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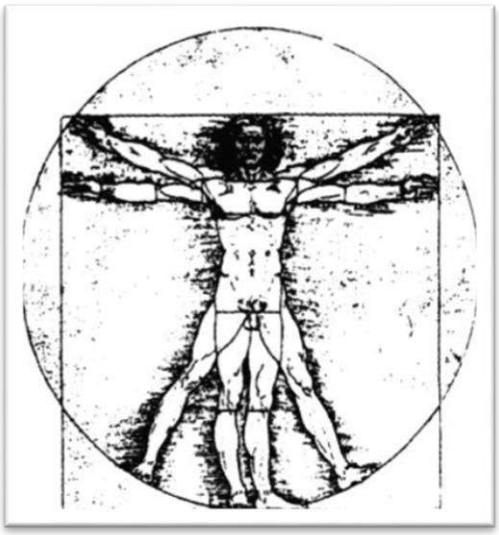

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ORIGINAL ARTICLE

PROCALCITONIN AS A PROMISING BIOCHEMICAL MARKER FOR EARLY DETECTION AND TREATMENT OF SEPSIS IN NEONATES AT INTENSIVE CARE UNIT AND ONCOLOGIC PATIENTS WITH FEBRILE NEUTROPENIA

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ABSTRACT

Introduction: Sepsis is a leading cause of death and a frequent complication in neonates at Pediatric Clinic Hospital in Skopje. Early diagnosis of sepsis as well as proper antibiotic treatment are crucial for this life-threatening condition.

Objectives: To determine the role of Procalcitonin (PCT) in establishing early diagnosis of sepsis, its sensitivity and specificity in correlation with other inflammatory markers, and blood culture as the gold standard.

Materials and methods: This retrospective-prospective study included 60 neonates and 40 oncology patients with febrile neutropenia. The control group included 50 patients with infection and sign of inflammation without sepsis according to the protocols for diagnosis, and examined group included 50 patients with two or three clinical signs of sepsis.

Results: PCT levels during the first 24h were evaluated in 44patients**.**The levels of C-reactive protein (CRP) were gradually increased after 12-36hours.The levels of white blood cells were increased in 21 neonates. In 16 patients' blood culture was positive. Seventeen patients developed severe sepsis, and 6 developed septic shock. After the start with antibiotics, PCT levels decreased, regardless of whether the blood culture was positive or negative. After 6-14 days, the levels of PCT and CRP decrease.

Conclusion: The level of PCT can provide rapid diagnosis of sepsis until the results of the blood culture are received. Increased PCT level allow to determine the severity of the infection in order to prevent worsening the condition, development of severe sepsis and septic shock and it helps in choice of appropriate therapy.

Keywords: Sepsis, C-reactive protein (CRP), procalcitonin (PCT)

INTRODUCTION

As a global health problem, sepsis is a leading cause of death in neonates and oncologic patients with febrile neutropenia, which is characterized with severe systemic inflammation, that affect all organs [1]. It is considered that sepsis leads to death, especially mortality is greatest in infancy and oncologic patients [2]. However, it has been shown that early detection and adequate antibiotic therapy significantly improve the outcome of the patients with infections that lead to sepsis. The initial treatment regimens should lead to stabilization and correction of metabolic, circulatory and respiratory disorders.

Adequate treatment with antibiotics and fluids should be started as soon as sepsis is clinically suspected. Inappropriate treatment of sepsis can lead to development of severe sepsis and septic shock.

Sepsis is manifested by early signs of circulatory disorders involving: tachycardia, tachypnea, peripheral vasodilation, and fever (or hypothermia), to circulatory collapse with multiple organic dysfunction syndrome and death [3]. Incidence of sepsis, severe sepsis and septic shock continues to increase. Although Gram positive bacteria remains the most common etiologic agent, infections of fungal etiology may also occur [3,4].

Over the years, much progress had been made in diagnosis and treatment of sepsis particularly in newborns and oncologic patients, and at the same time the mortality rate decrease. However, due to the increasing incidence of sepsis, the number of newborns and oncologic patients' death each year continues to increase. However, due to the increasing incidence of sepsis, the number of newborns' and oncologic patients' death continues to increase each year, 10%-20% sepsis, 20%-50% severe sepsis and 40%-80% septic shock [5].

Of fundamental importance is diagnosing an infection caused by bacteria or other microbiological organisms, for effective treatment and prognostic evaluation [5,6].

Current clinical and laboratory methods for diagnosing bacterial infections are either nonspecific or require longer time to develop the agent. Procalcitonin (PCT) is a biomarker that exhibits greater specificity than other proinflammatory markers in identifying pediatric patients with sepsis and can be used to diagnose bacterial infections [6]. Production of this gene prePCT, undergoes to proteolytic cleavage that produces PCT, which is further processed into mature calcitonin molecules [7]. Transcription and translation of CALC-1 gene is normally done in the thyroid C-cells and other neuroendocrine cells. PCT is the precursor for the hormone calcitonin (CT), composed of 116 amino acids with a *molecular weight of 14,5kDa*. Structure of PCT is divided into 3 sections: aminoterminus (57 aminoacids), immature calcitonin (33 aminoacids) centrally placed, and calcitonin carboxypeptide1 (CCP-1) the most distal end known as catacalcin (21 aminoacids). Its production is regulated, by calcitonin 1 gene (CALC-1). Nonetheless, production is activated, in response to bacterial infection in all parenchymal tissues, mediated by cytokines interleukin-6 (IL-6), tumor necrosis factor-α (TNFα) and interleukin-1β (IL-β). Other tissues don't have the ability to attach PCT to its mature form, that leads to accumulation of PCT [7,8]. Besides, PCT production is attenuated by interferon-γ primarily secreted in response to viral infection. This feature makes PCT a more specific marker for bacterial infection [9]. The level of PCT, in healthy pediatric patients is below 0.05 ng/ml. Blood concentrations of PCT increase during the systemic inflammation, mostly when it is caused by a bacterial infection [10]. When the PCT concentration go beyond 0.25ng/ml, the risk of local bacterial infection occurs. The risk of systemic bacterial infection occurs when the PCT level exceed 0,5ng/ml. High levels of PCT is common to be found in patients with severe sepsis and septic shock [11]. PCT is helpful marker for early detection of sepsis in newborns and oncologic patients. However, it has to be compared to the common inflammatory markers (WBC, Neutr%, CRP) and blood culture as a gold standard, for monitoring the infection. Results from cultures usually take at least 24 hours to become available and even then yield positive identification in only about 50% of cases [12], because of sampling error, previous antimicrobial treatment, or the presence of fastidious or slow growing pathogens. The dynamics of PCT concentration determine the duration of antibiotic therapy or its modification [13].

OBJECTIVES

The aims of this study are:

1. To determine the role of PCT in establishing early diagnosis of sepsis in neonates and oncology patients with febrile neutropenia
2. To determine the sensitivity and specificity of PCT in correlation with other inflammatory markers (WBC, Neutr%, CRP), blood culture as the gold standard for sepsis.
3. To determine the role of PCT in at-risk neonates and hematooncologic patients with febrile neutropenia in order to assess the prognostic value of this diagnostic method.

MATERIALS AND METHODS

A retrospective-prospective study performed at our Pediatric Clinic Hospital in Skopje, included 60 neonates and 40 oncology patients divided into two groups: Experimental and Control group. Experimental group of 50 pediatric patients diagnosed with sepsis according to the standards protocols on disease diagnosis.

The Experimental group was divided into two subgroups:

1. Subgroup of 30 newborns diagnosed with sepsis at the Intensive Care and Therapy Department.
2. Subgroup of 20 hematooncology patients with febrile neutropenia diagnosed with sepsis at the Hematology and Oncology Department.

Control group consisted 30 neonates who were admitted for other perinatal condition as indirect hyperbilirubinemia, intrauterine growth restriction or hypoglycemia and 20 oncologic patients who had no diagnosis of sepsis during the hospitalization period. The diagnosis of sepsis concerning the pediatric patients, is diagnosed according to the standard protocols on disease diagnosis. The clinical criteria for systemic inflammatory response syndrome (SIRS) are: fever>38.0°C or hypothermia <36.0°C, tachycardia >90 beats/minute, tachypnea >20 breaths/minute, leukocytosis>$12*10^9$/l or >10% immature neutrophils [14,15].

Criteria for Sepsis are presence of SIRS and confirmed infection, and positive blood culture.

Sepsis complicated by organ dysfunction (hypotension, hypoperfusion, acute renal failure and acute hepatic failure) is termed severe sepsis. Septic shock is a result of multiple organ dysfunction with persisting hypotension. Having a neutrophil count of less than <0.5 x10^9/L is defined as febrile neutropenia. The PCT and the other markers of inflammation (WBC, Neu%, CRP) are produced at the Clinical Laboratory of JZU University Clinic for Pediatric Diseases – Skopje.

Blood culture (BC) remains the gold standard for detection of pathogens that cause sepsis. At our Clinical Laboratory, the blood culture was evaluated with the *FilmArray blood culture* identification (BCID) panel. First BC sample is obtained before starting antibiotic therapy and the cause of sepsis is obtained using the Film array method, to identify a positive blood culture. Second BC sample is obtained within 3-5 days from starting with antibiotics and the third sample of BC is obtained within 6-14 days, only if the level of PCT after the third measurement is increased.

The PCT is determined through an immunological method: patented ELFA (Enzyme-linked fluorescent assay) technology, automated immune analyzer VidasBiomerieux (ng/ml).

The PCT value of healthy persons is under 0.05 ng/ml. Sepsis occurs when the PCT value is over 0.5 ng/m; high PCT values of over 2 ng/ml appear in severe sepsis and septic shock. First PCT sample was obtained within 24 hours from hospitalization.

Second PCT sample was obtained within 3-5 days and with commenced appropriate antibiotic treatment. Third sample of PCT was obtained within 6-14 days. We start with Broad-Spectrum antibiotics. The therapy starts immediately upon the first hemoculture.

RESULTS

This study included 100 pediatric patients divided into 50 patients in examined group in which sepsis was diagnosed, and 50 patients in the control group, according to the protocols for diagnosis of the disease.

Table 1. Number of patients with Severe Sepsis, Septic Shock and Lethal Outcome

	Neonates	**Oncologic patients**
Sepsis	8	12
Severe Sepsis	12	5
Septic Shock	4	2
Lethal outcome	6	1

During the first 24h, the values of PCT were significantly elevated ($p<0,05$) in 44 patients from the experimental group. Levels of CRP was significantly increased ($p<0,05$) after 12-36h after admission at the hospital. White Blood Count (WBC) was increased in 21 newborns (9000-30000 mm3). In 9 newborns as well as all 20 oncologic patients, the levels of WBC was low (WBC <4000mm3). In 9 newborns and 7 oncologic patients, the blood culture was positive. During the second measurement, 3-5 days after the start of appropriate antibiotic therapy, the PCT values were significantly decreased ($p<0,05$) but the blood culture remained positive in 7 newborns and 5 oncologic patients. CRP and Leukocytes levels were without significant changes ($p>0,05$). After the third measurement, levels of PCT and CRP were significantly decreased ($p<0,05$) that resulted in negative blood culture result. Twelve newborns and 5 oncologic patients developed severe sepsis, 4 newborns and 2 oncologic patients developed septic shock. Six newborns and one oncologic patient resulted in lethal outcome.

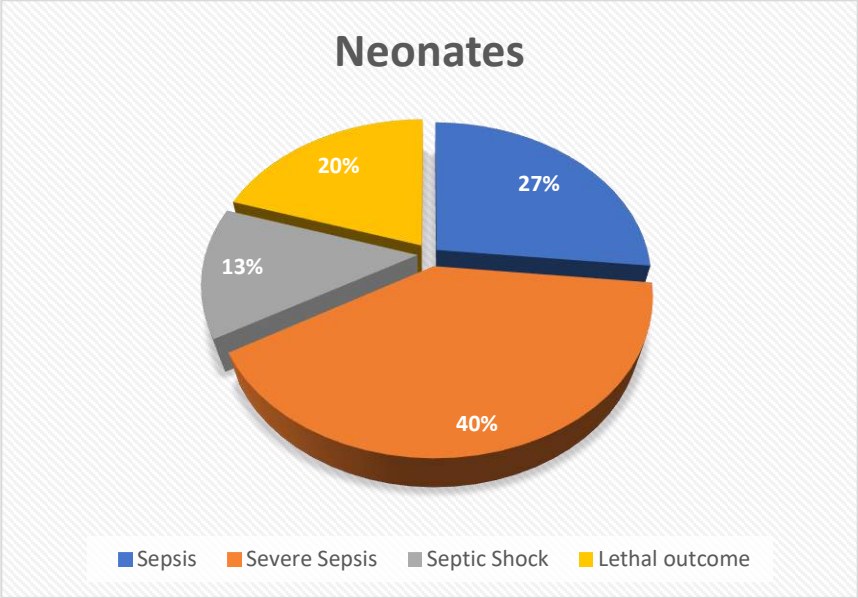

Fig. 1. Neonates with Sepsis, Severe Sepsis, Septic Shock and Lethal Outcome

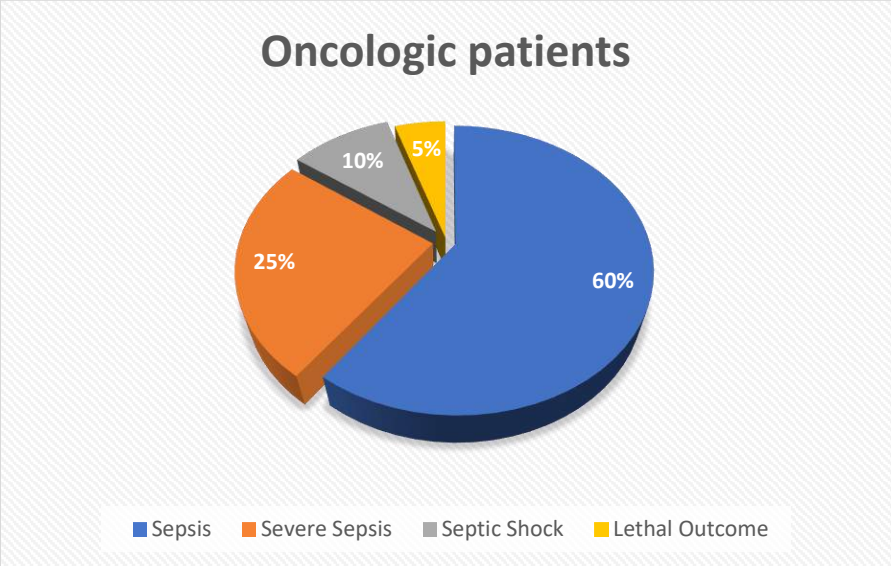

Fig. 2. Oncologic Patients with Sepsis, Severe Sepsis, Septic Shock and Lethal Outcome

For inflammatory markers for sepsis PCT and CRP, sensitivity, specificity, positive and negative predictive values were determined. The sensitivity (Sn) and specificity (Sp) of PCT was 88% and 92% respectively; the positive predictive value (PPV) was 91%, and the negative predictive value (NPV) was 88%.The Snand Spof CRP was 58% and7% respectively; the PPV was 65%, and the NPV was 62.5%.

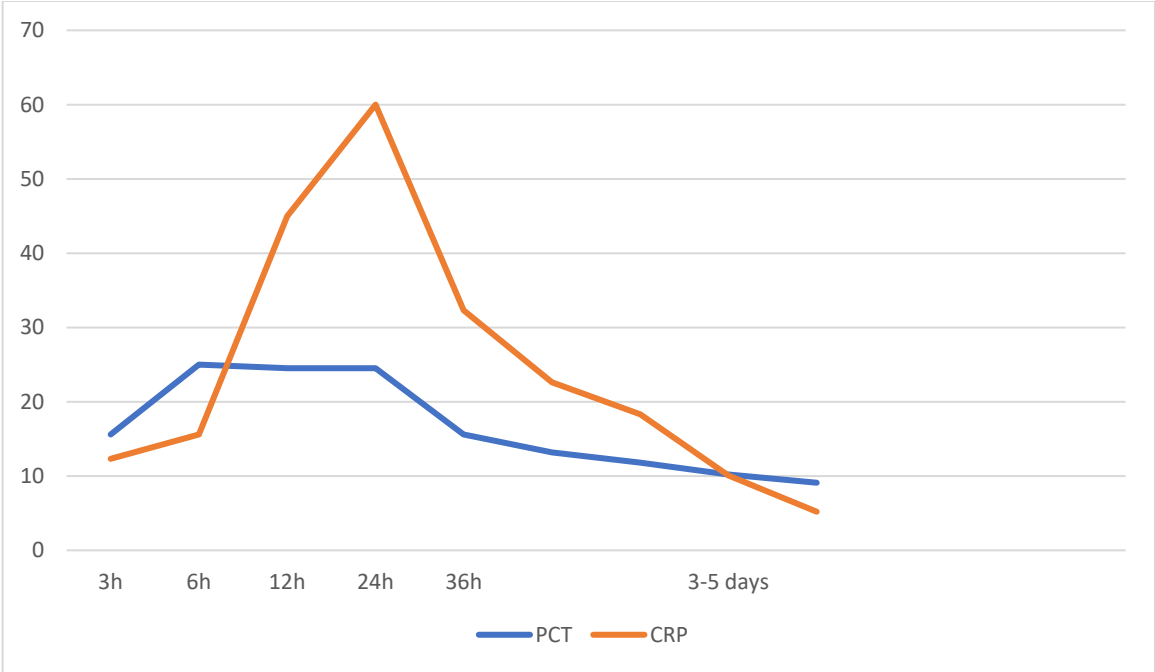

Fig. 3. The levels of PCT(Procalcitonin) and CRP(c-reactive protein) in the organism detected 3-4h, 6h, 24h and 3-5days after infections.

DISCUSSION

New findings from current studies on PCT levels and their sensitivity and specificity for early diagnosis of sepsis in pediatric patients have been described in a number of studies [16]. It is shown that bacterial lipopolysaccharide (LPS) is a potent inducer of PCT release in systemic circulation. In the organism the PCT is detected 3 to 4 hours after the infection, reaches its peak after 6 hours and the plateau for up to 24 hours.[**17]** On the other hand, the level of CRP increases 12 to 36 hours after bacterial infection. The half-life of PCT in the blood is between 25 and 30 hours [12]. Concentrations of PCT in the blood increase with systemic inflammation, especially when it is caused by a bacterial infection [18]. The risk of local bacterial infection occurs when the level of procalcitonin exceeds 0.25 ng/ml.The risk of systemic bacterial infection occurs when the level of PCT exceeds 0.5 ng / ml, high levels of PCT are present in severe sepsis and septic shock [18,19]. Studies show that early diagnosis and response to the therapy improve the outcome in pediatric patients with infections leading to sepsis. This opens up prospects for the use of PCT as an early marker for the selection and duration of antibiotic therapy for the treatment of sepsis in pediatric patients [17,19].

These findings are consistent with the findings in our study. In our study, at the beginning of therapy, the subjects had significantly elevated levels of PCT and CRP, while only 30% of the subjects had positive results from blood culture, which emphasizes the importance of procalcitonin in the early detection of sepsis. The effectiveness of the given PCT therapy in our study proved to be the first indicator of the reduction of inflammation compared to other parameters such as CRP, leukocyte count, and blood culture. This may indicate the need for regular determination of PCT after 3-5 days of initiating therapy to monitor the effects of it and its possible modification.The results of the level of PCT in both subgroups showed that PCT levels are increased in pediatric patients with sepsis comparing to the control group. Together, with other inflammatory markers of sepsis, PCT has an important role as an early marker in the diagnosis of sepsis, and will allow to monitor of therapy, duration, and response to the medication and prevent worsening of the condition. From the our study and aforementioned studies we can notice the importance of PCT as an early biochemical marker for diagnosis and prognosis of sepsis in pediatric patients, that shows substantial specificity and sensitivity than other proinflammatory markers. The dynamics of PCT concentration determine the duration of antibiotic therapy or its modification.

CONCLUSION

Sepsis in newborns and oncologic patients is a severe systemic infection and it remains a leading cause of morbidity and mortality. Early detection and treatment are pivotal for the outcome in neonates and oncology patients with infections leading to sepsis. PCT is an early marker for the diagnosis of sepsis in neonates and oncology patients. Two parameters were examined in newborns with two or three clinical signs for sepsis. The level of PCT was increased at the time of admission, while the CRP level increased gradually. Measurement of PCT level can provide a rapid diagnosis of sepsis until the results of the blood culture are received.The results obtained from the PCT levels in both subgroups of the examined group allow to determine the role of the increased PCT level during the infection in order to prevent worsening the condition, development of severe sepsis and septic shock. Additionally, it helps in choice of appropriate antibiotic, combination of antibiotics, and duration of response to the therapy in the treatment of sepsis.

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ORIGINAL ARTICLE

TOOTH DECAY IN EARLY CHILDHOOD

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ABSTRACT

Introduction: Circular caries occurs in the early childhood (1-1.5 year), immediately after the eruption of primary (deciduous) teeth. According to the latest world glossary, this term is also known as early childhood caries (tooth decay in early childhood).

Objective: The aim of this study was to determine the level of the glucose values in the saliva samples taken at different time intervals: 5, 15, 30 and 60 minutes after consuming two types of liquid food (sweetened milk and fruit juice).

Materials and methods: The study included a test-group of 40 children, age 3-3.5 years, with baby bottle caries that were still using baby bottles for feeding, and a control group of 40 children at the same age, but without caries. The glucose concentrations in the saliva samples were determined with the enzyme method GOD/PAP (Berhan and Trinder, 1972) at the Institute of Biochemistry of the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia.

Results: The analysis of the results showed statistically significant differences in determined values of saliva samples in the test-group and in the control group ($p<0.01$). These differences were more expressed after consuming fruit juices.

Conclusion: Study results has led us to the conclusion that fruit juice is stronger caries causing liquid than milk ($p<0.001$).

Keywords: baby bottle caries, saliva, glucose, sweetened milk, fruit juice

INTRODUCTION

Circular caries is a dynamic group of complex physical and chemical processes in the primary teeth [1]. The alternating periods of demineralization and remineralization as well as numerous interreaction processes of deciduous teeth surface lead to imbalance and mineral loss [2]. During this process, calcium and phosphate ions from hydroxyl apatite crystal pass into the plaque and saliva. In fact, the carious lesion occurs in two separate stages: The early stage is known as initial lesion - macula alba, or white spot, which is reversible [3, 4]. At this stage the caries has not completely penetrated the enamel border. At the moment when caries penetrates, it spreads along the border and then cavitation occurs, *i.e.*, caries, due to cracking of the enamel surface.

Circular caries is a specific type of caries found in the primary teeth, which can be detected, even in very young children (age1-1.5), immediately after the teeth eruption and reaching its culmination between the ages of 2 to 5 years [5]. This type of caries is often found in the literature as "baby bottle syndrome", *i.e.*, syndrome of children fed with the bottle and "nursing bottle caries" [6, 7, 8, 9]. In the modern world terminology, this caries is also known as "baby bottle caries" [8] while the latest scientific literature uses the term *Early Childhood Caries* (Tooth decay in early childhood [9, 10].

In the everyday dental practice, due to an unbalanced diet and the lack of oral hygiene in the early childhood, we are often faced with the problem of diagnosing of the advanced forms of early childhood caries [11, 12].

The increase of this prevailing caries [11, 13], as well as the unclear etiopathogenic mechanisms, were the main reasons for this study [12]. The appearance of baby bottle caries is characteristic for those who use the feeding bottle as passive, prolonged and inappropriate means of feeding [7, 8]. A content rich in fermentable carbohydrates such as sucrose, which in the oral environment is decomposed in glucose and fructose, simultaneously decrease the pH level favouring the disease [16]. In one study, it has been shown that less acidogenic sugars were lactose and galactose [17].

OBJECTIVE

The aim of this study was to determine the sucrose concentrations by evaluation of the concentration of glucose in saliva samples.

