

The first mechanism involves the functioning of these microbial domains based on principles of interrelation and interdependence, aimed at maintaining the activity and persistence of two or three domains.

Through the second mechanism the persistence processes are realized through the activation of

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only one domain of microorganisms, which, under conditions of increased persistence, suppresses the growth of the other two domains.

It is important to note that in many pathological conditions, microbial interactions primarily function through the first mechanism. Thus, bacterial-fungal resident interactions operate according to this mechanism when, under conditions of fungal reproduction, a biologically active network (a pellicle) is created in the tissues of the macroorganism. This network provides a surface to which bacteria can adhere, thereby creating favorable conditions for their activity and persistence. In this case, the pathogenic potential of bacteria is significantly enhanced.

In our opinion, one important aspect should be noted. The presence of bacterial and fungal communities in the form of a structurally organized biofilm is primarily considered from the perspective of pathology, especially in the context of a wound inflammatory process caused by various provoking factors.

This raises the question: What are the interbacterial-fungal relationships that, in our view, developed in the normal organism during its evolutionary process? This concerns the possible formation of either symbiotic or, contrarily, antagonistic relationships between the macroorganism and resident microorganism.

Undoubtedly, their functional interaction in physiologically established ecological niches (such as the oral cavity, digestive system and possibly the organs of immunogenesis) should only be activated after the structural organization is established, i.e., the formation of a bacterial-fungal matrix, which ensures their positive role in enabling the simultaneous survival of both biosystems (both macro- and micro-).

Unfortunately, similar studies in this sphere have not been conducted. However, we believe that studying the symbiotic activity of bacteria and fungi will significantly advance our understanding of the processes that occur under normal conditions at both the macro- and microbiological levels.

Over the past 70 years, studies have shown that a wide range of resident opportunistic-pathogenic microorganisms (both bacteria and fungi) colonizing the digestive system realize their pathogenic functions due to the presence of aliphatic polyamines – putrescine, spermidine, and spermine, which are essential for their activity and reproduction within the macroorganism [*Merali S, Clarkson A, 1966; Pfaller M et al., 1988; Cervantes Olivares R, 2003; Degreef H, 2008; Gerner E, Meykens F, 2009; Hullar M, 2013; Zilfyan A, et al., 2020; Hakobyan E et al., 2023*].

This circumstance indicates the necessity of significantly correcting the known, ineffective treatment regimens for bacterial and fungal infectious diseases, and, primarily, the wound inflammatory process.

Considering a number of informative studies that have evaluated the biological effectiveness of α-difluoromethylornithine in suppressing polyamine synthesis in bacteria and fungi [*Boyle S et al., 1988; Walters D, 1995; Heby O et al., 2003; Flamigni F et al., 2007; Mounce B et al., 2016; Huang M, 2020*], we believe that further studies should be conducted using α-difluoromethylornithine and/or its analogs (such as the widely used commercial drug “Eflornithine”).

Moreover, in the process of palliative wound treatment, we propose using a polyamine-free or polyamine-deficient diet. A recommended list of suitable food products can be found in our previous studies [*Avagyan S et al., 2023*].

These therapeutic and preventive measures should only be implemented following a thorough bacteriological analysis, which will result in polyamine-containing pathogenic and/or opportunistic bacteria and fungi isolated from the wound (and possibly from blood and urine).”

In conclusion, I believe it is both appropriate and, perhaps, even necessary to share several of our considerations regarding the biological significance of resident (i.e. intracorporeal) microorganisms that persist in ecological niches, not only under pathological conditions but also during the normal functioning of our body.

For many years, it has been believed that microorganisms found in human organs and tissues are primarily pathogenic for humans, responsible for a wide range of infectious diseases. Many infectious diseases, especially in the Middle Ages, resulted in fatal outcomes for humans. A similar situation occurred when humans faced zoonotic infectious diseases. In many cases, the same pathogens were harmful to both humans and animals. Massive fatal outcomes were

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observed in diseases such as leprosy, plague, anthrax, tuberculosis and diphtheria. Often, the widespread nature of these infectious diseases was expressed in the form of epidemics and pandemics.

It never occurred to our ancestors, both ancient and modern, that the bacteria, viruses and fungi living in our bodies, under normal physiological conditions, perform functions that are fundamentally different from those described by many infectious disease specialists, immunologists and pathologists when interpreting the etiology of various infectious diseases. Currently, we believe that scientists, especially those working in various spheres of medicine and biology, should adopt an objectively justified approach based on a single fundamental principle of development of macro- and microorganisms: the evolutionary approach. This perspective highlights the long-established relationship between the macroorganism and the microorganisms living in it. Over centuries, this relationship has evolved into a mutually beneficial one, benefiting both the human and the resident (intracorporeal) microorganisms.

The maintenance of symbiotic relationships between the macroorganism and its resident microorganisms was supported by mutually beneficial functions. On one hand, the integrative functions of the macroorganism are essential for its survival, while, on the other, the participation of intracorporeal resident microorganisms in these functions plays a crucial role in the proper functioning of various organs and systems. Unfortunately, it was only in the last two centuries that modern scientists began to embrace this perspective. During this period, informative data demonstrated that resident microorganisms, through their activity, produce a wide spectrum of biologically active substances of diverse natures. These substances have a profound impact on many physiological functions, including those of the immune, nervous, endocrine and cardiovascular systems. The synthesis of these endogenously active compounds is essential for maintaining the functional activity of integrative systems while also supporting the survival of resident microorganisms within the host organism [*Zilfyan A, 2016*].

A well-founded hypothesis by Professor Aleksanyan A.B., an academician of the Academy of Medical Sciences of the former USSR, which is presented to colleagues in a strictly authorial version: “The diphtheria microbe, when in the unusual conditions of an immune (vaccinated) organism, loses its aggressive properties. As a result of changes and the emergence of new conditions for the existence of the diphtheria pathogen, now it’s the time when its pathogenic nature is transforming towards saprophytization, in other words, into a harmless state for children [*Aleksanyan A, 1957*].

In our opinion, the microbe-host relationship, under normal conditions of the organism’s activity, should be based on at least two fundamental principles:

The principle of synergistic relationships among resident microorganisms within a single interaction. Inter-associative connections between different microorganisms, such as bacteria and fungi, are possible, particularly within various target organs where they persist.

The development of the infectious process is due to the disruption of the “dynamic” equilibrium between the evolutionarily established relationships in various microbial interactions.

The principle of biological expediency in the “coexistence” of microbial interactions and the host organism, developed through evolutionary processes, involves micro- and macro-structures, optimized for the growth and vitality of microorganisms, while also supporting the integrative functions of the host organism.

Ten years ago, one of the co-authors of this publication proposed the hypothesis that “intracorporeal interactions of resident microorganisms should be considered as an independent system in the integrative activity of the mammalian organism” [*Zilfyan A, 2016*].

The problem of long-term persistence of opportunistic-pathogenic and pathogenic bacteria and fungi in a macroorganism remains a topic of special study. Under pathological conditions, resident microorganisms initiate interactions within their ecological niches to ensure their survival utilizing the host’s energy resources. We propose that pathogenic and opportunistic-pathogenic fungi, persisting in the same ecological niches, not only contribute to the development of infectious processes but also carry out their functional activity under normal conditions through the formation of

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their hyphae (mycelium and pseudomycelium).

There is no alternative method for resident fungi to adhere to the tissues that form the ecological niches within the host's organism. Under normal conditions, intracorporeal resident fungi seem to function based on the unique features of their structural organization – creating mesh-like microstructures. In the same ecological niches and under the same conditions of normal functioning, intracorporeal resident opportunistic-pathogenic and pathogenic bacteria perform their multifaceted physiological functions.

In our view, when resident bacteria "interact" with the fungal network within each specific ecological niches, bacterial-fungal interactions, i.e., "biofilms", are formed.

A completely different situation arises under pathological conditions, when a local and/or diffuse infectious process occurs, triggered by a variety of provoking factors. In such cases, the virulent potentials of not only pathogenic but also opportunistic-pathogenic bacteria and fungi are significantly activated.

In our view, a structural and functional rearrangement of biofilms occurs, with their components, as their virulent potentials increase, beginning to exert an alternative (damaging) effect on somatic cells and the interterritorial matrix, both at the site of the wound and on the cellular and extracellular structures of internal organs. In this case, the infection spreads through the penetration of microorganisms into internal organs via hematogenous and lymphogenous pathways.

Moreover, it is not excluded that other virulent bacteria, migrating from different ecological niches within the macroorganism, may adhere to the surface of the fungal reticular structures.

Given this, we pose a very legitimate question, the answer to which would allow us to characterize, from a qualitatively new perspective, the evolutionarily formed "mutually beneficial" symbiotic relationships between the macroorganism and the intracorporeal (resident) bacterial-fungal interactions.

More specifically, the question is as follows: Is the principle of "biofilm" formation in ecological niches involved under the conditions of normal functioning of the macroorganism?

Our answer to the question we posed can be found in our monograph published in 2016: "The System of Intracorporeal Resident Microorganisms Involved in the Normal Functioning of the Organism" [*Zilfyan A, 2016*]. This definition should be understood as the associative activity of resident microorganisms (bacteria, fungi, and viruses) that persist in the host's ecological niches. These microorganisms function in a healthy organism through a mechanism that is evolutionarily fixed and well-justified: they maintain the host's existence by utilizing energy resources of the macroorganism, while simultaneously participating in the physiological activity of integrative systems at all levels of their structural organization.

Conclusion

In the organism of vertebrate mammals, under pathological conditions, especially in case of a chronic infectious process, biofilms of varying structural organization begin to form, depending on the types of resident bacteria and fungi. While bacterial biofilms have been widely described, fungal biofilms are less studied, and bacterial-fungal interactions in pathological lesions are relatively underexplored. However, this distinction is somewhat subjective, as mixed bacterial-fungal interactions often persist in pathological lesions, especially in newly formed ecological niches. Both resident opportunistic and pathogenic bacteria, as well as fungi, participate simultaneously in biofilm formation. The proportion of bacteria and fungi within the biofilm matrix can vary widely, depending on several factors, including the nature of the infectious process, its chronicity and the degree of immune suppression of the organism. The state of the biocenosis formed by resident bacteria and fungi within an ecological niche is largely determined by the specific relationships formed between them.

The formation of biofilms and their functional dynamics in pathological conditions depend on several factors, including:

The taxonomic characteristics of the specific bacteria and fungi.

The symbiotic and antagonistic relationships persisting in bacterial ecological niches.

The symbiotic and antagonistic relationships persisting in fungal ecological niches.

The virulence potentials acquired by both bacteria and fungi as they persist within a single ecological niche.

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The emergence of bacterial translocation, wherein bacteria persisting in one ecological niche begin to migrate and colonize new ecological niches within the macroorganism.

As previously noted, when a matrix is formed solely by bacteria, it is composed of the products of their metabolic activities (and potentially their decay), such as proteins, glycoproteins, extracellular (free) DNA, and other substances. The structure of the bacterial matrix is highly variable, depending on the taxonomic and virulent potentials of various bacteria. It depends on the nature and intensity of the catabolic products they produce that facilitate the adhesion of bacteria to the matrix.

It is noteworthy that a range of advanced researchers have tried to characterize the specific stages of bacterial biofilm formation, often referred to as "biofilms" [*Carniello V et al., 2018*], which were divided into four stages:

Transfer of bacterial mass to the surface.

Reversible bacterial adhesion. This stage is characterized by weak desmoplastic connections between the bacterial matrix and surrounding somatic cells, as well as the extracellular environment.

The stage of irreversible adhesion, characterized by more intense production of bacterial biofilm material for matrix formation, leads to the development of more pronounced desmoplastic processes between the resident bacteria adhered to the matrix surface and the peripherally oriented cellular and extracellular structures of the host organism.

Deformation of the bacterial cell wall and acquisition of emergent (atypical) properties.

It's interesting what the authors mean by this dynamic process of bacterial biofilm formation by using the terms "deformation" and "emergent properties of bacteria".

Both expressions do not fully capture the structural and functional changes that bacteria undergo during this stage. Regarding "deformation", it is unlikely that bacteria persisting in biofilms undergo "mass" destruction of their cell walls, leading to general deformations of the cytoplasmic membrane at the initial stages of biofilm development. It is unlikely that bacteria forming biofilms can acquire new properties that are not characteristic of them. In this context, we believe that the focus is on the enhancement or, conversely, the weakening of the virulent potential of each bacterial associate, as well as the adhesive capacity of the outer membranes of bacterial cells, which enable these bacteria to move not only within the matrix but also migrate to neighboring ecological niches.

It is possible that this migration process, where bacteria leave one biofilm and colonize new areas, might represent the beginning of bacterial translocation. Unfortunately, this process, through which bacteria colonize new ecological niches by migrating from established biofilms, has not been thoroughly studied before the publication of this article.

A similar gradation of reversible and irreversible adhesion processes has also been described in relation to the formation of biofilms (matrices) involving resident pathogenic fungi [*Gaffarova A, Khaytovich A, 2017; Abdulkair W, 2018; Enokaeva O et al., 2021*].

As early as 30 years ago, some leading scientists – microbiologists and bacteriologists expressed a prophetic thought for that time: "Even monomorphic bacteria within a single colony can differentiate into cells of different types, and that is most important, they form multicellular structures with highly organized architectures; the associative activity of such bacteria is subject to chronological control" [*Shapiro J, 1988*].

Moreover, there is an opinion that certain bacterial interactions exhibit specific skills characteristic of highly organized organisms. In the process of colonizing a macroorganism, some bacterial populations facilitate the advancement of the entire microbial community through biological substrates, while others ensure the functional activity within the macroorganism (e.g., production of biologically active substances with physiological and alterational effects, or by acquiring virulence properties). Some populations also provide the adhesive properties necessary for the persistence of other populations within the macroorganism's ecological niches.

In our view, the specific populations of bacteria that undergo adhesion are precisely those that function within the host organism by forming biofilms, i.e. biofilms.

As a result of analyzing the literary sources, it becomes evident that the structure of the matrix on which resident bacteria and fungi adhere is not straightforward, as it depends on the specific types of resident microorganisms involved in the forma-

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tion of biofilms.

In cases where only bacteria participate in the formation of the matrix, it is composed of their metabolic and degradation products (such as proteins, polysaccharides and free DNA). However, when both resident bacteria and fungi are involved in biofilm formation, the matrix structure becomes more complex. Thus, the statement that the hyphae of resident fungi, due to their anatomical features, merely serve as a substrate on which resident bacteria adhere (i.e., undergo adhesion) appears somewhat unjustified. That's why, the focus should not only be on the products of bacterial catabolism, as resident fungi themselves possess significant synthetic potential, particularly in producing a wide range of biologically active compounds, which also play a direct role in biofilm formation.

According to our hypothesis, under normal physiological conditions, opportunistic-pathogenic and pathogenic resident bacteria and fungi function within the biological ecological niches of the macroorganism. If our hypothesis is valid, then the structure of resident bacterial-fungal biofilms in a healthy organism should be markedly different from those formed during pathological conditions.

Thus, under normal conditions, the structural and functional organization of bacterial-fungal biofilms must adhere to the following criteria:

Firstly, the ecological niches in which resident bacteria and fungi collectively form biofilms must be strictly determined.

Secondly, only the bacterial-fungal interactions characteristic of each specific ecological niche should persist within it.

Thirdly, the functional activity of such ecological niches, in which resident bacteria and fungi persist, is selectively directed toward supporting many integrative functions of the macroorganism through the synthesis of a range of physiologically active substances that play a direct role in regulating organs and systems. Apparently, such synchronized activity of resident intermicrobial interactions is embedded in the evolutionary development of macro- and microorganisms, functioning on the principles of interdependence.

In pathological conditions, bacterial-fungal interactions in the macroorganism exhibit distinct structural and functional characteristics that differ significantly from those present during normal conditions. These interactions must meet the following criteria:

Firstly, the types of bacteria and fungi significantly differ from those that persist in the ecological niches under normal functioning conditions of the macroorganism.

Secondly, such resident microorganisms exhibit pronounced virulent properties.

Thirdly, high-virulence strains of microorganisms begin to function not only in evolutionarily formed ecological niches but also as the pathological process progresses.

Fourthly, in such a situation, the "phenomenon of bacterial translocation" starts to function, whereby resident microorganisms start migrating from their ecological niches and colonizing new ones.

Fifthly, the acquisition of new virulent potential largely depends on the production of biologically active substances, particularly with toxic effects, by resident bacteria and fungi, which has a profoundly negative impact on the course of pathological processes of various origins, including both infectious and non-infectious etiologies.

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EFFECTIVENESS OF THERAPEUTIC PLASMA EXCHANGE IN COMPARISON WITH STANDARD OF CARE IN THE TREATMENT OF YELLOW PHOSPHORUS POISONING: AN OBSERVATIONAL STUDY IN SOUTH INDIAN POPULATION

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Abstract

Yellow phosphorus poisoning is commonly seen in India, predominantly due to intentional self-harm. It is known to cause acute liver failure, which is a major contributory factor for its high mortality. Liver transplantation has been the definitive treatment in patients fulfilling the King's College Hospital Criteria for liver transplantation. Therapeutic plasma exchange is an alternative treatment option that has shown promising results with significantly improved outcomes, in various studies.

We conducted a single centre, prospective observational study in a tertiary care hospital in Southern India from August 2022 to March 2024. 46 patients were included in the study, 23 of whom underwent therapeutic plasma exchange. Patients were monitored throughout their hospital admission and were further grouped into those that fulfilled the King's College Hospital criteria and those that did not.

Fulfilment of King's College Hospital criteria was found to be a poor prognostic indicator, with a mortality rate of 84% ($p<0.001$). Survival rate of 65% was seen in patients who underwent therapeutic plasma exchange ($n=15$), when compared to 57% ($n=13$) in those that did not ($p=0.546$). Among patients who met King's College Hospital criteria, a mortality rate of 100% was seen without therapeutic plasma exchange ($n=8$), which was reduced to 72% ($n=5$) with the usage of therapeutic plasma exchange ($p=0.228$). Survival, among patients who underwent therapeutic plasma exchange, was directly proportional to number of cycles of therapeutic plasma exchange that they underwent ($p=0.007$). Early initiation of therapeutic plasma exchange (within 5 days of yellow phosphorus poisoning) had a survival rate of 73% ($n=8$), when compared to 58% ($n=7$) in those whom therapeutic plasma exchange was initiated after 5 days ($p=0.46$).

Improved survival rates were seen in yellow phosphorus poisoning patients that underwent therapeutic plasma exchange, especially in those that met the King's College Hospital criteria. However, statistical significance could not be established. Larger multicentric randomized controlled trials are needed to further analyse outcomes with the usage of therapeutic plasma exchange.

Keywords: *yellow phosphorus poisoning, plasmapheresis, therapeutic plasma exchange, King's College Hospital criteria, acute liver failure*

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Introduction

Yellow phosphorus is a commonly found ingredient in products such as rodenticides, fireworks, fertilizers, etc. Accidental or intentional consumption of such compounds is commonly seen in the Indian subcontinent [*Mark K et al., 2021*]. Yellow phosphorus poisoning is a challenging menace due to its widespread availability compounded by its toxic effects on the human body. The compound, once it enters the circulation, gets concentrated primarily in the liver, and to a lesser extent in other organs such as kidneys, heart, brain and pancreas [*Soni J et al., 2020*]. As a result, acute liver failure is the most common manifestation of yellow phosphorus poisoning [*Gopalakrishnan S et al., 2020*].

Clinical course after consumption of yellow phosphorus can be divided into 3 phases [*Mishra A et al., 2017*]. Gastrointestinal symptoms predominate usually within the first 24 hours which include perioral burns, nausea, vomiting, loose stools, and abdominal pain. During the second phase which lasts between 1-4 days, the patient may remain asymptomatic. After systemic absorption into the blood, the third phase starts wherein symptoms and signs of organ failure develop after 4-7 days. yellow phosphorus is considered highly toxic. There are scarce epidemiological data regarding the ingestion of yellow phosphorus in the subcontinent. Objectives: This study aimed to identify the clinical profile of rodenticide-poisoned patients and delineate mortality predictors. Design: Prospective observational study. Setting and participants: Study was conducted at the Department of Internal Medicine, Government Villupuram Medical College and Hospital. All adult inpatients with a history of rodenticide poison exposure were eligible participants. A total of 99 patients completed the study protocol. Main outcome: Survival with or without morbidity and death. Results: In all, 90.91% of patients consumed the paste formulation of rodenticide [yellow phosphorus (67.2%These include jaundice, bleeding manifestations due to coagulopathy, renal failure and encephalopathy. Monitoring of lab parameters show leukopenia along with coagulopathy which may be seen as early as 2 days. This is followed by rise in liver enzymes along with increasing bilirubin levels. Serum creatinine concentrations can go up in those cases where significant amounts of yellow phosphorus have reached the kidneys leading to acute kidney injury.