MATERIALS AND METHODS

The test group comprised 40 children with baby bottle caries, aged 3-3.5 years who were still using a baby bottle for feeding. The group was divided into two subgroups according to the type of food used through baby bottle: 20 examined patients were consuming milk with 2 tablespoons of sugar and the other 20 were consuming fruit juice. The control group was consisted of 40 children at the same age (3-3.5 years), with no detected caries, nor used baby bottle for their feeding. They were divided under the same criteria into two subgroups: 20 patients consumed sweetened milk and 20 consumed fruit juice, respectively. We examined the saliva samples from each examinee 5, 15, 30 and 60 min after the liquid intake, as well as one saliva sample in the morning before consuming any food or liquid (on empty stomach). The samples were taken by the parents according to our instructions and morning samples were used for comparison. The saliva samples were used for determination of the glucose concentrations. The laboratory procedures were done at the Institute of Biochemistry, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of Macedonia. The concentrations values were determined with the enzyme method GOD/PAP (Berhan and Trinder, 1972). The glucose values were compared with the basic values taken in the mornings, before consuming any food or drink (Fasting Saliva Sugar on Awakening-FSSA value). The obtained data were analysed with the statistical package SPSS version 23, with the basic statistical methods: X (average arithmetic); SD (standard deviation); SE (standard error); X^2 test; Student's T-test.

RESULTS

Data analysis considering glucose values (concentration - μmol/L) in the saliva samples taken at different time intervals: 5, 15, 30 and 60 min. after consuming sweetened milk in test and control group and FSSA values are presented in Table 1.

Results analysis shown significant differences in the concentration of saliva samples taken in the morning on empty stomach between test and control groups.

Also, statistically significant differences among test group and control group 5minutes after consuming sweetened milk.

Significant differences among test group and control group 15 minutes after consuming sweetened milk.

There were significant differences among test group and control group 30 minutes after consuming sweetened milk.

As shown in Table 1, significant differences among test group and control group 60 minutes after consuming sweetened milk.

Table 1. Glucose values (concentration - μmol/L) in the saliva samples taken at different time intervals: 5, 15, 30 and 60 min. after consuming sweetened milk in test and control group and FSSA values

Glucose in saliva sample (μmol/L)	N (number)	Average glucose value (X)	Difference X to FSSA* value	T-test	SD	p-value
FSSA value	Test group (20)	317.215 = B			62.62	
	Control group (20)	239.275 = B	/	4.49	45.72	<0 .001
after 5 min	Test group (20)	779.0	461.785		308.01	
	Control group (20)	639.0	399.725	1.73	187.94	<0.05
after 15 min	Test group (20)	470.8	153.585		127.79	
	Control group (20)	267.65	28.375	6.59	51.52	<0.001
after 30 min	Test group (20)	344.25	27.035		68.13	
	Control group (20)	242.9	3.625	5.52	45.67	<0.001
after 60 min	Test group (20)	320.7	3.485	4.54	63.60	<0.001
	Control group (20)	241.0	1.725		45.89	

*FSSA value-saliva samples taken in the morning on empty stomach (BASAL value of saliva)

Data analysis considering differences in glucose values in saliva samples taken at different time intervals after consumption of sweetened milk in the study and control group in relation to the basal value of saliva are shown in Figure 1.

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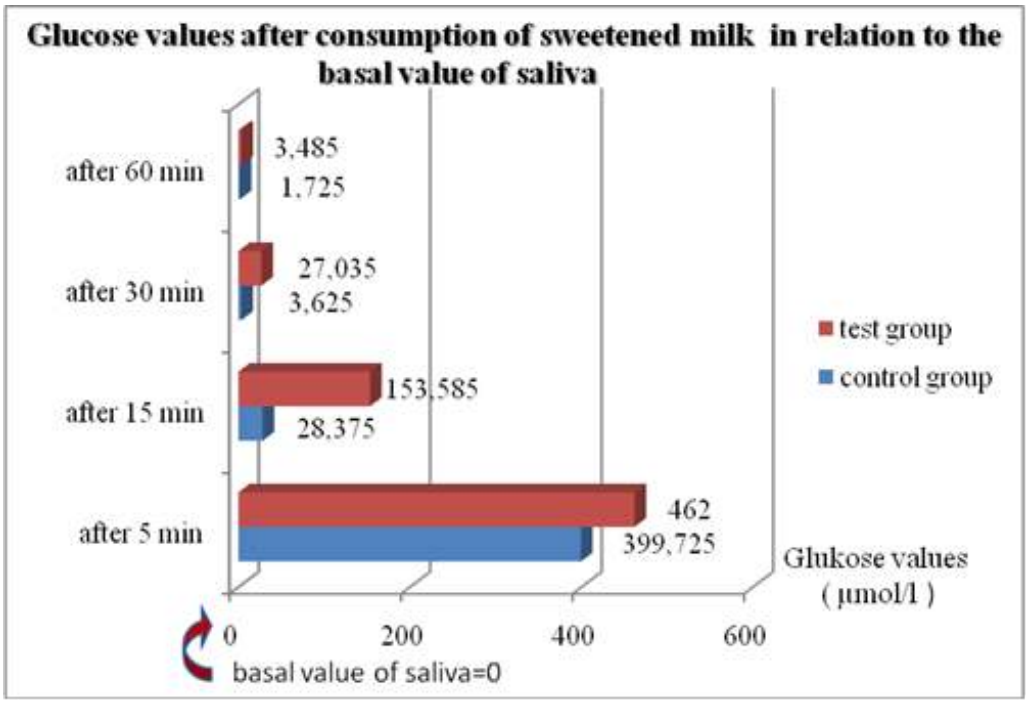

Fig. 1. Differences in glucose values in saliva samples taken at different time intervals after consumption of sweetened milk in the study and control group in relation to the basal value of saliva

Data analysis considering glucose values (concentration - μmol/L) in the saliva samples taken at different time intervals: 5, 15, 30 and 60 min. after consuming fruit juice, in test and control group and the differences in the FSSA value are presented in Table 2.

Table 2. Glucose values (concentration - μmol/L**)** in the saliva samples taken at different time intervals: 5, 15, 30 and 60 min. after consuming fruit juice, in test and control group and the differences in the FSSA value

Glucose in saliva sample (μmol/L)	N (number)	Average glucose value (X)	Difference X to FSSA	T-test	SD	p-value
FSSA value	Test group (20)	330.8	/	4.56	70.505	**< 0.001**
	Control group (20)	243.25	/		48.915	
after 5 min	Test group (20)	1460.5	1129.7	7.72	291.954	**< 0.001**
	Control group (20)	836.2	592.95		213.204	
after 15 min	Test group (20)	892.95	562.15	8.57	123.97	**< 0.001**
	Control group (20)	524.4	281.15		146.923	
after 30 min	Test group (20)	578.7	247.9	15.3	80.159	**< 0.001**
	Control group (20)	257.25	14.0		48.337	
after 60 min	Test group (20)	338.45	7.65	4.89	67.604	**< 0.001**
	Control group (20)	**246.25**	**3.2**		49.861	

*FSSA value-saliva samples taken in the morning on empty stomach (BASAL value of saliva)

Data analysis considering **d**ifferences in glucose values in saliva samples taken at different time intervals after consumption of sweetened milk in the study and control group in relation to the basal value of saliva are shown in Figure 2.

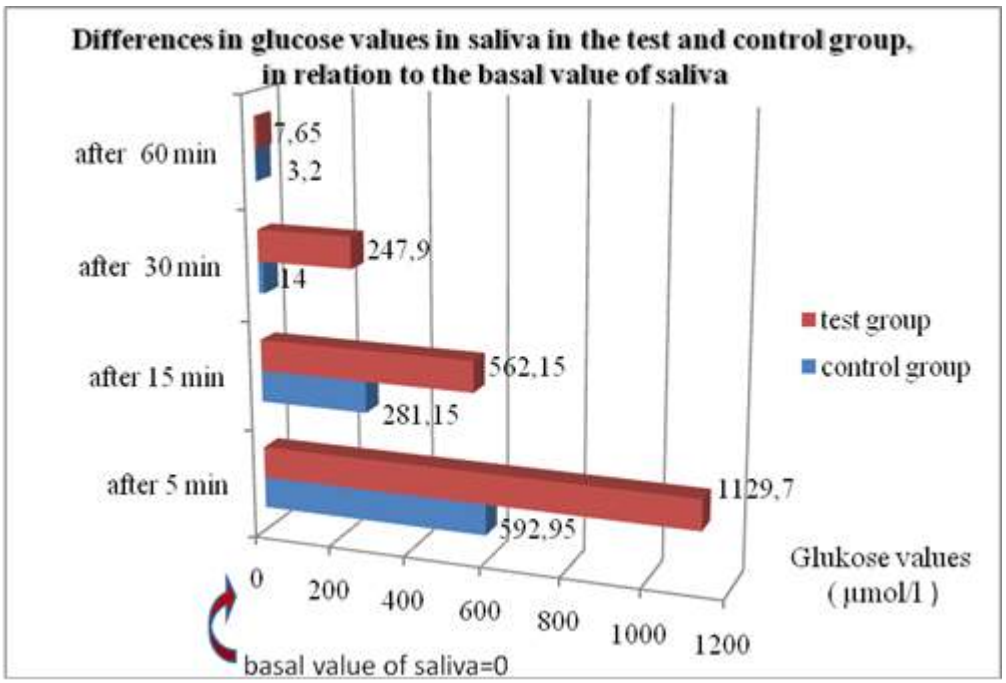

Fig. 2. Differences in glucose values in saliva samples taken at certain time intervals after consumption of juice in the study and control groups in relation to the basal value of saliva

Data analysis considering Glucose values between the test and control group in saliva samples taken at different intervals, depending on the type of content consumed are presented in Figure 3.

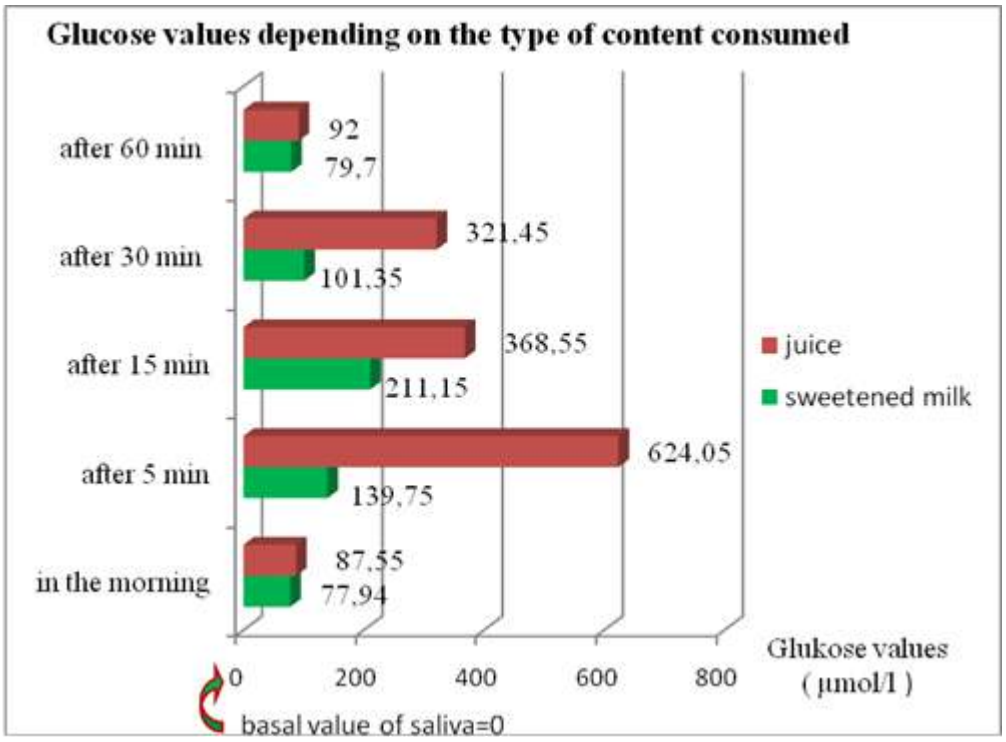

Fig. 3. Glucose values between the test and control group in saliva samples taken at different intervals, depending on the type of content consumed

DISCUSSION

According to some studies [18, 19] the high concentration of glucose in the oral cavity remained longer in the younger children compared to the older ones (over 1.5), due to the slower process of the salivary self-cleaning, which is in agreement with our findings.

The comparison of the salivary pH values in the examinees from the 2 subgroups of the test group, obtained 5 min after the consumption of sugared milk and juice, showed that they decreased to the value of 5.51 in the subgroup of children consuming juice, while they were 5.91 in the subgroup that consumed sugared milk [20]. These results corresponded to the higher average concentrations of glucose obtained in the same saliva samples after consumption of juice (1460 µmol/L), compared to milk (836.2 µmol/L). The obtained results referred to the fact that the juice that children consume, besides the glucose and fructose from the fruit, known as internal or natural carbohydrates, contains also refined sucrose which in the process of its fermentation decomposes to its monomers: glucose and fructose [20, 21]. Thus, the glucose and fructose are present in the juice in much higher concentration (coming from the fruit as well as from the added sucrose) [22, 23]. Our results correspond to those that examined the pH level of saliva, plaque and glucose values after consumption of different types of sweetened drinks. The salivary pH value in the examinees with Decayed-Missed-Filled (DMF) = 3-5 decreased below the critical level of 5.5 after the consumption of the sweetened liquid. During the night these values decreased even more (5.5) [19]. Considering the fact that during the night the salivation is declining and reaches minimum, there is a self-cleaning of the carbohydrates from the oral cavity [16,17,18). In the second group of the examined subjects without detected caries (DMF = 0), the same author obtained pH values higher than the critical level of 5.5 [21].

Bobinac [16] obtained similar results while studying the influence of the juices [16,20,21,24,25] (sweetened and non-sweetened) on the salivary pH at different time intervals after consumption, and he concluded that juices were highly cariogenic liquids. When the cariogenic liquids are taken often, one by one, the process of demineralization is speeding up [9, 15, 17, 20, 21]. The same process was detected in our study in patients consuming sweet liquids from the baby bottle often, especially before falling asleep or during the night [22]. The pH of the saliva decreased below the critical value of 5.5 because the salivation flow also decreased during the night, while children were sleeping and there was no self-cleaning of the mouth [17, 21]. The result was accelerating and emphasizing the demineralization process [6, 8, 9] in children feed with sweetened liquids and the development of a specific type of caries, the so-called: "Caries caused by a bottle diet" [5, 10, 21, 24, 27] "Baby bottle caries", *i.e.*, "Baby bottle syndrome".

CONCLUSION

The FSSA values in saliva samples were higher in the tested group, showing a statistically significant difference with coefficient $p<0.001$ compared to the control group. The glucose concentration in the saliva samples was higher in the patients with baby bottle caries in the first 5 min. after the consumption of the milk, with a statistical significance ($p<0.05$) compared to the control group, while after 30 min. we found a higher statistical significance ($p< 0.001$). At the same intervals of 5 and 30 minutes after the consumption of fruit juice, the glucose concentrations were higher in the patients with baby bottle caries compared to those from the control group, showing a very high statistical significance ($p< 0.001$). Also, the difference in the glucose concentrations was more intensified after the consumption of juice, which confirmed the conclusion that the juice has more cariogenic effect compared to the sugared milk ($p<0.01$). The general recommendations for our patients were to avoid feeding with bottle with sweetened liquids, especially before the sleeping time and overnight in order to prevent this highest caries risk etiological factor.

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ORIGINAL ARTICLE

AXIAL LENGTH AS A RISK FACTOR FOR GLAUCOMA IN MYOPIA

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ABSTRACT

Introduction: Glaucoma, one of the leading causes of irreversible blindness in the elderly population worldwide, is progressive optic neuropathy. Because glaucoma ranks second on the list of ophthalmic diseases with the highest morbidity, there are many definitions that characterize its importance in the scientific world and in the seeking of opportunities for its treatment, and thus reducing the percentage of blindness that glaucoma can cause. Eyes with high myopia are less tolerant of IOP fluctuations than other eyes. In them the bulbus oculi is larger, the lamina cribrosa is thinner, and the scleral wall is thinner with varying elasticity. Even the slightest increase in IOP can cause damage to the posterior pole because the myopic eye has an elongated elliptical shape, the posterior wall is stretched much more, unlike the non-myopic eye, so with each increase in IOP, the optic nerve is more prone to damage.

Objective: The aim of this study was to determine whether the increased axial length encountered in myopic eyes is associated with glaucoma, i.e. whether it is a risk factor for glaucoma.

Materials and methods: A retrospective case-control study was performed, which included patients aged 25 to 70 years. The study was conducted at the Ophthalmology Clinic, in Skopje, in the Glaucoma Cabinet, in the period from 2015-2019.The study included 100 patients, who were divided into two groups. The axial length of the bulbus and axial length of the vitreous were measured in all patients using the echobiometry method. VC-axial length of vitreous, the normal value is 15.37mm for men, 15.48mm for women.AX-axial length of the bulb, the normal value is 23.0-23.9mm. The obtained values were compared between the examined and the control group.

Results: In determining the significance of the contribution to the prediction of glaucoma, it was found that the axial length (ax) (Wald = 0.48 / p> 0.05 (p = 0.49) has a greater influence than the length of the vitreous (vc) (Wald = 0.03 / p> 0.05 (p = 0.87). When increasing the axial length (ax) by 1 mm. the risk of glaucoma increases by 16.40% (Exp (B) = 1,164), the effect of the axial length (ax) is not significant / 95% C.I: 0.76-1.79 / p> 0.05.When increasing the length of the vitreous (vc) by 1 mm the risk of glaucoma increases by 3.30% (Exp (B) = 1.033), the effect of the vitreous length (vc) is not significant / 95% C.I: 0.69-1.54 / p> 0.05.

Keywords: axial lenth, glaucoma, myopia

INTRODUCTION

Is it glaucoma? Or is it a condition that resembles glaucoma? Ophthalmologists often face a diagnostic dilemma when examining patients with high myopia. "High myopia may not have glaucoma, but the condition looks like glaucoma. Or they may have glaucoma, but we are not sure." (Simon K. Law, MD). Simon K. Law once stated that the difficulty in diagnosis lies in the fact that the papilla of the optic nerve (PNO) in myopia usually looks abnormal, and its changes are one of the parameters for the diagnosis of glaucoma.

And eye pressure may not be very high. There may even be visual field defects that do not progress, or progress differently. This causes a diagnostic problem and the problems that follow.

Glaucoma, one of the leading causes of irreversible blindness in the elderly population worldwide, is progressive optic neuropathy [1].

According to the European Glaucoma Association, glaucoma is a group of chronic progressive neuropathies most commonly characterized by morphological changes in the papillae of the optic nerve and retinal nerve fibers, without the presence of other eye diseases or congenital anomalies.

Because glaucoma ranks second on the list of ophthalmic diseases with the highest morbidity, there are many definitions that characterize its importance in the scientific world and in the seeking of opportunities for its treatment, and thus reducing the percentage of blindness that glaucoma can cause [2]. According to Murray-Lewis, about 67 million people were diagnosed with glaucoma in 2000, making glaucoma the second leading cause worldwide. The number of patients with visual acuity in both eyes equal to or less than 0.1 is about 6 million [3]. The risk factors for glaucoma can be divided into systemic and local. Systemic risk factors are blood pressure: hypotension or hypertension, vasospasm, diabetes, chronic heart disease, hypercholesterolemia, thyroid disease, etc. Local risk factors are: parapapillary atrophy, intraocular pressure, papillary escalation, certain diseases of the anterior or posterior segment of the eye, central corneal thickness, fluctuations in IOP, etc. According to the pathophysiological mechanism of myopia, it is divided into bulbar and lental. Bulbar myopia occurs as a result of increased axial length of the bulb. In these eyes the axial length of the bulb is greater than 24 mm. Every millimeter of increase in length over the normal manifests as myopia of 2.5 D (e.g. axial length of 26 mm, myopia -5D). A much rarer type of myopia is lental myopia, which is a refractive anomaly resulting from increased fracture power of the lens [4]. One of the theories for the occurrence and development of myopia refers to the effect of increased IOP, and insufficient scleral resistance in a number of young people. Their sclera stretches under the action of slightly increased IOP, and the eye as a whole becomes larger. This mechanism, called minihydrophthalmos, has not yet been established in most myopias [4]. Eyes with high myopia are less tolerant of IOP fluctuations than other eyes. In them the bulbus oculi is larger, the lamina cribrosa is thinner, and the scleral wall is thinner with varying elasticity. Even the slightest increase in IOP can cause damage to the posterior pole because the myopic eye has an elongated elliptical shape, the posterior wall is stretched much more, unlike the non-myopic eye, so with each increase in IOP, the optic nerve is more prone to damage [4].

OBJECTIVE

The aim of this study was to determine whether the increased axial length encountered in myopic eyes is associated with glaucoma, i.e. whether it is a risk factor for glaucoma.

MATERIALS AND METHODS

A retrospective case-control study was performed, which included patients aged 25 to 70 years. The study was conducted at the Ophthalmology Clinic, in Skopje, in the Glaucoma Cabinet, in the period from 2015-2019.

The study included 100 patients, who were divided into two groups:

1. The first group of respondents (group 1 / Patients with primary open-angle glaucoma) included: 60 patients diagnosed with glaucoma. Entry criteria for this group were the following:
   - increased intraocular pressure (IOP) -over 24mmHg. without therapy;

- changes of the optic nerve papilla (PNO);
- vision field defects;
- anti - glaucoma therapy.

2. The second group of respondents (group 2 / Control group) included: 40 patients without glaucoma. Entry criteria for this group were the following:
   - patients without glaucoma;
   - normal IOP;
   - normal vision field;
   - orderly results of PNO.

The axial length of the bulbus and axial length of the vitreous were measured in all patients using the echobiometry method. This method is performed on the ALCON Ocu Scan RXP device and consists in measuring the length of the bulbus and the vitreous with the help of A-scan.

The method itself consists of instilling the anesthetic Tetracaine (Alcaine) into both eyes of the patient in a supine position. After a few minutes, the measurement is performed by using the immersion method. The device automatically takes ten measurements. For the needs of the study was taken the average value of all measurements.

Normal values for emmetropic eye echobiometry:

- VC-axial length of vitreous, the normal value is 15.37mm for men, 15.48mm for women.
- AX-axial length of the bulb, the normal value is 23.0-23.9mm. The obtained values were compared between the examined and the control group.

Descriptive statistics (Mean ± Std.Dev.; ± 95.00CI.; Minimum; Maximum; Data distribution was tested on: Kolmogorov-Smirnov test; Lilliefors test; Shapiro-Wilks test (p); The difference in the series of numeric marks between the examined * control group was analyzed using T-tests; Grouping (t / p) and Mann-Whitney U Test (Z / p).

RESULTS

1. Vitreous length (vc) & Axial length (ax)

Table 1 and Graph 1 show descriptive statistics of vitreous length (vc) and axial length (ax). The length of the vitreous (vc) varies in the range 17.55 ± 2.14 mm, ±95.00% CI: 17.13-17.98; the minimum value is 13.87 mm. and the maximum value is 24.42 mm.

The axial length (ax) varies in the range 25.31 ± 2.02 mm, ±95.00% CI: 24.90-25.71; the minimum value is 21.20mm and the maximum value is 30.49 mm.

Table 1. Vitreous length (vc) & Axial length (ax)

Variable	Valid N	Mean	Confidence-95,00%	Confidence+95,00%	Minimum	Maximum	Std.Dev.
Vitreous length (vc)	100	17,55	17,13	17,98	13,87	24,42	2,14
Axial length (ax)	100	25,31	24,90	25,71	21,20	30,49	2,02

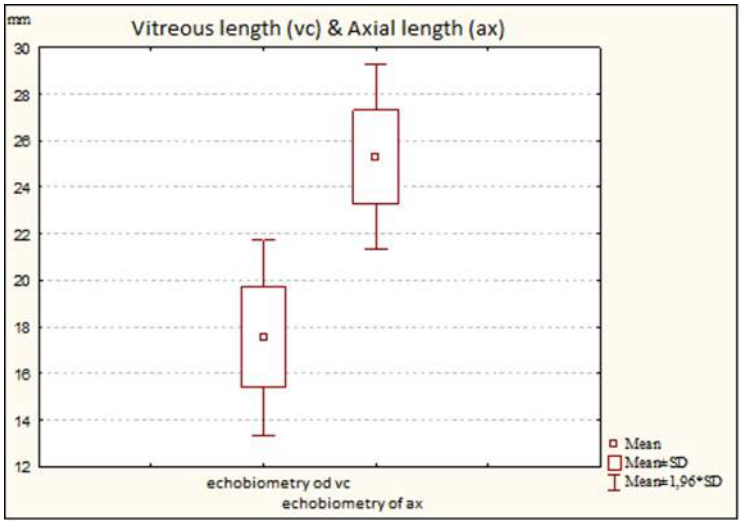

Fig. 1. Vitreous length (vc) & Axial length (ax)

2. Glaucoma prediction / Glass body length (vc) & Axial length (ax)

The enter method was used to determine the predictive values of vitreous length (vc) and axial length (ax) for glaucoma. The global accuracy of this model for predicting glaucoma is 62.00%. The sensitivity is 91.70% and the specificity is 17.50% (Table 2).