As there is no antidote available to neutralise the effects of yellow phosphorus in the body, treatment mainly revolves around supportive management in the form of early gastric lavages followed by usage of N-acetyl cysteine which acts as an antioxidant, thus attempting to minimize the toxicity [*Bhat S et al., 2015*]. As part of standard treatment, N-acetyl cysteine is administered in most centres, using doses similar to that which have been approved for use in paracetamol poisoning, that is, the 21-hour regimen which consists of 3 doses; loading dose of 150 *mg/kg* infused over 1 *hour*, then followed by second dose of 50 *mg/kg* infused over 4 *hours*. The third dose of 100 *mg/kg* is then infused over 16 *hours*.

Outcomes have, however, been very poor as many patients present to the hospital once the signs and symptoms of liver failure have already developed [*Fernandez O et al., 1995*]. Liver transplantation has been traditionally considered the only definitive treatment option in patients who develop acute fulminant liver failure [*Saraf V et al., 2015*].

Plasmapheresis or therapeutic plasma exchange is a process, which in principle, involves the removal of unwanted compounds or proteins present in or bound to other proteins in the plasma, by exchanging significant volumes of plasma itself and replacing it with other colloids such as albumin or fresh frozen plasma [*Kohli R et al., 2022*]. Various centres have attempted using therapeutic plasma exchange for mitigating the effects of yellow phosphorus poisoning, with variable outcomes. Review of literature has shown various studies that have demonstrated significant biochemical improvements after therapeutic plasma exchange when compared with standard treatment, without any major adverse events [*Varghese J et al., 2020; Angraje S et al., 2021; Radhakrishnan K et al., 2023; Rohini R, Routray M, 2024*]. It has been postulated that one mechanism by which therapeutic plasma exchange can be beneficial in yellow phosphorus poisoning cases is by providing physiologically important compounds that are present in fresh frozen plasma used as a replacement fluid. Another hypothesis suggests that PLEX helps by removing toxic compounds such as Damage Associated Molecular Patterns and other toxins in plasma, and

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thus reducing the toxic effects of yellow phosphorus that has already been absorbed in blood with an ongoing pathological response by the body [*Varghese J et al., 2020; Angraje S et al., 2021*].

In patients that fulfil King's College Hospital criteria for liver transplantation, outcomes are seen to be worse than in those patients that do not meet the criteria [*Varghese J et al., 2020; Mathew J et al., 2021; Mohanka R et al., 2021*]. However, therapeutic plasma exchange has shown to benefit these patients even without liver transplantation and overall survival benefit is seen. No guidelines have, as of yet, been established for the treatment of acute liver failure due to yellow phosphorus poisoning using plasmapheresis. Our study aims to establish the role of therapeutic plasma exchange in yellow phosphorus poisoning by comparing primary outcome of death in those that underwent therapeutic plasma exchange with those that received standard treatment alone, to help develop concrete guidelines for treatment of yellow phosphorus poisoning and incorporating therapeutic plasma exchange as part of standard care for yellow phosphorus poisoning. Subgroup analysis between those that fulfilled King's College Hospital criteria and those that did not, was also done.

Material and methods

We conducted a single centre prospective observational study in a tertiary care centre in South India between August 2022 to March 2024. Institutional ethics committee approval was obtained (IEC2:444/2022). Adults >18 years of age who consumed yellow phosphorus were included in the study. Exclusion criteria included those who consumed a mixture of poisons, those who had an underlying chronic liver disease of any aetiology.

With 80% power and mortality difference taken as 20% (based on available studies) and 5% level of significance the sample size calculated was minimum of 23 in each group. It was calculated using PASS software for two independent differences of proportions

The study involved the comparison of standard treatment in yellow phosphorus poisoning with that of therapeutic plasma exchange. Currently, there are no definitive guidelines for treatment of yellow phosphorus poisoning and standard treatment involves supportive care with gastric lavage, vitamin K supplementation, fresh frozen plasma (if needed) and N-Acetyl Cysteine infusion (regimen given for paracetamol poisoning). All the permissions and clearances were obtained before starting the study. Consent was taken from every participant included in the study. Those 46 participants adhering to the selection criteria were taken for the study and routine lab investigations that were sent as standard of care were observed serially. The treatment protocol that was initiated for every patient was observed. No audio/video recording was involved in this study and the study involved only thorough examination of the patient and routine investigation (Complete Blood Count, Renal Function Test, Liver Function Test, Prothrombin Time/ International Normalized Ratio, Ultrasound of Abdomen, Hepatitis B Surface Antigen, Anti-Hepatitis C Virus Antibodies). All the participants who were selected for the study were classified into different groups based on the treatment that they undergo (standard/therapeutic plasma exchange). No additional tests were done. Participants were followed up for up to 90 days, via a phone call, for determining survival.

Data collection was by taking proper clinical details, history, relevant investigations and entering them into predesigned proforma. Data analysis was carried out using SPSS version 29. Mean, median and standard deviation were used for summarizing data with continuous variables while categorical data was analysed using frequencies and percentages. Further contrast and correlation between different groups of participants was done using student's t tests, Pearson's Chi-square tests and Fischer's exact tests, with a p value below 0.05 considered to be statistically significant. The outcome was then compared in the two groups in terms of duration of hospital stay, King's College Hospital criteria, mortality.

Results

A total of 46 participants were part of the study out of which 23 underwent therapeutic plasma exchange and 23 that did not. The youngest patient was 18 years while eldest being 76 years, and with the mean age being 31 ± 13 years. Average duration of hospitalisation was 10.4 ± 7.2 days. Other patient characteristics are mentioned in table 1.

An overall mortality rate of 39% (n=18) was

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seen in our study. The mean day of initiation of therapeutic plasma exchange was 5.4 ± 2.2 day. Overall, 65% of participants in the therapeutic plasma exchange group survived (n=15), while 57% of participants in the standard treatment group (n=13) survived (p value – 0.546), as shown in table 2.

The 73% of participants who were initiated on therapeutic plasma exchange within 5 days survived while only 58% of participants, who were initiated on therapeutic plasma exchange 6th day onwards, survived (p value – 0.469). Average number of cycles of therapeutic plasma exchange done per patient were 3.1 ± 1.5 cycles. Survival rates were directly proportional to the number of cycles of therapeutic plasma exchange that participants underwent (p value – 0.007) (Table 3,Figure).

Out of 46 participants 27 were fulfilling the King's College Hospital criteria for acute liver failure; 84% of participants who fulfilled the criteria died, while only 7.4% of participants who did not meet the criteria died (p<0.001). Among the 19 participants who fulfilled the criteria, mortality was 100% if participants received standard treatment alone (8 out of 8 died). However, 3 out of 8 participants who underwent therapeutic plasma exchange survived (Table 4). Hence mortality rate was reduced to 72% (p-value 0.228).

Discussion

Intentional self-harm with yellow phosphorus poisoning has been reported widely with high incidence among South Indian population due to its easy availability and lethal toxicity. Mortality rates have been high as majority of the population belonging to low socio-economic status can neither afford liver transplantation nor have access to centres that perform it. Therapeutic plasma exchange has thus emerged as a cheaper, and more widely available alternative to liver transplantation in treating patients of acute liver failure.

Our study showed improvement in survival rates among those that underwent therapeutic plasma exchange when compared to those that did not (65% vs. 57%), which was in accordance with previous studies that have shown survival rates of 70-79% in those that undergo therapeutic plasma exchange [*Varghese J et al., 2020; Angraje S et al., 2021*]. Interplay amongst various factors that

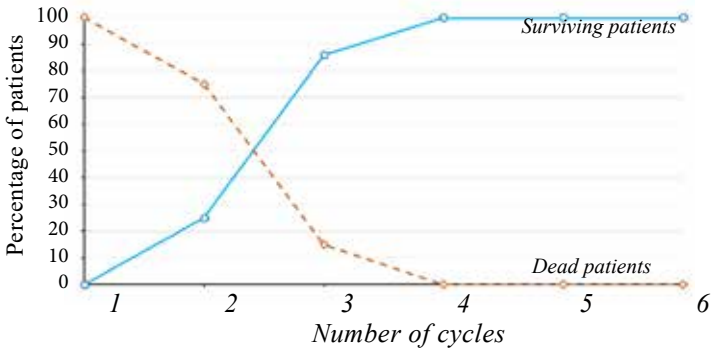

Figure. *Survival rate correlation with number of cycles of therapeutic plasma exchange*

Table 1

Descriptive Characteristics of Study Participants

Characteristics		*Groups*	
		TPE (N=23)	*Standard Treatment (N=23)*
Age (years)		33 ± 15	30 ± 10.8
Males - n(%)		15 (65%)	10 (43%)
Females - n(%)		8 (35%)	13 (57%)
Amount of Poison Consumed (grams)		12.5 ± 5.8	10.2 ± 7.6
Duration Before Seeking Medical Help - n(%)	*≤1 day*	13 (57%)	10 (43%)
	1-3 days	7 (30%)	11 (48%)
	≥3 days	3 (13%)	2 (9%)
Intubated - n (%)		5 (22%)	7 (30%)
Required Haemodialysis - n(%)		3 (13%)	-
No. of Days of Hospital Stay (days)		13.8 ± 7.9	7 ± 4.2
Meeting KCH Criteria - n(%)	*Yes*	11 (48%)	8 (35%)
	No	12 (52%)	15 (65%)
Outcome - No. (%)	*Survived*	15 (65%)	13 (57%)
	Died	8 (35%)	10 (43%)

Notes: *TPE - Therapeutic plasma exchange, KCH - King's College hospital , N(%) - Total number(перцент) of patients. n(%) - number (percent) of patients in a group.p.*

Table 2.

Subgroup Analysis of Primary Outcome

Characteristics		Survived (N=28)	Died (N=18)	p-value
Gender	Male	18 (72%)	7 (28%)	0.091
	Female	10 (48%)	11 (52%)	
	No	26 (60%)	17 (40%)	
TPE	Yes	15 (65%)	8 (35%)	0.546
	No	13 (57%)	10 (43%)	
KCH Criteria	Yes	3 (16%)	16 (84%)	<0.001
	No	25 (93%)	2 (7%)	
Day of Initiation of TPE	≤5 days	8 (73%)	3 (27%)	0.469
	>5 days	7 (58%)	5 (42%)	

Notes: *TPE - Therapeutic plasma exchange, KCH - King's College hospital , N(%) - Total number(перцент) of patients. n(%) - number (percent) of patients in a group.*

Table 3.

Comparison of Mean Characteristics Between Participants Who Survived vs. Died

	Survived (M ± SD)	Died (M ± SD)	P-Value
Dose (grams)	10 ± 6	13.9 ± 7	0.054
Duration before seeking help (Days)	1.3 ± 1.6	1.5 ± 1.5	0.746
Day of Initiation of TPE	5.1 ± 2.5	6.1 ± 1.3	0.317
Noumber. of cycles of TPE per patient	3.9 ± 1.1	1.6 ± 0.7	<0.001
Duration of hospital stay (days)	14.2 ± 6.7	4.7 ± 2.9	<0.001

Notes: TPE - Therapeutic plasma exchange, M - Mean, , SD - Standard Deviation

Table 4.

Survival Benefit with Therapeutic plasma exchange (TPE) in King's College hospital criteria (KCH) Group

KCH Criteria	Outcome	TPE Done	TPE Not Done
Yes	Survived	3 (27%)	0 (0%)
	Died	8 (73%)	8 (100%)
No	Survived	12 (100%)	13 (87%)
	Died	0 (0%)	2 (13%)

may influence the outcome were not independently analysed, such as day of initiation of therapeutic plasma exchange, duration of seeking medical help after consumption of yellow phosphorus, incidence of secondary infections, etc. As therapeutic plasma exchange involves siphoning of blood volume through an extracorporeal device, hypotension is a known complication of the procedure which can be compounded in the presence of sepsis leading to worsening of shock. Effect of this confounding factor and cause of death in these patients was not analysed separately.

Overall mortality rate due to yellow phosphorus poisoning was 39% in our study while mortality rates as high as 76.2% have been seen in previous studies [*Appavu V et al., 2019*]. Fulfilment of King's College Hospital criteria was found to be an independent poor prognostic factor with a mortality rate of 100% in those who do not undergo therapeutic plasma exchange (p value <0.001), affirming the findings of various previous studies as mentioned in the introduction. However, this was brought down to 72% in the subgroup of participants who underwent therapeutic plasma exchange. This was in line with similar studies conducted previously [*Varghese J et al., 2020*]. The King's College Hospital criteria includes variables such as prothrombin time, bilirubin levels and time to develop encephalopathy, which are all individual markers of advanced liver failure. Thus, combining such variables portends a grave prognosis in all patients with acute liver failure, irrespective of the cause.

Survival, among participants who underwent therapeutic plasma exchange, was directly proportional to the number of cycles of therapeutic plasma exchange that they underwent (p value – 0-007). This can be explained by increased removal of plasma cytokines and adhesion molecules, replacement of plasma factors, and immune modulation [*Larsen F et al., 2016*]. Yet other postulated mechanisms include removal of Von Willebrand factor from the patient's plasma and supplying ADAMTS13 which improves perfusion in the microcirculation of the liver and other vital organs, and promoting functional recovery of these organs [*Sardar D et al., 2019*]. However, it is prudent to note that correlation between survival and number of cycles of therapeutic plasma exchange in our study can be skewed, as participants who were admitted in a critically ill state could undergo comparatively lesser cycles of therapeutic plasma exchange before they expired.

Information obtained regarding time of consumption of poison and dose of poison consumed is anecdotal and approximate, as told by either patient themselves or by patient bystanders. This information could not be independently verified and hence analysis done based on this information alone may not be accurate.

There was no statistically significant correlation found between survival and earlier presentation to the hospital (p value – 0.746), as the study was conducted in a tertiary care centre that received referred cases from other primary health centres where many patients had received initial care involving stomach washes and gastric lavages. Effect of such interventions could have thus impacted the outcomes, which was not separately analysed in the current study.

Mean dose of poison consumed was 11.6 ± 6.7 grams. No significant correlation could be found between mortality and the dose of poison consumed (p value – 0.054), as opposed to previous studies [*Mohanka R et al., 2021; Angraje S et al., 2021*], where they found improved outcomes in those patients that had consumed <10g of yellow phospho-

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rus. In our study, dose of poison consumed by participants could not be verified objectively, and was only as estimate claimed by the patients themselves or the bystanders. Thus, expected results such as worsening of outcomes with increasing dose of yellow phosphorus consumed were not seen.

Majority of patients who were intubated in this study were moribund and underwent emergency intubation at a very late stage of the disease/peri-arrest state. Hence further studies are required to establish intubation as an independent poor prognostic marker for mortality.

The above-mentioned findings hint towards better outcomes in patients of yellow phosphorus poisoning that undergo therapeutic plasma exchange. With better formulated guidelines regarding optimal number of cycles and timely initiation of therapeutic plasma exchange, we can hope to improve liver transplantation-free survival in yellow phosphorus poisoning patients.

Conclusion

Acknowledgments: *Mohammed ND contributed to the background literature review, data acquisition and manuscript preparation. Arjun B and Aishwarya C contributed to manuscript preparation, design of the study and data analysis. Raghavendra R contributed to the conception and design of the study, background literature review, statistical analysis and revision of the manuscript. Sujatha BS contributed to statistics and overall editing of the manuscript.*

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CONSUMER BEHAVIOR IN ACUTE DIARRHEA TREATMENT: ANALYZING TRUST IN PHARMACY EMPLOYEES

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Abstract

Pharmacy employees play a critical role in managing minor ailments, including acute diarrhea and they are essential to ensuring the safe use of medicines and contribute significantly to public health education.

The study aim is to evaluate the role of pharmacy employees in managing minor ailment, with a specific focus on acute diarrhea. The study also explored consumer behavior in seeking treatment for diarrhea, the extent of reliance on pharmacy employees for advice, and the factors influencing consumer trust in community pharmacy services.

A survey was conducted between 2019 and the first trimester of 2021, involving 383 pharmacy consumers from various regions of Republic of Armenia, including Yerevan. The study was quantitative and the questionnaire developed based on the World Health Organization's standard guidelines. Data were analyzed using the SPSS statistical software package, employing descriptive statistics and Pearson's correlation coefficient to examine relationships between characteristics.

The results of research indicated that a considerable proportion of respondents had doctor-diagnosed health disease, while others reported no such diagnoses despite believing they had various diseases. Frequent community pharmacy visits were common, with a significant number of respondents purchasing medicines multiple times per month. Acute diarrhea and gastrointestinal disorders emerged as some of the most commonly self-managed conditions, often without specialist consultation. Only a small percent of consumers sought advice from pharmacy employees during case of diarrhea, with many relying instead on previous experience or non-professional sources.

The study highlighted general reluctance to seek professional advice from pharmacy employees, largely due to mistrust and past negative experiences. This behavior can result in irrational treatment, delayed medical intervention, and the misuse of medicines, particularly antibiotics. The findings underscore the need for enhanced public education and stronger engagement between community pharmacy employees and consumers to promote safer and more informed use of medicines.

Keywords: *pharmacy employee, acute diarrhea, pharmacy consumer, trust*

Introduction

As the most accessible healthcare professionals, pharmacy employees have significant potential to provide primary healthcare services. The role of pharmaceutical consultation is becoming increasingly important, especially with the growing prevalence of self-medication, where the phar-

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macy employee often becomes the main healthcare consultant for the patient [*Loh, P. et al., 2023*].

Diarrhea is one of the most common reasons for visiting a pharmacy, as many patients seek relief through over-the-counter medicines. To ensure optimal patient care and promote the rational use of drugs, it is crucial that pharmacy employees possess the necessary professional knowledge [*Sancar M et al., 2015; Watson M et al, 2020*]. One of the key responsibilities of pharmaceutical care is to provide consumers with the necessary advice regarding medicines. Within the pharmacist-patient professional framework, pharmacy employees are expected to deliver pharmaceutical care that is grounded in care, trust, communication, collaboration, and shared decision-making [*FIP/WHO, 2012*].

Many developed countries, such as the United Kingdom, Canada, New Zealand, have successfully integrated pharmacists into various public health programs, including the provision of treatment and counseling for managing minor illnesses [*Aly M et al., 2017; Dineen-Griffin S et al., 2020*]. In contrast, in developing countries like Armenia, the role of pharmacy employees remains largely confined to the traditional function of dispensing medicines, with public health services rarely being provided. Literature from several countries indicates that many patients are more likely to seek advice from pharmacy employees than from doctors. The primary reasons for this preference are the greater availability of pharmacy services in terms of time and financial affordability, as well as the convenience of local access [*Berenbrok L et al., 2020; Berenbrok L et al., 2022; Valliant S et al., 2022*]. Studies conducted in various countries suggest that consumers are even willing to pay for pharmacist consultations in the future, underscoring the invaluable role of pharmacy employees in ensuring the safe and effective use of medicines [*Lakić D et al., 2017; AlShayban DM et al., 2020*]. In this context, pharmacy employees are seen as key qualified professionals who educate consumers on proper medication use, thereby managing the self-medication process [*Veiga P et al., 2021*].

Acute diarrhea is a common condition in the Republic of Armenia, and in some cases, it can be effectively managed with the advice of a pharmacy employee. It is critical to assess whether pharmacy staff are adequately prepared to manage such cases. Pharmacists play a vital role in treating acute diarrhea and providing appropriate counseling, particularly in developing countries where resources are limited, and patients often prefer free medical consultations and obtaining medicines without visiting a doctor.

Raising public awareness that minor illnesses can be effectively managed in pharmacies with over-the-counter drugs and proper counseling is essential, as is increasing consumer confidence in pharmacy employees [*Aly M et al., 2017; Tew M et al., 2023*]. Properly managed pharmacy care is emphasized and even encouraged for some minor illnesses, as it can lead to optimal resource use, reduced healthcare costs, increased patient empowerment, greater consumer satisfaction, and overall improved healthcare outcomes.

In some countries, the management of minor illnesses by pharmacy staff is included in government policies, enhancing access to primary care, reducing the demand on general practitioners and emergency services for minor conditions [*Paudyal V et al., 2018; Dineen-Griffin S et al., 2020; Tew M et al., 2023*]. These programs enable pharmacy staff to provide basic primary care services in a structured manner, offering necessary counseling, treatment, or referral to a physician when needed. This approach increases access to health services, promotes rational workforce utilization, and places a strong emphasis on self-medication [*Dineen-Griffin S et al., 2020*]. Therefore, in resource-limited settings, the management of minor illnesses by pharmacy staff is considered valuable and has the potential to reduce primary care costs [*Yusuff KB et al., 2021; Nazaryan L et al., 2024*].

The aim of this study is to evaluate the role of pharmacy employees in managing minor ailment, with a specific focus on acute diarrhea. The study also explored consumer behavior in seeking treatment for diarrhea, the extent of reliance on pharmacy employees for advice, and the factors influencing consumer trust in community pharmacy services.

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MATERIAL AND METHODS

From 2019 through the first trimester of 2021, the Department of Pharmaceutical Management at YSMU conducted a comprehensive survey aimed at understanding consumer behavior related to the treatment of minor ailments, particularly acute diarrhea, in the Republic of Armenia. The survey explored key areas including medicine selection, consumer satisfaction and the role of community pharmacy employees in guiding treatment choices.

The study involved 383 pharmacy consumers across various regions of the Republic of Armenia , including Yerevan. The sample size was determined using The Survey System Version 11.0, with parameters set for a reliability coefficient of t=1.96, the first type error is with 5% probability ($\alpha = 0, 05$) and the evaluation accuracy is 3% ($\Delta = 3\%$). We considered the worst case scenario - P = 0.5, since the results of similar studies conducted in the Republic of Armenia were not found. The questionnaires were based on standard WHO survey instruments and adapted to address the specific context of the Armenian healthcare system [*WHO, 2006*].

Given the breadth of the survey, four distinct questionnaires were developed—Pharm Test A, Pharm Test B, Pharm Test C, and Pharm Test D—each focusing on different aspects of consumer behavior and community pharmacy interaction [*Avagyan et al., 2019*]. These questionnaires were approved by the YSMU Ethics Committee and were administered during consumer visits to pharmacies across the Republic of Armenia .

The survey focused on several key areas:

- Consumer demographics, including age, education level place of residence
- Presence of medically diagnosed health conditions and frequency of community pharmacy visits
- Sources of advice consulted for treating diarrhea and other minor ailments
- The role of community pharmacy employees in providing advice and recommendations for treatment
- Reasons for consumer trust or distrust in community pharmacy employees, particularly in relation to medicine purchases

The data collected were analyzed using the SPSS statistical software package (version 23.0). Descriptive statistics (frequencies and percentages) were used to summarize consumer characteristics and behaviors. Pearson's correlation coefficient was applied to assess relationships between characteristics.

RESULTS

The study involved the participation of 383 pharmacy consumers. Their characteristics are summarized in Table 1. The majority of participants were in the 51 to 60 age group, with the lowest level of involvement of those aged 18 to 29. Among the participants, 58% were from the capital and 42% were from rural areas. The majority of participants (38.7%) held postgraduate degrees.

The survey revealed that 45% of participants had various health problems diagnosed by a physician, including cardiovascular problems, gastrointestinal disorders, nervous system problems, endocrine disorders, oncological diseases.

Among the respondents, 55% reported not having any health problems diagnosed by a physician. However, some of these individuals believed they had various diseases. Additionally, 28% of respondents reported making purchases at the community pharmacy three or more times in the last month, 25%

TABLE 1

Demographics of pharmacy consumers (N = 383)

Variable	Groups	Frequency (%)
Age (years) (*N* = 383)	18–29	63 (16.4)
	30–40	70 (18.3)
	41–50	76 (19.8)
	51–60	91 (23.7)
	>61	83 (21.6)
Level of education (*N* = 383)	None	0
	High school	84 (22%)
	Postgraduate degree	148 (38.7%)
	Undergraduate degree	151 (39.3%)
Place of Residence (*N* = 383)	Central city	222 (58%)
	Rural areas	161 (42%)

NOTES: *Response rate = 100%, N- The number of respondents completing the item*

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shopped twice, and 28% shopped once (Fig. 1).

In the course of the work, the most frequently self-medicated conditions were identified, where patients often relied on the advice of a pharmacy employee. The most common conditions were acute respiratory disorders of the upper respiratory tract, which accounted for 30.7% of cases, gastrointestinal disorders, including diarrhea, constipation, heartburn, and flatulence, were the second most common at 26.5%. Pain symptoms (21.8%) and various nervous system problems (21.1%) were next commonly self-medicated.

The survey revealed that most consumers use medicines in case of diarrhea independently, without any consultation, 19% use medicines according to the pharmacy employee consultation, 11% apply to hospital physician, 10% apply to primary care physician, 4% use internet resources and only 1% use advertising source. In fact, it turns out that when it is required to take a medicine in case of acute diarrhea, most consumers resort not to the pharmacy employee but decide what medicines must be taken by themselves, so they use self-medication. And this fact indicates that the self-medication process is not controlled, as only direct consultation with community pharmacists provides efficient professional guidance for safe and appropriate OTC use. It turns out that more than half (55%) of participants use medicines without specialist consultation.

The study found that 59.8% of consumers never agree to buy drugs on the advice of a pharmacy em-

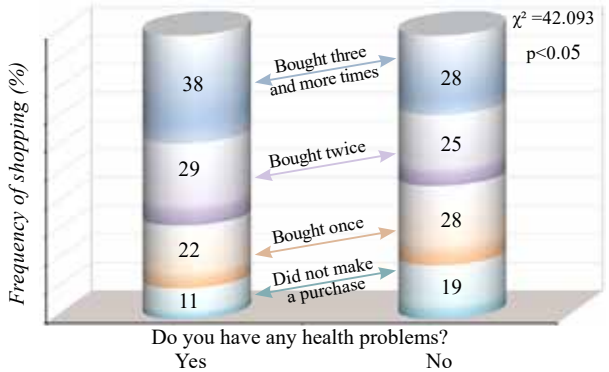

Figure 1. *Relationship between consumers having health problems and frequency of shopping at a pharmacy (N=383)*

ployees and the main reasons for such behavior for consumers were: their previous unsuccessful experience (37.8%), not trusting the pharmacy employee (30.6%), the pharmacological group of the recommended drug (15.3%), the price of the drug (16.3%)

Discussion

The management of minor ailments, particularly symptoms like acute diarrhea, is greatly influenced by consumer behavior and their interactions with pharmacy services. Interestingly, while 55% of participants did not report having doctor-diagnosed health problems, many believed they were suffering from various diseases. This perception, combined with frequent community pharmacy visits (with 28% shopping three or more times a month), suggests a tendency towards self-diagnosis and self-management of health problems. This behavior can have serious consequences, as consumers may misinterpret symptoms, leading to irrational treatment or delays in seeking necessary medical intervention. Pharmacy employees play a important role in this scenario. Given the high frequency of consumer interaction with community pharmacies, these employees are in a unique position to guide consumers effectively. It is imperative that pharmacy employees engage with consumers to clarify their health concerns through targeted questions about their problem, provide appropriate advice, and, importantly, refer them to a doctor when necessary. This approach can help mitigate the risks associated with self-diagnosis and ensure that consumers receive the professional care about need.

The study identified the most frequently self-managed diseases, with gastrointestinal disorders, including diarrhea, being the second most common, following acute respiratory disorders of the upper respiratory tract. During diarrhea symptoms, a significant percent of respondents admitted that they independently decide what medicine to take, relying on their previous experience and only 9.7% consulted hospital doctors, even fewer relied on the internet or advertising as information sources. Notably, only 16.8% of consumers sought advice from a pharmacy employee when experiencing diarrhea symptoms.

It is important to note that gastrointestinal disorders, including diarrhea, are classified as minor ailment for which the WHO encourages self-man-

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agement if done under the advice and supervision of a pharmacy employee. However, the majority of respondents use medicines without consulting a specialist, often relying on non-specialist sources during diarrhea symptoms. This is concerning because it can lead to complications, delayed detection of serious conditions requiring immediate medical intervention, irrational drug pairings, and other health risks. A lack of consultation with community pharmacy employees can also contribute to the overuse and misuse of antibiotics, a problem observed in many other studies. Research in other countries has shown that consumers often have misconceptions about antibiotic use, believing that antibiotics should be initiated as soon as possible during acute diarrheal symptoms, without understanding that antibiotics should be avoided or discontinued if a viral infection is confirmed [*Leelakanok N et al., 2021*].

To make the research more comprehensive, we also examined the reasons why consumers choose to manage their health problem without consulting a pharmacy employee. It was found that 59.8% of consumers never agree to purchase medicines based on the advice of a pharmacy employee. The primary reasons for this behavior were previous negative experiences and a lack of trust in community pharmacy employees. As a result, there is irrational drug use among pharmacy consumers, leading to various health problems.

There is a weak pharmacist-consumer relationship, characterized by mistrust and misconceptions about the pharmacist's role. Improving pharmaceutical care and enhancing consumer education in managing acute diarrhea symptoms can prevent unnecessary and irrational drug use, unwanted combinations, overdosing, and other risks.

Pharmacy employees need to be actively involved in public education to disseminate reliable, authoritative information and guide rational decision-making during diarrhea symptoms. The effectiveness of public education efforts depends heavily on public trust in community pharmacy employees. It is crucial to elevate the role of pharmacy employees in managing minor ailments. The study's results also confirm the need for a reliable, professional, publicly accessible source of information that enables them to manage their treatment process effectively, even when they cannot wait in long queues at the community pharmacy. Such an information system would empower consumers to be more informed and involved in their healthcare decisions about minor ailments.

CONCLUSION

This study provides valuable insights into the behaviors and attitudes of pharmacy consumers, particularly in the context of managing minor ailment such as acute diarrhea. The findings highlight a significant reliance on self-management and a general reluctance to seek professional advice from community pharmacy employees, driven by factors such as mistrust and past negative experiences. Despite the critical role that community pharmacy staff can play in guiding appropriate treatment, a large proportion of consumers continue to rely on non-professional sources or their own judgment, which can lead to irrational or delayed treatment. The study underscores the need for enhanced public education and stronger engagement between community pharmacy employees (pharmacist/pharmacy technician) and consumers. By improving the level of trust and communication, community pharmacies can become more effective partners in health management, ensuring safer and more informed use of medicines. Additionally, the development of accessible, reliable information source could support consumers in making better health decisions, particularly when direct access to healthcare professionals is limited.

Overall, this research calls for a concerted effort to bridge the gap between consumers and community pharmacy professionals, thereby improving health outcomes and promoting rational drug use in the management of minor ailments.

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THE FIRST REPORT OF GRAPHITE TATTOO IN THE SOFT PALATE: A NOVEL CASE WITH A REVIEW OF ARTICLES

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Abstract

Pigmented lesions of the oral mucosa can have intrinsic or extrinsic origin. Pigmentation with extrinsic origin occurs by extrinsic agents such as carbon, silver (amalgam), graphite, and iron dust. Graphite tattoos usually appear in response to incidental or intentional thrust of an external object such as pencil lead. Their common site of incidence is the gum and hard palate, and usually occurs in boy children and adolescents. If the dentist cannot make a precise diagnosis for the pigmented lesions considering its clinical conditions, biopsy is necessary for ruling out malignancies including melanoma. In this study, a graphite tattoo has been presented in the soft palate of a 47-year-old woman, which is unique to the best of our knowledge.

A well-defined blue-black symmetric round macule was observed on the left side of the soft palate adjacent to the midline of a 47-year-old woman, 3 mm in diameter, who had referred to oral and maxillofacial medicine ward of faculty of dentistry with complaint of "black nevus has appeared in my soft palate". After extensive reviews across all databases, excisional biopsy was done for the patient using a punch with 3 mm diameter, and a piece of graphite was extracted.

In this case presentation, a unique case to the best out of knowledge was reported according to searches done from 1940 to 2023 in the soft palate of a 47-year-old woman. Surprisingly, the patient had no memory of trauma or thrust of a pencil or breakage of its lead in her oral mucosa.

Keywords: *graphite tattoo, soft palate, pigmented lesions*

Introduction

Pigmented lesions of the oral mucosa can have intrinsic or extrinsic origin [*Meleti M et al., 2008; De Giorgi V et al., 2009*]. Pigmentation with extrinsic origin occurs by extrinsic agents such as carbon, silver (amalgam), graphite, and iron dust. From among them, amalgam tattoos have the highest prevalence [*De Giorgi V et al., 2009*]. Graphite tattoos usually appear in response to incidental or intentional thrust of an external object such as pencil lead [*Adel K et al., 2004*]. Their common site of incidence is the gum and hard palate, and usually occurs in boy children and adolescents. The size of graphite tattoos can range from 1 to 15 *mm* which present as blue-grey-black macules [*Anderegg Jr C, Lyles M, 1992; Phillips G et al., 2005; Moraes R et al., 2015*]. If the dentist cannot make a precise diagnosis for the pigmented lesions considering its clinical conditions, biopsy is necessary for ruling

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Figure 1. *Well-defined blue-black symmetric round macule on the left side of the soft palate adjacent to the midline.*

out malignancies including melanoma [*Anderegg Jr C, Lyles M, 1992*]. In this study, a graphite tattoo has been presented in the soft palate of a 47-year-old woman, which is unique to the best of our knowledge.

Case report

A 47-year-old woman referred to the oral and maxillofacial medicine department of faculty of dentistry with the complaint of "a black nevus has appeared in my soft palate" in October 2022. This woman found out about this pigmentation in December 2021 through examination by a general dentist.

The patient stated that this pigmentation has had no size change so far compared to the previous year when she found to have it, and has not had any symptoms including burning sensation, pain, or discomfort.

In the medication history of the patient, taking levothyroxine 25 *mg* was reported to manage her hypothyroidism.

In the intraoral examination, a well-defined and well-bordered blue-black symmetric round macule was observed on the left side of the soft palate adjacent to the midline (Fig. 1). The diameter of this lesion was 3 *mm*, was not sensitive upon palpation, and did not blanch upon applying pressure.

Material and methods

First, search was done from 1940 to 2023 with keywords ("amalgam tattoo" AND "hard palate"), ("amalgam tattoo" AND "soft palate"), ("graphite tattoo" AND "hard palate"), and ("graphite tattoo" AND "soft palate") across databases including Google Scholar, PubMed, Embase, Ovid, Cochrane, Scopus, and Science Direct. The goal was to find similar papers that have given reports on graphite tattoo or amalgam tattoo in the soft or hard palate region. Table 1 summarizes the found case reports.

As can be seen, only two cases of amalgam tattoo had been reported in the soft and hard palate and two cases of graphite tattoo had been reported in the hard palate, while no case report existed on presence of graphite tattoo in the soft palate.

Table 1 shows the search results. Table 2 presents a summary of the cases that had reported graphite tattoo in the hard palate.

For taking biopsy and diagnosis, first an infiltrate injection of lidocaine with epinephrine was done around the pigmented lesion in the soft palate. Excisional biopsy was done for the patient with 3 *mm* diameter. After punching, the extracted surface was still pigmented. Through examination by probe, its consistency was hard.

Table 1

Papers that have given reports on graphite tattoo or amalgam tattoo in the soft or hard palate region

	Exogenous pigmentations			
Location	Soft palate		Hard palate	
Substant	GT	AT	GT	AT
Pubmed	1	0	0	0
Google scholar	1	0	1	2
Cochrane	0	0	0	0
Embase	0	0	0	0
Scopous	0	0	1	0
Science direct	0	0	0	0
Ovid	0	0	0	0
Result	1	0	1	2

Notes: *GT - Graphite tattoo, AT - Amalgam tattoo*

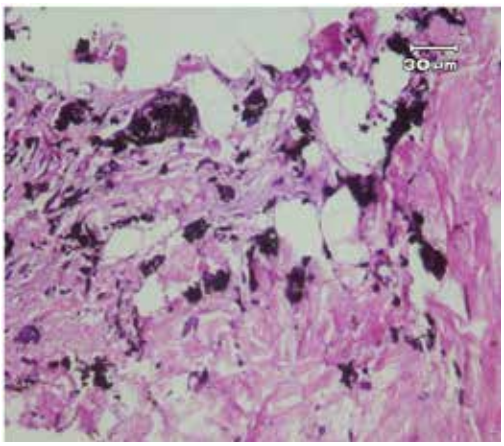

Figure 2. *Excision of a piece of graphite in the excisional biopsy.*

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Table 2

Summary of published articles on graphite and amalgam tattoos (1940-2023)

Article design	Treatment	Color	Size	Location	Age	Sex	Authors/Year
Graphite tattoos							
Case report	Biopsy	Blue-black	0.5 *cm*	Right hard palate	27	F	Molini 2016
Case report	Biopsy	Bluish gray	0.3x0.1x0.5 *cm*	Left hard palate	62	F	Moraes RM 2015
Amalgam tattoos							
Case report	Biopsy	Brownish black	Extensive	Right hard palate	56	M	Kamal FM 2019
Case report	Biopsy	Black	0.8 *cm*	Soft palate	66	F	Mayall FG 1992

Notes: *F – female; M – male*

Upon applying pressure to its surroundings, the pigmented object was detached off the surrounding mucosa, whereby a 3 *mm* piece of graphite was seen. The tissue isolated from the soft palate was sent for pathology examination (Fig. 2). Tissue sectioning in the pathology reported "unremarkable oral mucosa with exogenous pigmentation" (Fig. 3-6).

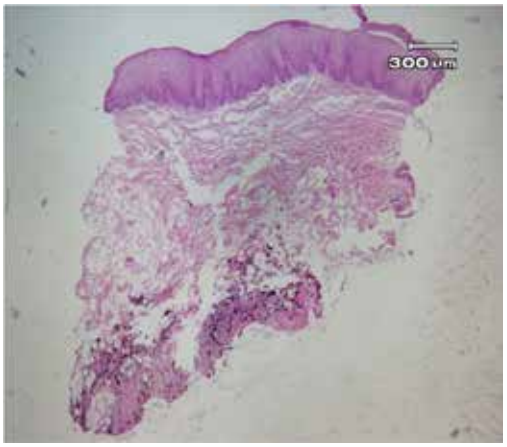

Figure 3. *Epithelium and connective tissue can be seen, diffuse fine black pigmentation located among the regional adipose tissues. Microscopic view (×40 magnification).*

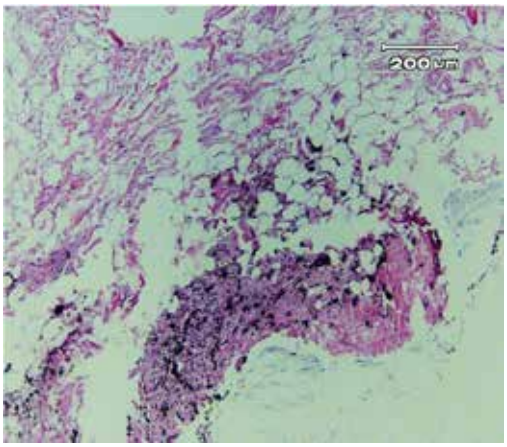

Figure 4. *Exogenous particles can be seen among regional adipose tissues. Microscopic view (×100 magnification).*

Discussion

Clinical differential diagnoses for pigmented lesions are very extensive, including intrinsic and extrinsic, single and multiple, local and systemic pigmentations [*Meleti M et al., 2008*]. This case was a local and single lesion, whereby no similar pigmentation was seen at any point of the mouth or skin, and was not reported by the patient. Intrin-

Figure 5. *Exogenous black pigmentations seen in both separately and small aggregations. High power field microscopic view (×400 magnification).*

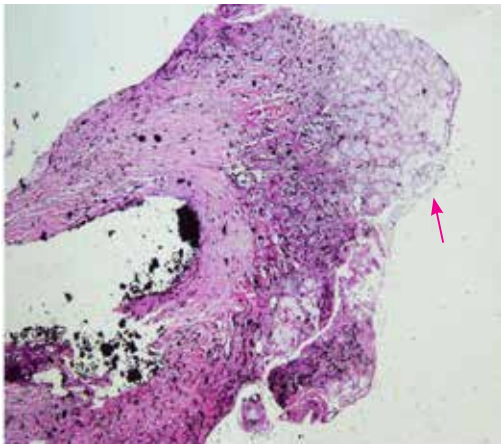

Figure 6. *Exogenous particles seen beneath the minor salivary glands. Mcroscopic view (×10 magnification).*

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sic pigmentations can have bilirubin, hemoglobin, or melanin origins [*Radhakrishnan S et al., 2021*]. Here, bilirubin origin was not applicable to this case. Also, due to the negative result of diascopy test, intrinsic pigmentations with hemoglobin origin were again not placed in the differential diagnosis [*Rudd M et al., 2001; Glick M, 2021*]. intrinsic pigmentations with melanin origin include melanotic macule, nevus, melanoacanthoma, and melanoma, in terms of order of prevalence. Extrinsic pigmentations include amalgam tattoo and graphite tattoo [*Meleti M et al., 2008*]. The most important condition in differential diagnosis of all pigmented lesions is melanoma, which necessitates taking a biopsy [*Anderegg Jr C, Lyles M, 1992*].