Table 2. Predictive values of vitreous length (vc) & axial length (ax) / for glaucoma / Model of discrimination

Observed		Predicted		
		Glaucoma		Percentage Correct
		yes	no	
Glaucoma	no	7	33	17.5
	yes	5	55	91.7
Overall Percentage				62.0

a. The cut value is .500

In determining the significance of the contribution to the prediction of glaucoma, it was found that the axial length (ax) (Wald = 0.48 / p> 0.05 (p = 0.49) has a greater influence than the length of the vitreous (vc) (Wald = 0.03 / p> 0.05 (p = 0.87) (Table 2.1).

When increasing the axial length (ax) by 1 mm. the risk of glaucoma increases by 16.40% (Exp (B) = 1,164), the effect of the axial length (ax) is not significant / 95% C.I: 0.76-1.79 / p> 0.05.

When increasing the length of the vitreous (vc) by 1 mm the risk of glaucoma increases by 3.30% (Exp (B) = 1.033), the effect of the vitreous length (vc) is not significant / 95% C.I: 0.69-1.54 / p> 0.05.

Table 2.1 Binary Logistic Regression Analysis for Glaucoma Prediction /vitreous length (vc) & Axial length (ax)

Variable	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Vitreous length (vc)	.032	.205	.025	1	.874	1.033	.692	1.543
Axial length (ax)	.152	.219	.481	1	.488	1.164	.758	1.786
Constant	- 3.985	2.979	1.789	1	.181	.019		

a. Variable(s) entered on step 1: Vitreous length vc, Axial length ax.

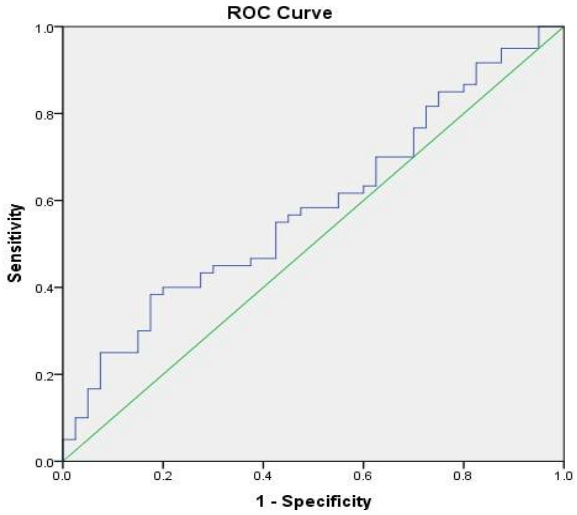

Fig. 2. Binary Logistic Regression Analysis for Glaucoma Prediction /vitreous length (vc) & Axial length (ax)

The ROC area is 0.583 which means that in 58.30% / 95% CI: 0.471-0.696 / p> 0.05 (p = 0.159) / of all possible pairs of patients in which one has glaucoma and the other has no glaucoma, this model will determine a higher probability of glaucoma (Figure 2).

DISCUSSION

Glaucoma, one of the leading causes of irreversible blindness in the adult population worldwide, is a progressive optic neuropathy. Primary open-angle glaucoma (POAG) is the most commonly reported type of glaucoma in prevalence studies based on the worldwide population. Increased intraocular pressure is a well-known major risk factor for POAG. In addition, there is evidence that other risk factors such as age, sex, race, refractive error, heredity, and systemic factors may play a role in the pathogenesis of glaucoma.

Regarding the values of the parameters obtained by echobiometry, the length of the vitreous varies in the interval 17.55 ± 2.14 mm and the axial length varies in the interval 25.31 ± 02.02 mm. When determining the predictive values, it was found that increasing the axial length by 1 mm increases the risk of glaucoma by 16.40% insignificantly by p = 0.488, and when increasing the length of the vitreous by 1 mm, the risk of glaucoma increases by 3.30% insignificantly by p = 0.874.

The results of the Singapore Eye Study [5] support our results for the axial length of the eye, which found that patients with longer axial bulb length were 3 times more likely to have POAG than patients with normal axial length (OR, 3.00).

A similar association was found in the Kandy Eye Study [6], where it was shown that there is an association between axial length and glaucoma (p = 0.003). Increasing axial length increases the possibility of glaucoma. This occurs as a result of thinning of the lamina cribrosa and peripapillary sclera in eyes with longer axial length, so that intraocular pressure of different values can cause damage.

A study conducted in the United States [7] at the Mayo Clinic found a strong correlation between axial length and RNFL thickness (p <0.001). In the study, which included eyes with moderate myopia (-2.96 + 1.36), it was shown that increasing the axial length by 1 mm reduced the thickness of the RNFL by 2.2 microns. The influence of the axial length on the RNFL thickness, as a major predictor of glaucoma, is essential for the monitoring and diagnosis of glaucoma patients. Often, in patients with longer axial length without knowing the refractive error, glaucoma can be misdiagnosed as a result of reduced RNFL thickness. Therefore, these patients should be monitored if they develop additional symptomatology of the disease.

In the Los Angeles Latino Eye Study [8], a direct association was found between axial length and glaucoma (p <0.001).

CONCLUSION

Based on the results of our study, the following conclusion was reached that there is no significant difference (p> 0.05) between the study (group with glaucoma) and the control group (group without glaucoma) in terms of axial bulb length and axial vitreous length.

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ORIGINAL ARTICLE

CORRELATION BETWEEN ECHOCARDIOGRAPHIC PARAMETERS AND THE SEVERITY OF STAGE IN PATIENTS WITH COPD

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ABSTRACT

Introduction: COPD is a disease of the modern society and it is a reason for high morbidity, early mortality and high rate of death and a big burden of the health system.

Materials ant methods: In this pilot study 22 patient were enrolled with COPD stage 3 and 4 (severe and very severe form). All 22 patient were clinically examined (anamnesis, clinical status, ECG of the patient) and echocardiography was performed. Demographic and correlation of risk factors was done in all 22 patients. Echocardiography analysis (with plenty parameters) such as calculation of dimensions, structural and functional changes of the right and left heart was made.

Objective: The aim of this study was to identify the echocardiographic parameters important for evaluation of COPD patients in stage 3 and 4 according to GOLD classification.

Results: In our study male gender dominated with 77,3 %, and average age was 66,4%. All 3 risk factors (HTA, HLP, DM) that were taken for analysis were noted with 54,5%. Smoking as risk factor for developing COPD was present with 59%. Left chamber had normal diastolic size and was 41,6% with average values for global systolic strength (in normal ranges) as EF% with 57%. Systolic strength of the left ventricle was preserved in all 22 patients. All 22 patients had reduced AT (acc. time), <105 m/sec. Value of the tricuspid regurgitation in 8/22 patients was significant and it was 36% and two patient had significant PAH (pulmonary artery hypertension). Tissue Doppler of the tricuspid valve as a marker of diastolic dysfunction was positive in 9 patients with reduction of S'. TAPSE was with normal values in all 22 patients.

Conclusion: Echocardiography as a noninvasive method and successful strategy for evaluation of right chamber parameters and function especially in patients with COPD. Our pilot study showed some early positive echocardiography parameters that were important for right heart evaluation (dimensions and function of the right heart). Small sample of patients was limited factor for the significance of our study.

Key words: COPD, echocardiography, right heart, pulmonary hypertension

INTRODUCTION

COPD is a disease of the modern society and is a cause of high morbidity, early mortality and high rate of death and a heavy burden on healthcare systems. It was on the sixth place in 1990, and it is assumed that in 2020 itis going to be one of the 3 leading causes of death worldwide [1, 2, 3]. COPD is a chronic disease, slowly progressive and is characterized with a chronic inflammation of the bronchi, which in most cases is an irreversible process.

By definition, it is a chronic obstruction of the airflow as a combination of inflammation of the bronchi and bronchiole and parenchymal destruction(emphysema) [4]. Smokers are the most commonly affected subjects and as the disease progresses it leads to chronic respiratory failure. Active, passive smoking and air pollution are the main causes of disease progression. Smoking is no longer privilege of the male population; the global perception has been changed in a direction of equalization of gender smokers [2]. COPD is a disease represented in different forms: chronic bronchitis, chronic respiratory failure and emphysema [2, 4].

COPD has its first expression between the age of 40 and 50. It is characterized with air-limitation, chronic morning cough, chronic fatigue, and mucous production. COPD causes weak immune system, possibility of frequent lung infections, chest tightness, and lack of energy.

The confirmation of COPD is done by taking anamnesis of the patient, physical examination, contemporary laboratory tests (NT-proBNP), spirometry, x-ray of the lungs [5]. Patients with COPD are prone to developing cardiovascular diseases, bronchiectasis, lung cancer, osteoporosis, metabolic syndrome, muscle weakness, depressive syndrome and other health conditions [6].

Early cardiovascular screening and echocardiographic evaluation of this vulnerable group of patients with COPD will improve the quality of life and define the parameters that are crucial for monitoring this group of patients, managing the disease and reducing the need for hospitalization.

Anatomic and pathophysiologic close relationship between the respiratory and cardiovascular system as well as the present risk factors were a great motivation for conducting a thorough research in this field. COPD affects the cardiovascular system, especially the right heart function.

Patients known to have the disease for a long period have already suffered from permanent changes of the vascular bed that subsequently lead to right heart dilatation and development of right heart failure. The right heart failure is the end-stage of the disease and results in an overall bad quality of life.

OBJECTIVE

The aim of this study was to identify the echocardiographic parameters important for evaluation of COPD patients in stage 3 and 4 according to GOLD classification.

We expect that this study will help in defining the echocardiographic parameters that are crucial for this group of patients with COPD.

MATERIALS AND METHODS

- 22 patients (with confirmed stage of COPD) were examined and classified by GOLD classification in stage 3 and 4.
- Standard classification consists of several stages such as:
- -Stage 1 of COPD (FEV1/FVC <70%)
- -Stage 2 of COPD (FEV1/FVC<80%) GOLD II/III, divided into two subgroups
- -Stage 3 of COPD (FEV1<30%)

Patients with coronary artery disease, congenital heart disease, cardiomyopathies (dilatate, hypertrophic), idiopathic pulmonary hypertension, asthma and pulmonary fibrosis were excluded from the study. This is a pilot study of the planned doctoral dissertation which is going to be a prospective (cross-sectional study) study in a two-year time interval. Recruitment of patients has started in January 2019 and is planned to be finished by the mid of 2020.The total number of planned patients to be included in the doctoral dissertation is 100, but in this study we present the results obtained in 22 patients. Patients enrolled in this study were with stage 3 and 4 of COPD.

In cooperation with the University Clinic for Pulmonology and Allergology, all patients were classified in stages of COPD in advance (with spirometry analyses) and sent to our University Clinic for Cardiology for further examination. On admission, anamnesis, status and clinical examination, ECG and assessment of risk factors were done in every patient.

Echocardiographic examination was done in all 22 patients with vivid 7 echomachine by general electronic. All the analyses were made on echopaque working station.

These echo findings were analyzed:

1. Dimensions and volumes of the left ventricle: end-diastolic dimension of the left ventricle (LVDd), dimension of the left atrium (LA) obtained by long parasternal axis in M-mode, end-diastolic volume of the left ventricle (LVEd), end-systolic volume of the left ventricle obtained by 4 chamber view.
2. Estimation of the muscle contraction expressed as EF (ejection fraction) in percentage obtained by Simpson method.
3. Estimation of the diastolic function of the left ventricle is expressed as E/e' relationship

In addition, attention to the parameters of the right chamber was given: Estimation of the basal diameter of the right chamber measured by 4 chamber view in the end-diastole. Estimation of the functional area of changes of the right chamber which gives information on the global function of the right ventricle (FAC %). It is measured by 4 chamber view and calculated as FAC(%)=100x(EDA-ESA)/EDA, which means as a difference between end-diastolic and end-systolic area divided by end-diastolic area multiplied by 100. FAC above 35% means neat right systolic function. Tricuspid annular systolic excursion (TAPSE) measured by M-mode is an indicator of the longitudinal systolic function of the right ventricle. Cursor in M-mode is placed on the lateral wall of the right ventricle at the level of the tricuspid annulus in 4 chamber view.

- Reduction under 16 mm is one of the markers of an impaired longitudinal function of the right ventricle.
- S'wave of tissue doppler of the right chamber is more toll for evaluation of the longitudinal function. Values below 10 met/sec are a marker of an impaired longitudinal function of the right ventricle.
- Estimation of pulmonary hypertension is made by Bernoulli equation: SPAP (Vmax TR)2+ pDAP (pressure that is added depending on the dimension of inferior vena cava and its collapse). Value equal or above 50mm/Hg is considered as pulmonary hypertension.
- diameter and collapse of inferior vena cava by which the pressure of the right atrium is measured
- acceleration time (AT) is calculated by pulse wave doppler through the right ventricle flow tract, and values above 105 m/sec are considered as normal.
-

RESULTS

Our study included 22 patients with COPD; 17 patients were male and 5 female. The average age was 66.4+6.3, range 55 to 76 years.

Table 1. Demographic features of patients with COPD

Variables	**Average**	**Minimum**	**Maximum**	**St.dev.**
age	66.4	55.0	76.0	6.31
body weight	82.0	56.0	130.0	20.04
height	169.1	155.0	186.0	8.98
gender	**No**		**%**	
female	5		22.7	
male	17		77.3	

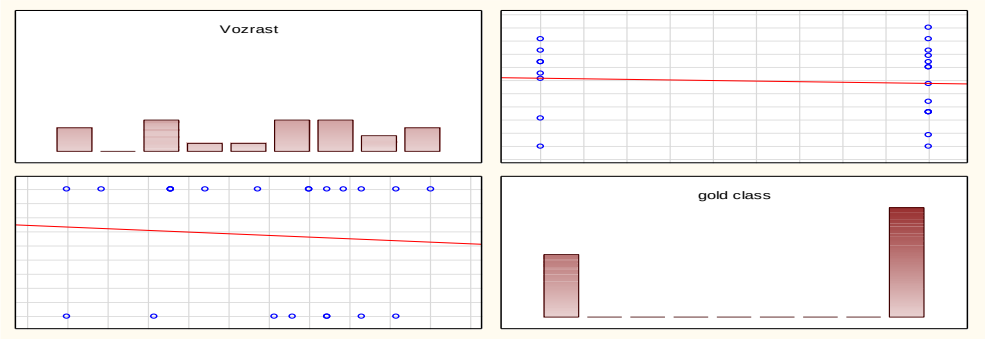

Fig. 1. Correlation between age and GOLD classification

There was a negative and weak rang correlation between age and GOLD classification (Spearman rank orders correlation)

Table 2. Stage of COPD 3/4

stage/GOLD	N	%
3/ heavy stage	8	36.4
4/ very heavy stage <30%	14	63.6

All patients examined in this study were in stage 3 and 4 of COPD, but stage 4 was prevalent.

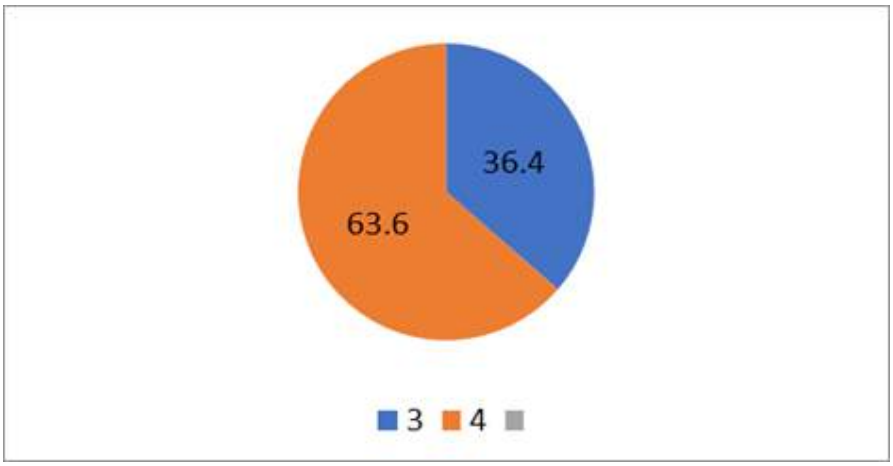

Fig. 2. Stage of COPD ¾

Table 3. Echocardiographic features obtained by measurment of the left atrium and ventricle

Variables/female	**N**	**average**	**minimum**	**maximum**	**St.dev.**
LVEDd	5	41.6	38.0	45.0	3.05
LVEDvol.	5	70.6	56.0	94.0	16.23
LVES vol	5	33.4	27.0	39.0	5.50
variables/male					
LVEDd	17	44.4	40.0	54.0	4.32
LVEDvol.	17	77.5	46.0	134.0	25.24
LVES vol	17	33.7	20.0	56.0	10.79
EF (%)	22	57,9	53	68	
E/e' sep/	22	6.9	2.7	11.9	1.88
left atrium					
Mm	**N**			**%**	
>40	3			13.6	
<40	19			86.4	
E/e' sep/					
>8m/sec	4			18.2	
<8m/sec	18			81.8	

The average value of LVEDd (mm) was 41.6+3.0, range 38 to 45 mm, in females and 44.4+ 4.3, range 40 to 54 mm,in males. The average value of LVED vol in female patients was 70+16.2, range 56 to 94 ml, and 46-134.0 ml in male patients. The average value of LVES vol in female patients was 33.4+5.5, range 27-39 ml, and 33.7+10.8, range 20.0-56 ml, in male patients. The analysis made in all patients showed diastolic dimension of left ventricle in both genders with low normal values as well as systolic and diastolic volume of the left ventricle. Dimensions of the left atrium above 40 mm were registered in 13.6% and below 40 in 86.4% (Table 2) of patients. In conclusion, the majority of patients were with normal dimensions of the left atrium.

Global systolic function expressed as ejection fraction (EF%) in all patients was above normal values (52%), average 57.9+4.2, range 53.0 to 68%. Correlation between mitral flow and e' wave determined by tissue doppler showed an average value of 6.9+1.9, range 2.7 to 11.9 m/sec. Above cut-off (>8m/sec) value was registered in 4 patients (18.2%) and below cut-off values in 19 patients. The analysis of data showed that most of the patients had normal diastolic function of the left ventricle, with weak correlation between diastolic function of LV and the stage of the disease. Dimensions of the aorta in all patients was below 38mm, average values were 32.2+2.7, range 26.0 to 37.0 mm.

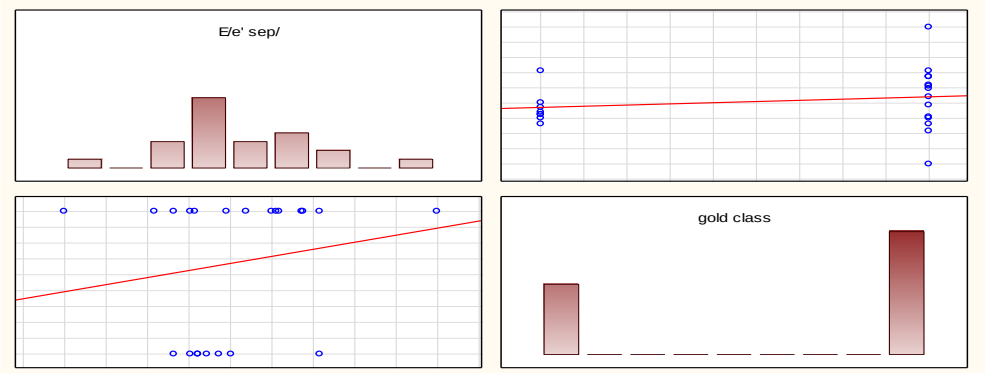

Fig. 3. Correlation between E/e' septal and GOLD classification

There was a weak positive rang correlation (Spearman rank order correlation) between E/e' septal and GOLD classification.

Table 4. Evaluated parameters of the right ventricle

variables	**average**	**minimum**	**maximum**	**St. Dev.**
RV base	32.0	27.0	39.0	3.26
S TDV RV	0.09	0.07	0.1	0.01
TAPSE	19.5	14.0	28.0	2.94
FAC(%)	44.5	32.0	55.0	6.19
AT a.pulm.	79.0	59.0	96.0	8.88
SPAP	39.6	26.0	53.0	7.14
variables	**No**		**%**	
RV base				
>30mm	13		59.1	
<30mm	9		40.9	
S TDV RV				
>0.10	13		59.1	
<0.10	9		40.9	
TAPSE				
>=16mm	22		100.0	
<16mm	0			
FAC(%)				
>35	20		90.9	
<35	2		9.1	
AT a.pulm				
<=105	22		100.0	
>105	0			
SPAP				
<=50	20		90.9	
>50	2		9.1	

The right ventricle basal segment average value was 32.0+3.3, range 25.0-41.0 mm. Values above >30 mm, but still within reference values were found in 13(59.1%), and below 30 mm in 9(40.9%) patients. Assessment of S wave obtained by tissue doppler above tricuspid anulus showed a starting reduction of the longitudinal function of the right ventricle. Nine of 22 patients had reduction of S wave below 0.09. According to TAPSE, which is a parameter that is used also for evaluation of the longitudinal function of the right ventricle, the average value was 19.5+2.9, range14 to 28, but all patients had values above 16 mm, which was considered as normal. The parameter that defines the global function of the right ventricle (FAC%) had the average value of 44.5+6.2, range from 32 to 55%. Above cut-off values (35%) were registered in 21 (90.9%) patients and below cut-off values in 2 patients (9.1%). These two patients had an increased SPAP leading to development of pulmonary hypertension. Mean artery pressure was obtained with a formula derived by maximum velocity of the tricuspid regurgitation, and the average value was 39.6+7.1, range 26 to 53. Above cut-off values (50 mm/hg) was registered in 2 patients (9.1%), and below cut-off values in 20 (90.9%) patients. The analysis of the collapsibility of inferior vena cava in patients with COPD is one of the parameters that shows an increased pressure in the right heart cavities. In 50% of our patients non-collapse of inferior vena cava was registered.

There was a statistically significant association between GOLD and inferior vena cava insp/exp (<50%;>50%) (Fisher Exact 2 tailed test =0.023375).

The time of acceleration (AT)obtained as a parameter from the tissue doppler record of the pulmonary artery had an average value of 79.0+8.9, ranging from 59 to 96 msec. Value below cut-off (<=105) was registered in all 22 patients (100%).

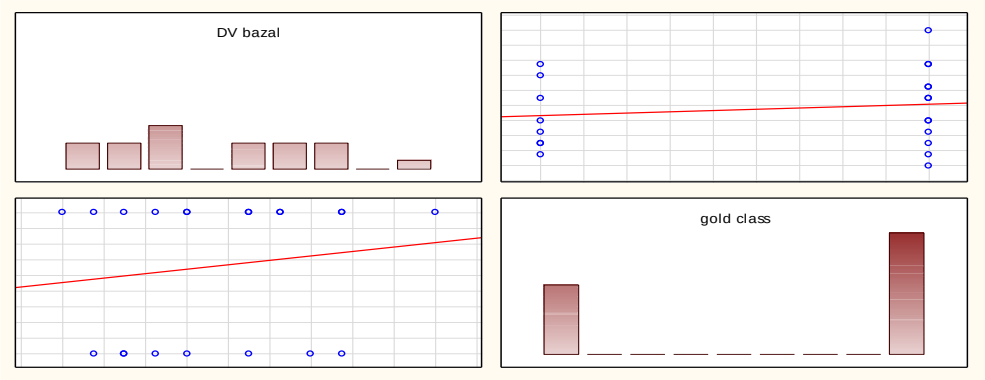

Fig. 4. Correlation between RV basal and GOLD classification

There was a weak positive rang correlation (Spearman rank order correlation) between RV basal and GOLD classification(r=0.146651).