Melanoma claims 0.5-1% of all oral malignancies and has the lowest prevalence, while being the most fatal one. Any oral mucousal region can be affected by melanoma, though the palate is the most common site of involvement. Oral melanoma has no specific clinical symptoms in the early stages, and emerges with pigmented macule which is poorly circumscribed, which was similar to the mentioned case in this regard [*Adel K et al., 2004; Meleti M et al., 2008; Femiano F et al., 2008; Muller S, 2010; Mohan M et al., 2013; Molini P et al., 2016; Glick M, 2021*]. Nevertheless, since around year had passed from initial diagnosis of the lesion for the patient, and the patient did not report any size change, thus this diagnosis was ruled out.

Another pigmented lesion with melanin origin is melanoacanthoma. This lesion is usually dark pigmented macular or plaque-like lesion which is poorly circumscribed and grows fast, and mostly occurs in women [*Rohilla K et al., 2013; Glick M, 2021*]. As the size of the lesion had not changed over the previous year, and it had well-defined borders, melanoacanthoma was also ruled out from the list of differential diagnosis.

Ultimately, melanotic macule, melanocytic nevus, amalgam tattoo, and graphite tattoo remained in the initial differential diagnoses, which could not be ruled out through clinical examination.

In order of prevalence, pigmented lesion in the mentioned case fell in the differential diagnosis with melanotic macule. In spite of ambiguous etiology, it seems that trauma can be involved in development of melanotic macule. Any mucosal region can be affected, through the gum and lower lip are more common regions. It is also more probable to occur in women and adults. Melanotic macule has well-defined borders and has usually homogenous pigmentation [*Buchner A et al., 2004*]. The gender and age of incidence of melanotic macule were similar to the mentioned case, while its common site of indigence was unlike this case.

Oral melanocytic nevus is rare, emerges as single lesions, and is more common in women. It is often asymptomatic, and exist as brown and blue macule, smaller than 1 *cm* [*Adel K et al., 2004; Muller S, 2010*]. Unlike the rarity of oral nevus, blue nevus has the second highest prevalence in oral nevi [*Meleti M et al., 2008; Lee H et al., 2010; Santos T et al., 2011; Molini P et al., 2016*]. Its most common site of incidence is the hard palate [*Pinto A et al., 2003; Santos T et al., 2011*]. The site, size, and color of melanotic nevus were similar to the mentioned case.

Extrinsic pigmentation includes amalgam tattoo and graphite tattoo, with the most common being amalgam tattoo. Amalgam tattoo is a pigmented lesion that occurs due to amalgam restorations or their replacement. They are often opaque in radiography, but if they are small, they may be radiolucent. Also, after one year, due to absorption of silver by the bloodstream, amalgam tattoo may not be observed as radiopaque [*Moraes R et al., 2015*]. No opacity was seen in soft palate area in her panoramic radiography (Fig. 7). In the extensive searches done across all databases, the site of incidence of amalgam tattoos had been reported the gum and hard palate, and there was only one report of soft palate.

As mentioned in the Methods section, after excisional biopsy through punch, we encountered a hard object; after examination with the probe, surprisingly a 3 *mm* piece of pencil lead was excised.

Figure 7. *Panoramic radiology.*

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Apparently, this piece of pencil should have been buried inside the soft palate with trauma and there should have been a memory of breakage of pencil lead in the oral mucosa of the patient. However, when she was asked about this matter, unbelievably and in spite of diploma level of education as well as having full awareness, she recalled no such memory, and no matter how much the dentist insisted, she expressed no past history of trauma.

Graphite tattoo was the last condition that could fall in the differential diagnosis of this pigmentation. Thrusting the pencil lead in the mouth especially during childhood and adolescence can result in graphite tattoo [*Rihani F, Da'ameh D, 2006*]. In reviewing papers, in spite of extensive searches done, so far no such a case had been reported, and to the best out of knowledge, this was a unique case of graphite tattoo in the soft palate.

Conclusion

In this case presentation, to the best out of knowledge, according to searches done from 1940 to 2023, a unique case of graphite tattoo was reported in the soft palate of a 47-year-old woman, who surprisingly recalled no memory of trauma or thrust of pencil and breakage its lead in her oral mucosa. Diagnosis of graphite tattoo for the pigmentation present in this study was unique considering the gender (female), site of pigmented lesion (soft palate), and relatively high age of the patient (47 years), as well as no recall of thrusting any external object such as pencil lead. Such a rare finding can resolve suspicion of existence of other pigmented lesions especially initial stages of oral melanoma.

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ASSESSMENT OF VITAMIN USE AND SELF-MEDICATION PRACTICES AMONG CONSUMERS

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Abstract

People often take vitamins and mineral supplements to improve their health, but excessive intake can lead to toxicity and adverse effects. Unregulated self-medication is common, especially in developing countries like Armenia, and poses significant risks, including harmful drug interactions. This underscores the importance of healthcare providers educating patients about the safe use of vitamins. The study aims to assess vitamin use and self-medication practices among consumers. This study used a descriptive, cross-sectional design involving 385 consumers from different regions of Armenia. The sample size was determined using Cochran's formula, providing a 95% confidence interval and a 5% margin of error. A structured questionnaire was utilized for data collection, which took place anonymously between September 2023 and April 2024. Data were analyzed using SPSS version 23.0, focusing on descriptive statistics, with a significance level set at $p < 0.05$.

A diverse group of participants with varying ages and educational backgrounds was surveyed in Armenia, with a gender distribution of 51% female and 49% male. The majority were aged 18-45, and 59% had higher education. Key motivations for self-administering vitamins included perceived trivial health issues (42%) and prior experience (37%). Notably, 78% self-medicated with vitamins over the past year, and 71% shared their experiences with others. However, 72% did not undergo screening tests before self-administering vitamins, primarily purchasing them from pharmacies (93%).

The findings indicate that consumers in Armenia actively engage in vitamin self-medication and often share their experiences, raising concerns about potential risks due to insufficient research or screening prior to use. The prevalence of pharmacy purchases offers an opportunity for healthcare professionals to enhance oversight and promote safer practices. Developing clear guidelines for pharmacists and launching public awareness campaigns are essential for encouraging responsible self-medication and mitigating risks.

Keywords: *self-medication, vitamins, public health, health risks, pharmacists.*

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Introduction

People choose to take supplements for various reasons, often motivated by a desire to improve their health and well-being. Interestingly, individuals who take vitamin and mineral supplements typically already get more of these nutrients from their diet compared to those who don't use supplements. However, excessive intake of certain vitamins and minerals can be harmful, and overdosing on supplements may cause illness. While small amounts of supplements are generally safe, they haven't been proven to offer significant health benefits [*Wierzejska, 2021*].

The consumption of vitamins and dietary supplements is widespread in developing countries. Fat-soluble vitamins, such as vitamins A and D, are more likely to cause toxicity because they accumulate in the body and are metabolized slowly. While hypervitaminosis is uncommon, it can pose serious health risks. The improper or excessive use of vitamins can lead to various health complications, making it important to avoid overdosing and misuse [*Dhyani A et al., 2019*].

Similar to conventional pharmaceuticals, dietary supplements may result in potential risks, including adverse reactions, pharmacokinetic interactions, financial burden, postponement of more efficacious treatments, unrealistic expectations, and increased polypharmacy. While the majority of vitamins and minerals are available without prescription, higher doses of certain substances are regulated under controlled drug schedules. The risk of toxicity or overdose arises when consumers inadvertently ingest the same active ingredient from multiple products. Healthcare professionals have a pivotal role in advising patients by evaluating the potential risks and benefits of supplements and directing them to credible, evidence-based sources of information [*Moses G, 2021*].

The World Health Organization defined self-medication as using pharmaceuticals to treat self-identified illnesses or symptoms without a doctor's prescription or as intermittently continuing to take medications that a doctor has previously prescribed for persistent or recurrent conditions [*WHO, 2000*]. Self-medication aims to achieve a therapeutic benefit, yet individuals often use supplements to address perceived deficiencies without consulting a doctor or obtaining a prescription. Vitamin-based supplements are commonly consumed to counterbalance an unhealthy lifestyle and enhance health or performance [*Ahmed N et al., 2023*].

Unregulated self-medication is highly prevalent across the Republic of Armenia, occurring frequently across all demographic groups, irrespective of age, gender, or educational background [*Avagyan S et al., 2019; Barseghyan A, et al., 2023*]. Research conducted in Germany also highlights the issue of self-medication with vitamins, which is particularly concerning due to potential drug-vitamin interactions [*Perlitz H, 2019*].

According to the study conducted by Ylinen and co-workers amongst Finnish children under age 12 years, they found out that a majority of medication taken by the children were through self-medication, especially the use of vitamins with vitamin D being recommended for use in children below 3 years. Although only a few parents adhered to this recommendation, Vitamin D was still the most common vitamin used [*Ylinen S at al., 2010*]. Knopf in his work which examined results from national health surveys -where adults aged 18 to 79 were interviewed on health-related themes- reported that 18.1% (95% confidence interval: 17.0-19.2%) out of all participants in the DEGS1 (German Health Interview and Examination Survey for Adults (2008-2011) survey, consumed vitamins, minerals and food supplements in self-medication with significantly higher prevalence in older people (60 to 79 years), women, people with high social status, ex- and non-smokers, people living alone, and those involved in sports. Comparatively, participants in the GNHIES98 (German National Health Interview and Examination Survey, 1998) study had decreased prevalence than those that were in the DEGS1 study by about 6% (5.8%, 95% confidence interval: 4.1-7.5%, $p < 0.001$) This indicated the need for further action to be taken on the increase in self-medication due to the potential for interactions with prescribed medicines the results show the importance of ascertaining self-medication with vitamins, minerals and food supplements [*Knopf H, 2017*].

In individuals with chronic conditions increased caution is necessary regarding vitamin supplementation. For instance, elevated doses of vitamin E have been associated with an increased risk of

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heart failure in diabetic patients, thus high-dose vitamin E should be avoided in this population. Furthermore, in individuals with a history of myocardial infarction (heart attack), excessive vitamin E intake has been linked to a higher mortality risk. Therefore, patients with a prior history of heart attack are advised to avoid high doses of vitamin E [*Korah M et al., 2017*].

The aim of this study is to assess the patterns of vitamin use and self-medication practices among consumers.

Material and methods

Study design and participants: This descriptive, cross-sectional, and analytical study was conducted among 385 consumers in various regions of Armenia, including the capital city, Yerevan. Participants were randomly selected without duplication, with a response rate of 99.99%.

Inclusion criteria: Participants in this study were consumers aged 18 and older, with permanent residency in Armenia, who could speak, read, and write in Armenian and had no mental problems.

Exclusion criteria: The study was conducted with participant consent and adhered to ethical guidelines. Individuals who were under 18 years old, unable to read or communicate in Armenian, lacked permanent residency in Armenia, or chose not to participate were excluded from the study.

Sampling technique and sample size calculation:The sample size was determined using the Cochran's Sample Size Formula [Nanjundeswaraswamy T, Divakar S, 2021]. In this study, a sample size of 385 was estimated with a 95% confidence interval and a 5% margin of error.

Study instruments: The questionnaire comprised 19 closed-ended questions, where respondents selected from a predetermined set of options. Closed-ended questions in a questionnaire are generally easier for respondents to answer and tend to have higher response rates. The questionnaire was developed in such a way that it is accessible and understandable for all adults and individuals of different educational levels in Armenia.

Instrument validity and reliability: The survey employed in this study assessed several key aspects of self-medication practices, including knowledge of vitamins and attitudes toward self-medication. To ensure reliability, the survey was pre-tested with a small sample of participants (pilot study) before the main data collection.

Data collection: The study was conducted from September 2023 to April 2024. Participants, selected using an Excel random number generator to maintain impartiality and reduce selection bias, completed an anonymous, structured questionnaire with their consent. The questionnaire was carefully designed based on a review of relevant literature to fully ensure content validity and relevance to the topic.

Statistical analysis: The survey data were entered into the SPSS statistical software package, version 23.0, for analysis. Descriptive statistics, including frequencies, percentages, were used to characterize the data. A significance level of $p < 0.05$ was considered statistically significant throughout the analysis. The use of such statistical tools allows to fully analyze the situation and draw correct conclusions.

Ethical approval: The questionnaire was formally approved by the Ethics Committee of Yerevan State Medical University during a scheduled session (Protocol no. 10, dated 17.06.2021), confirming that the study complies with the ethical guidelines set forth in the Declaration of Helsinki.

Results

A diverse cohort of participants of varying ages and educational backgrounds was involved in the research conducted among the population of the Republic of Armenia. The gender distribution of the sample was 51% female and 49% male. The majority of respondents fell within the age groups of 18-25 years (22%), 26-35 years (27%), 36-45 years (32%), and 46-55 years (10%). The 56-64 (5%) and 65 and above (4%) age groups had the smallest shares.

In terms of education, the majority of participants (59%) had attained higher education, including bachelor's and master's degrees. Additionally, 27% had completed vocational (secondary professional) education, while 11% had completed secondary education. A smaller proportion, 3%, had obtained postgraduate qualifications.

The study examined the reasons behind the self-administration of vitamins, revealing that the most common factors were the perceived triviality of the health issue (42%) and previous personal

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experience with vitamin use (37%). Financial barriers also played a role, with 12% of participants citing the high cost of medical consultations as a reason for self-administration. Additionally, 7% of respondents reported a lack of time to see a doctor. Only a small proportion (2%) indicated that the urgency of their health condition influenced their decision to self-medicate.

The research findings revealed that a significant majority (78% of consumers) independently chose to self-administer vitamins over the past year. This indicates a widespread tendency among the population to manage their own vitamin intake without professional guidance, reflecting both a reliance on personal judgment and possibly a lack of consultation with healthcare providers. The 22% who did not take vitamins may include individuals who rely on dietary sources for essential nutrients or those who do not trust supplements. A deeper understanding of these factors could contribute to more effective public health strategies aimed at promoting balanced nutrition and responsible supplementation practices.

The study revealed that consumers not only engage in self-medication through the use of vitamins but also actively share their personal experiences and promote this practice among others. Notably, 71% of participants admitted that they have indeed shared their treatment experiences, talked about the vitamins they used, and offered advice to others, while 29% of participants indicated that they do not share their experience of using vitamins.

According to the survey data, a significant 72% of the population did not undergo vitamin screening tests before self-administering vitamins, indicating that most individuals engage in vitamin use without any prior medical assessments. In contrast, only 28% of respondents reported having their vitamin levels monitored before taking vitamins.

The majority of the population obtains vitamins predominantly from pharmacies (93%), which reflects positively on consumer behavior in terms of ensuring accessibility and availability of vitamins through regulated sources

Discussion

This study demonstrates that self-medication with vitamins as a form of self-administered therapy is prevalent among the citizens of Armenia. The findings indicate a notable trend where individuals are increasingly relying on vitamins for personal health management without consulting healthcare professionals. This behavior reflects broader patterns of self-medication within the population, highlighting the need for further investigation into the implications of such practices on public health and the potential risks associated with unsupervised vitamin intake.

One reason cited by 7% of respondents for not consulting a doctor is a lack of time. In today's fast-paced world, many individuals prefer to make their own health decisions and opt for over-the-counter vitamins and medications to address their concerns. Additionally, 42% of respondents perceive vitamin deficiencies or specific health issues as minor problems that do not require a doctor's visit. As a result, they choose to self-medicate, believing that vitamins can effectively solve their health problems without professional guidance. However, this belief can lead to the risks associated with improper dosages or potential interactions with other medications being overlooked.

Financial barriers also play a role in the decision to self-medicate. Twelve percent of participants reported that the high cost of a doctor's consultation forces them to self-medicate and purchase vitamins independently. For those without access to affordable healthcare, over-the-counter supplements often seem like a cost-effective solution for maintaining health. However, this approach is not always the safest or most effective and highlights the need for clear regulation.

Interestingly, prior experience plays a significant role for 37% of individuals. Having previously taken vitamins, many rely on their past self-medication, assuming that the same product or dosage will yield similar results again. This behavior underscores a common issue in self-medication: the assumption that an effective treatment will continue to work without adjustment. Conversely, the urgency of the health problem is a minor factor for only 2% of respondents. This suggests that self-treatment with vitamins is typically reserved for non-urgent health issues, where individuals feel comfortable addressing

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perceived deficiencies without immediate medical intervention. Vitamin intake should be closely monitored by healthcare professionals, including pharmacists and physicians, due to the potential for adverse effects and toxicities associated with excessive or inappropriate vitamin consumption [*Korah M, 2017*].

The research findings revealed that 78% of consumers independently utilized vitamins over the past year. This trend is echoed in a study conducted in Iraq, where participants reported self-administering vitamin D supplements without medical supervision, frequently relying on information obtained online. However, the use of social media as a source of health information may pose significant risks to consumers due to the potential for misinformation. To address these concerns, it is essential that pharmacy employees are well-informed and capable of guiding consumers in the appropriate use of vitamins. They should also educate consumers about the risks associated with inadequate vitamin intake, thereby promoting safer practices in self-medication [*Shanshal A et al., 2023*].

The self-use of bioactive supplements and vitamins by consumers saw a significant rise in 2020 during the COVID-19 pandemic. During this time, television and internet advertisements began promoting certain supplements for protecting against and aiding in the treatment of COVID-19. Notably, sales of vitamin C, vitamin D, and zinc surged within a single week in March 2020 [*Adams K et al., 2020*].

While vitamins are essential for the normal functioning of the body, their improper use can lead to adverse effects, including hypervitaminosis, a condition caused by excessive vitamin intake, which can severely harm various body systems. Hypervitaminosis related to fat-soluble vitamins is particularly concerning, as these vitamins can accumulate in body tissues, potentially leading to severe toxicity. In contrast, the risk associated with water-soluble vitamins is generally lower, as they are more easily excreted and less likely to cause life-threatening conditions [*Roop J, 2018; Grebow J, 2020*].

Ordinary consumers are often unaware of these risks and lack the knowledge to choose the right vitamins for themselves, a task that can be better managed with the guidance of a pharmacist. The survey results revealed that 72% of consumers do not conduct research before using vitamins, increasing the risk of developing hypervitaminosis. This lack of awareness highlights the urgent need for improved education on the safe use of vitamins and the potential consequences of misuse. Assessing consumer knowledge about vitamins and bioactive supplements can help develop more relevant informational tools, raise awareness, and promote better health outcomes [*Karbownik M et al., 2021*].

The observation that a significant majority of the population (93%) purchase vitamins primarily from pharmacies suggests a favorable trend in consumer behavior toward pharmacies, which can be leveraged to manage self-medication effectively. However, many consumers do not inform healthcare providers about their use of vitamins and other bioactive supplements, increasing the risk of adverse drug interactions [*Ronis M et al., 2018; Sirico F et al., 2018*].

Encouraging this behavior through pharmacies can improve public health outcomes by ensuring individuals have access to essential vitamins while minimizing the risks associated with unregulated sources. Overall, these findings underscore the critical role pharmacies play in promoting the safe and informed use of vitamins among the population.