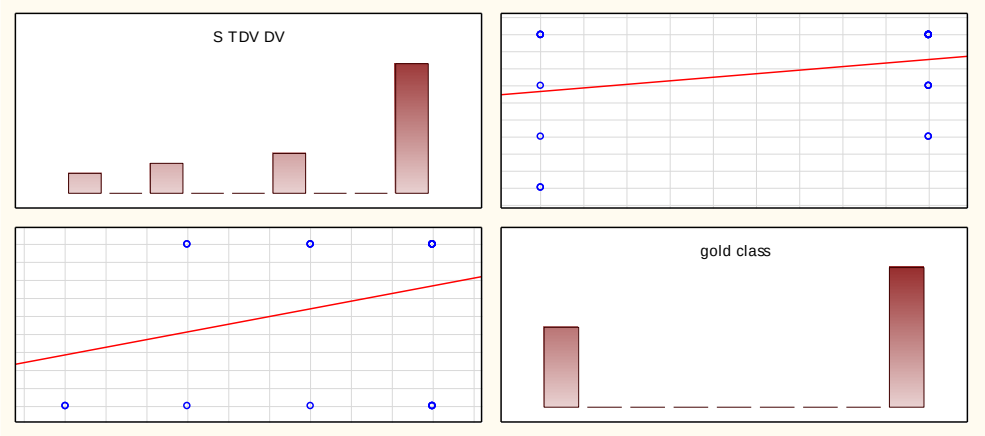

Fig. 5. Correlation between S TDV RV and GOLDclassification

There was a weak rank correlation (Spearman rank order correlation) between S TDV RV and GOLDclassification.

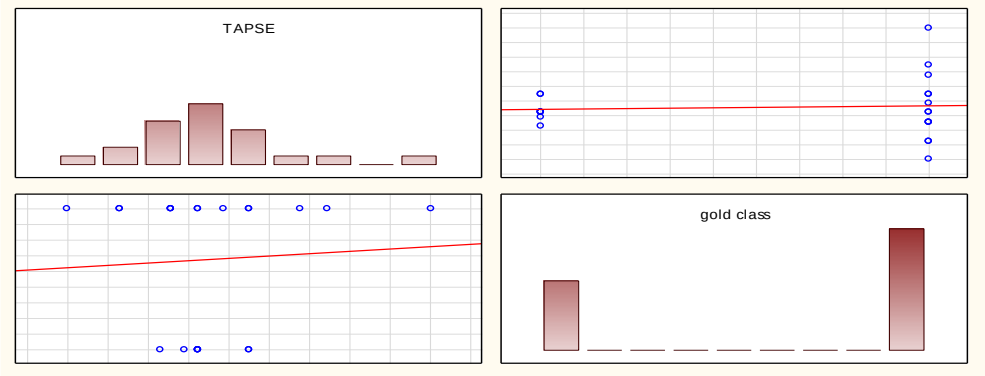

Fig. 6. Correlation between TAPSE and GOLD classification

There was a positive weak rank correlation (Spearman rank order correlation) between TAPSE and GOLD classification (r=-0.015109).

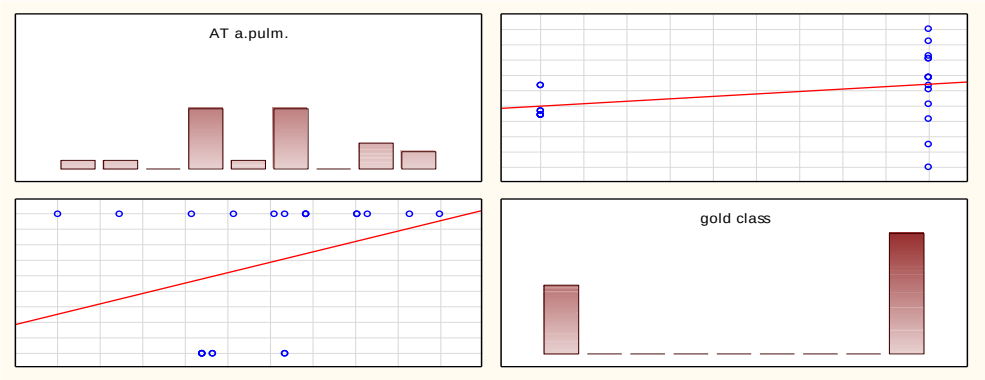

Fig. 7. Correlation between AT *a.pulm* and GOLD classification

There was a positive moderate rank correlation (Spearman rank order correlation between AT *a.Pulm.* and GOLD classification (r.0.404425).

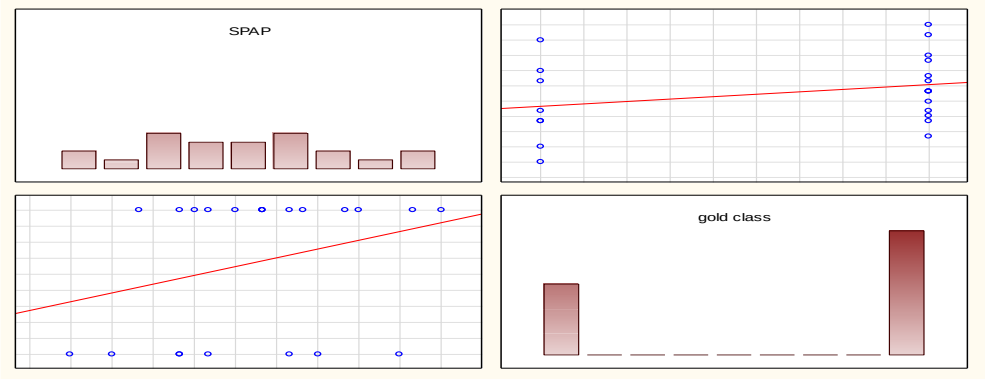

Fig. 8. Correlation between SPAP and GOLD classification

There was a positive moderate rank correlation (Sprearman rank order correlation) between SPAP and GOLD classification(r=0.283786).

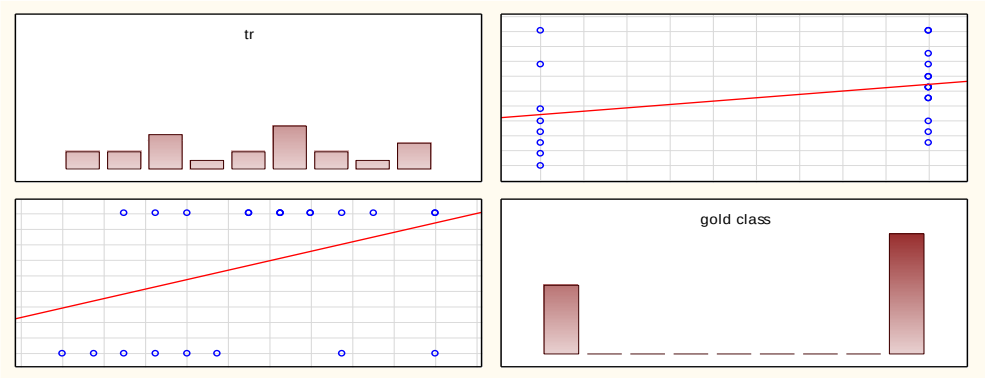

Fig. 9. Correlation between tricuspid regurgitation(m/sec) and GOLD classification

There was a positive moderate rank correlation (Spearman rank correlation) between tricuspid regurgitation m/sec and GOLD classification (r= 0.373828).

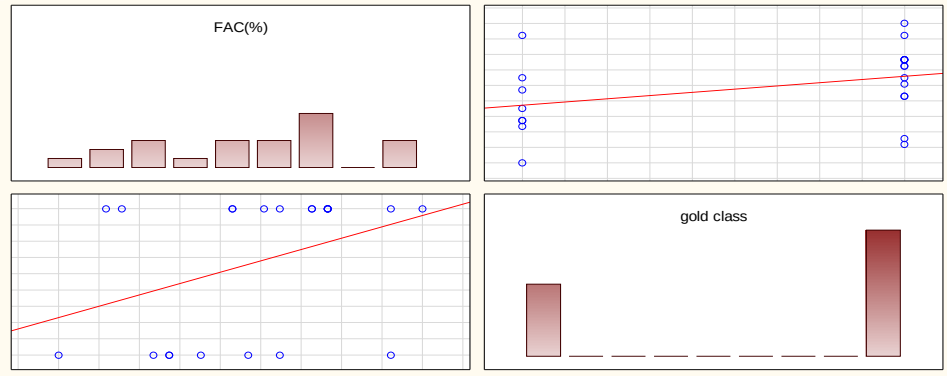

Fig. 10. Correlation between FAC (%) and GOLD classification

There was a positive moderate rank correlation (Spearman rank order correlation) between FAC (%) and GOLD classification (r.0 388891). The analysis showed a weak positive correlation between echocardiographic parameters, right ventricle dimension, velocity of S wave of RV and TAPSE and the stage of the disease, but there was a moderate correlation between AT, sPAP and tricuspid regurgitation according to the stage of the disease, GOLD 3 and 4 classification.

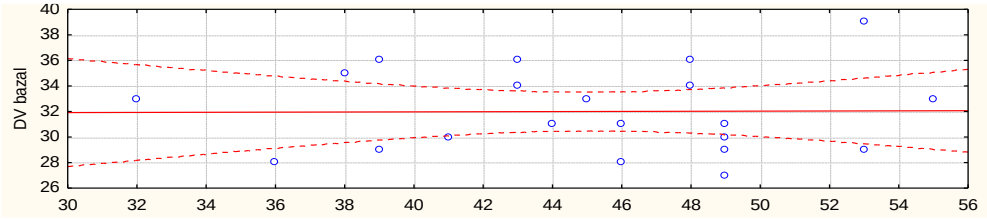

Fig. 11. Correlation between FAC (%) and RV basal dimension

There was a weak correlation between FAC (%) and RV basal dimension(r=0.0103).

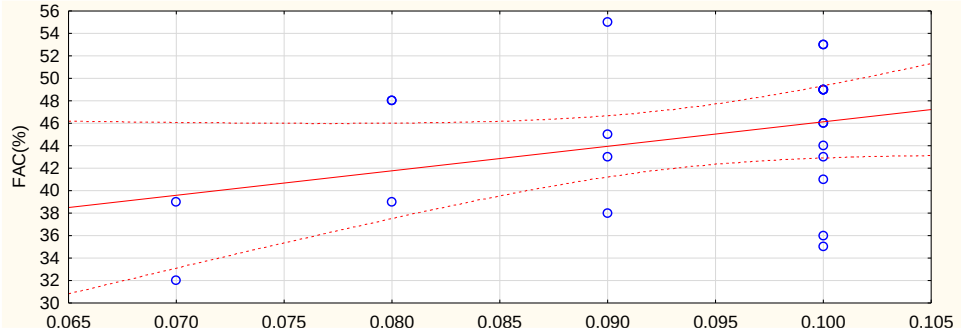

Fig. 12. Correlation between FAC (%) and S TRV RV

There was a weak moderate correlation between FAC (%) and s TRV RV(r=0.363513).

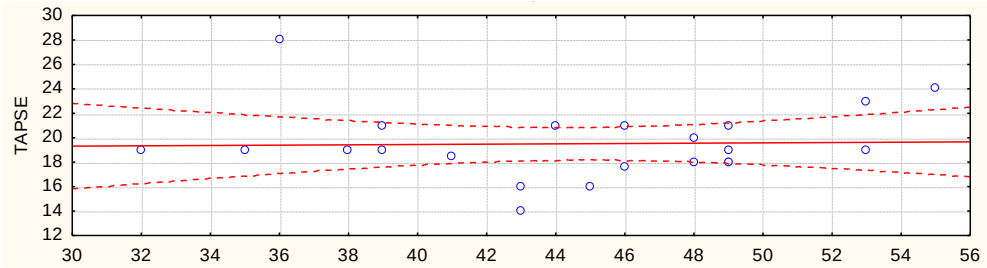

Fig. 13. Correlation between FAC (%) and TAPSE.

There was a weak, negligible correlation between FAC (%) and TAPSE (r=0.0279). The analysis showed a weak correlation between FAC as a parameter for global function of the right ventricle and the dimension of RV and TAPSE, but there was a moderate positive correlation between S wave of the tissue doppler as a parameter of the longitudinal function of RV.

DISCUSSION

Early cardiovascular screening done by echocardiographic evaluation in patients with COPD, class 3 and 4 (defined by Gold) will contribute to defining the parameters that are important for monitoring the course of the disease, improvement and decrease of the frequency of hospitalization. In our pilot study we made analysis of all the parameters, both demographic and echocardiographic, but the accent was put on the right heart parameters. Male gender was prevalent (77.3%), and the average age was 66.4+6.3. In most of the studies male is a predominant gender as was the case in our study, too. This trend had tendency to decrease in later years of the observation period. One more scientific work published in 2019 in China. [7]. The right ventricle is necessary for obtaining the normal pump strength by the maintenance mechanism of normal perfusion in different pressure and loading conditions of the right ventricle [8]. The right ventricle mass is nearly 1/6 of the mass of the left ventricle due to physiological role of pumping in conditions with not so high pulmonary pressure. COPH results with different implications on the pulmonary artery pressure and different levels of right ventricular disfunction [9]. Wall movement of the right ventricle is complex and it is a result of shortening of the muscle fibers in three dimensions: circumferential, longitudinal and radial. During systole of the right ventricle there is a longitudinal shortening from the base to the apex and radial movement of the septum. Additionally, circumferential movement gives an opportunity for rotation and torsion of the right ventricle [8].

Echocardiography as a non-invasive and easily available method allows analysis of the morphology and function of the right ventricle, qualitative and quantitative. Estimation of RV is often hard to evaluate because of its complex anatomy, due to which the evaluation must be done in more echocardiographic windows and cross sections [8, 10]. In our study, the average values of diastolic dimension of the left ventricle expressed in mm was 41.6+3.0, ranging from 38 to 45 mm. The analysis showed that all patients had left ventricle dimension with low normal values, as well as the systolic and diastolic volumes of the left ventricle. Patients with severe /very severe degree of COPD have right ventricular overload that makes flattening of the interventricular septum which disables completely opening of the left ventricle. All that speaks in favor of small dimensions of the left heart cavities. The left ventricular systolic function expressed as ejection fraction (EF%) in all patients was above normal values, the average being 57.9+4.2, range from 53.0 to 68.0%. Despite the longevity and severity of the disease the pumping force for long time is with preserved ejection fraction EF%. In one meta-analysis that summarized the results from 15 studies in 2019 where primarily an estimation of the diastolic function was made, COPD patients were found to have normal EF% [11].

In our pilot study E/e’ obtained by tissue doppler of the mitral valve showed an average value of 6.9+1.9, range 2.7 -11.9 m/sec. Normal values of diastolic function of the left ventricle was found in 18 patients (81.8%). The analysis of our data showed that most of the patients had normal diastolic function of the left ventricle, with weak correlation between diastolic function of LV and the severity of the disease. Two studies published these last years have different interpretations about diastolic dysfunction in patients with COPD. One study by a group of authors from Egypt published in 2016analyzed the correlation and the stage of diastolic dysfunction of LV in different groups of COPD.

According to the severity of the disease, lower E/A ratio was found, extended deceleration time in patients with more severe form of COPD, but not all echocardiographic parameters for diastolic disfunction were analyzed. Reduction of E/E' ratio in patients with COPD is considered due to lower filling pressure of the left ventricle that is supported by the concept of reduced preload in patients with COPD. E/E' is a parameter that is independent of age, preload and heart rate and itis an early marker for diastolic dysfunction [11, 12]. In one meta-analysis conducted in 2019(case control study) including patients with COPD and diastolic dysfunction was found that patients with COPD versus control group had increased E/E' ratio, lower E/A ratio and extended DT.

Our study showed that all 22 patients had normal TAPSE values (>16 mm). In 9 of the 22 patients, the value of S wave obtained from tissue doppler was below 0.09 cm/sec, that is, below the normal value. Having in mind the fact that these 2 parameters speak about assessment of longitudinal function of the right ventricle that are disturbed before global systolic function of RV, the estimation of longitudinal function of RV by tissue doppler was more sensitive than TAPSE determined by M-mode. In the literature, there are no studies on comparison of these two parameters and their sensitivity. The parameter for defining global function of RV(FAC%) in our study had an average value of 44+6.2, and below cut-off value was found in two patients (9.1%). These two patients had increased SPAP in addition of development of pulmonary hypertension. In Guptas *et al.* study from 2014, an analysis of 17 male patients with severe stage of COPD was made and all of them had neat global function of RV, FAC 56.8+10.6, average value of TAPSE was 27 mm and SPAP 35 mm/Hg. Basal diameter of RV was 35.194+9.184 mm. These results are similar to ours presented in this study [1]. Eight patients had a significant tricuspid regurgitation with values above 2.7 m/sec or 36.4%. This data indirectly gives us information about the degree of expression of pulmonary pressure and the development of pulmonary hypertension. Expression of PAH in our pilot study was calculated based on the velocity of the tricuspid regurgitation, diameter and collapsibility of inferior vena cava as a marker for determining the pressure in the right atrium, placed in Bernoulli equation. An analysis of non-collapsibility of inferior vena cava in a patient with COPD is one of the parameters indicating an increased pressure in the right heart chambers. In the examined series in 50% of the patients non-collapse of vena cava more than 50% was registered [13]. A group of authors published a study showing enlarged dimensions of inferior vena cava >20 mm and inspiratory collapse <50%, which were significantly associated with an increased mortality in patients with PAH [13]. In patients with significant pulmonary hypertension, the left ventricle dimensions in systole and diastole might be reduced as a result of septum deviation towards the left ventricle because of the right ventricle overload [13]. In our study the left ventricle dimensions were low- normal. A review paper published in 2009 demonstrated that pulmonary hypertension in most of the cases was mild to moderate in patients with a severe form of COPD. A small sample of patients with COPD demonstrated a significant PAH and worse prognosis [14]. Pulmonary artery hypertension was often found in COPD patients that were in an advanced stage of the disease, in approximately 20% of patients. Most of the cases were with mild to moderate form of pulmonary hypertension and 5% of them with a severe form. Patients with a severe form of COPD had an average survival of 40 months [14, 15]. Acceleration time (AT) at the level of the right ventricle outflow tract above valves of the pulmonary artery in the literature has been reported of no significance as is tricuspid regurgitation. In our study it was below 105 m/sec in all patients that might lead us to an increased pulmonary pressure, but still with no significance. Disadvantage of this parameter is that it has to be corrected if the heart rate is above >100/beats/min, that is 60-100 beats/min, but in our study, there were no patients whose heart frequency was above 100/beats/min at the time of examination.

The appearance of mid-systolic notch is associated with a significant PAH and right ventricular involvement, which was present in 2 of our patients [13].

CONCULSION

Echocardiography as a noninvasive method with all its modalities has shown superiority in assessing and monitoring of patients with COPD. Echocardiography can give us in a fast and accurate way many parameters in different stages of the disease, which will help in assessment and prognosis of patients with COPD.

The limitation of this study is the small number of patients in order to estimate many parameters, but this problem will be overcome with increasing the number of patients in the final study. According to the results obtained so far, we can conclude that echocardiography parameters for evaluation of the right and left ventricle remained within the reference limits long after the onset of the disease. Some of the echocardiographic parameters might be harbinger for initial deterioration of the right ventricle function and the development of pulmonary hypertension, such as tricuspid regurgitation, reduction of collapse of inferior vena cava, shortening of AT from tissue doppler of pulmonary valve, S wave from tissue doppler and many other parameters and derived parameters which in the final study will find their role.

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UDC: 616.31/.32-006.6-085.849.1

ORIGINAL ARTICLE

DEFINITIVE TREATMENT OF PLANOCELLULAR OROPHARYNGEAL CARCINOMA WITH MODERATE ACCELERATION OF INTENSITY MODULATED RADIATION THERAPY WITH SIMULTANEOUS INTEGRATED BOOST

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ABSTRACT

Introduction: Intensity Modulated Radiation Therapy (IMRT) represents a new technique in the treatment of head and neck carcinomas. Oropharyngeal carcinomas are ideal target for this radiation technique, because of their anatomic localization close to critical healthy organs. Simultaneous Integrated Boost (SIB) in IMRT allows in at the same time at the same fraction delivering of different dose to different structures to irradiated volume with distribution on higher dose to the tumor and lower dose in elective irradiated regions. Moderate acceleration of IMRT-SIB allows in shorter time delivering of higher dose to planning target volume and achieving bigger tumor control.

Objective: Aim of this study was to present planning technique IMRT – SIB with 95% dose coverage of volume of planning target volumes referring to primary oropharyngeal carcinoma, metastatic lymph nodes, elective region in head and neck which is irradiated and to critical organs near the tumor, achieving in the future better tumor control and fewer side effects on healthy tissues.

Materials and methods: This retrospective study included 31 patients with advanced planocellular oropharyngeal cancer treated at University clinic of Radiotherapy and oncology-Skopje, with modulate acceleration of IMRT-SIB and weekly concurrent cisplatin.

Results: With IMRT SIB we achieved high 95% dose coverage of the planned target volumes (PTV54 = 99,46 % and PTV66 = 98.74 %) and successfully spare the spinal cord as the most important organ at risk.

Conclusion: IMRT- SIB fulfill the goal of better tumor coverage and moderate acceleration achieve dose reduction in critical healthy organs.

Keywords: IMRT-SIB, oropharyngeal carcinoma, target volume

INTRODUCTION

Head and neck carcinomas represent global health issue, causing significant morbidity and mortality despite modern medical techniques in early diagnostics and therapy. Oropharyngeal planocellulare carcinomas as rare solid tumors in head and neck regions and are 8th most common cancers in Europe [1]. In this group of oropharyngeal carcinomas belongs carcinoma originating from soft palate, tonsils, base of tongue, and posterior pharyngeal wall.

Risk factors that contribute to development of oropharyngeal cancers are: excessive consumption of cigarettes and alcohol, and an association with HPV infection in the oropharyngeal region [2,3].

The association between the occurrence of planocellular carcinoma, especially of the tonsils, and the base of the tongue and high-risk human papillomavirus (HPV) infection has been confirmed [4,5]. Treatment approach for oropharyngeal carcinoma is multidisciplinary starting from its diagnosis, treating and follow- up. Concurrent chemoradiotherapy is preferred treatment option for locally advanced planocellular head and neck cancers, especially oropharyngeal cancer, enabling better progression free survival and overall survival in relation to other treatment options [6]. Intensity Modulated Radiation Therapy (IMRT) represents a new technique in the treatment of oropharyngeal carcinomas. This advanced radiotherapy technique allows more precise cancer targeting with reduced dose delivered to the healthy surrounding tissues [7]. It is useful in reducing long-term toxicity in radiotherapy by reducing the radiation dose to one or both parotid glands, temporomandibular joints, mandible, glottic larynx, and pharyngeal constrictors [8]. It shows superiority according to other preexistent radiotherapy techniques with its consistency, homogeneity and conformity in delivering the dose in PTV (Planning Target Volume) in patients with oropharyngeal carcinoma [9,10]. Simultaneous Integrated Boost (SIB) in IMRT allows at the same time in the same fraction delivering of different dose into different targeted regions. The advantage of this technique is a better target conformity, lower dose to critical structures, and possibility for acceleration of treatment which would reduce the total time of therapy by dose escalation in the tumor volume [11]. Many different schedules of dose fractionation have been published but still there is no universally accepted standard dose for IMRT-SIB fractionation [12]. The dose that is not standardized in this newly established technique in radiotherapy with weekly concurrent cisplatin in the treatment of locally advanced planocellular oropharyngeal carcinomas is still a research challenge in the field of radiotherapy and oncology.

OBJECTIVE

Aim of this study was to present planning technique IMRT-SIB with 95% dose coverage of volume of planning target volumes referring to primary oropharyngeal carcinoma, metastatic lymph nodes and elective region in head and neck which is irradiated. Also, we analyzed received doses to organs at risk nearby tumor that affect the quality of life of the patients with oropharyngeal cancer treated with definitive concurrent chemoradiotherapy.