Conclusion

The results of the study indicate that consumers in the Republic of Armenia actively engage in vitamin self-medication and frequently share their personal experiences, contributing to the widespread and rapid adoption of self-medication practice. Most consumers do not undergo necessary tests or screenings before taking vitamins, raising concerns about the potential risks of hypervitaminosis. Given that most vitamin purchases are made through pharmacies, there is an opportunity to regulate vitamin self-medication by involving pharmacy staff in monitoring and managing the self-medication process, leading to a more structured and safer approach for this problem.

To address this issue, it is essential to develop and implement clear and well-designed guidelines for health professionals, particularly pharmacists, regarding the appropriate use and administration of vitamins. Additionally, public information campaigns should be organized to raise awareness among consumers about the potential risks of uncontrolled and unnecessary vitamin use and to promote responsible and safe self-medication practices.

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SEX DETERMINATION USING CONFOCAL RAMAN MICROSCOPE WITH CHEMOMETRIC METHOD FROM DENTAL SAMPLE AND CONFIRMATION BY AMELOGENIN GENE

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Abstract

Lack of evidence in DNA analysis for sex determination of dental samples, with burned teeth. Resulting in the field of forensic dentistry looking for alternatives in sex determination for personal identification efforts. One alternative is to use spectral analysis from the Raman Microscope Confocal with the chemometric method. This alternative method uses samples of 20 teeth from 10 male and female dental samples, which are then analyzed on the enamel, dentin, and pulp surfaces with Raman spectra and also gold standards in the form of amelogenin DNA analysis, accompanied by chemometric tests using machine learning in the form of principal component analysis, restricted Boltzmann machines, support vector machine and artificial neural network, and comparative analysis based on the dental surface and signal fragments in the form of full width at half maximum.

The results obtained the accuracy value of the artificial neural network and support vector machine comparison, namely 75% on the Dentin surface using artificial neural network principal component analysis and Enamel using support vector machine, radial basis function, restricted Boltzmann machines. This means that the enamel and dentin surfaces are potential areas that can be used to predict gender using the chemometric method. Analysis of signal fragments at wave numbers 920-1080 cm-1 with the main molecule (PO_4) symmetric stretching (960 cm-1) analyzed in the form of full width at half maximum has a p value of 0.001, this means that there is a significant difference between men and women based on signal fragments. The examination of the gold standard DNA amelogenin has the same results between men and women in each layer of teeth. It can be concluded that the analysis of the confocal raman microscope in sex determination with the chemometric method of dental samples confirmed by DNA amelogenin gene can be used as an alternative method of gender identification.

Keywords: *Raman, artificial neural network, support vector machine, restricted Boltzmann machines Amelogenin, dental sample.*

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INTRODUCTION

In criminal cases, there is usually no evidence that can be used as a source for DNA (deoxyribonucleic acid) examination in large quantities, this makes DNA samples require careful handling. DNA analysis can use bones and teeth which are the strongest tissues of all human organs, which can be used as a source of forensic identification [*Yudianto A, dan Setiawan F, 2020*]. In dental samples, more accurate sex determination methods based on dental samples include Barr identification of dental pulp and amplification of specific DNA sequences of the X or Y chromosome using polymerase chain reaction or identification of sex-specific differences in the enamel matrix protein, amelogenin [*Gamulin O et al., 2021*]. Some literature shows that DNA examination of dental samples has shortcomings, most researchers have reported that it is very difficult to extract DNA from teeth that have been exposed to temperatures of 200°-400°C, and no consensus has been reached on the level of cremation at which teeth will still produce nuclear DNA signals [*Peral D et al., 2021*].

Forensic odontologist uses various analytical tools for sex determination, one alternative for sex determination can also be done using Raman spectral analysis [*Banjsak L et al., 2023; Kristanto R et al., 2023*]. Raman spectroscopy and Fourier Transform InfraRed analysis, which was shown in the study of Sekhaneh W. et al. (2021), namely Raman spectroscopy can identify and classify several elements of content in ancient teeth with burnt conditions or those that have been buried for a long time. Raman mapping can help to explore archaeological samples for well-preserved organic material, thereby identifying the best candidates for further analysis (DNA extraction) [*Sekhaneh W et al., 2021*].

This is also proven in the study of Rubio L. et al. (2018) that Spectrophotometry can be used to measure the color of 40 teeth heated at temperatures of 100°, 200°, and 400°C for 60 minutes. Spectrophotometric analysis of the color of burned teeth can predict the feasibility of human DNA extraction for identification purposes [*Rubio L et al., 2018*]. Raman Spectroscopy is a sophisticated analytical method that provides detailed and specific information at the molecular level. In terms of its versatility, this method can provide information that may be below the capabilities of other spectroscopic methods. Raman spectroscopy has properties that are useful for forensic applications [*Kristanto R et al., 2023*].

Raman Spectroscopy can provide fast, accurate, sensitive, and in situ detection analysis. It can sensitively and accurately reflect changes in the composition and structure of the material. With the advent of nanotechnology, advanced optical microscopes and miniaturized lasers have been developed, and problems such as weak signals, low signal-to-noise ratios, and strong autofluorescence backgrounds have also been overcome to enable Raman Spectroscopy to be gradually applied in the biomedical field. Raman Spectroscopy has been used to detect bacteria and the composition of cells, tissues, and biofluids, and in recent decades has received increasing interest for medical prognosis and diagnosis [*Zhang Y et al., 2022*].

This study aims to determine the accuracy of the spectral analysis results of confocal Raman microscopy in sex determination using human tooth samples with chemometric methods and data confirmation with the amelogenin gene.

MATERIAL AND METHODS

This study used 20 human dental samples from tooth extraction, consisting of 10 male tooth samples and 10 female tooth samples. The tooth elements used were random, consisting of incisors, premolars, and molars. This study has obtained ethical clearance from the ethics committee of the Faculty of Dentistry, Airlangga University No: 634/HRECC.FODM/V/2023.

Research samples with permanent teeth conditions, no caries, or fractures. The samples were then vertically sectioned using a disk diamond bur, so that 2 sides of the teeth were obtained. Then the results of the first side of the tooth section were subjected to spectral analysis using Confocal Raman Microscope and while the other side was subjected to confirmation analysis using the amelogenin gene.

The Spectral Analysis Examination using Confocal Raman Microscope was carried out in the Chemical Engineering Lab, Faculty of Chemical Engineering, Gadjah Mada University, Yogyakarta. The Raman Spectra tool used was the HORIBA brand (Kyoto, Japan) with a laser length of 785 *nm* (Max 100 *mW*). Laser with a wavelength of 100-

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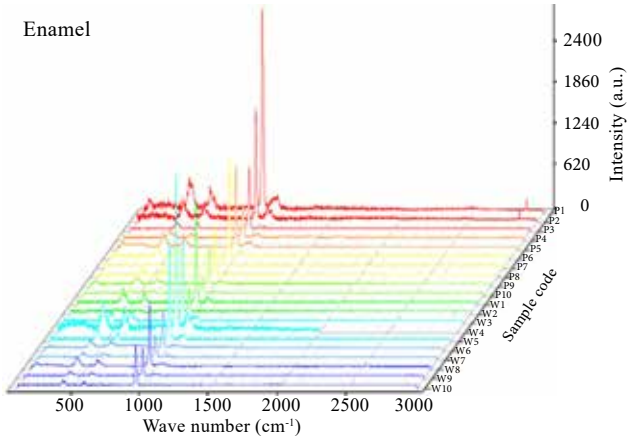

Figure 1*. Results of Raman Spectra Graph on the enamel surface in the wave number range of 100-3400 cm-1 from all samples between men and women*

3400 *cm*-1 was fired at 3 areas of the tooth surface consisting of enamel, dentin, and tooth pulp. The results of the recorded spectra examples are shown in figure 1.

Amelogenin Gene Analysis Examination at the Institute of Tropical Disease, Universitas Airlangga, Surabaya. This study used Sigma primers with leaders X: 542 *bp* and Y: 358 *bp* which were analyzed on extra samples from the enamel, dentin, and tooth pulp tooth surfaces.

Chemometric analysis using principal component analysis, restricted Boltzmann machines, support vector machine and artificial neural network using the Matlab application, and continued comparative analysis using SPSS Statistics 27.

Results

The results of the spectral analysis of Confocal Raman Microscope showed the highest spectral peak between the wave numbers 920-1080 *cm*-1 with the molecule (PO_4) symmetric stretching (960 *cm*-1). This can be done by cutting the signal fragments at these numbers to carry out more detailed analysis based on the enamel, dentin, and pulp tooth surfaces, as well as the signal fragments in figure 2.

The results of the Chemometric analysis in this study used principal component analysis and restricted Boltzmann machines on the 920-1080 *cm*-1 wavenumber signal fragment shown in figure 3. Principal component analysis is a method designed to reduce the spatial dimensions of data to find and interpret dependencies between vari-

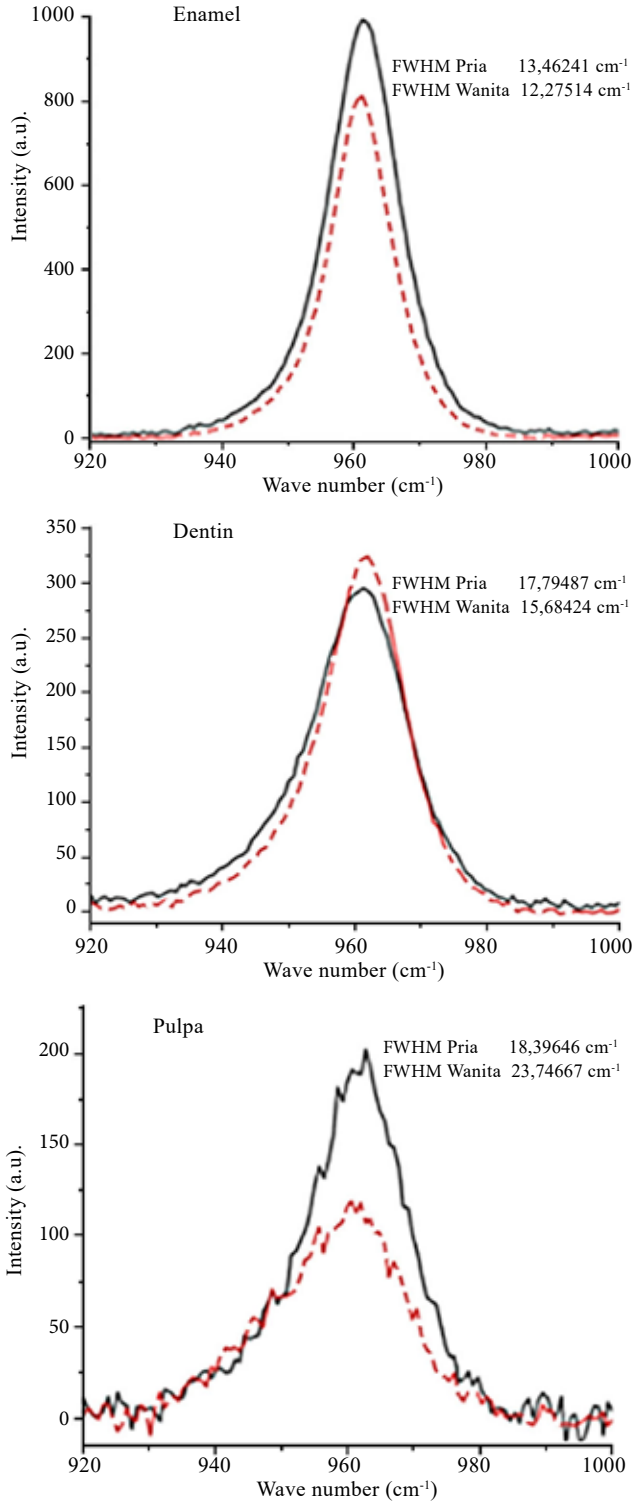

Figure 2. *Spectral graph results of signal fragments with wave numbers 920-1080 cm-1 from enamel, dentin and dental pulp samples*

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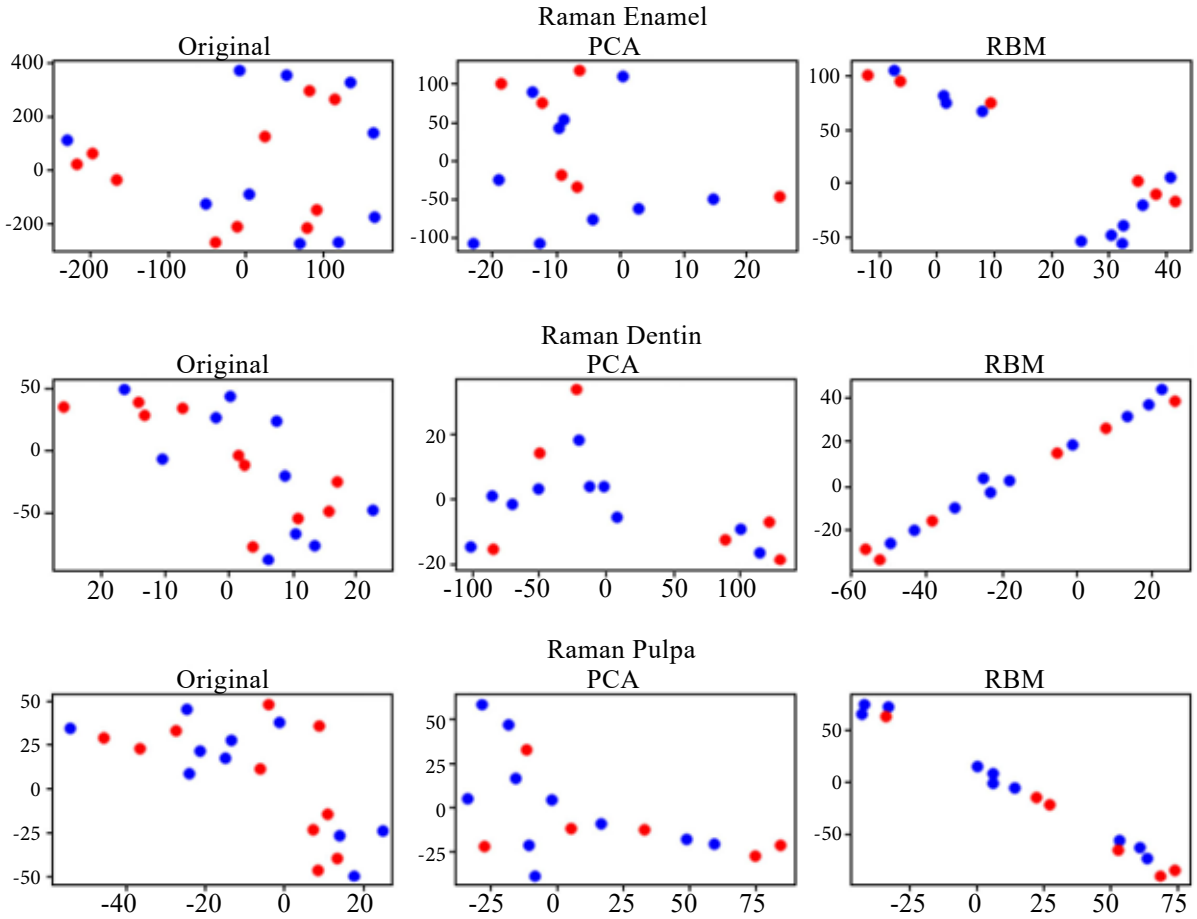

***FIGURE 3**. Clustering results on Raman data using principal component analysis and restricted Boltzmann machines on the enamel, dentin, and pulp surfaces with blue indicating male gender and red indicating female gender*

ables or to help stabilize measurements in statistical analysis such as regression analysis or cluster analysis [*Haryati A, Sugiyarto S, 2021*]. Restricted Boltzmann machines are unsupervised energy-based generative models (neural networks), which are directly inspired by statistical physics. Restricted Boltzmann machines attempts to represent complex interactions (or correlations) in the visible layer (data) by introducing new hidden (latent) variables [*Vrabel et al, 2020*].

Comparison results of artificial neural network and support vector machine analysis accuracy, and confusion matrix of dental samples using confocal Raman microscope

Table 1 shows the artificial neural network principal component analysis using 1 principle component that has 95% accuracy has the highest value in the Dentin surface area with a value of 75%, while the support vector machine restricted Boltzmann machines analysis using 8 principle

TABLE 1

Comparison results of artificial neural network and support vector machine accuracy

Area	Artificial neural network		Support vector machine, radial basis function,	
	principal component analysis	restricted Boltzmann machines	principal component analysis	restricted Boltzmann machines
	1 PCA 95%	1 PCA 95%	8 PCA 95%	8 PCA 95%
Enamel	25%	50%	50%	75%
Dentin	75%	25%	50%	25%
Pulp	50%	0%	50%	0%

NOTES: PCA - principal component, accuration 95%

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components that have 95% accuracy has the highest value in the Enamel area with a value of 75%. This means that gender determination with Raman Micro Confocal can be used in the Enamel and Dentin areas. The results of the artificial neural network and support vector machine are based on the Confusion Matrix Analysis shown in table 2.

Table 2

Results of Confusion Matrix Analysis from Confocal Raman Microscope in male and female gender

Analysis	Confusion Matrix			Confusion Matrix			Confusion Matrix			Confusion Matrix		
Raman Enamel	TRUE	M	F	TRUE	M	F	TRUE	M	F	TRUE	M	F
	M	0	2	M	0	2	M	1	1	M	2	0
	F	1	1	F	0	2	F	0	2	F	2	0
Raman Dentin	TRUE	M	F	TRUE	M	F	TRUE	M	F	TRUE	M	F
	M	0	2	M	0	2	M	0	2	M	0	2
	F	1	1	F	0	2	F	1	1	F	0	2
Raman Pulp	TRUE	M	F	TRUE	M	F	TRUE	M	F	TRUE	M	F
	M	0	2	M	0	2	M	0	2	M	0	2
	F	0	2	F	0	2	F	2	0	F	2	0

Statistical Analysis SPSS version 27 using non-parametric Kruskal Wallis Test based on gender with enamel, dentin, and pulp tooth surfaces (Table 3). Based on gender with fragment-signal size analysis of molecules (Area, center of gravity, max height, and full width at half maximum). Showing have different result in parameter of fragmen signal, that can be using to sex determination of male and female by confocal Raman spectra.

DNA analysis of amelogenin

The results of the confirmation of the amelogenin gene analysis showed significant results on the enamel surface of each sample between men and women. This shows that the sigma primer with a large leader has good accuracy as a gold standard in determining male and female sex.

Discussion

Raman spectroscopy has been shown to be important in the forensic analysis of trace evidence [*Tague T, Leona M, 2013*], and Raman Spectrometry has proven its value in the field of forensic dentistry. This is a very practical method of tissue chemical analysis because it can be performed on living specimens and "ex vivo" [*Ionita I, 2009*].

Based on the results of the Raman study, it is predicted that teeth contain inorganic (PO4 and CO3) in the wave number range of 400 and 1100 c*m*−1 [*Gamulin O et al., 2021*]. The data obtained in this study which are in the wave number range are 853 cm^{-1} (CCH) aromatic and (CC) proline; 876 cm^{-1} (CC), (CO3) lydroxypoline; 960 cm^{-1} (PO_4) symmetric stretching.

Based on the spectral data produced in this study, the (PO_4) symmetric stretching molecule (960 $cm^{-1)}$ is the strongest signal as in figures 1 and 2, which will be used in this study to analyze the differences between male and female gender. This is supported by the results of research conducted by Banjsak L. et al. (2023), showing that the average Raman spectrum of male and female teeth shows the main differences at wave numbers 964 *cm*-1 (PO_4), 1006 *cm*-1 (PH_3), 1453 *cm*-1 (CH_2). However, there is no clear characteristic of these types of molecules as a differentiator between male and female gender, so it is necessary to conduct a non-parametric statistical test of differences as seen in table 3. The results of the analysis show that the enamel, dentin and pulp surfaces of men and women do not have significant differences with a p value. 0.605 ± 0.271, which is significantly greater than p>0.05.

Based on the signal fragments on the enamel, dentin and pulp surfaces, there is a significant difference with a p value of 0.001, which is smaller than p<0.05. This indicates a significant difference between male and female gender based on full width at half maximum. In accordance with the research of Greco et al (2023), the wave number 960cm^{-1} shows the main spectroscopic parameters that can be obtained from dentin samples. Based on the signal fragment (full width at half maxi-

Table 3

Analysis of differences in non-parametric Kruskal Wallis test

	Comparation	
	Layer	Fragmen signal
Group	Male – Female	Male - Female
Chi Square	1.005 ± 2.610	99.568 ± 90.518
Asymp. Sig	0.605 ± 0.271	0.001
P	> 0.05	< 0.05

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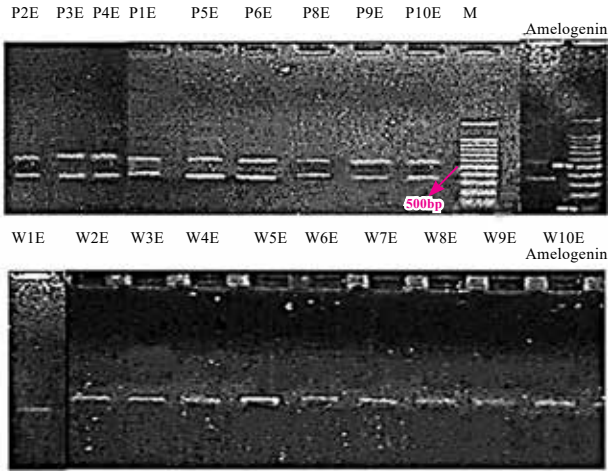

Figure 4. *The result of Amelogenin Gen analysis of male and female gender use dental sample (Enamel)*

mum) of the band located at around 960 *cm*-1, and associated with the v1 vibration of PO_3^4, it can be used to estimate the crystallinity of the hydroxyapatite phosphate group.

To find out which areas are significant in the analysis of gender on the enamel, dentin, and pulp surfaces, this study was continued using chemometrics with the support vector machine and artificial neural network algorithms to classify male and female gender [*Mohammad N et al., 2022*]. For the purposes of support vector machine and artificial neural network analysis, where the wavelength and wave number data are limited to a certain Raman range (920 - 1080 *cm*-1). This limitation is based on the content of the main elements and main molecules owned by teeth called host elements [*Birzhandi P et al., 2022*]. Apart from that, this limitation is also intended to reduce over-fitting when classifying with the chemometric algorithm [*Trisnawati N et al., 2019*].

Before being analyzed with support vector machine and artificial neural network, the data underwent initial data processing (preprocessing) in order to eliminate variables that were very different from the average or to reduce/reduce the data dimension (data sets with large numbers into simpler data sets) when classifying [*Yang H et al., 2015*]. Initial data processing used principal component analysis and restricted Boltzmann machines in each section (enamel, dentin, and pulp) and the results are as in figure 3.

Figure 3 shows the distribution of the initial Raman data (original) and the results of the transformation with principal component analysis and restricted Boltzmann machines in the enamel, dentin, and pulp sections. In the enamel and dentin sections, the distribution of principal component analysis results is better than the distribution of the initial data, while in the pulp section, the principal component analysis distribution is still mixed between gender and women like the initial distribution and data groups have not yet formed. The results of the restricted Boltzmann machines transformation in the enamel and pulp sections show that data groups are formed for each gender. However, for the dentin section, the data distribution tends to form a linear line with the data still mixed. The results of this study show that the support vector machine restricted Boltzmann machines value on the enamel surface has a good accuracy of 75%, this result is different from the research conducted by Gamulin O. et al. (2021) and Banjsak L. et al. (2023) which showed that the dentin surface is more accurate with a value of 70-75%.

The results of this study were confirmed by DNA analysis of the amelogenin gene, showing the suitability of samples on the enamel surface of men and women with the amelogenin gene marker X = 542 bp and Y = 358 bp with the Elmrghni S. and Kaddura M. method (2019). In this study, the polymerase chain reaction results showed 2 bands which meant male gender and 1 band which meant female gender. In the polymerase chain reaction analysis of amelogenin, if the male control DNA produced two clear peaks, while in the female control DNA, there was only one peak [*Ahmad A, Khan S, 2022*]. Basically, amelogenin is the main protein component found in the enamel matrix and is involved in amelogenesis, which is the development of enamel. Amelogenin is a type of extracellular matrix protein that, together with ameloblastin, enamelin, and tuftelin, directs enamel mineralization to form a highly organized matrix of rods, interstem crystals, and proteins [*Bansal A et al., 2012; Habelitz S, Bai Y, 2021*]. The amelogenin system is very important in forensic science, especially in the context of genetic sex determination [*Cardoso I et al., 2024*].

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Conclusion

Sex determination can be done using a Confocal Raman Microscope with 75% accuracy on support vector machine and artificial neural network on the surface of enamel and dentin. All samples showing 100% accuracy or significance with gold standard analysis use DNA Amelogenin Gene either enamel, dentin, or pulp area.

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COMPARATIVE OUTCOMES FOLLOWING PERCUTANEOUS CORONARY INTERVENTION AND CONSERVATIVE TREATMENT IN ELDERLY PATIENTS WITH ACUTE MYOCARDIAL INFARCTION: SINGLE CENTER RETROSPECTIVE COHORT ANALYSIS

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Abstract

Cardiovascular diseases remain the leading cause of morbidity and mortality among the elderly population, defined as individuals aged 65 years and older. As this demographic continues to expand globally, with projections indicating that by 2030, one in every five individuals will be over 65, the burden of cardiovascular diseases is anticipated to rise correspondingly. Among the various manifestations of cardiovascular diseases, coronary artery disease is particularly prevalent, often leading to acute coronary syndromes such as myocardial infarction and unstable angina. Elderly patients who present with acute myocardial infarction are at increased risk for adverse outcomes owing to higher comorbidity burden and complicated coronary anatomy. We evaluated the three-year outcomes following coronary revascularization compared to conservative management among elderly patients presenting with acute myocardial infarction.

Totally 155 patients over 75 years of age who were admitted for acute myocardial infarction underwent invasive treatment with coronary angioplasty (n=58) or only medical treatment (n=97). In the Invasive Treatment group cohort, 3-year survival probability was 74.1% as compared to 29.9% in the Conservative treatment group cohort (p<0.001). Mean survival time at 3 years of follow up was 31.50 (95% CI 29.35-33.65) months among the patients of Invasive treatment group versus 24.65 (95% CI 22.71-26.59) months among the patients of Conservative treatment group (p<0.001). Mean time to rehospitalization at 3 years was 34.05 (95% CI 32.37-35.72) in the Invasive treatment group cohort compared to 30.03 (95% CI 28.13-31.93) in the Conservative treatment group cohort (p=0.004).

Coronary revascularization in elderly patients with acute myocardial infarction significantly reduces all-cause mortality and cardiovascular events over a three-year follow-up period. However, rehospitalization rates remain comparable between treatment groups. Given the need for a thorough clinical assessment before determining treatment, coronary revascularization should be strongly considered as a strategy to enhance overall survival probability.

Keywords: *elderly patients, acute myocardial infarction, coronary angioplasty, pharmacologic treatment, comorbid diseases, mortality, rehospitalization.*

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Introduction

Cardiovascular diseases remain the leading cause of morbidity and mortality among the elderly population, defined as individuals aged 65 years and older. As this demographic continues to expand globally, with projections indicating that by 2030, one in every five individuals will be over 65, the burden of Cardiovascular diseases is anticipated to rise correspondingly. Among the various manifestations of Cardiovascular diseases, coronary artery disease is particularly prevalent, often leading to acute coronary syndromes such as myocardial infarction and unstable angina. Percutaneous coronary intervention, a minimally invasive procedure aimed at revascularizing occluded coronary arteries, has become a cornerstone in the management of coronary artery disease. However, its application in the elderly presents unique challenges and considerations [*Mehta R et al.,2005; Bossi I et al., 2006; Shan L et al., 2014*]. The elderly population often presents with a higher prevalence of comorbid conditions, including hypertension, diabetes mellitus, chronic kidney disease, and cerebrovascular disease. These comorbidities not only increase the complexity of clinical management but also elevate the risks associated with interventional procedures like Percutaneous coronary intervention. Anatomical factors, such as increased vascular calcification and tortuosity, further complicate the procedure, potentially leading to lower success rates and higher complication rates. Despite these challenges, several studies have demonstrated that Percutaneous coronary intervention can significantly improve health-related quality of life in elderly patients [*Feldman D et al., 2006; Devlin G et al., 2008; Johnman C et al., 2011; Tegn N et al., 2016*]. However, increasing age poses a higher risk for periprocedural complications secondary to age-related physiological changes, frailty, and comorbidities [*Moscucci M et al., 2003; Guagliumi G et al., 2004; Ciszewski A et al., 2008*]. The decision-making process for Percutaneous coronary intervention in elderly patients is further complicated by the underrepresentation of this age group in clinical trials. Historically, elderly patients have been excluded from many randomized controlled trials evaluating Percutaneous coronary intervention, leading to a paucity of high-quality evidence to guide therapy in this population. This exclusion has contributed to a "treatment-risk" paradox, where elderly patients, despite being at higher risk for adverse outcomes from acute coronary syndromes, are less likely to receive invasive treatments like Percutaneous coronary intervention. Community studies have shown that elderly patients are less likely to undergo revascularization, perhaps due to this paradox. Recent studies have sought to address this gap by specifically examining the outcomes of Percutaneous coronary intervention in elderly cohorts. For instance, a study analyzing data from the Thai Percutaneous Coronary Intervention Registry found that while elderly patients (≥75 years) had higher rates of comorbidities and in-hospital mortality compared to younger patients, age itself was not an independent predictor of increased mortality after Percutaneous coronary intervention. Instead, factors such as acute coronary syndrome and heart failure were more strongly associated with adverse outcomes. The complexity of coronary artery disease in the elderly often involves multivessel disease, necessitating decisions between complete revascularization versus culprit-only Percutaneous coronary intervention [*Gnanenthiran S et al., 2017*]. Common comorbid conditions among the elderly population including cancer, peptic ulcer disease, gastritis, chronic obstructive pulmonary disease, diabetes mellitus, chronic kidney disease, and congestive heart failure are considered independent risk factors for coronary angiography and may provoke periprocedural complications [*Graham M et al., 2002; Avezum A et al., 2005; Capodanno D, Angiolillo D, 2010; Chhatriwalla A et al., 2013; Bogomolov A et al., 2013*]. Moreover, data obtained from several investigations have shown that age ≥ 75 years is a negative predictor of undergoing percutaneous coronary intervention [*Gnanenthiran S et al., 2017*]. Thus, the clinical decision on whether to proceed with invasive therapy continues to be controversial and requires an individualized approach in contemporary practice [*Vlaar P et al., 2008; Shan L et al., 2014; Gnanenthiran S et al., 2017*].

The aim of this study was to compare the invasive and conservative strategies in elderly patients admitted with acute myocardial infarction and to analyze the overall survival and rehospitalization rates by Kaplan-Meier analysis.

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Material and methods

Study design: We retrospectively investigated 155 patients ≥75 years old admitted with acute myocardial infarction to the Department of General and Invasive Cardiology at the University Hospital of Yerevan State Medical University between 2014 and 2018. Patients had either received invasive or conservative management. Medical treatment was comparable in both groups except of antithrombotic pre- and postprocedural treatment.

Patient demographics and treatment data were collected through chart reviews, while follow-up was conducted via telephone communication. The study protocol received approval from the Ethics Committee of Yerevan State Medical University.

Study Endpoints: The primary endpoint was the evaluation of all-cause mortality after 3 years of follow-up. Cardiac death was defined as death resulting from myocardial infarction, stroke, or sudden cardiac death.

Secondary endpoints included rehospitalization, the need for coronary revascularization, and bleeding complications.

Statistical analysis: Categorical variables are presented as number (percent) and continuous variables are presented as mean ± standard deviation. Mean survival times are displayed with standard error and 95% confidence intervals.

Categorical variables were compared between groups using the Chi-square (x^2) test. In cases where expected frequencies were below the threshold for validity, Yates' correction for continuity was applied. For continuous variables, we assessed normality using the Kolmogorov-Smirnov test. Based on normality, group comparisons were conducted using the Student's t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables.

Survival probability and rehospitalization rates were assessed using the Kaplan-Meier method, and comparisons between groups were performed using the log-rank test. Mean survival time was compared between groups using the Mantel-Cox method. To assess predictors of survival, we conducted a Cox proportional hazards regression analysis to adjust for confounding variables. All statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). A two-tailed p-value < 0.05 was considered statistically significant.

Table 1

Baseline characteristics

		Invasive treatment group N=58	Conservative treatment group N=97
Average Age		79±3.8	80±4.1
Sex	M n(%)	22 (37.9%)	48 (49.5%)
	F n(%)	36 (62%)	49 (50.5%)
Comorbidity	Chronic kidney disease [a] n(%)	20 (34%)	31 (32%)
	HFrEF n(%)	32 (55.2%)	58 (59.8%)
	Diabetes mellitus n(%)	12 (20.7%)	25 (25.8%)
	Hypertension n(%)	50 (86.2%)	87 (89.7%)
	Anemia [b] n(%)	11(19%)	11 (11.3%)
Smokers n(%)		6 (10.3%)	9 (9.3%)
STEMI n(%)		29 (50%)	24 (24.74%)
non-STEMI n(%)		29 (50%)	54 (55.67%)
Acute heart failure n(%)		21 (36.2%)	31 (32%)
In hospital bleeding n(%)		6 (10.3%)	3 (3.1%)
Multivessel disease n(%)		46 (79.3%)	71 (73.2%)

Notes: *M - Male, F - Female, [a] - patients with glomerular filtration rate <60, [b] - Patients with Hemoglobin <100 g/l) HFrEF - Heart Failure with reduced Ejection Fraction, STEMI - ST- elevation Myocardial Infarction , N - Total number of patients. n(%) - number (percent) of patients in a group.*

Results

Between 2014 and 2018, 155 patients aged over 75 years were admitted for acute myocardial infarction. Of these, 58 patients (22 male and 36 female) received invasive treatment involving coronary angioplasty, while 97 patients (48 male and 49 female) were managed conservatively. The average age was 79±3.8 years in the Invasive treatment group and 80±4.1 years in the Conservative treatment group. Among all patients, 53 (34.2%) had ST-elevation myocardial infarction, and 102 (65.8%) had non-ST-elevation myocardial infarction. Baseline patient characteristics are displayed in Table 1.

Medical treatment was comparable in both groups except of antithrombotic pre- and postprocedural treatment (Table 2).

The 3-year survival probability was significantly higher in the Invasive treatment group at 74.1% compared to 29.9% in the Conservative treatment group (p<0.001). In the Invasive treatment group, 26% of patients died, with 60% of these deaths at-

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Table 2

Pharmacological treatment in two groups

	Invasive treatment group n(%)	Conservative treatment group n(%)
Aspirin	56 (96.6%)	90 (92.8%)
Clopidogrel	57 (98.3%)	71 (73.2%)
Angiotensin-converting enzyme inhibitor	39 (67.2%)	60 (61.9%)
Beta blocker	47 (81%)	78 (80%)
Unfractionated Heparin/ low molecular Heparin	54 (93.1%)	87 (89.7%)
Spironolactone	35 (60.3%)	59 (60.8%)

tributed to myocardial infarction. In the Conservative treatment group, 70% of patients died, with 54% of these deaths due to myocardial infarction. Kaplan-Meier curves were used to estimate survival from all-cause mortality, stratified by the type of intervention performed (invasive treatment vs. conservative management) and the age of patients at the time of myocardial infarction. The analysis revealed significant differences in survival probabilities between the two treatment groups. Patients who underwent invasive treatment (coronary angioplasty) demonstrated higher survival rates compared to those managed conservatively. Additionally, age at the time of myocardial infarction further influenced survival outcomes, with younger patients generally showing better survival probabilities than older patients within each treatment group. These findings highlight the impact of both intervention type and age on long-term survival following acute myocardial infarction (Fig. 1).

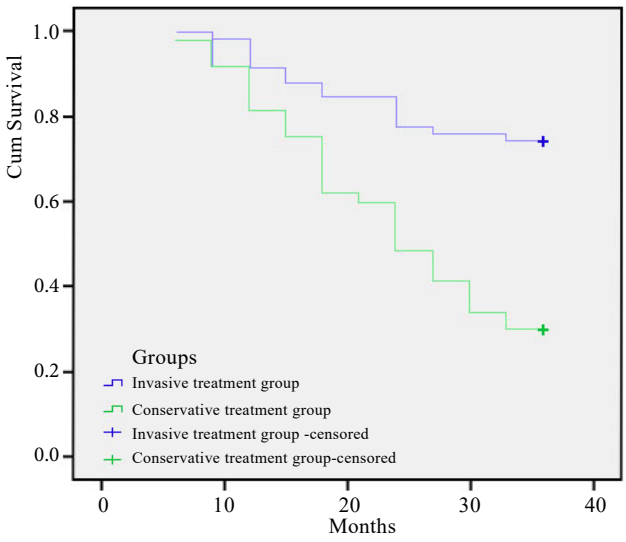

FIGURE 1. *Kaplan-Meyer curves for estimated survival from all-cause death stratified based on the interventon performed and age at the time of myocardial infarction*

The mean survival time was 31 months (95% CI 29.35-33.65) in the Invasive treatment group and 24.6 (95% CI 22.71-26.59) months in the Conservative treatment group ($p<0.001$) (Table 3).

Rehospitalization probability at 3 years was 51.7% in the Invasive treatment group compared to 33.2% in the Conservative treatment group ($p<0.01$). Of the 41% of patients that were rehospitalized in the Invasive treatment group, 42% were due to myocardial infarction and of the 44% of that were rehospitalized in the Conservative treatment group, 49% were due tomyocardial infarction. Stent thrombosis was reported in 1.7% of cases. Kaplan-Meier curves were generated to estimate rehospitalization rates, stratified by the type of intervention performed (invasive treatment vs. conservative management) and the age of patients at the time of myocardial infarction. The analysis revealed that patients who underwent invasive treatment (coronary angioplasty) had a higher probability of rehospitalization compared to those managed conservatively. Additionally, age at the time of myocardial infarction played a role, with younger patients generally experiencing lower rehospitalization rates than older patients within each treatment group. These findings underscore the influence of both intervention type and age on the likelihood of rehospitalization following acute myocardial infarction (Fig. 2).

Mean time to rehospitalization across the entire study population was 31.67 months (95% CI

TABLE 3

Means and Medians for Survival Time

Groups	Mean[a]			
	Estimate	Std. Error	95% CI LB	95% CI UB
Invasive treatment group	31.500	1.098	29.348	33.652
Conservative treatment group	24.649	0.988	22.713	26.586
Overall	27.213	0.789	25.667	28.759

Note: [a] *- Estimation is limited to the largest survival time if it is censored, CI* - Confidence Interval, LB - lower bound, UB - upper bound

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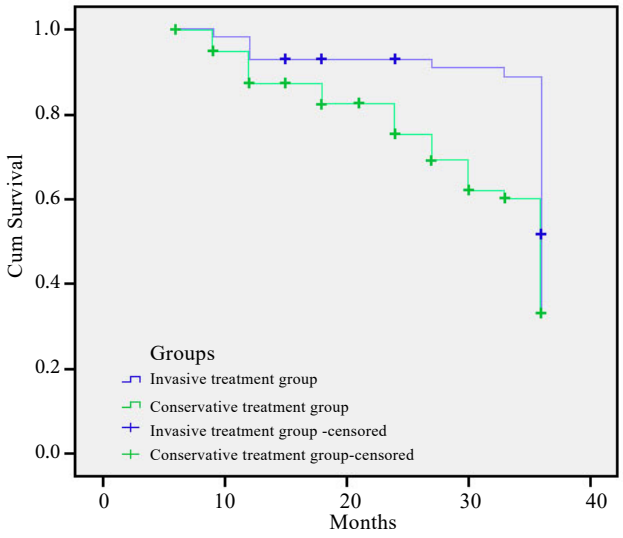

Figure 2. *Kaplan-Meier curves for estimated rehospitalizations based on the interventon performed and age at the time of myocardial infarction*

30.32-33.21). Mean time to rehospitalization in the Invasive treatment group was 34.05 (95% CI 32.34-35.72) compared to 30.03 (95% CI 28.13-31.93) in the Conservative treatment group (p=0.004) (Table 4).