MATERIALS AND METHODS

This research represents non randomized controlled study in order to obtain dosimetry results from the realized IMRT-SIB technique on which the treatment of patients with oropharyngeal cancer depends, as well as the impact of this treatment on their quality of life due to side effects that are an inevitable part and which can be prevented and minimized with this method. The study group consists of 31 patients who started treatment from October 2017 till December 2019, using moderate acceleration of IMRT-SIB with competitive weekly cisplatin.

Immobilization and CT simulation

Preparation for the treatment starts with immobilization and Computed Tomography (CT). Patient is in the supine position and for immobilization is used a thermoplastic mask which fixes the head, neck and shoulders, so we can always be able to reproduce a completely identical position at the time of treatment compared to CT simulation. For CT simulation we used 2,5 mm transverse cross-sections.

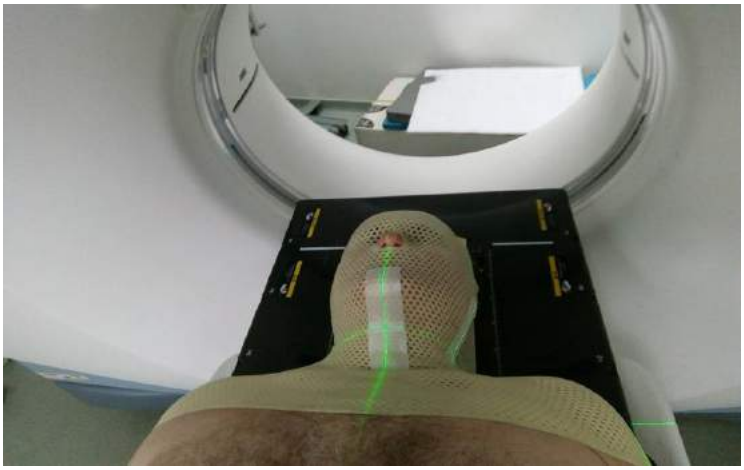

Fig. 1. Immobilization with thermoplastic mask

Delineation of targeted volumes

Once the computed tomography is performed, the target volumes are delineated. The delineation of targeted volumes and critical organs was performed according to the protocol of the study RTOG H-0022 [13].

Gross Tumor Volume-t66 (GTV-t66) presents the primary tumor which is determined by clinical examination, endoscopic diagnostic methods, CT, PET CT or magnetic resonance. Gross Tumor Volume-n66 (GTV-n66) metastatic lymph nodes in head and neck region determinate with the same diagnostic methods as primary tumor. With integration of GTV-t66 and GTV-n66, GTV66 is obtained which refers the primary disease. With an expansion of 5mm from GTV-t66 and GTV-n66, is obtained Clinical Target Volume - CTV66. Then we delineated elective regions with lymph nodes in the neck (CTV-n54) which include metastatically altered lymph nodes (GTV-n66) with 1cm expansion as well as CTV-t54 which is obtained by 1cm expansion of GTV-t66.

CTV 54 is obtained by integrating CTV-n54 and CTV-t54. Planning Target Volume - PTV66 is geometrical margin obtained with 0.5mm expansion from CTV66 and PTV54 with 0,5- mm margin from CTV54.

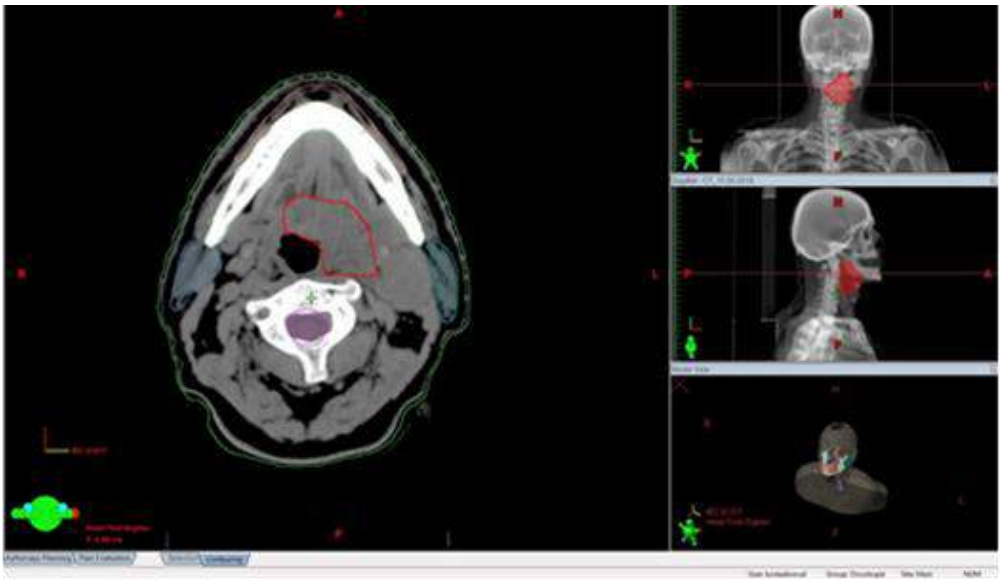

Fig. 2. Delineated Gross Tumor Volume - t66 (GTV-t66)

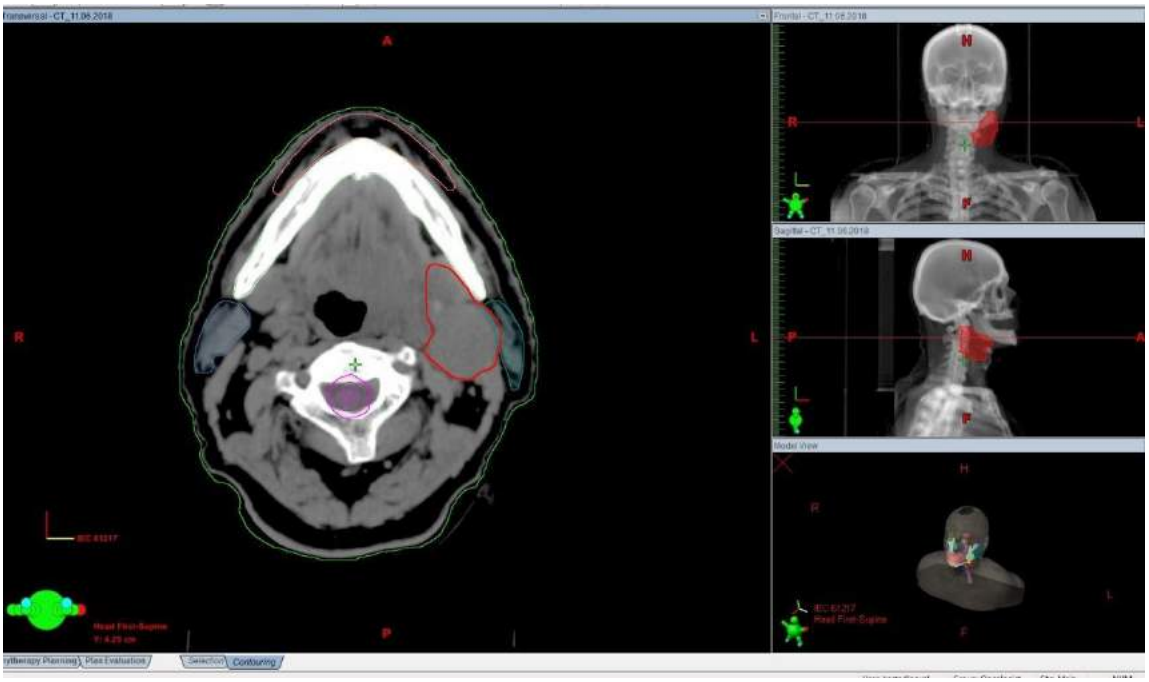

Fig. 3. Delineated Gross Tumor Volume - n66 (GTV-n66)

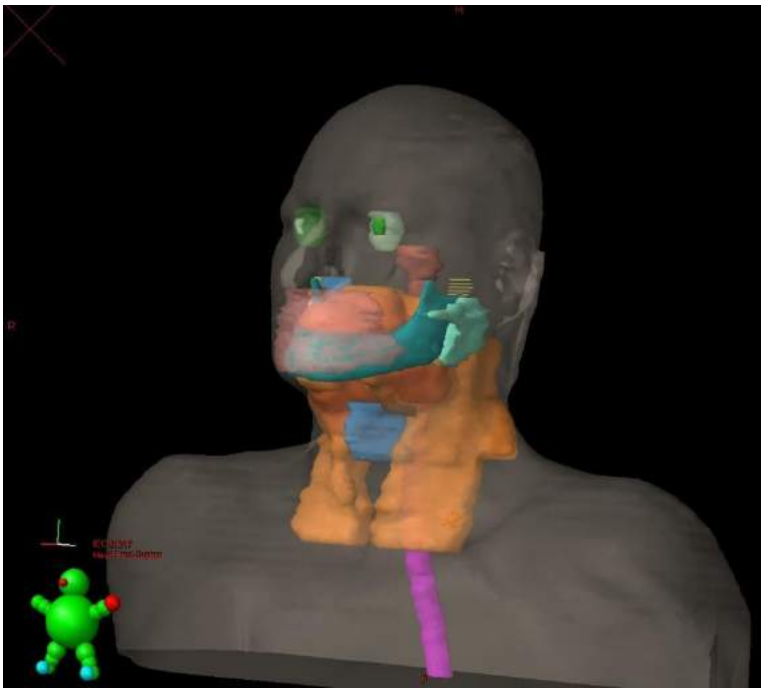

Fig. 4. Three-dimensional view of planned volumes for irradiation and organs at risk

Delineation of organs at risk

Radiotherapy in oropharyngeal cancer is planning challenge because of the close relation of this region with healthy surrounding tissues between. The healthy organs need to be spared from radiation. They need to receive minimum dose of the maximum permissible dose that would not cause organ damage anatomically and functionally monitored in the short or long term.

Organs at risk of interest that were delineated and taken into the parameters for examination in this study, in patients planned according to IMRT-SIB technique, were:spinal cord, parotid gland (bilateral parotid) and glottic larynx.

Table 1. Dose constraints of organ at risk according to Radiation Therapy Oncology Group 0225

Organ at risk	**Dose constraints**
Spinal cord	≤ 45 Gy
PRV Spinal cord	< 50 Gy
bilateral parotid gland	Mean dose < 26 Gy and/or if possible to keep dose in 50% from volume of each parotid gland ≤ 20
glottic larynx – mean dose	≤ 45 Gy
Other healthy tissue outside from target	< from 110 % from prescribed dose of PTV66

Dose fractionation

IMRT-SIB is based on precise computed planning and precise controlled delivering of the dose to target with dose distribution approximate to the shape of the target tumor volume with a minimal dose delivered to healthy tissues. SIB in IMRT allows simultaneous delivery of a different dose to different structures of the radiation volume in one fraction by distributing a higher dose in the tumor and a lower dose in the electively irradiated regions [10,11]. SIB technique, which applies in this research, is according RTOG H-0022 study [13]. Patients received radiotherapy fraction once a day, five days in the week and total tumor dose of 66 Gy in 30 fractions, 2,2 Gy in one fraction, in GTV as the highest risk area is realized. Low risk region is covered with 54 Gy of 1.8 Gy in fraction as area for elective irradiation [7]. Higher dose in tumor tissue (2.2 Gy) versus standard dose of 2 Gy in fraction in conventional radiotherapy, presents moderate accelerating of IMRT-SIB and allows for short time distribution of total tumor dose in planned targeted regions achieving greater tumor control [11,12]. In elective region dose is delivering in 30 fractions, 1,8 Gy in fraction while in the same time tumor tissue received 2,2 Gy in fraction. Planning and dosing schedule in this technique allows better preservation of healthy tissues and provides a better life quality for patients with oropharyngeal cancer.

RESULTS

In the period from October 2017 to December 2019 at the University Clinic for Radiotherapy and Oncology – Skopje, 31 patients with advanced oropharyngeal cancer were treated with moderate accelerated IMRT-SIB and weekly cisplatin 30 mg/m². We analyzed tumour localization, stage, 95% dose coverage of volume from prescribed dose of PTV66 and PTV54, spinal cord, PRV spinal cord, bilateral parotid gland and glottis larynx. Patients' characteristics are present in Table 2 with stratification by gander, localization of primary tumour and stage according to the eighth edition of American Joint Committee on Cancer [14]. Most of the patients were male, most common place of primary oropharyngeal origin were tonsils and most common stage was IVA.

Table 2. Patients characteristics (n = 31)

Characteristics	Number of patients (%)
Gender	
Male	27 (87)
Female	4 (13)
Localization of primary tumor	
Tonsils	15 (48.4)
Base of tongue	10 (32.3)
Posterior wall of hypopharynx	4 (12.9)
Soft palate	2 (6.4)
Stage	
III	4 (12.9)
IVA	14 (45.2)
IVB	13 (41.9)

Table 3 and Fig. 5 contains statistically obtained results from 95% dose coverage of planned targeted volume PTV66 and PTV54 per patient with planocellular oropharyngeal cancer, presented in percentage. Results show a high 95% dose coverage of the planned target volumes PTV66 and PTV54 which is close to 100%.

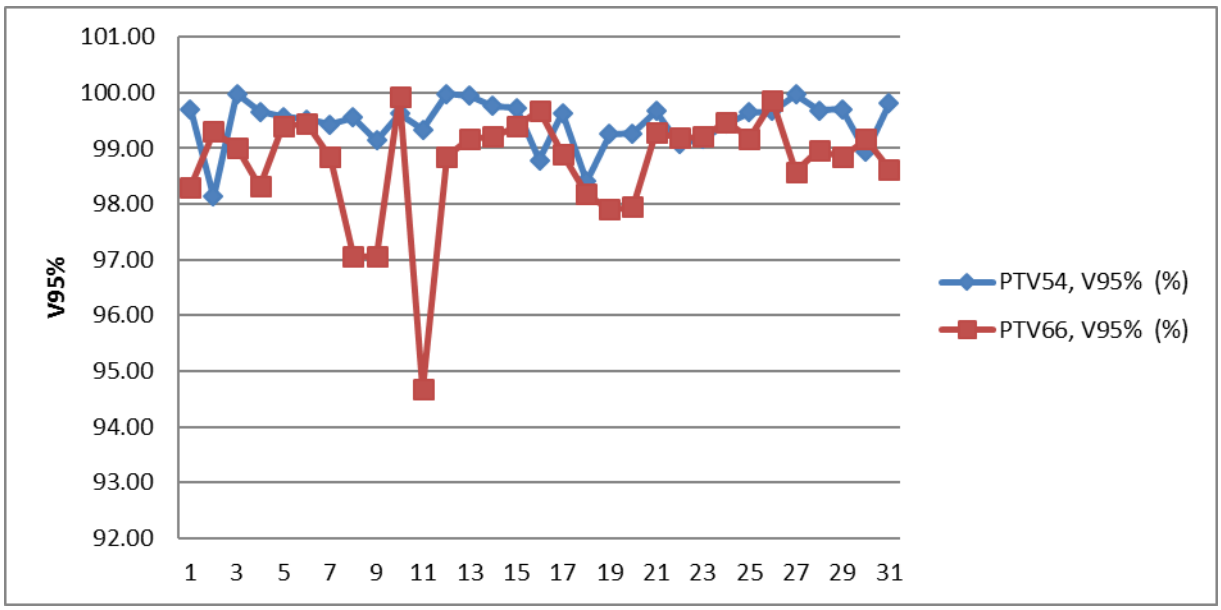

Fig. 5. 95% dose coverage of planned targeted volume PTV66 and PTV54 in 31 patients

Table 3. Average value of 95% dose coverage of targeted volume PTV66 and PTV54

95% dose coverage of targeted volume	average value ± SD (%)
V95%, PTV54 (%)	99,46 ± 0,43
V95%, PTV66 (%)	98,74 ± 1,02

SD:Standard deviation

Results for received mean dose in organs at risk in this study are presented in Table 4 and refer to bilateral parotid gland, , glottic laryngs, spinal cord and PRV Spinal cord (0.3 mm margin for structure of spinal cord).

IMRT-SIB technique successfully spares the spinal cord by meeting the criterion of RTOG 0225 for dose limits of organs at risk in the region that is irradiate. We did not manage to spare other organs at risk according to RTOG 00225 because of the advanced stage of disease.

Table 4. Average value of volume of interest (organs at risk)

Organs at risk	Average value±SD (%)
Dmean bilateral parotide gland (Gy)	42.48 ± 7.49
Dmean glotic larynx (Gy)	53.80 ± 9.57
PRV_spinal cord(Gy)	48.86 ± 0.57
spinal cord (Gy)	39.15 ± 4.09

SD: Standard deviation

DISCUSSION

Purpose of each radiotherapy technique is maximally targeting of tumor cells with minimal damage of healthy surrounding tissues which are a limiting factor for determination of prescribed radiotherapy dose. Results in this study show that with IMRT-SIB we achieved a high 95% dose coverage of the planned target volumes PTV66 and PTV54 which is close to 100%. This is not common with older radiotherapy techniques (3D conformal radiotherapy) [9].

From the results of Table 4, IMRT-SIB technique successfully spares the spinal cord by meeting the criterion of RTOG 0225 for dose limits of organs of risk in the region that is irradiate. Radiotherapy affects the quality of patient's life disturbing swallowing and speech. All of the treated patients (Table 2) have advanced stage of planocellular oropharyngeal cancer. Due to the size of the tumor volume and the positive metastatic lymph nodes in the cervical lymph pool region, the criteria for sparing healthy organs (parotid gland and glottic larynx) were not met in these 31 patients. With delineating organs at risk and trying to protect them, they receive a lower dose than they would with 3D conformal radiotherapy where they are not delineated and protected at all. According to our results parotid gland and glottic larynx received higher dose from prescribed dose for their spearing Table 4.

CONCLUSION

IMRT-SIB fulfill the goal of better tumor coverage and together with the prescribed dose and fractionation regiment (moderate acceleration) achieve dose reduction in critical healthy organs. In the future, with this technique, following up the patients with advanced oropharyngeal cancer, we can research whether it will result in high rate of overall survival and reduce of long-term side effects of radiotherapy (xerostomia, impaired swallowing and speech) achieving better quality of life.

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ORIGINAL ARTICLE

THE RISK FACTORS FOR POSTOPERATIVE OUTCOMES IN NEONATAL CARDIAC SURGERY

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ABSTRACT

Background: In the last three decades a neonatal cardiac surgery has improved the approach and methods for adequate treatment of complex congenital heart defects. Although we have advances in fetal cardiac imaging and improved perioperative cardiac procedures, the postoperative outcomes in neonatal care (neonates) are still present.

Objective: To present our experience of operated neonates with congenital heart defects in a tertiary referral center, Neonatal Intensive Care Unit (NICU) in Skopje N. Macedonia.

Materials and methods: We conducted a retrospective study including neonates who underwent surgical intervention between January 2013 and December 2015 at the University Clinic for Pediatric Diseases in Skopje. We analyzed perioperative and postoperative variables. The main outcomes were duration of cardiopulmonaly bypass (CPB), and x-cross of aorta, duration of mechanical ventilation, intensive care unit stay and postoperative complications. Fourteen (14) neonates were discharged from our Clinic.

Results: Out of a total of 85 children, 15/85 (17.6 %) were neonates; males 12/15(80%) females 3/15 (20 %). The overall mortality was 1/15% (6.6%). There were 13/15 (86.6%) corrective procedures and 2/15 (13.3%) palliative ones. The mean duration of CPB was 46.6 min. (18±296 min.), the mean duration of x-cross of aorta was 17.5 min. (10±65 min.). The mean duration of mechanical ventilation was 3.4 days (1±15 days), duration of inotropic support was 4.2 days (1± 16 days) and ICU stay was 5.8 days (7.9 ± 14days). After the operation, 2/15 (13.3%) neonates required reintubation in the ICU. Postoperative complications were confirmed in 3 neonates (intracranial bleeding with seizures, block nodes AV and pneumonia). The incidence of all postoperative complications was 6.6%. Fourteen (14) patients were discharged from the Clinic.

Conclusion: Due to adequate cardiac surgery, significant technological advances, devices and increasing experience in neonatal cardiac surgery we have improved postoperative outcomes.

Keywords: cardiac surgery, neonates, cardiopulmonaly bypass, inotropic support, ICU length of stay

INTRODUCTION

Congenital heart defects (CHD) are the most common birth anomalies, occurring in approximately 6 per 1000 live births [1,2]. Developed countries have reported expectedly good postoperative outcomes after neonatal cardiac surgery, with mortality of less than 5% [3,4]; for example, in USA 6.61 per thousand live births, in Australia 4.31 and in Canada 12.5 per 1000 live birth. In the European Union the annual occurrence of congenital heart defects is 7.97% per 1000 newborns (in the UK 3.17, in Finland 1.95, in Denmark 6.18, in Sweden 3.57, etc.). This was a result of the improvement in technological devices and advances, and understanding the physiology and pathophysiology of CHD. In the present study the mortality was 1/15 (6.6%). Neonates with complex cardiac diseases characterized by ductal dependency usually undergo cardiac surgery in the first days of their life. Unfortunately, the same cardiac defects are still associated with a high risk of morbidity and mortality.

Thus, there is a need to examine potential risk factors for poor outcomes. Inadequate antenatal recognition of CHD and delayed surgical intervention are common for prolonged duration of mechanical ventilation, inotropic support and NICU stay [4,5,6].

Neonates with complex congenital heart defects, who undergo congenital cardiac surgery, requiring cardiopulmonary bypass (CPB) and x-cross of aorta are at a higher risk of significant postoperative outcomes due to the worsening of myocardial perfusion, increase of inflammatory cells level, damage of myocardial function and in some cases delayed and minimized postoperative improvements [7,8].

Although the latest innovations in medical devices have been developed to improve cardiac surgery, still postoperative outcomes in neonatal patients are associated with in-hospital mortality of 5-10%.

OBJECTIVE

The aim of our study was to report the postoperative outcomes after cardiac surgery, total corrective and palliative cardiac surgery, in 15 neonates in a tertiary neonatal intensive care unit in N. Macedonia.

MATERIALS AND METHODS

The present retrospective study was conducted at the University Clinic for Pediatric Surgery in Skopje between January 2013 and December2015. In this study all neonates (age <30 days) who underwent corrective and palliative surgery for CHD during this period were included. The diagnosis of CHD was established by antenatal recognitions and examinations, clinical examinations (hyposaturation, general and acrocyanosis, presence of murmur) and echocardiography. The analysis included neonatal's age, cardiac diagnosis, palliative procedures, corrective operative procedures with duration of cardiopulmonary bypass (CPB), and x-cross of aorta. Additionally, duration of mechanical ventilation, and inotropic support, ICU stay, necessity of reintubation and postoperative complications were evaluated. Mortality was defined as death within the first 7 days after surgery or before hospital discharge.

RESULTS

Of the total of 85 patients,15/85 (17.6%) were neonates who underwent corrective or palliative cardiac surgery at our center during the study period from January 2013 through December 2015. Male patients were 12/15(80%) female 3/15 (20%). Overall mortality was 1/15% (6.6%). Preoperatively, 9/15 neonates were with hyposaturation (75-80%), 4/15 neonates were with general cyanosis, acrocyanosis was confirmed in 3/15 neonates, and presence of murmur in 6/15 neonates.

Details of the cardiac diagnoses and numbers of patients are presented in Table 1. There were 13/15 (86.6%) corrective procedures and 2/15 (13.3%) palliative procedures (aortopulmonary shunt and pulmonary artery banding).

Table 1. Cardiac diagnoses and number of patients

Cardiac diagnoses	**Number of patients**
Patent ductus arteriosus	2
Coarctation of aorta	2
Ventricular septal defect	3
Single ventricle	2
Transposition of the great arteries	3
Tetralogy of Fallot	1
Total anomalous systemic venous connection	1
Hypoplastic left heart syndrome	1

Details of the cardiac diagnoses, palliative and corrective operative procedures and number of patients are summarized in Table 2.

Table 2. Cardiac diagnoses, palliative and corrective operative procedures and number of patients

Cardiac diagnoses	**Palliative and corrective operative procedures**	**No. of patients**
Patent ductus arteriosus	PDA closure	2
Coarctation of aorta	coarctation repair	2
Ventricular septal defect	VSD closure	3
Single ventricle	Norwood procedure	2
Transposition of the greatarteries	arterial switch operation	3
Tetralogia of Fallot	closure of the ventricular septal defect with a patch	1
Total anomalous systemic venousconnection	complex intracardiac repair with rerouting of the veins	1
Hypoplastic left heart syndrome	**Norwood procedure**	1

The mean duration of CPB was 46.6 min. (18±296 min.) and the mean duration of x-cross of aorta was 17.5 min. (10±65 min.). Details of intraoperative procedures are summarized in Table 3.

Table 3. Mean duration (expressed in minutes) of intraoperative procedures among patients

Intraoperative procedures	**Mean duration in minutes**	**Minimum-maximum**
cardiopulmonary bypass (CPB)	46.6	18-296
x-cross of aorta	17.5	10-65

Postoperatively, the mean duration of mechanical ventilation was 3.4 days (1±15 days), duration of inotropic support was 4.2 days (1± 16 days) and ICU stay was 5.8 days (7.9 ± 14 days). After the operation, 2/15 (13.3%) neonates required reintubation in the ICU. Details of postoperative variables are presented in Table 4.

Table 4. Mean duration (expressed in days) of postoperative procedures among patients

Postoperative procedure	**Mean duration in days**	**Minimum-maximum**
mechanical ventilation	3.4	1-15
Inotropic support	4.2	1-16
ICU stay	5.8	7.9-14

Postoperative complications were confirmed in 3 neonates (intracranial bleeding with seizures, block nodes AV, and pneumonia). After 5.8 days in the ICU, 14 neonates were discharged.

Postoperative complications are presented in Table 5.

Table 5. Postoperative complications frequency in patients

Postoperative complications	Number of patients
intracranial bleeding with seizures	1
block nodes AV	1
pneumonia	1

DISCUSSION

In this retrospective study we have presented postoperative outcomes in 15 neonates in a tertiary intensive care unit in Skopje, after they underwent neonatal cardiac surgery. The overall mortality of all 15 operated neonates was 1/15 (6.6%).

In the last several decades, development of devices, significant advances in cardiac surgery and postoperative care, reduced the rate of mortality among operated neonates [9]. To improve the outcomes the collaborative efforts of neonatal surgeons, intensive care doctors and cardiologists acting as "one team" are also very important. In addition, the growing experience and clinical practice have been reported as essential in improving the postoperative outcomes in some studies [10,11]. The proportion of palliative procedures in 2/15 (13.3%) neonates compared with 13/15 (86.6%) neonates with corrective procedures did not affect morbidity and mortality. In our study we presented the practice and experience in performing corrective operations, such as arterial switch operation, coarctation repair, VSD closure, complex intracardiac repair with rerouting of the veins, etc.

The utilization of CPB and x-cross of aorta are main surgical procedures during an open-heart surgery with different effects on body organs. Although the safety and development of devices during the past decades has significantly improved, there are still a large number of complications [12].

Kansy *et al.*[13] in their study revealed that prolonged intraoperative procedures (duration of CPB and x-cross of aorta) and cardiac defect complexity were significant risk factors for an increased number of postoperative complications. These observations are partly in agreement with those published in previous studies.

In our study we confirmed that neonates with complex heart defects had prolonged duration of CPR and x-cross of aorta. As mentioned earlier, they are recognized as risk factors associated with prolonged postoperative variables such as ICU stay with mechanical ventilation and duration of inotropic support [14]. Longer ICU stay is a major indicator of postoperative management of neonates. Some studies have demonstrated that impact and effects of mechanical ventilation require prolonged inotropic support and additionally cause worsening of the general condition and complicated postoperative outcomes [15,16]. Our study results revealed that ICU length of stay caused prolonged need of mechanical ventilation and inotropic support.

After surgery, 2/15 (13.3%) neonates with postoperative complications in the first 48 hours required reintubation. The first neonate had intracranial bleeding with seizures, and the second one had massive pneumonia, which required prolonged ICU stay and inotropic support.

Although in the last few decades the progress in technology and devices has been improved, the correlation between usage of CPB and intracranial bleeding with clinical sings of seizures was manifested. In many cases, the disorder is caused by damage of brain during surgery. The incidence of neurological disorders in some studies was in range of 4-15% [12,17]. In our study the incidence of postoperative clinical seizures was 6.6%.

Several studies confirmed that one of the most common complications was pneumonia. In the study by Maryam *et al.* [12], the incidence of pneumonia was 6.24% and in the study by Hornick *et al*. [18] the incidence of postoperative pneumonia was much less (2.2%).

Unfortunately, the incidence of nosocomial pneumonia after cardiac surgery in some studies varied between 9.6 to 21.5% [19], which is a very unpredictable outcome. In our study the incidence of pneumonia was 6.6%. Another recorded postoperative complication that had been confirmed without reintubation was block nodes AV as a result of atrio-ventricular canal defect repair. In the study by Hornik*et al.* [18, 20] the incidence was 2.8%. In our study the incidence of block nodes AV was 6.6%.

The main purpose of the present study was to examine the correlation between perioperative procedures and postoperative outcomes. The postoperative outcomes are poor, having in mind the number of operated neonates (3/15). In the future we should focus on larger multicenter studies to confirm the correlation between perioperative risk factors and postoperative complications in neonatal cardiac surgery.

Our study of postoperative outcomes after cardiac surgery even though a small one confirmed that with adequate, timely realized cardiac surgery, appropriate usage of perioperative devices and postoperative treatment improvement of postoperative outcomes was achieved. However, we need larger studies in order to confirm the postoperative outcomes.

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ORIGINAL ARTICLE

ANTIBIOTIC SUSCEPTIBILITY OF *CLOSTRIDIOIDES DIFFICILE* STRAINS ISOLATED FROM FECAL SAMPLES

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ABSTRACT

Introduction: *Clostridioides difficile* is one of the most important intra-hospital pathogens. The major risk factor for acquiring an infection with *Clostridioides difficile* (CDI) is a long-term antibiotic treatment. Although all the antibiotics can lead to CDI, studies have shown that the mostly involved are wide spectrum antibiotics like fluoroquinolones, penicillins, third generation cephalosporins and clindamycin. On the other hand, the treatment of severe CDI cases involves application of antibiotics like vancomycin or metronidazole.

Objective: Our aim with this study is to investigate the percentage of resistance to eight antibiotics (vancomycin, metronidazole, tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin) among the *Clostridioides difficile* isolates, indirectly evaluating the risk of acquiring CDI (by using the last six of them) or the risk of therapeutic failure in treating CDI (by using the first two).

Materials and methods: Eighty isolates of *Clostridioides difficile,* collected from fecal samples from as many patients during a four-year period (1. 2015 - 1. 2019), were later subject to PCR ribotyping and to antibiotic susceptibility testing to the eight antibiotics mentioned before, by using the E test.

Results: Ribotyping of the 80 isolates of *C.difficile* showed that they belong to 20 different ribotypes. The most common one was 001/072, with 40 % (32) of the isolates. Ten isolates belonged to the ribotype 014/020 (12.5%). Five isolates belonged to each of the ribotypes 002, 017 and 027. Other 15 ribotypes 005, 255/258, SLO 046, SLO 047, 003, 012, 015, 023, 046, 070, SLO 069, SLO 110, SLO 120, SLO 160, and SLO 187 were represented by 3 or less isolates. All 80 of the *C.difficile* isolates in this study showed good sensitivity towards vancomycin and metronidazole. Resistance percentages towards tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin were 1,25%, 49%, 55%, 57%, 100% and 45% respectively. The highest antimicrobial resistance percentages were detected in isolates taken from patients of surgical clinics. The highest antimicrobial resistance percentages were detected in isolates belonging to the dominant ribotype 001/072 and hypervirulent ribotypes 017 and 027.

Conclusions: Vancomycin and metronidazole should remain the first option therapy for CDI. Therapy with clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin could be a risk factor for acquiring CDI. Patients on ciprofloxacin are at especially high risk. Excessive use of a particular antibiotic plays a major role in selecting and multiplying resistant clones of *Clostridioides difficile* strains. Acquiring such characteristics contributes subsequently to the distribution of the ribotypes, but also contributes to the originating of the new *Clostridioides difficile* ribotypes. Surveillance of such genotypic and phenotypic characteristics of the *Clostridioides difficile* isolates can be of great value in controlling this modern epidemic of CDI.

Keywords: *Clostridioides difficile* infection, *C. difficile,* antimicrobial susceptibility

INTRODUCTION

Clostridioides difficile is one of the most important intra-hospital pathogens. This sporogenic anaerobic bacterium has commonly been isolated from feces, mostly from elderly hospitalized patients on antibiotics and has been associated with several clinical manifestations ranging from diarrhoea to pseudomembranous colitis [1].

The major risk factor for acquiring an infection with *Clostridioides difficile* (CDI) is a long-term antibiotic treatment. Although all antibiotics can lead to CDI, studies have shown that the mostly involved are the wide spectrum antibiotics, like: fluoroquinolones, penicillins, third generation cephalosporin and clindamycin [2]. Non-severe cases of CDI can be solved by terminating the use of the given antibiotic and by using probiotics [3]. On the other hand, the treatment of severe CDI cases involves application of antibiotics, such as vancomycin or metronidazole [4]. In many hospitals, including the University Clinical Complex "Mother Teresa" in Skopje, this therapy is given empirically, although there are few reports of emerging resistance worldwide [5].

OBJECTIVE

Our aim with this study is to investigate the percentage of resistance to eight antibiotics (vancomycin, metronidazole, tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin) among the *Clostridioides difficile* isolates, indirectly evaluating the risk of acquiring CDI (by using the last six of them) or the risk of therapeutic failure in treating CDI (by using the first two).

MATERIALS AND METHODS

All fecal samples received in the 1. 2015-1. 2019 period at the Institute of Microbiology and Parasitology, Faculty of Medicine, Skopje, in order to diagnose *Clostridioides difficile* infection (CDI), were subject to immunochromatographic detection of glutamate dehydrogenase (GDH) antigen and toxins A and B of *Clostridioides difficile*. In order to cultivate them, the same samples were planted on two plates: directly on Cycloserine-Cefoxitin-Fructose agar (CCFA) and on Columbia blood agar after performing the alcohol shock test. Such planted plates were incubated anaerobically for 48 hours at 37^0C in order to isolate *Clostridioides difficile*. The grown colonies were identified by characteristic macroscopic appearance and also microscopically by Gram staining. The definitive identification was made by using the automated system VITEK 2.

Eighty isolates of *Clostridioides difficile* from as many patients were collected from the cultures and were later typed using the PCR ribotyping method as the most commonly used typing method for this bacterium in Europe [6]. The antimicrobic susceptibility towards the eight antibiotics: vancomycin, metronidazole (according to EUCAST breakpoints) and tetracycline, clindamycin, erythromycin, imipenem, ciprofloxacin and moxifloxacin (according to CLSI break points), was also determined by using the E test on all eighty isolates. Interpretation criteria (breakpoints) for the susceptibility testing of the isolates are shown in table 1.

Table 1. Interpretation criteria for the antimicrobial susceptibility testing of the *Clostridioides difficile* isolates according to their minimal inhibitory concentrations (MIC)

	Vancomycin**	**Metronidazole****	**Tetracycline***	**Erythromycin***	**Clindamycin***	**Ciprofloxacin***	**Moxifloxacin***	**Imipenem***
Susceptible (μg/ml)	**≤2**	**≤2**	**≤4**	**≤2**	**≤2**	**≤2**	**≤2**	**≤4**
Intermediate (μg/ml)	**-**	**-**	**8**	**4**	**4**	**4**	**4**	**8**
Resistant (μg/ml)	**>2**	**>2**	**≥16**	**≥8**	**≥8**	**≥8**	**≥8**	**≥16**

**Interpretation is based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST);
* Interpretation is based on Clinical and Laboratory Standards Institute (CLSI).

RESULTS

In the examined period of 2016 – 2020, we have received 1380 fecal samples from symptomatic patients for CDI and in 182 of them presence of *Clostridioides difficile* was confirmed. After keeping only, the first isolate from the patients that had multiple samples tested and eliminating the isolates that had not survived the laboratory manipulation and subcultivation, we finally collected 80 isolates of *Clostridioides difficile* for further examination.

The origin of the isolates (clinics where the symptomatic patients have been hospitalized) is shown in table 2.

Table 2. Origin of the Clostridioides difficile isolates

Origin of fecal samples (isolates) sent		**Collected isolates (percentage of total isolates collected)**	**Total samples received (percentage of positive samples received)**
Surgery clinics	Number	22	70
	%	27,5%	31,4%+
Internal diseases clinics	Number	31	256
	%	38,8%	12,1%+
Pediatrics clinic	Number	7	413
	%	8,7%	1,7%+
Infectious diseases clinic	Number	10	442
	%	12,5%	2,3% +
Private ambulance outpatients and the rest	Number	10	199
	%	12,5%	5%+

The isolates originated from 41 male and 39 female patients. The average age of the patients was 54. Fifty six percent of the patients were over 60 years old. The percentage of toxigenic strains among the isolates of *Clostridioides difficile* was 92. In order to classify the isolates in groups, for better correlation with their antimicrobial susceptibility, we have performed the PCR ribotyping as the most widely used *Clostridioides difficile* typing method in Europe. PCR ribotyping results are shown in table 3.

Table 3. Ribotypes confirmed among the isolates of *Clostridioides difficile*

	***C. difficile* ribotypes**	**Number (N)**	**Percentage (%)**
1	001/072	32	40,00%
2	002	5	6,25%
3	003	1	1,25%
4	005	3	3,75%
5	012	1	1,25%
6	014/020	10	12,50%
7	015	1	1,25%
8	017	5	6,25%
9	023	1	1,25%
10	027	5	6,25%
11	046	1	1,25%
12	070	1	1,25%
13	255/258	3	3,75%
14	SLO 046	3	3,75%
15	SLO 047	3	3,75%
16	SLO 069	1	1,25%
17	SLO 110	1	1,25%
18	SLO 120	1	1,25%
19	SLO 160	1	1,25%
20	SLO 187	1	1,25%
Total		80	100%

Antimicrobial resistance of the 80 *Clostridioides difficile* isolates toward the 8 examined antibiotics is shown in table 4.

Four of the examined antibiotics, which the isolates showed variable susceptibility to, were also analysed in terms of associating the resistance of the strain with its origin. This association is shown in table 5.

Table 4. The resistance of *Clostridioides difficile* strains towards the eight examined antibiotics

Antibiotic	Result Interpretation	Ribotype						
		001/072 **n=32**	014/020 **n=10**	002 **n=5**	017 **n=5**	027 **n=5**	Others* **n=23**	Total **n=80**
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Vancomycin	Susceptible	32 (100)	10 (100)	5 (100)	5 (100)	5 (100)	23 (100)	80 (100)
	Resistant	-	-	-	-	-	-	-
	Intermediate	-	-	-	-	-	-	-
Metronidazole	Susceptible	32 (100)	10 (100)	5 (100)	5 (100)	5 (100)	23 (100)	80 (100)
	Resistant	-	-	-	-	-	-	-
	Intermediate	-	-	-	-	-	-	-
Tetracycline	Susceptible	32 (100)	10 (100)	5 (100)	2 (40)	5 (100)	22 (95,65)	76 (95)
	Resistant	-	-	-	-	-	1 (4,35%)	1 (1,25)
	Intermediate	-	-	-	3 (60%)	-	-	3 (3,75)
Clindamycin	Susceptible	2 (6,25)	1(10)	3(60)	-	1 (20)	13 (56,52)	20 (25)
	Resistant	28 (87,50)	1(10)	-	5 (100)	-	5 (21,74)	39 (48,75)
	Intermediate	2 (6,25)	8 (80)	2 (40)	-	4 (80)	5 (21,74)	21 (26,25)
Erythromycin	Susceptible	4 (12,50)	9 (90)	5 (100)	-	-	18 (78,26)	36 (45)
	Resistant	28 (87,50)	1 (10)	-	5 (100)	5 (100)	5 (21,74)	44 (55)
	Intermediate	-	-	-	-	-	-	-
Imipenem	Susceptible	6 (18,75)	4 (40)	-	-	-	11 (47,83)	21 (26,25)
	Resistant	23 (71,88)	3 (30)	3 (60)	5 (100)	4 (80)	8 (34,78)	46 (57,5)
	Intermediate	3 (9,38)	3 (30)	2 (40)	-	1 (20)	4 (17,39)	13 (16,25)
Ciprofloxacin	Susceptible	-	-	-	-	-	-	-
	Resistant	32 (100)	10 (100)	5 (100)	5 (100)	5 (100)	23 (100)	80 (100)
	Intermediate	-	-	-	-	-	-	-
Moxifloxacin	Susceptible	6 (18,75)	9 (90)	4 (80)	1 (20)	-	23 (100)	43 (53,75)
	Resistant	26 (81,25)	1 (10)	-	4 (80)	5 (100)	-	36 (45)
	Intermediate	-	-	1 (20)	-	-	-	1 (1,25)

Table 5. Antibiotic resistance of the *Clostridioides difficile* isolates to clindamycin

Antibiotic (Percentage of Resistant Isolates)	**Origin of the isolate**					
	Surgery Clinics	**Internal Diseases Clinics**	**Pediatric Clinic**	**Infectious Diseases Clinic**	**Private Ambulances Outpatients and the Rest**	**Total (%)**
Clindamycin	77	51	14	30	20	49
Erythromycin	77	65	16	30	30	55
Imipenem	86	58	43	20	40	57
Moxifloxacin	68	55	14	20	10	45

DISCUSSION

Metronidazole and vancomycin are still considered as first option for treatment of CDI [7]. In many diagnostic laboratories worldwide, *Clostridioides difficile* isolates are not routinely tested for their antibiotic susceptibility, which means that these two antimicrobials are applied empirically. Although our results showed no resistance among the isolates to these two antibiotics, which favours the practice mentioned before, there is still place for concern. In few studies [8,9,10] it is noted that resistance to metronidazole and vancomycin in *Clostridioides difficile* isolates can be present, especially among the ones belonging to the ribotype 027. This suggests that introducing the regular susceptibility testing to all isolates to these two antibiotics can help in preventing the therapeutic failures which could be expected in our hospitals in the future. This practice can also trigger the introduction of the new therapeutic drugs for CDI in our hospitals such as fidaxomycin [10], which unfortunately are still not available.

Of the other six antibiotics tested here, only tetracycline showed good action against *Clostridioides difficile* isolates. Resistance of the isolates to tetracycline was only 1,25%, which means that this drug has low potential for CDI. In some articles, it is also mentioned as a possible therapeutic option for CDI [11]. Clindamycin was considered as a very high risk antimicrobial for inducing CDI in the past and that resulted in reduction of its use [12]. In our study 49% of the isolates showed resistance to clindamycin, which is in the frame of the world average [7]. Especially high resistance showed the isolates belonging to the hyper-virulent ribotype 017 and the dominant ribotype 001/072. Isolates originating from the surgery clinics showed higher resistance to clindamycin than the ones from the other locations.

The resistance of the isolates to erythromycin was 55%, which means that using this antibiotic brings almost the same risk of CDI as clindamycin, probably as a result of the same resistance mechanisms [7]. Especially high resistance to erythromycin was shown by the isolates belonging to the hyper-virulent ribotypes 017 and 027 and the dominant ribotype 001/072. Isolates originating from the surgery clinics showed higher resistance to erythromycin than the ones from the other locations.

Although imipenem had not been mentioned as a risk for CDI in the past, it should be taken into consideration now. Out of the 80 isolates in this study, 57% showed resistance to imipenem, a much higher percentage than in most of the European countries [8]. In our opinion, excessive use of imipenem in our hospitals contributed to the wide distribution of such resistant strains. Same as with clindamycin, the isolates belonging to the hyper-virulent ribotype 017 and the dominant ribotype 001/072 showed the highest resistance. Isolates originating from the surgery clinics showed higher resistance to imipenem than the ones from the other locations.

Acquiring a resistance to fluoroquinolones is considered as a key moment in the evolution of the hyper-virulent ribotype 027 [13]. Currently, the application of ciprofloxacin is considered as the greatest risk for CDI, considering the 100 % resistance of the strains in this study, as well as in many others from all over the world.

However, the application of moxifloxacin is considered not as risky as ciprofloxacin. Forty five percent of the isolates showed resistance to moxifloxacin. This is the case especially among the isolates belonging to the hyper-virulent ribotypes 017 and 027, and the dominant ribotype 001/072. This resistance is very rare among other ribotypes. Like in all cases from before, with antibiotics with variable action against *Clostridioides difficile,* isolates originating from the surgery clinics showed higher resistance to moxifloxacin.

CONCLUSION

Finally, we would like to conclude that the excessive use of a particular antibiotic plays a major role in selecting and multiplying resistant clones of *Clostridioides difficile* strains. Acquiring such characteristics contributes subsequently to the distribution of the ribotypes, but also contributes to the originating of the new *Clostridioides difficile* ribotypes. Surveillance of such genotypic and phenotypic characteristics of the *Clostridioides difficile* isolates can be of great value in controlling this modern epidemic of CDI.

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CASE REPORT

GIANT CORPORAL LIPOMA TREATED WITH LIPOSUCTION

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ABSTRACT

Introduction: Lipomas are the most frequent benign neoplasms originating from the adipose tissue. Usually arising from the subdermal fat, they deform body contour and affect natural appearance. Therefore, main indication for their removal is of cosmetic reason. Traditionally, open classical surgical ablation is a mainstay of their treatment. In order to achieve scar- less removal and decreased postoperative morbidity, many new techniques have been innovated in the last decades. Out of them, liposuction has gained popularity with increasing rate of utilization due to simple applicability, safety, high patient compliance, but mostly because of small incision/scar and eventual total removal. Subcutaneous lipomas are the most common indication for non-cosmetic application of liposuction worldwide.

Case presentation: In this paper, we present a case of giant lipoma of the body that has been removed successfully with liposuction. Total tumor removal is achieved as shown in the postoperative period without a recurrence in a period of 36 months follow-up.

Conclusion: Appropriate preoperative diagnostic and evaluation is crucial when selecting lipomas that can be treated in this way.

Keywords: lipoma; liposuction; lipectomy

INTRODUCTION

With an incidence of 1-2.1/1000, lipomas are the most common neoplasms in human body, being benign tumors of mesenchymal origin from the adipose tissue [1]. Mainly emerging from the subcutaneous fat, usually located on the trunk, extremities and nuchal area, they are easy to be palpated and inspected. These most frequent forms have classic appearance of soft, circumscript, painless and mobile solitary lesions, that concern patients typically in 4^{th} and 5^{th} decade of their life, after a period of long lasting slow growth [1,2]. However, lipomas can develop at any age, at any region, at different organs and can have deeper and atypical localizations. They can vary in dimensions; if larger than 10cm in diameter they are referred to as giant [2].

According to WHO classification of soft tissue tumors, histologically, there are many forms of lipoma, depending on the tissue that is co-present beside the adipose cells. These comprise conventional lipoma, angiolipoma, lipomatosis of the nerve, lipoblastoma, hybernoma, mielolipoma, chondroid lipoma, spindle cell and pleomorphic lipoma [3]. Conventional lipoma, simple lipoma or common lipoma is the same term that reflects the clinical entity of the most common form composed of mature fat cells – adipocytes without atypia, arranged in lobules divided by fibro-vascular septa. Fine capsule enveloping the tumor is usually present [4]. Anamnesis (history, pain growth), physical examination (mobility, bordering, tenderness) and tumor's features (location, consistency, smoothness, margins) are usually sufficient for getting the diagnosis done. However, basic work-up consisted of ultrasound investigation with linear probe and fine needle aspiration biopsy can be used to conclude it [5,6]. In superficially located cases, it is often enough to get the data needed.

Further investigations, such as MRI, can be used in doubtful cases, as their rarer malign counterparts, especially atypical lipoma and low grade liposarcoma, can present clinically quite similar [7]. Patients seek operation mainly because of cosmetic concerns, body disfigurement, pain (when nerve is compressed), increased growth or due to cancer phobia. Treatment traditionally comprises open surgical ablation often requiring larger incisions, longer postoperative morbidity and eventual postoperative complications [1,4]. Based on the idea of less scarring and less postoperative morbidity, new modalities have emerged lately [8-10]. Among them, liposuction raised popularity since its introduction in mid-1970s as a minimal invasive approach with high patients' compliance and high satisfaction rate, being a safe and effective method as well [11]. However, there are few reports of efficient treatment of giant lipomas [12-14]. This paper presents a case of successful removal of conventional lipoma with liposuction and postoperative follow-up of 36 months.