Discussion

In this single-center retrospective cohort analysis of 155 patients aged over 75 years presenting with acute myocardial infarction, we aimed to evaluate the impact of an early invasive treatment approach on mortality and rehospitalization rates. Our findings indicate that invasive treatment with coronary angioplasty is associated with a significantly improved survival probability compared to conservative treatment in this elderly population. However, the Kaplan-Meier survival function for rehospitalizations showed statistically similar probabilities between the two groups. While invasive treatment demonstrated a reduced frequency of myocardial infarction-related rehospitalizations, comorbidities and the requirement for long-term dual antiplatelet therapy may partially explain the somewhat increased rehospitalization rates in the Invasive treatment group, leading to similar probabilities with the Conservative treatment group.

Table 4

Means for Survival Time for the Rehospitalization

Groups	Mean[a]			
	Estimate	Std. Error	95% CI	
			LB	UB
Invasive treatment group	34.045	.856	32.366	35.723
Conservative treatment group	30.029	.970	28.128	31.929
Overall	31.672	.691	30.317	33.027

Note: *[a] - Estimation is limited to the largest survival time if it is censored, CI* - Confidence Interval, LB - lower bound, UB - upper bound

The management of acute myocardial infarction in elderly patients poses unique challenges, as this population often presents with a higher burden of comorbidities. These factors likely contribute to an increased risk of mortality during acute myocardial infarction and peri-procedurally following coronary revascularization. Despite these challenges, our study highlights the potential survival benefits of an invasive treatment approach in this high-risk group [*Hasdai D et al., 2000; Achenbach S et al., 2008; Krishnaraj R, Charles K, 2013; Alfredsson J, Alexander K, 2016; Walker D et al., 2018; Damluji A et al., 2020*]. Moreover, a higher prevalence of frailty in the elderly population likely further worsens the poor prognosis following acute myocardial infarction and revascularization in older patients. Indeed, a general trend has been observed wherein an invasive treatment approach with either angioplasty or stenting becomes more frequently delayed or withheld with increasing age [*Alfredsson J, Alexander K, 2016; Walker D et al., 2018; Damluji A et al., 2020*]. Current recommendations from the US emphasize the need to individualize patient treatment, taking into account both the patient's clinical status along with their comorbidity burden [*Graham M et al., 2002; Bach R et al., 2004; Dangas G, Singh H, 2010*]. It is important to emphasize the lack of robust randomized-controlled clinical trial data examining invasive interventions in elderly patients presenting with acute myocardial infarction. This gap in evidence complicates clinicians' ability to weigh the benefits and risks of coronary revascularization in this population, particularly as age increases. The absence of high-quality data makes it challenging to establish clear, evidence-based guidelines for managing acute myocardial infarction in older adults, who often present with unique clinical complexities and comorbidities. Further research is urgently needed to provide stronger evidence and inform decision-making in this vulnerable pa-

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tient group [*Lefèvre T et al., 1998; Fach A et al., 2010; Kolte D et al., 2013*].

The improved survival probability associated with coronary angioplasty observed in our study population occurred in the setting of statistically similar age and comorbidity burden between cohorts. Several studies have shown that coronary revascularization during acute myocardial infarction may be associated with increased mortality in the elderly population [*Dynina O et al., 2003; Wang T et al., 2011; Antonsen L et al., 2013*]. Reassuringly, our findings suggest that the mortality benefit achieved through improved coronary blood flow via invasive treatment likely outweighs the risks of periprocedural complications and death associated with coronary angioplasty, even in elderly patients with multiple comorbidities. This underscores the potential value of an invasive approach in this high-risk population, despite the challenges posed by advanced age and comorbid conditions. These results provide important insights for clinicians when considering the balance of risks and benefits in the management of acute myocardial infarction in older adults.

It should be mentioned that an invasive treatment approach in older patients with acute myocardial infarction is associated with an increase in both minor and major bleeding events, possible contributors to mortality at long term follow-up [*Graham M et al., 2002; Moscucci M et al., 2003; Spoon D et al., 2014; Shanmugam V et al., 2015*]. However, recently, the frequency of bleeding events has decreased, likely because of more selective approaches to antithrombotic treatment following angioplasty [*Bossi I et al., 2006; Capodanno D, Angiolillo DJ, 2010; Schulz S et al., 2010*]. For example, international guidelines recommend the use of glycoprotein IIb/IIIa only for bailout and emergency coronary interventions. More recently, the statement of American College of Cardiology, American Heart Association, European Respiratory Society on antithrombotic therapy for patients with permanent atrial fibrillation and acute myocardial infarction with subsequent angioplasty recommended short-term triple antithrombotic therapy followed by dual antiplatelet therapy, revised compared to previous recommendations [*January C et al., 2019*]. Accordingly, the adoption of these new recommendations may have played a role in reducing periprocedural bleeding complications. This improvement highlights the importance of updated guidelines and best practices in enhancing patient outcomes, particularly in high-risk populations such as elderly patients undergoing invasive procedures for acute myocardial infarction. By minimizing bleeding risks, these advancements further support the safety and feasibility of invasive treatments in this vulnerable group.

Limitations: *This study is a single-center, retrospective analysis with a relatively small sample size. As with any retrospective study, there is a potential for unmeasured confounders that could influence the results. While randomized controlled trials focusing on myocardial infarction outcomes in the elderly population are needed to provide more robust evidence, we believe the findings from this study hold clinical significance in guiding treatment approaches for this patient group. However, it is important to note that the data used in this study are from over six years ago and may not fully reflect advancements or changes in contemporary clinical practice. Future research incorporating more recent data and larger, multicenter studies is essential to validate and update these findings.*

Conclusion

Routine invasive intervention is associated with improved survival and a reduction in cardiovascular events in elderly patients with acute myocardial infarction at three-year follow-up. The findings of this study support the adoption of an interventional approach for managing acute myocardial infarction in this population. However, a thorough assessment of the patient's clinical status at presentation is crucial to guide the decision-making process and determine the most appropriate treatment pathway. This individualized approach ensures that the benefits of invasive intervention are balanced against the patient's overall health and risk factors.

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KNOWLEDGE AND PERCEPTIONS ABOUT THE DIGITAL RECTAL EXAMINATION: EXPERIENCES IN COLOMBIA

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Abstract

Prostate cancer is the leading cause of malignancy in Colombian men. Early detection through the combination of prostate antigen and digital rectal examination has been important; however, men refuse digital rectal examination on account of discomfort, embarrassment and fear of detection of cancer, among others.

Therefore, this study aimed to describe and analyze the knowledge and perceptions of the Colombian male population regarding digital rectal examinations.

A descriptive study was conducted through an anonymous and virtual survey of adult men between 2023 and 2024. The survey included variables such as age, sexual orientation, number of children, history of prostate diseases, relatives with prostate cancer, and experience and perceptions of digital rectal examination.

The study included 791 men with an average age of 36.85 years. The majority (84.20%) were heterosexual. Concerning prostate medical history, 0.76% had prostate cancer, 3.79% had benign hyperplasia, and 2.53% had prostatitis. A total of 16.69% had undergone a digital rectal exam at some point. The decision not to perform the digital rectal examination was mainly due to a lack of knowledge (49.32%), fear (5.61%), or other reasons (45.07%).

Although the Colombian male population has basic knowledge about the importance of digital rectal examination in the detection of prostate cancer, negative perceptions persist regarding the performance of this screening test. The main reasons for avoiding this test include ignorance, lack of medical advice, sociocultural barriers, fear, shame and misogyny. Therefore, health promotion and disease prevention programs must be strengthened based on the reeducation of medical professionals and patients to deconstruct self-destructive masculinity.

Keywords: *rectal examination, prostate cancer, man, disease, health.*

Introduction

Prostate cancer is the second most common cause of malignancy in the male population and the eighth most common cause of mortality worldwide [*World Health Organization, 2024a*], with emotional and psychological burdens on the affected population, generally derived from stigmas that persist with treatment and related to hegemonic social construction of masculinity [*Medina-Coello E et al., 2009; Rivero A, Berríos R, 2016*]. In Colombia, prostate cancer is the most common cancer in men and the second most common cancer in the general population, slightly surpassed by breast

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cancer. In addition, prostate cancer represents the fifth cause of mortality due to malignancy in the country, after stomach, lung, colorectal and breast cancer [*World Health Organization, 2024b*]. Because approximately 50% of the world's population are men, prostate malignancy, owing to its high incidence, is a public health problem that generates significant costs for the health system and territorial socioeconomic dynamics [*Gordon L et al., 2018*]. There are biochemical, semiological and exploratory screening tools that facilitate early detection of prostate alterations by clinicians, avoiding fatal outcomes. Although prostate cancer is a serious and prevalent disease, it has a favorable survival rate of approximately 100% when it is treated in the early stages, indicating the importance of early diagnosis of this pathology [*Ministerio de Salud y Protección Social, 2013; Villegas C et al., 2015; Bratt O, 2024*].

Currently, the clinical practice guidelines for the early detection, diagnosis, treatment, follow-up and rehabilitation of prostate cancer in Colombia recommend early detection through the combination of the prostate-specific antigen test and digital rectal examination. For all symptomatic or asymptomatic men over 50 years of age who attend medical consultation, the above screening strategies can be carried out by any medical professional regardless of their level of specialization or referral center [*Ministerio de Salud y Protección Social, 2013*].

Rectal examination as a diagnostic tool dates back to ancient civilizations that used it to diagnose anorectal pathologies [*Tebala G, 2015*]. Later, owing to its anatomical relationship with the prostate, a simple, cost-effective and affordable strategy was found to evaluate alterations of this gland [*Ying Y et al., 2023*]. The biomarker prostate-specific antigen, also known as kallikrein-3, is a glycoprotein explicitly secreted by the prostate gland that participates in the liquefaction of semen after ejaculation, promoting sperm mobility, and a biomarker that, under benign or malignant pathological conditions, can increase its release and blood levels higher than 4 *ng/dL* [*Sánchez-Martínez L et al., 2013; Ying Y et al., 2023*]. Since its discovery around the 1980s and its subsequent authorization for the monitoring and early diagnosis of prostate cancer, this glycoprotein has become a routine screening strategy for this malignancy with significant socioeconomic implications due to its increased sensitivity detection and decreased patient discomfort during digital rectal examination [*Sánchez-Martínez L et al., 2013; Ying Y et al., 2023*].

Both screening strategies (digital rectal examination and prostate-specific antigen), combined or independent, still present controversies before different health institutions around the world; some consider that they sometimes lead to unnecessary overdiagnosis and treatment, whereas others recommend that rectal examinations should be excluded because of patient discomfort and low sensitivity [*Romero C et al., 2021; Ying Y et al., 2023*].

Colombian medical regulations establish good clinical practice from the combined performance of both screening strategies [*Ministerio de Salud y Protección Social, 2013*]. Specifically, Colombian men reject digital rectal examination due to discomfort, shame, pain, and fear of cancer detection, as well as misinformation and lack of promotion in health entities [*Arbeláez J, Montealegre N, 2012; Fajardo-Zapata A, Jaimes-Monroy G, 2016*]. Therefore, this study aimed to describe and analyze the knowledge and perceptions of the Colombian male population regarding digital rectal examinations.

Material and methods

Study design: A descriptive study was conducted through an anonymous and virtual survey of the adult male population via Google Forms and distributed through social networks between September 2023 and February 2024. The Bioethics Committee of the Medical Research Institute of the Faculty of Medicine, University of Antioquia, approved the study, all patients gave their consent before involving in the study.

The questions included variables such as age, sexual orientation, number of children, history of prostate diseases, family history of prostate cancer, history of digital rectal examination, and knowledge and perceptions of digital rectal examination. The inclusion criteria were male and over 18 years old, whereas the exclusion criteria were transgender men under 18 years old and answering the survey incompletely.

Statistical analysis: The median and range of each variable were used as measures of central tendency, and the Kruskal–Wallis and chi-square tests were used to compare the different groups via the statistical programs GraphPad Prism 9.0 (GraphPad, San Diego, CA, USA) and Jamovi (The Jamovi project, version 2.5), a $p<0.05$ was considered significant.

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Results

Seven hundred ninety-one men participated in the study; the average age was 36.85 ± 14.77 years (18-88 years), 84.20% of the volunteers (666/791) were heterosexual, and 40.46% (320/791) of the volunteers had children (1-8 children).

Six participants (0.76%) stated that they had been diagnosed with prostate cancer, 30 (3.79%) had been diagnosed with benign prostatic hyperplasia, and 20 (2.53%) mentioned having a medical history of prostatitis at some point in their life. Similarly, 59.17% did not know anyone close with prostate cancer (468/791), and 40.83% mentioned knowing someone close (323/791), including friends (57.28%, 185/323) and relatives (44.27%, 143/323). However, only 2.48% of those surveyed had an active diagnosis or suffered from the disease (8/323).

A total of 16.69% of the respondents (132/791) stated that they had ever had a digital rectal examination; this group of individuals was 20 years older than the group of men who had never had it (53, 81 ± 15, 77 *vs.* 33.46 ± 11.98, <0.0001). The age at screening initiation was 43.55 ± 13.37 years. The average number of times that the digital rectal examination was performed was 2.4 ± 2.04 (1-10 times), which is positively correlated with the age of the volunteers (r = 0.30, p = 0.0006). On the other hand, among those who had not undergone a digital rectal examination (659/791), 49.32% were due to a lack of knowledge (325/659), 5.61% mentioned that it was out of fear (37/659) and 45.07% was for other reasons (297/659), among which it stands out not being indicated either by age (34%, 101/297) or by the decision of the doctor (14.81%, 44/297). In addition, 47.91% of the men (379/791) expressed that they feared being diagnosed with a disease through screening.

Additionally, when men are grouped by age (less than 29 years old, between 30 and 59 years old and over 60 years old), as expected, the percentage of men who undergo digital rectal examination increases. However, the percentage of men over 60 years of age who do not have digital rectal examination is high (Fig. 1).

A total of 66.75% (528/791) of the participants knew the digital rectal examination. A total of 96.97% (128/132) of the men who underwent the exam know what the exam consists of, but only 60.70% (400/659) of the men who have not yet had the digital rectal examination know what it is about (p <0.0001) (Fig. 2).

When the participants were asked about their opinions about the relationship between performing a digital rectal examination and losing sense of masculinity, 91.53% said they disagreed with this relationship (724/791), whereas 8.47% answered affirmatively (67/791). The percentage of men who have taken the exam and believe that their masculinity is not affected was 93.94% (124/132), which is similar to its counterpart since 91.05% of those who have not yet performed the examination do not associate it with the loss of masculinity (600/659, p=0.2838). Under the above, if the doctor requests a digital rectal examination, 89.38% of the respondents would not feel violated (707/791), and 85.21% (674/791) would not feel ashamed of the procedure either.

Of the individuals who said they felt violated (84/791), 10 had the existence of other screening mechanisms, such as prostate-specific antigen, as their main reason; 17 reported that the digital rectal

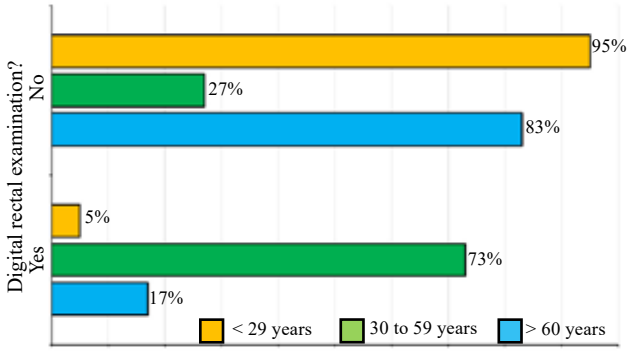

Figure 1. *Relationship between age and digital rectal examination results*

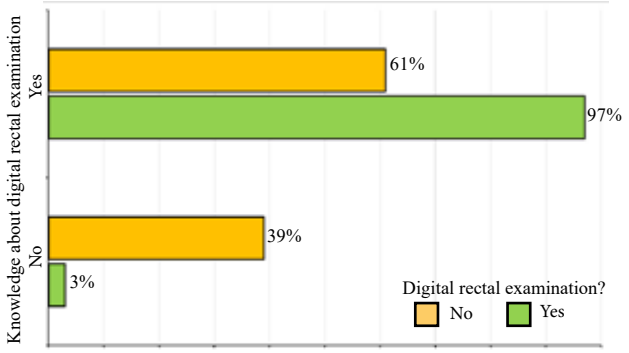

Figure 2. *Relationships between knowledge about digital rectal examination and the population that has or has not undergone the procedure*

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examination was embarrassing, uncomfortable and invasive; and 7 stated that they would feel violated by the fear of being touched or sexually abused.

When the participants were asked if the medical staff adequately explained the procedure on any occasion, 73.32% of the men (580/791) never received education from their treating physician, and 52.47% (415/791) of those surveyed said they did not know the benefits of digital rectal examination.

47.53% (376/791) of the participants answered affirmatively that the digital rectal examination could benefit them. However, more than a third of those surveyed believe that performing a digital rectal examination is no longer necessary due to the measurement of prostate antigen levels in the blood, transrectal ultrasound or other diagnostic methods. In this order of ideas and concerning the perception of the need to perform a digital rectal examination sometime in life, 86.98% of the participants (688/791) perceived it as necessary; however, paradoxically, 13.02% of participants (103/791) believed that it was not necessary, and these men were included in all age ranges 37.91 ± 15.67 years (18-68 years).

Regarding the discomfort and pain that they would expect to have during the prostate exam, 64.1% of the participants said that they could have mild discomfort (507/791), 28.32% (224/791) reported no discomfort, and 7.59% (60/791) reported severe discomfort. In addition, when the groups were divided into those who did or did not undergo digital rectal examination, the percentages of patients with mild and severe discomfort decreased (Fig. 3).

On the other hand, when inquiring about the gender preferences of the treating physician when performing the digital rectal examination, 65.36% of the population is indifferent if the examination is performed by a man or a woman (517/791); 21,62% preferred a woman to do it (171/791), whereas only 13.02% preferred a man to do it (103/791).

Finally, when comparing the previous results in relation to the two most prevalent sexual orientations in the surveyed population, heterosexual and homosexual, the heterosexual male population (n=666) is indifferent to the gender of the examiner in 65.92% (439/666) of the participants, whereas 10% (67/666) prefer that a man perform it, and 24.02% (160/666) favor women. On the other hand, among those who identify as homosexuals (n=75), a similar percentage shows indifference toward the professional's gender (60%, 45/75), but 34.7% of this population (26/75) opts for men (p = 0.00046 *vs.* 10% heterosexuals), and only 5.3% (4/75) prefer women Table 4).

Discussion

This study assessed knowledge and perceptions about digital rectal examination in 791 mostly heterosexual men from Colombia. Less than one-fifth of the volunteers had ever undergone a digital rectal examination. Of all the men, only 0.76% had a history of prostate cancer, 3.79% of benign hyperplasia, and 2.53% of prostatitis

Similar research has been conducted in Colombia but with a smaller sample size, including only the population over 40 years of age [*Muñoz Astudillo M et al., 2011; Arbeláez J, Montealegre N, 2012; Fajardo-Zapata A, Jaimes-Monroy G, 2016; Castro Avendaño J, 2024*]. The mean age in our

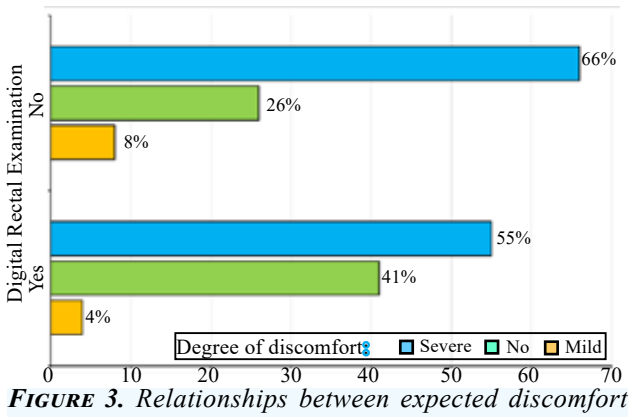

Figure 3. *Relationships between expected discomfort during rectal examination in the population that has or has not undergone the procedure*

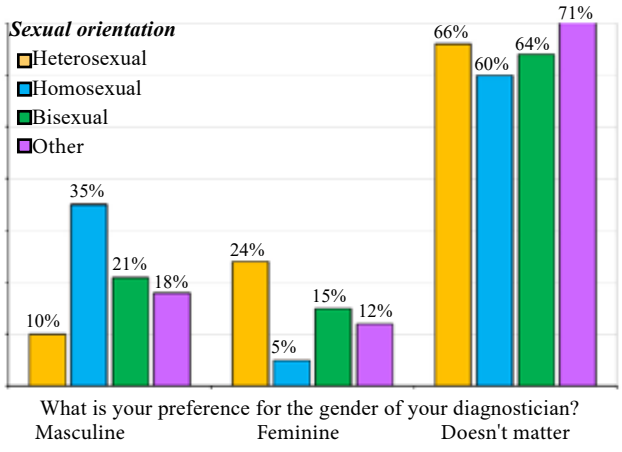

Figure 4. *Relationship between the sexual orientation and gender preference of the examiner*

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study was lower (36.85 ± 14.77) than that reported in previous studies, and nearly 50% of the population in these studies reported having undergone a digital rectal examination [*Muñoz Astudillo M et al., 2011; Arbeláez J, Montealegre N, 2012; Fajardo-Zapata A, Jaimes-Monroy G, 2016; Castro Avendaño J, 2024*], while in the present study, it was only 16.65% probably the participants in this study were men who were younger with no medical indication for routine screening.