CASE REPORT

We present a case of 61-years old male patient, with a corporal conventional lipoma treated with liposuction. The patient noticed the condition twenty years ago, as a small lump with slow painless growth over the years. No other symptoms were reported except for the disfiguration of the body. Location is lateral aspect of thoraco-abdominal wall, with a portion on the back. He wanted the removal because of fear of its growth, which has been accelerated in the last years. He noted impairment with garments and aesthetic mutilation as well. Clinically, it is a mobile, subcutaneous, well – bordered tumor, soft and painless on pressure which is 26x14cm gross. (Figure 1). Ultrasound examination with linear probe showed well – encapsulated soft tissue tumor superficially located over the muscles. Fine needle biopsy showed Ist classification group. These additional data were in consistency with the clinical evaluation and assumed benign tumor of lipomatous origin. Preoperatively, usual blood count was done and operation was planned in local anesthesia with mild sedation. Single shot wide spectrum antibiotic was given intravenously 30 minutes before the operation.

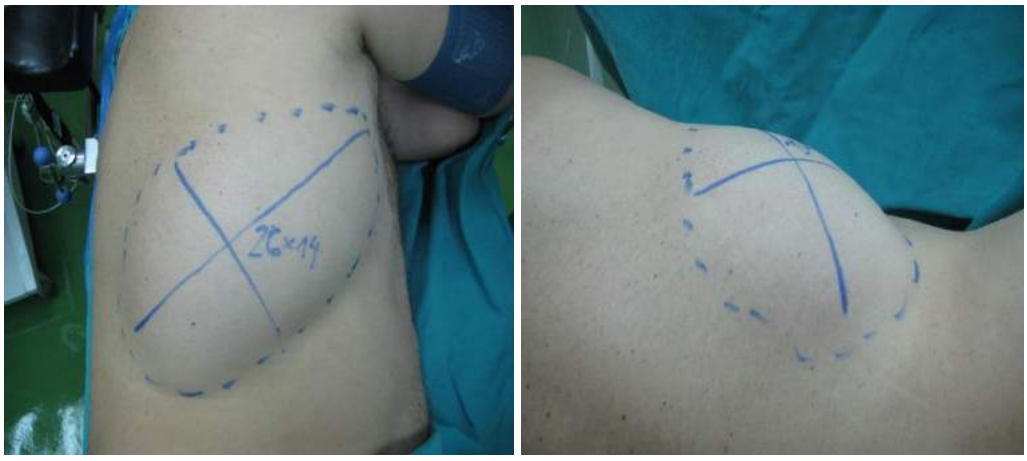

Fig.1. Preoperative photography and markings

Infiltration of 2ml 1% Lidocaine and 0.01% adrenaline in the planned 0.5cm incision was done. Few moments after, incision was made and infiltrating cannula was incorporated into the tumorous tissue. Tumescent infiltrating technique was used: about 200 ml of Klein's solution (0.1% lidocaine and 1:1 milion adrenalin in 1000ml 0.9% saline solution) was infiltrated under pressure in the lipoma with a blunt 3mm cannula until skin started getting the aspect of orange peel. (Figure 2a) After a waiting period of 10-15 minutes while gentle massaging, liposuction was conducted using 30cm long, blunt Mercedes ǿ3mm cannula (Byron®) and manually made negative pressure with 60 cc syringe that fits the cannula.

Manually made vacuum with syringe is enough for the liposuction of a lipoma (Figure 2b); no expensive equipment like powered suction device or ultrasound is required. Liposuction is done until reaching the smoothness of the overlying skin and when bloody aspirate predominates in syringe as a typical sign of end point. Aspirate was filtrated and the hard part sent for pathohistological examination. Incision was closed with one resorptive subcutaneous suture and compressive dressing was applied. The patient was discharged the same day from hospital. Checkup was scheduled in 3 days with advice to wear compressive dressing and to continue usual obligations meanwhile. Wearing compressive garment (shirt with elastin) for 3 weeks was advised. Analgesics were prescribed in case of necessity.

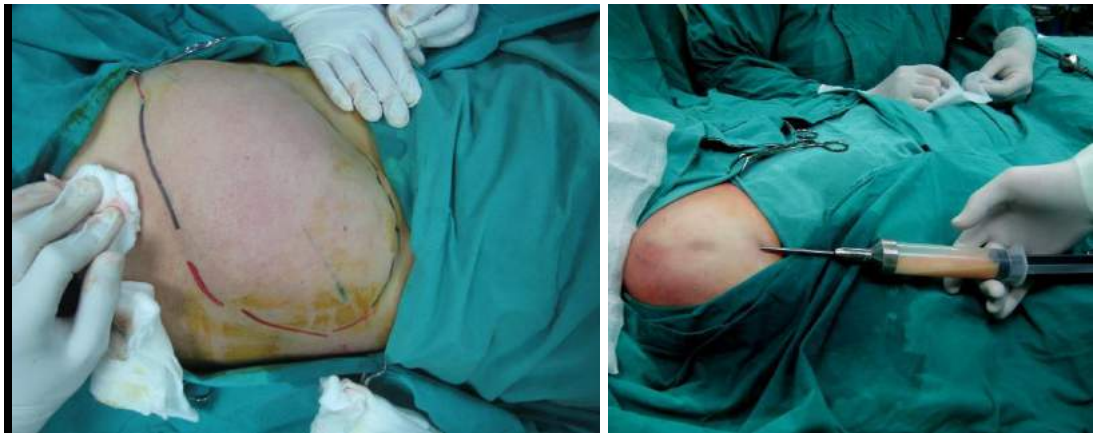

Fig. 2a. Left. Infiltration of the lipoma until orange peel aspect of the overlying skin.
Fig. 2b Right. Liposuction with syringe made vacuum

Patient generally felt well during the first critical days. He complained about mild pain that was increasing when changing posture, but not as strong as to take painkillers. On the first checkup, there was bruising and swelling over the plane that diminished in 2 weeks. (Figure 3) Lipoma was removed totally. Over the period of one- and three-months checkups, there were no signs of recurrence, pain or skin irregularities. Incisional scar was almost invisible. Smoothness of the skin contour was achieved and the patient was satisfied. After a year, excellent results can be seen (Figure 4): no signs of recurrence, superior skin alignment and almost invisible incisional scar. The same findings were noted on 36th month of follow up. Pathohystology results were in favor of lipomatous benign lesion - lipoma.

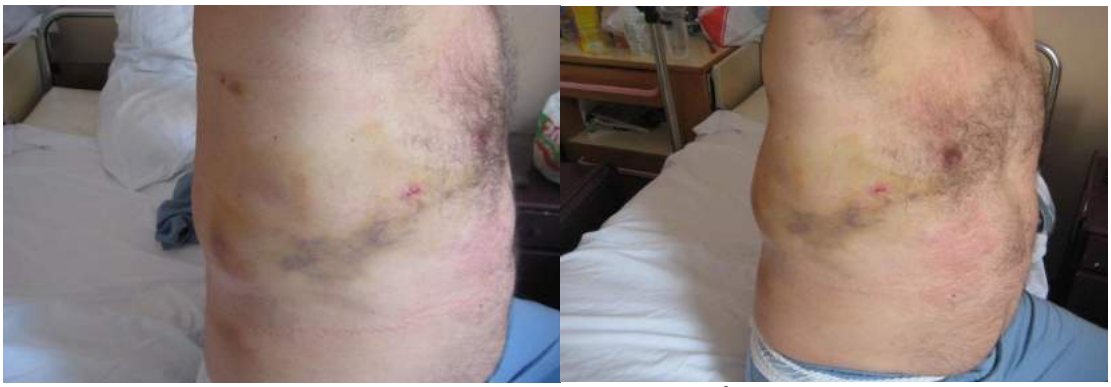

Fig.3. Postoperative bruising and swelling on 3rd postoperative day

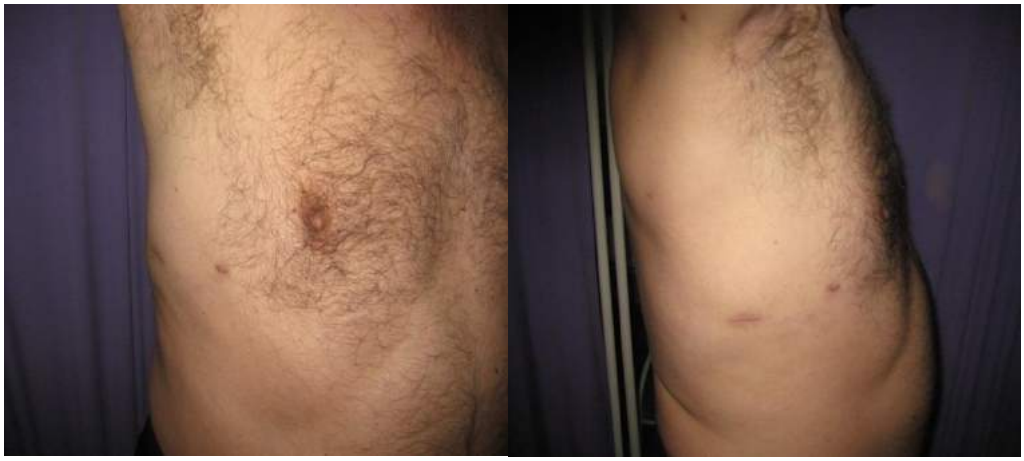

Fig.4. Appearance and result one year after operation

DISCUSSION

To diagnose a lipoma usually is not difficult. Clinical presentation is of biggest importance prior diagnosis. Encapsulated and well-defined, as benign lesions they are painless and slow growing. Still when the tumor is fast growing, painful and larger than 10 cm and/or has atypical or deeper location, one has to bear in mind the possibility of sarcoma, most of which are atypical lipomas (or well-defined liposarcomas) [15]. Imaging techniques such ultrasonography, CT and MRI as well as fine needle/core biopsy are useful applications [4-6]. MRI is highly sensitive in the detection of well-differentiated liposarcomas and highly specific in the diagnosis of simple lipomas. Accurate diagnosis before any attempt for liposuction - assisted lipectomy is imperative. If accuracy is doubtful, tissue samples prior liposuction can be taken in the manner of open biopsy. However, fine needle aspiration biopsy is a highly sensitive method for differentiating benign and malign soft tissue tumors [6].

Aspirate from the liposucted lipoma should be sent for pathologic examination. Studies have demonstrated that cell integrity in lipoaspirate is not damaged thus adequate pathohistology can be done accordingly [16]. Hereby, misdiagnosing liposarcoma of any type can be annulled successfully. Diagnostic pathway of our patient comprised anamnesis, clinical examination, ultrasonography, fine needle biopsy and microscopic examinations of the lipoaspirate.

In 1985, Rubenstein et al. were first to treat lipoma with liposuction [10], and so far, lipoma is the most frequent entity of its non – cosmetic application [17]. Advantages are smaller scar, less pain, good cost – effectiveness, shorter operative time, better final surface contour, high patient compliance, ability to remove more lipomas through fewer incisions, small complication rate, ability to remove a tumor from distant operative site aesthetically acceptable [18]. Liposuction is indicated in suprafascial/subcutaneous, lipomatous masses, uni- or multilateral with size greater than 5 cm where diagnosis is well established. Some authors encountered difficulties when removing giant lipomas [13]. Our lipoma is giant and we did not have any problem; we had cannula long enough as to reach the very end border of the lesion. Difficulties might arise when a shorter cannula is used and, in this case, an additional counter incision is reasonable solution.

Minor sequels such are bruising, hematoma, immediate dimpling, light pain and swellings are concomitant to liposuction. They are mainly self-resolving. Infection is unusual. Liposuction is safe procedure when following guidelines [11]. On the other hand, open surgery includes larger scars, risk of hematoma, infections, seroma formation, dehiscence, hypertrophic scar, skin invaginations.

The main concern about liposuction in giant lipomas is its questionable ability to achieve radical removal of the lipomatous tissue and higher recurrence rate. Rubistein noted that it's difficult to remove the fibrous capsule with liposuction [10]. Raemdonck *et al.*, in the only comparative study in the literature, showed higher recurrence risk in giant lipomas treated with liposuction compared to open surgery. This study consists of 30 cases [12]. Case reports of giant lipomas treated with liposuction showed no recurrences in 2 years follow up period [13,14]. Al-basty proposed capsule extirpation with forceps through liposuction incision or additional counter incision in larger lipomas as to prevent recurrences. In 6 years follow-up period of 16 patients, there were no recurrences [18]. The proposed modification has also been applied in the largest and most recent published study that reports no recurrence in 44 treated lipomas for a mean period of 6 years follow-up [19]. In our case, there are no signs of recurrence in a period of three years follow up. In non-fibrotic lipoma, as in our case, it's possible to destroy capsule mechanically and then perform liposuction. Otherwise forceps usage seems reasonable.

As comparative studies about treatment of giant lipomas comparing open surgery versus liposuction - assisted lipectomy are lacking, one has to inform the patient about the probable higher risk of recurrence. The recurrence risk of open surgery is about 2%; there are not many cases in the literature about liposuction - assisted lipectomy in giant lipomas that can estimated with a risk of 2% or higher [19]. Therefore, these statements about higher recurrence rate are based on small series or are observational in nature. Nevertheless, in order the scientific truth to be concluded finally, larger randomized prospective studies are needed.

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CASE REPORT

SYMPATHETIC OPHTHALMIA AS A RESULT OF PENETRATIVE EYE INJURY

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ABSTRACT

Introduction: Sympathetic ophthalmia was first clinically described by William Mac Kenzie in the middle of the 19th century. Sympathetic ophthalmia (SO) is very rare, specific, typically bilateral asymmetric granulomatous autoimmune inflammation of the uveal tract. Signs and symptoms vary in their severity and onset. Sympathetic ophthalmia is non-necrotizing panuveitis that can be developed either postsurgical or after accidental trauma to one eye.

Objective: To present a very rare case of sympathetic ophthalmia.

Case report: A 51-year-old man came at our Clinic for an eye examination. He complained of severe decreasing of visual acuity on one eye. In anamnesis he reported that 7 months ago he had injured his left eye with a sharp metal object. He was admitted to our Clinic with corneal-scleral penetrative wound, prolapsed iris, anterior chamber hemorrhage and suspected intraocular foreign body. After full examination vitrectomy was performed with foreign body extraction. His last examination revealed that his left eye was in hypotonia with pupillary membrane sub phthisis of the eye ball and mild anterior chamber reaction. Sympathetic ophthalmia is a clinical diagnosis based on clinical examination and a patient history of prior penetrating ocular trauma. The latent period of appearance is usually between 2 weeks and 3 months, although it can appear as early as 5 days and as late as 66 years after the initial incident. Ocular investigations like fundus fluorescein angiogram and PS OCT are useful in establishing the diagnosis. Our patient had a typical inflammatory finding on the anterior and the posterior segment. Sympathetic ophthalmia sometimes may be difficult to distinguish from VKH or sarcoidosis. Although there are differences between these choroidal inflammations, still they should be considered especially if there is no history of prior eye injury or if it develops postoperatively.

Conclusion: The best still known treatment for SO in patients with low visual outcome on the injured eye is the surgical treatment. Enucleation performed immediately before, concomitant with, or subsequent to the development of SO results with good visual outcome.

Keywords: sympathetic ophthalmia, OCT, injury

INTRODUCTION

Sympathetic ophthalmia (SO) is very rare specific, typically bilateral asymmetric granulomatous autoimmune inflammation of the uveal tract [1]. Sympathetic ophthalmia was first clinically described by William Mac Kenzie in the middle of the 19th century [2]. As a separate disease entity from other ocular inflammatory disorders with detailed histopathologic analysis was first presented by Ernest Fuchs. He and Dallen described independently the Dalen Fuchs nodules as inflammatory nodular aggregations.Until recently, accidental penetrating ocular trauma was the most common event for development of SO. According to Liddy and Stuart the incidence of SO ranges from 0.2 to 0.5% after penetrating ocular injuries and Mark estimated the incidence to be less than 0.01% after intraocular surgery [3,4].

Usually, the inflammation is more severe in the exciting eye than in the sympathizing eye. Signs and symptoms vary in their severity and onset. Sympathetic ophthalmia is non-necrotizing panuveitis that can be developed either postsurgical or after accidental trauma to one eye [5]. The pathology of the inflammation is not clearly understood, but it seems that disrupting the integrity of tissue of the inciting eye and the released ocular antigens through the HRB leads to autoimmune reaction in the injured eye (the *exciting* or *sympathogenic eye*), as well as an inflammatory reaction to the uninjured eye (the *sympathizing eye*) [6]. The eye is an essential organ that provides vision and therefore normal life function as well as its tissue minimal ability to regenerate when damaged makes it one of the immune privileged organs in our body. In the injured eye tissues like the uveal tract, lens and retina can act as antigens and provoke an autoimmune response in the unaffected eye. Optical Coherence Tomography, or OCT, is a technique for obtaining sub-surface images of translucent or opaque materials at a resolution equivalent to a low-power microscope and is used to present this case.

However, recent studies suggest a higher risk of developing SO when vitrectomy has been performed as a procedure in context to other penetrating ocular injuries.

OBJECTIVE

The main objective of our study was to present a very rare case of sympathetic ophthalmia.

CASE REPORT

A 51-year-old man came at our Clinic for an eye examination. He complained of severe decreasing of visual acuity on one eye. In anamnesis he reported that 7 months ago he had injured his left eye with a sharp metal object. The patient was admitted to our Clinic with corneal-scleral penetrative wound, prolapsed iris, anterior chamber hemorrhage and suspected intraocular foreign body. After full examination vitrectomy was performed with foreign body extraction. The last examination revealed that his left eye was in hypotonia with pupillary membrane sub phthisis of the eye ball and mild anterior chamber reaction. On the right (healthy) eye the best corrected visual acuity (BCVA) was 0.1 s.c., and light with no projection on the left. Right intraocular pressure was 12.2 mmHg and the left was in hypotonia. On anterior segment he had perilimbal injection, mutton-fat KPs, flare in anterior chamber (2+), and pigment epithelial cells on anterior lens capsule. Fundus inspection found exudative posterior retinal ablation, inflammatory cells in vitreous, swelling of the surrounding retina with the optic nerve head. Mid equatorial choroidal yellowish white lesions (*Dalen-Fuchs nodules*) were present. Optical coherent tomography on the posterior segment (PS OCT) was done showing the choroidal cell infiltration, retinal thickening posterior poll exudative retinal ablation, moderate flare in vitreous, and optic nerve swelling (Fig.1-lower tomogram), (Fig.2 left).

The treatment was started with topical corticosteroids together with cycloplegic and mydriasis agents and systemic corticosteroids. Two weeks later he was also given corticosteroid-sparing agent, Azathioprine.

He was followed closely and 4 weeks later the vision on the right eye was fully recovered with BCVA 1.0 s.c.

He had no more signs of inflammation on the anterior segment; there were few cells in the vitreous body, mild persistent optic nerve head edema with intra-retinal small cystoid fluid filed in the macular region.

On the control OCT performed 1 month later he had a complete subretinal fluid (SRF) absorption and still persistence of small cystoid intraretinal fluid collections (Fig. 1 - upper tomogram). There was mild disc edema and no edema in the nasal sector (Fig. 2 right).

On the first visit, eye ultrasonography on AB-scan showed posterior serous retinal detachment and cells in the vitreous body. One month later, the finding was normal (Fig. 4, 5).

He continued with topical and systemic therapy with lower doses. He is still under observation until full remission.

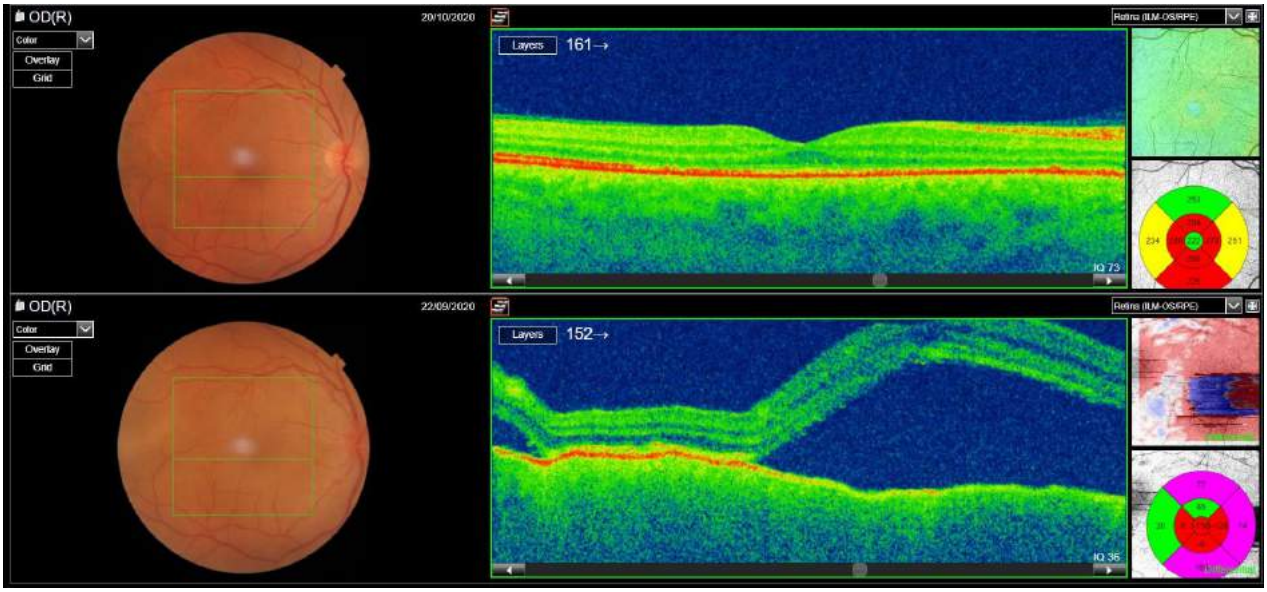
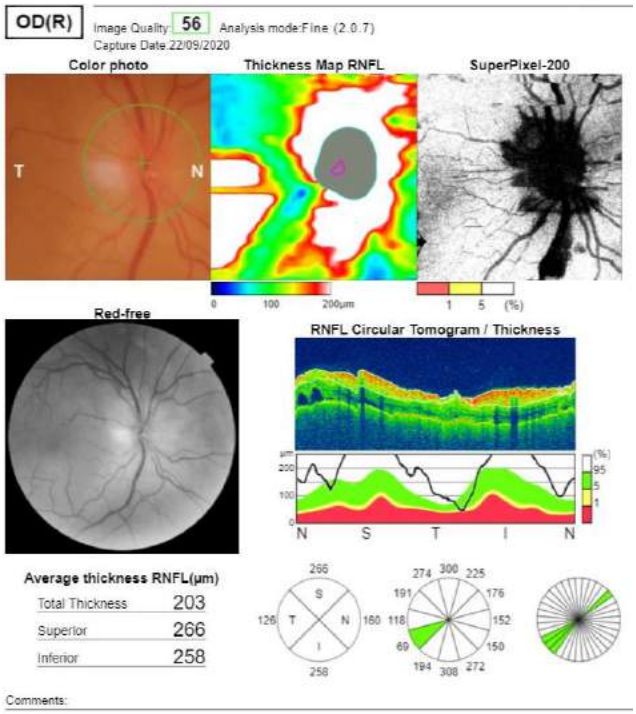


Fig.1. PS OCT comparative analysis: on the picture below there is a serous retinal detachment on the posterior pole with collection of SRF, CMT: µ; one month later on the upper tomogram there is a normal flat retina with absorbed SRF and persistence of minor intra-retinal cystoid fluid collections.