The performance of the digital rectal examination was directly and positively correlated with the age of the participants; that is, the frequency of the digital rectal examination increased as the age range increased, a finding congruent with that reported by a recent study in the city of Bogotá [*Castro Avendaño J, 2024*] and that is based on current Colombian policies that determine the target population for prostate cancer screening 6.

On the other hand, among those who have not undergone a digital rectal examination, the reasons are similar to those reported by other studies. In Medellín [*Arbeláez J, Montealegre N, 2012*], ignorance, lack of money, discomfort and carelessness were the main reasons, which go hand in hand with those found in a study in Santa Rosa del Cabal, where the above reasons, in addition to misogyny, fear and religious beliefs [*Muñoz Astudillo M et al., 2011*] are added, and a more recent one in Bogotá [*Fajardo-Zapata A, Jaimes-Monroy G, 2016*], where the lack of medical advice and the feeling of denigration attracted attention. In the present study, lack of knowledge or information represented the highest percentage, followed by other reasons, such as lack of medical indication and age. Despite a low percentage, the manifestation of fear was present, which reinforces the community's need to receive timely and adequate information on the prevention of prostate cancer and digital rectal examination as a fast, safe and cost-effective medical tool to achieve an early diagnosis of prostate alterations and reduce negative perceptions and fears towards it.

Likewise, it should be noted that the fear of a possible diagnosis of prostate cancer is an essential determinant of the behavior of the male population, and this finding is shared with the results obtained through national and international investigations [*Muñoz Astudillo M et al., 2011; Rivero A, Berríos R, 2016; Fajardo-Zapata A, Jaimes-Monroy G, 2016; Castro Avendaño J, 2024; Castaldelli-Maia J et al., 2024*]. Fear of a prostate cancer diagnosis is linked to the perceptions of severity that the male population has about the disease and the uncertainty generated by the lack of information, as well as taboos towards treatment [*Muñoz Astudillo M et al., 2011; Rivero A, Berríos R, 2016; Almeida É et al., 2020; Castaldelli-Maia J et al., 2024*].

Regarding the social construction of hegemonic masculinity and the sensation of loss of masculinity linked to digital rectal examination, the results are similar to those found in the available literature [*Arbeláez J, Montealegre N, 2012; Fajardo-Zapata A, Jaimes-Monroy G, 2016*]. Although the vast majority of respondents expressed disagreement with this relationship, a percentage of the population still feels their masculinity attacked by the performance of the test, a finding that is important in the present study, where the number of men who feel their masculinity violated by the digital rectal examination is found both in men who have not yet had it as in those who have already done it; this situation reflects misogynistic social dynamics independent of age in our society, a fact that is reinforced in various studies in Latin American countries, where the conception of hegemonic masculinity behaves as a risk factor for the early detection and treatment of chronic diseases, such as prostate cancer, and for the lack of disposition towards digital rectal examination [*Rivero A, Berríos R, 2016; Paredes Ajalla AM et al., 2022; Contreras-Sánchez L et al., 2023; Castaldelli-Maia J et al., 2024*]. The above represents the cornerstone of prevention and education campaigns, where the social deconstruction of self-destructive masculinities that end up going against self-care [*Rivero A, Berríos R, 2016; Mendoza J, 2019; Reyes Vasconcelos L et al., 2020; Castaldelli-Maia J et al., 2024*], should prevail in the education of the patient in search of men stop associating a preventive medical procedure with the feminization of their virility [*Reyes Vasconcelos L et al., 2020; Castaldelli-Maia J et al., 2024*] and the feeling of abuse.

The findings of this work are similar to those of other investigations in the Colombian territory and Latin America [*Arbeláez J, Montealegre N, 2012; Fajardo-Zapata A, Jaimes-Monroy G, 2016; Mendoza J, 2019; Contreras-Sánchez L et al., 2023*]. However, the percentage was slightly lower than

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that reported in a study in Bogotá [*Castro Avendaño J, 2024*], where approximately 70% of the participants were aware of digital rectal examinations and prostate-specific antigens as screening strategies. Although the majority of respondents stated that they knew about the digital rectal examination procedure, reaching almost 100% of those who had already done so in the past, the fact that, among those who had not yet been screened, only a 60% was found to have basic knowledge, this accounts for the lack of dissemination from the public health area to ensure that the prevention message reaches the young male population, thus promoting the repeal of all social constructions towards digital rectal examination that are culturally perpetuated.

In addition, more than 70% of those surveyed, including all age ranges, stated that they had never received an explanation from health personnel about digital rectal examination. Additionally, more than 50% lacked knowledge about its benefits. However, as previously highlighted, prostate cancer is one of the leading causes of death for the male population in Colombia and the world [*WHO, 2024a; 2024b*].

Interestingly, although Colombian regulations are clear when establishing prostate cancer screening via prostate-specific antigen measurement and digital rectal examination as complementary strategies [*Ministerio de Salud y Protección Social, 2013*], a considerable percentage of respondents say that they do not consider digital rectal examination necessary because of the existence of a laboratory test. Thus, the role of the general practitioner is highlighted as a source of information for health and a fundamental axis in the prevention of diseases [*Heins M et al., 2013*]. These findings reveal the need for a doctor-patient relationship based on health communication, which, rather than treating diseases, seeks to educate individuals who are empowered by their health-disease processes and who act guided by current regulations.

On the other hand, the majority of the population did not show a gender preference towards the treating physician, which is compatible with the results of a study carried out in a German territory where 75.4% did not show a preference for the urologist's gender [*Tamalunas A et al., 2021*]; however, this finding differs from later findings reported by the same author, where more deeply, through a questionnaire that included 14 questions about preferences, it was found that only 35% of the participants consistently answered that they had no preference about practitioner gender [*Tamalunas A et al., 2022*]. Recent studies reported that the preference for the gender of the urologist in consultation goes hand in hand with the gender of the patient; that is, men tend to lean towards professionals of the same gender [*Orom H et al., 2014; Tamalunas A et al., 2021; Tamalunas A et al., 2022*]. This was associated with the fact that the male population included in that study considered that men could better understand their bodies, had practical skills and that communication was more comfortable. However, and interestingly, this did not occur in the present study, since the population that preferred women was more remarkable. Only the homosexual population expressed a statistically significant inclination for male professionals. Notably, the aforementioned works were carried out in Europe, since data for Latin America have yet to be reported in this area. In this way, possible sociocultural aspects could play an essential role in the differences previously described. Consequently, this topic is presented as a field that has been little explored in Latin American urology and would benefit from further research.

In short, men are presented as a risk population for the late diagnosis of pathologies related to sexual and reproductive health owing to the perceptions and prejudices that they have about medical consultations and their association with "weakness" and loss of virility, added to the risk behaviors that their own social conceptions and gender roles generate in them. The foregoing is evident from what was found in this research, where the majority of the population considered that women are the ones who consult health services the most and who reinforce the need for health education in the male population, with the engagement of strategies that consider that the roles assigned to each sex significantly shape behaviors and that Latin American social dynamics continue to be strongly marked by the paternalistic culture and hegemonic masculinity [*Abou Orm Saab K, Camacaro Cuevas M, 2013; Heins M et al., 2013; Reyes Vasconcelos L et al., 2020; Restrepo Gil E et al., 2022*].

The limitations of this study are the self-reported characteristics of the questionnaire used, this may be linked to various types of bias that depend

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on the participant's memory and introspection; however, recent evidence suggests that self-reports are a friendly and practical strategy that favors the sincerity of the participants on sensitive issues such as those related to sexual and reproductive health [*Del Valle M, Zamora E, 2021*].

Conclusion

The performance of digital rectal examinations is more common in older men than in young men, which is consistent with the current regulations of the Comprehensive Health Care Routes.

The Colombian male population surveyed stated that they had basic knowledge about the importance of digital rectal examination in prostate cancer screening; however, negative perceptions and remarks regarding the performance of this practice and its relationship with the loss of masculinity continue to be present. Thus, the main reasons for not undergoing this test in Colombian men include ignorance, lack of indication by age or by a doctor, various sociocultural barriers, fear, shame and misogyny.

Points for improvement are highlighted in communication and education for health, as well as in the doctor-patient relationships of health personnel. Health promotion and disease prevention programs must be strengthened based on the re-education of medical professionals and patients to deconstruct self-destructive masculinity models, which are contrary to the early detection of diseases. Self-care and reducing these sociocultural barriers affect the health of men in Colombia.

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ROLE OF CORTISOL IN THE CARCINOGENESIS OF LARYNGEAL CANCER

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Abstract

Laryngeal precancerosis is a pathogenetic borderline condition including a wide range of risk factors that increase the likelihood of laryngeal epithelial cell malignancy. The induction of tumour growth is without fail associated with the precancerous changes in the mucous membrane of the larynx. Many tumours are hormone-dependent.

The study of the pathogenetic role of hormones in precancerous diseases and laryngeal cancer remains a very relevant problem. Cortisol protects the human body from any stress; however, its role in carcinogenesis is not fully understood. Cortisol is involved in all processes in the body, so almost all cells are equipped with specific receptors. The purpose of our study is to determine the concentration of the cortisol hormone in the blood of patients with laryngeal precancerosis and cancer, to determine its role in malignant transformation and laryngeal carcinogenesis.

The article provides an analysis of the pathogenetic role of cortisol in the genesis of various precancerous diseases and laryngeal cancer. The blood concentration of the stress hormone cortisol was analysed in 56 patients with laryngeal precancerosis and 26 patients with malignant neoplasms of the larynx. The level of cortisol concentration in the blood of patients with laryngeal cancer is significantly higher than in patients with precancerous larynx. Scientific novelty: for the first time, a comparative analysis of cortisol levels in patients with precancerous diseases and laryngeal cancer was carried out.

The results obtained during the research create prospects and generate great interest in studying cortisol receptors in the tumour tissue of the larynx.

Keywords: *larynx, hormone, precancerous diseases, laryngeal cancer, cortisol, malignancy, carcinogenesis.*

Introduction

Currently, there are no objective and reliable markers for assessing the risk of malignant transformation of precancerous laryngeal diseases other than determining the degree of dysplasia [*Gorban N et al., 2013; Rivera C et al., 2017*]. Precancerous and cancerous diseases of the larynx are most often registered in men over the age of 40-50 years [*Antoniv V et al., 2011; Naydyon A et al., 2012*]. Women are characterized by an increase in the incidence of dyskeratosis and laryngeal cancer after 51-55 years, or at the age of 40 years. A number of authors associate such findings in this age group with hormonal

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changes in the body [*Nazhmudinov I, 2018*]. Precancerous and cancerous diseases of the larynx can develop at young age and in childhood [*Probst R et al., 2012; Andratschke M et al., 2015*].

It has been proven that psychosocial factors can influence the development and persistence of human papillomavirus primarily by affecting the immune response [*Chor J et al., 2016; Bates J et al., 2019; Lugović-Mihić L et al., 2021*].

The adrenergic pathway and activation of the sympathetic nervous system support carcinogenesis through tumour growth, malignant transformation, macrophage infiltration, angiogenesis, inflammation, spread, etc. Stress hormones (corticosteroids) affect signalling pathways involved in the transformation of malignant cells caused by human oncogenic viruses [*Smeets D et al., 2011; Lu D, 2017; Lugović-Mihić L et al., 2021*]. It is noteworthy that Th1 cells participate in cellular immunity, release IL-2, IFN-γ and TNF-β and activate macrophages, while Th2 cells stimulate humoral immunity, activate the B-cell response and antibody production, and also promote the secretion of IL-3, IL-5, IL-10 and IL-13.

Back in 1989, Ogoltsova E.S. and Matyakin E.G. demonstrated the results of experiments by various researchers, which indicate that the process of developing laryngeal cancer is hormone-dependent. For instance, experiments involving prolonged lubrication with a benzene solution on the laryngeal mucous membrane of rabbits with altered sex hormone levels (due to castration or extended administration of estrogenic drugs) revealed that papillomas developed later compared to experiments using only a carcinogen [*Berstein L, 2000; Zaridze D, 2004*].

Kalyi V.V. (2008, 2010) has also studied the pathogenetic role of sex hormones in laryngeal cancer. As a result, it is known that in men with laryngeal cancer, dysfunctions of the pituitary-gonadal system have been revealed both at the level of peripheral endocrine glands synthesizing sex hormones and at the level of central mechanisms regulating steroidogenesis. The nature and degree of severity of the observed changes are associated primarily with the stage of the neoplastic process and with the anatomical localization of the tumour, most often manifested in stage IV lesions of the supravascular larynx [*Kalyi V, Chertova N, 2010*].

Various stressful events are recognized by the paraventricular nucleus of the hypothalamus, which participates in a biological chain that combines personal experience, physiological signals and the release of corticotropin-releasing hormone [*Iftikhar A et al., 2021*]. Corticotropin-releasing hormone influences the pituitary gland, prompting it to release adrenocorticotropic hormone, which in turn signals the adrenal cortex to release glucocorticoids [*Iftikhar A et al., 2021*]. Thus, the body's adaptive response to stress depends on many interrelated neuroendocrine, immune, cellular and molecular mechanisms.

The adrenergic pathway and activation of the sympathetic nervous system support carcinogenesis through tumour growth, malignant transformation, macrophage infiltration, angiogenesis, inflammation, spread, etc. Stress hormones (corticosteroids) affect signalling pathways involved in the transformation of malignant cells caused by human oncogenic viruses [*Lugović-Mihić L et al., 2021*].

The results of research on hormonal changes in people under stress, especially changes in cortisol levels, are also important. Hypocortisolemia is manifested in individuals experiencing severe stress, as well as in those with chronic fatigue syndrome, fibromyalgia, and rheumatoid arthritis. On the other hand, elevated glucocorticoid levels are associated with an increase in white blood cells, a decrease in circulating lymphocytes and in the neutrophils-lymphocytes ratio [*Lugović-Mihić L et al., 2021*].

Previous studies on corticosteroids in patients with various cancers have indicated the described relation [*Magnon C et al., 2013; Lu D, 2017; Iftikhar A et al., 2021*]. For example, in patients with prostate cancer, cortisol stimulates carcinogenesis by activating the androgen receptor in the absence of androgens. The findings from the literature review highlight the need for further investigation into hormonal changes in patients with precancerous laryngeal diseases and cancer to enhance our understanding of the role of hormones in carcinogenesis.

Material and methods

Totally 88 patients aged 25 to 62 years (average age: 55.2 ± 2.08 years old) with malignant neoplasms and precancerous diseases of the larynx were examined. At the first stage of our research, in view of the data of physical examination, complaints, video laryngoscopy results, morphological and histological analysis of the tumours, 56 patients (main group) with precancerous lesions of the larynx were selected from the general cohort,

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of which 31 patients have had chronic hyperplastic laryngitis, 10 patients – a polyp of the vocal fold and larynx; leukoplakia of the vocal fold was documented in 8 cases, laryngeal pachyderma – in 7 cases. The comparison group included 26 patients (comparison group) with laryngeal cancer T1-3N0M0G1-3. All patients with a malignant laryngeal invasion had not received any special treatment prior to the examination.

Blood samples for hormone determination in peripheral serum were collected in the morning from the cubital vein, with 10 *ml* drawn after an overnight fast. The serum was cooled with ice water and centrifuged at 1500 *rpm* for 10 minutes with cooling no later than 30-60 minutes after sampling. Cortisol levels were measured using an ELISA kit (EIA-1887, Cortisol ELISA) for direct quantitative determination through immunoenzyme assay in plasma. A blood serum sample with endogenous cortisol was incubated in a slot together with an enzyme conjugate. After incubation, the unbound conjugate was washed out with water. The amount of bound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample. Upon the addition of the substrate, the intensity of colour is inversely proportional to the concentration of cortisol in the sample. This research was conducted at the State Budget Institution of the Ministry of Healthcare of the Czech Republic "Republican Oncological Dispensary".

Results

The cortisol level was studied in 56 patients of the main group and in 26 patients of the comparison group. Considering that the reference range of cortisol in women and men does not differ, the level of this hormone was assessed jointly by the comparison groups.

It was found that the average concentration of cortisol equal to 577.0 ± 11.1 *nM/l* (11.5%) in patients with laryngeal cancer was significantly ($p<0.05$) higher than in the examined individuals with precancerous diseases of the larynx, in whom the average concentration of cortisol was determined to be 528.4 ± 14.2 *nM/l*. It was also determined that in patients of the comparison group, an increase in the cortisol level was observed significantly more often (11.5%) compared to the main group (5.4%, $p<0.05$).

Discussion

The presence of markers that correlate with precancerous disease or early stage laryngeal cancer may be crucial for clinical decisions on monitoring or aggressive treatment of such lesions. To achieve the research goal, several tasks were undertaken: cortisol levels in the blood were measured, and the hormonal status of the patients was evaluated. Additionally, a comparative analysis of cortisol concentrations in patients with precancerous laryngeal conditions and laryngeal cancer was conducted. It should be noted that the average cortisol concentration (577.0 ± 11.1 *nM/L*) in patients with malignant neoplasms was significantly ($p<0.05$) higher than that of the examined people with precancerous diseases of the larynx (528.4 ± 14.2 *nM/L*). Comparison group patients were significantly more likely to have an increase in cortisol levels, compared to the main group (11.5% and 5.4%, $p<0.05$). The findings can be explained by the fact that the stress-activated hypothalamic-pituitary axis and the sympathetic nervous system act through glucocorticoid and adrenergic pathways that affect immune regulation, including stress-induced immunosuppression, oncogenic infection, as well as other potentially stress-induced neuroendocrine transmitters affecting carcinogenesis (for example, serotonin promotes the growth of tumour cells and angiogenesis; dopamine has the opposite effect) [*Lu D, 2017*]. A number of authors have provided data on the effect of corticosteroids on carcinogenesis in patients with various types of cancer [*Magnon C et al., 2013; Lu D, 2017; Iftikhar A et al., 2021*]. We were able to supplement the previously provided data on the relationship between glucocorticoids and carcinogenesis in patients with malignant laryngeal neoplasms.

Conclusion

The assessment of the hormonal status, primarily cortisol levels, in patients with precancerous laryngeal conditions and cancer and its connection to the malignant transformation of precancerous diseases of the larynx is of great practical and scientific importance. The data on the role of hypercortisolemia in the process of laryngeal carcinogenesis have been clarified. An increase in cortisol concentration in patients with laryngeal cancer compared with to those with precancerous diseases proves the role of cortisol and stress in the malignancy of laryngeal epithelial cells. Patients with precancerous diseases of the larynx are recommended to measure the level of cortisol in the blood. An increase in cortisol levels can be considered a marker of malignancy. The results obtained can be applied to the work of otolaryngologists, endocrinologists and oncologists.

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Ստեփան Ա. Ավագյան (Երևան. ՀՀ)

Խմբագրական կոլեգիա

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