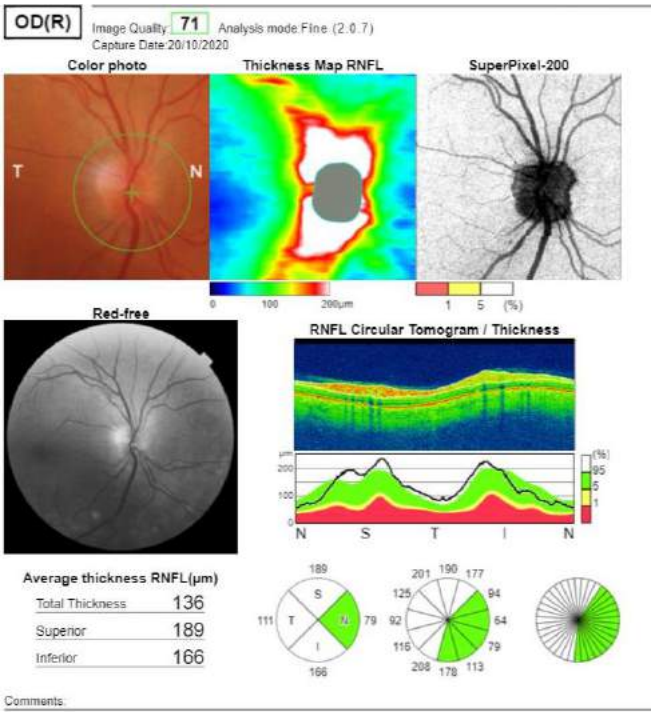


Fig.2. (left) OCT analysis RNFL AT 203µ with thickening in 4 quadrants

Fig.2. (right) OCT analysis RNFL month later AT 136µ, with regression on disc edema and no edema in nasal quadrant

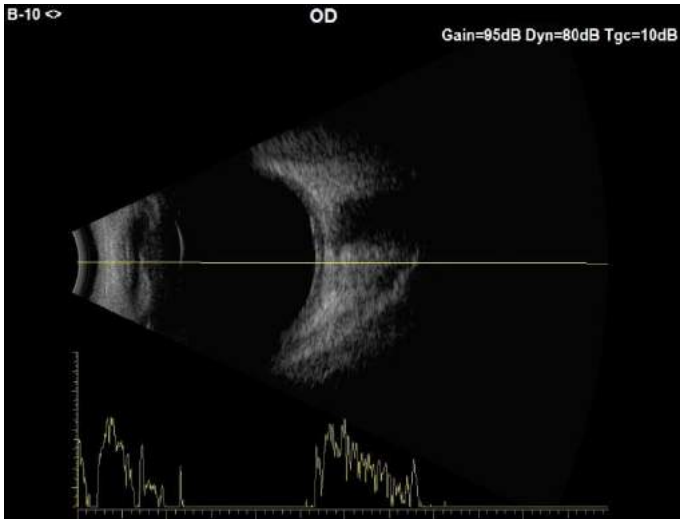


Fig. 4. Right eye US AB-scan with posterior retinal detachment

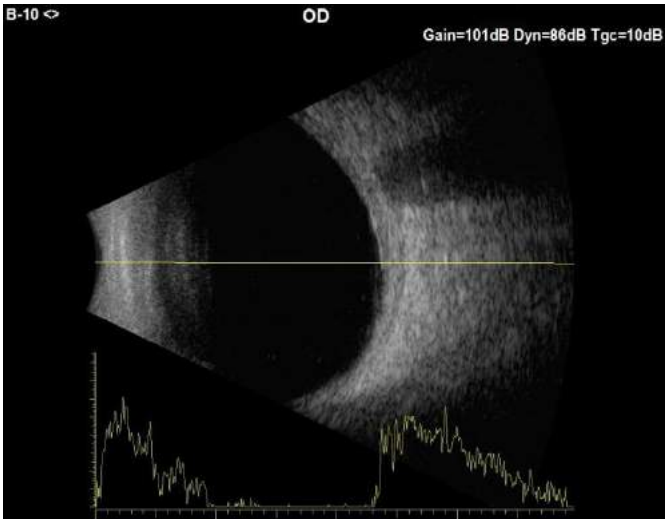


Fig.5. Right eye, month later normal US

DISCUSSION

Sympathetic ophthalmia is a clinical diagnosis based on clinical examination and a patient history of prior penetrating ocular trauma. The latent period of appearance is usually between 2 weeks and 3 months, although it can appear as early as 5 days and as late as 66 years after the initial incident [7,8]. Ocular investigations like fundus fluorescein angiogram and PS OCT are useful in establishing the diagnosis [9].

Our patient had a typical inflammatory finding on the anterior and posterior segment. Histopathology of the inflammatory changes in cases with sympathetic ophthalmia has shown to be identical in both eyes, the exciting and sympathizing eye [10].

However, in the history of SO atypical histopathologic features have been reported. In SO there is a diffuse granulomatous infiltration throughout the uveal tract. Jakobiec *et al.* [11] in eyes removed after initial surgical trauma reported predominance of CD8 cells in the choroid, while Chan *et al.*[12] in an eye that was enucleated several months after the initial nonsurgical trauma found predominance of CD4 T cells. These changes in T cells subsets over time are probably an attempt of the eye to down-regulate the immune response with the influx of suppressor T cells. Rao *et al.* compared a situation with intraocular antigen presentation (situation comparable to no penetrating trauma) and extraocular antigen presentation (comparable to penetrating wound with uveal prolapse) [13]. The result was absence of SO in the contralateral eye in the first group and presence of inflammatory uveal reaction after 14 to 16 days in the second group of patients. These findings show that aqueous humor outflow tissues have similar characteristics like lymphatic vessels. Genetic predisposition to sympathetic ophthalmia and increased frequency of (HLA) A11 was also presented by Reynard *et al.* suggesting the genetic important role in SO pathogenesis [14]. Sympathetic ophthalmia sometimes may be difficult to distinguish from VKH or sarcoidosis. Although there are differences between these choroidal inflammations, still they should be considered especially if there is no history of prior eye injury or if it develops postoperatively.

CONCLUSION

The best-known treatment of SO ophthalmia in patients with low visual outcome on the injured eye is the surgical one. Enucleation performed immediately before, concomitant with, or subsequent to the development of SO results in good visual outcome. Prompt medical treatment with topical and systemic corticosteroids, cyclosporine or cytotoxic agent as well as prompt diagnosis can lead to remission of the uveal inflammatory reaction and save the patient from potential blindness.

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REVIEW ARTICLE

MATRIX METALLOPROTEINASES AND BIOFILM IN CHRONIC WOUNDS: THERAPEUTIC OPPORTUNITIES

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ABSTRACT

Wound is a type of injury to the skin and underlying structures. Impaired healing is the result of changed systemic or local microenvironmental factors. Biofilm and matrix metalloproteinases are local factors which are among most important and interesting to evaluate. Chronic wound infections typically form biofilms, which are usually resistant to conventional antibiotics. High levels of matrix metalloproteinases are also typical for chronic wounds. We discuss non-conventional antimicrobial therapeutics of chronic wound biofilms and treatment of high levels of matrix metalloproteinases.

Keywords: biofilm, matrix metalloproteinases, chronic wounds, therapy

INTRODUCTION

Wound is a type of injury to the skin and underlying structures [1]. Chronic wound is defined as a secondarily healing wound that does not heal within a period of 3 months [2]. Classification of wounds is based on etiology, by Rank-Wakefield system, duration of wound healing, skin integrity, degree of contamination, and morphologic characteristics [3]. According to etiology wounds can be blunt trauma wounds, burn injuries, penetrating injuries, incisional wounds. By Rank –Wakefield system wounds are classified as tidy and untidy whether to suture or not. According to the duration of wound healing there are acute and chronic wounds. Acute wounds heal immediate for few weeks, chronic wounds fail to heal in time, usually they heal within a period of 3 months. Wounds can be open and closed according to integrity of the skin. By degree of contamination, they are classified as clean, clean contaminated, contaminated and dirty wounds. Morphologically wounds are divided to bruises, abrasion wounds, lacerated wounds, incisional, crush injury, degloving injury, penetrating injury.

Chronic wounds can be classified as pressure wounds (decubitus and neuropathic ulcers), inflammatory wounds (autoimmune and primary cutaneous disorders), vascular insufficiency wounds (venous, arterial and mixed ulcers), malignant wounds (primary and secondary cutaneous malignancies), miscellaneous wounds (burns, radiation injury, frostbite, vasculitic ulcers, insect bites) [3].

After breaking the skin protective barrier, response is migration of many cells which are involved in the repairing process of wound [4, 5]. Usually, the main reasons for skin injury are trauma or surgical procedures, but most commonly it is associated with diabetes, hypertension, obesity, malignancies, peripheral vascular disease, prolonged immobilization and advanced age [4]. Skin repairing process is well organized and requires immobilization of multiple cells from every skin layer. Wound healing process consists of four overlapping phases of hemostasis, inflammation, tissue proliferation and tissue remodeling [5].

The first stage of wound healing is hemostasis which has the role to stop bleeding after vascular damage. There are 3 steps in this stage vasoconstriction, primary hemostasis and secondary hemostasis. Vasoconstriction of the vessel walls stops bleeding. Primary hemostasis is formation of the platelet plug. Secondary hemostasis is activation of the coagulation cascade when the fibrin mesh is formed. Thrombus made from platelet plug and fibrin mesh stops bleeding, induces releasing of growth factors and complement which are scaffold for cells important and necessary for wound healing [6].

Within 24 hours after injury, wounds progress to the next phase of wound healing known as inflammatory phase. It involves several steps and lasts until post injury day 4. First signals are increased intracellular Ca^{2+} at wound edges which propagates to the center of the wound [7]. Other signals are damage-associated molecular patterns, hydrogen peroxide, lipid mediators, and chemokines which are released from injured cells. These signals first recruit neutrophils as first line inflammatory cells. After that macrophages, mast cells, and dendritic cells are recruited [7]. This phase leads to removing bacteria and devitalized tissue with neutrophils and macrophages [8]. Neutrophils phagocytize bacteria and tissue debris producing reactive oxygen species and release matrix metalloproteinases which digest injured tissue. Macrophages are cells which take place in inflammatory phase and the next phase of wound healing. Their function is phagocytosis and releasing proteases for digestion of necrotic tissue.

Third stage of wound healing is proliferative phase. It occurs between days 4 and 21. Key role in this phase have fibroblasts. Characteristic for third phase is synthesis of collagen, deposition and cross linking. In this phase extracellular matrix is reconstituted by adding proteoglycans. Other cells in proliferative phase are myofibroblasts responsible for contraction of wound [9].

The last stage is remodeling phase and it lasts for years. In this phase collagen type III is replaced by collagen type I, which is followed by reorganization of extracellular matrix [10]

In most cases healing restores barrier function of the skin, but sometimes wounds fail to heal and the healing process phases are prolonged. That leads to persistent, non-healing state which is defined as chronic wounds [11].

Infection, as a leading cause of delayed wound healing, with pathogenic microbes, bacteria and fungi, invades the wound bed and forms biofilm. Factors that make proliferation of microbes in chronic wounds are devitalized tissue, moist and inflammatory processes which are dysregulated [12].

Biofilm is defined as communities of surface-attached or self–attached microorganisms, embedded within a hydrated matrix of extracellular polymeric substances which provides protection against antimicrobial agents and host defenses [13].

Biofilms in chronic wounds

Bacteria can colonize and infect wounds, resulting in prolonged wound healing process. Most chronic wounds are polymicrobial. The structure of biofilms is made of microbial aggregates, that are encased within an extracellular matrix (ECM). ECM consists of polysaccharides, proteins, and glycoproteins, referred to as the extracellular polymeric substance (EPS) figure 1 [14, 15].

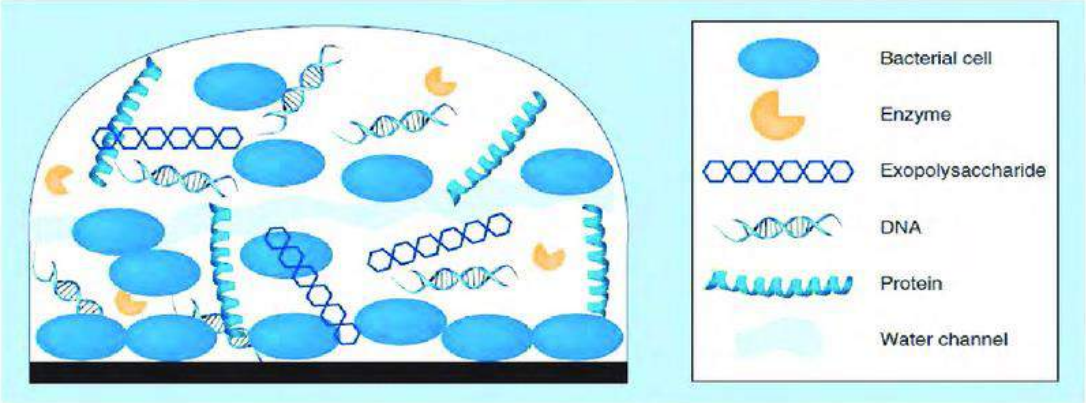

Fig. 1. Structure of biofilm (Sangwan S, Pratibha P, Hemender T. Anti-biofilm enzymes: a strategy to remove biofilms. Agrobios. 2019;17(12):35–75. [14])

Biofilm-forming bacteria include *P. aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Streptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus,* and the yeast *Candida albicans,* Figure 2 [16,17].

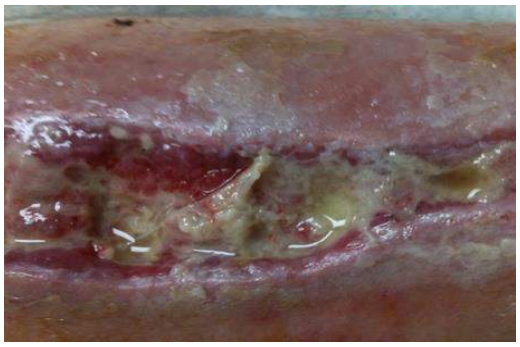

Fig. 2. Biofilm with antibiotic resistant pathogens in chronic, non-healing wound (Bowler PG. Antibiotic resistance and biofilm tolerance: a combined threat in the treatment of chronic infections. J Wound Care. 2018;27(5):273–277. [16])

It is thought that high levels of oxidative stress (OS) are one of the reasons for chronic wounds promoting the colonization of biofilm forming bacteria over commensal beneficial bacteria [18].

Healing wounds, usually have low levels of OS, and are colonized with diverse bacterial microbiome that never makes biofilms [18]. There are biofilm forming bacteria in this microbiome but commensal beneficial bacteria are predominant and that lead to healing. Some bacteria, such as *Cutibacterium acnes, Achromobacter sp., Delfia sp.,* and *Escherichia coli* are associated with healing wounds. They are bioindicators of healing and prevent colonizing the wound with pathogenic bacteria [18].

On the other hand, chronic wounds have high levels of OS, low level bacterial diversity with predominance of biofilm forming bacteria such as *Pseudomonas aeruginosa, Enterobacter cloacae, Corynebacterium frankenforstense, and Acinetobacter sp.* [18].

The concept of "biofilm based wound care" has the aim to prevent biofilm formation in wound tissue. The problem with this concept is the polymicrobial nature of biofilm, where fungi are used as a scaffold for attachment of bacteria and protect each other from therapeutic modalities [19, 20].

It has been reported that *Candida spp.* usually interact with bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, Burkholderia cenocepacia, Streptococcus* spp, *Acinetobacter baumannii, Enterococcus faecalis,* and *E. coli* [21].

Fungal microbiome in diabetic foot ulcers (DFUs) is represented with phylotypes which belong to the phylum *Ascomycota* or *Basidiomycota.*

Cladosporidium herbarum and *Candida albicans* (from phylum *Ascomycota)* and *Trichosporon* and *Rhodosporidium* spp (from phylum *Basidiomycota*) are the most frequent fungi in mycobiome. Impact of antibiotic therapy on diversity of mycobiome is evident after antibiotic administration [22, 23]. *Cladosporium* spp. with other allergic fungi such as *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Pleospora* spp., and *Fusarium* spp. are very frequently found in DFUs. These fungi same as pathogenic species have impact on wound chronicity, but they are negatively associated with hemoglobin A1c and white blood cell levels, contrary to *Candida* spp., *Trichosporon asahii*, and *Rhodotorula* spp. That results in different immune response depending on diversity of the fungal microbiome [22, 23].

Biofilms are more resistant to antimicrobial therapy than bacteria alone. It is result of impaired host inflammatory response. Leucocytes are with low ability to produce reactive oxygen species and they have difficulties while penetrating through the biofilm [24]. This results in impaired phagocytosis of bacteria. The exopolymer of the biofilm block complement activation, suppress the lymphoproliferative response, and impairs the ability of opsonins on bacterial walls to be detected by phagocytes [25].

Bacteria in biofilms, have decreased metabolic activity. Metabolically active cells are usually targets for antimicrobial agents, so bacteria are resistant to these agents [26]. Exopolysaccharide in the biofilm is a mechanical protector of bacteria from antimicrobials and the immune cells [27]. In biofilms bacteria can transfer plasmid-mediated antimicrobial resistance genes among them which lead again to resistance to treatment [25].

In chronic wounds host immune response is impaired, infection cannot be eradicated, which leads to chronic inflammation. In the wound bed there is protease mediated degradation of receptors and cytokines, oxidative stress and inhibition of mitosis and apoptosis.

The standard antimicrobial tests which are used in laboratories usually can detect planktonic microorganisms. That is the reason why these tests are not used in detection of biofilms [28]. There are several methods used for detection of biofilms. Instruments used as a model system are modified Robbins device, Calgary biofilm reactor, perfused biofilm fermenter, and model bladder. These model systems provide information about biofilm mechanisms. Substratums for model systems are various: silastic disks, cellulose acetate filters, urinary catheters, plastic pegs. There are many parameters that adjust rate of biofilm formation. That parameters are composition of medium, time of retention, flow rate, shear force, roughness, and chemistry of substratum and species of organisms [28, 29]. Methods used for detection of biofilm are tube method, congo red agar, microtiter plate, real time PCR, mass spectrometry, confocal laser scanning microscopy. Aim of these methods is qualitative and quantitative detection of biofilm, and detection of biofilm genes. Methods are based on viable counting, direct examination by scanning electron microscopy or transmission electron microscopy or by chemical analysis.

Matrix metalloproteinases (MMPs)

Matrix metalloproteinases are zinc endopeptidases and they degrade all components of extracellular matrix [30]. These enzymes and their inhibitors TIMP (tissue inhibitor matrix metalloproteinases) play key role in all the phases of wound healing. MMPs are produced by many cell types, including MMPs based on their substrate are divided into 7 groups: collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, membrane-type MMPs, and other MMPs.

In nonhealing wounds there is overproduction of MMPs and that leads to destruction of growth factors necessary for wound healing. Inflammatory phase is prolonged and the healing process is delayed.

MMPs are secreted from lymphocytes and granulocytes, activated macrophages, fibroblasts, keratinocytes, endothelial cells and vascular smooth muscle cells as response to cytokines, hormones and other cells from extracellular matrix [31, 32]. Growth factors as transforming growth factor-beta, vascular endothelial growth factor, epidermal growth factor, interleukins and interferons play key role in healing process and they activate MMPs in the same time [33].

In chronic wounds, macrophages don't phagocyte neutrophils and apoptosis is inhibited. Recruitment of inflammatory cells continues and the inflammatory phase is prolonged. Extracellular matrix releases reactive oxygen species and proteases, which results in defect in collagen deposition and impaired phase of re-epithelization [34].

Function of proteases is regulated with protease inhibitors. Degradation of alfa-1 antitrypsin contributes to high activity of serine protease in chronic wounds.

Fibronectin is a glycoprotein, which is important in many cell-cell and cell-matrix interactions, clotting process, phase of granulation. Its degradation depends of levels of elastase, alfa-1-proteinase inhibitor and alfa-2-macroglobulin. There are high levels of fibronectin in chronic wounds, and it is structurally different from fibronectin in acute wounds [35].

Treatment of chronic wounds

An overall approach to manage patient's wounds consists of debridement, control of infection/inflammation, moisture and edge/environment management [36]. Debridement is removal of devitalized tissue with bacteria, proteases, inflammatory mediators and hyper granulated tissue. It can be autolytic with hydrogels and hydrocolloid dressings, sharp debridement, mechanical with negative pressure wound therapy and enzymatic debridement [36]. Controlling inflammation means to look for underlying causes, such as vasculitis, vasculopathy and malignancy. Moisture management is important because moist environment is necessary to promote growth factors, cytokines and chemokines and too much moisture can lead to maceration and prolonged wound healing process [36].

Therapy in chronic wounds targeting MMPs

Treatments that target MMPs in chronic wound healing have the ability to reduce their activity. MMPs with their high-level activity in wound are one of the reasons which are associated with impaired healing process. Many studies research effects of various treatment options for treatment of chronic wounds. There are treatments targeting MMPs in chronic wounds to reduce their negative effects.

Tetracyclines are antibiotics that inhibit MMPs activity. These antibiotics have anti-inflammatory, anti-apoptotic, anti-proteolytic, anti-angiogenic, and anti-metastatic function [35]. There are studies with doxycycline and minocycline as semi synthetic tetracyclines with good response in the treatment that target MMPs, but the problem is resistance of bacteria on these antibiotics [37, 38]

Because of this problem there are attempts of treatment with chemically modified tetracyclines (CMTs). The aim is to remove antimicrobial activity, so the bacteria resistance will be excluded. Effects of this treatment are still not well documented [35].

Wound dressings with silver nitrate and silver sulfadiazine are very frequently used in treatment of chronic wounds. Their mechanisms of action are directed against infection. They also have anti-inflammatory properties and target MMPs decreasing activity of gelatinases (MMP-9 mostly).

Because of the possible cytotoxicity of silver, the need for frequent changes of dressings, new therapeutic alternatives were developed. Treatment with these novel nanocrystalline silver (NCS) increase efficacy of action against fungi and bacteria but they are still not well documented [35].

Collagen as the main component of the ECM, produced by fibroblasts is studied in collagen-based dressings in the treatment of chronic wounds. These dressings provide alternative substrates for MMPs, so the degradation of endogenous collagen is reduced. These treatment options need more research studies for definitive use in practice [35].

Negative pressure wound therapy is a technique when reticulated open-cell foams, are placed onto the wound bed and covered with a semi-occlusive film, connected to a therapy unit that delivers vacuum pressure to the wound [35]. This method has many mechanisms of action and one of them is reducing levels of MMPs with reduction of protease –rich wound fluid.

Superabsorbent hydrogel dressings are another option for reducing MMP activity. They directly bind these enzymes and Zn and Ca ions that are needed for activity of MMPs.

Therapy in chronic wounds targeting biofilms

These treatments include conventional antibiotic treatment and non-conventional therapeutic options. The use of conventional antibiotics and antimicrobials in treatment of chronic wound biofilms is with very poor respond. The reason for that is construction of biofilm with thick EPS which act as mechanical barrier for antibiotics, bacteria cells with low metabolic activity, poor oxygenation, variation in pH, high oxidative stress. Because of that the new non-conventional therapeutic options are in progress [39].

Antimicrobial therapies that directly target microbial processes target microbial structure and function of microbes and they are described in the following text.

Phage therapy - bacteriophages are viruses that infect bacteria by injecting their DNA into bacterial cells. They multiply and new virus particles are formed. These new viruses are released by lysis and killing of the bacterial cell. This process is dependent of high specificity of bacterial strains that can be infected by phage. That means elimination of pathogenic bacteria which are predominant in biofilms and beneficial flora is preserved. Enzymes produced from phages that break down ECM are depolymerase and alginase. This treatment is effective in early phases of formation of biofilm and usually is used prophylactically [39].

Nano-based technologies are the nano antimicrobials-based therapies and usually use metals such as silver, zinc and copper or combination treatments such as magnetic hyperthermia-based technology with iron oxide nanoparticles and D-amino acids. Metals have bactericidal activity and the exact mechanism of action is still not known exactly. Possible explanations are that this treatment acts by interactions with cell membrane, production of oxygen species, lipid peroxidation, protein oxidation and DNA damage. They also activate macrophages and migration of fibroblasts. There are in vitro studies for treatments with nano system based on magnetic hyperthermia, nanohybrid enzymes or nanozymes and chitosan encapsulated ferulic acid nanoparticles. In vivo studies are needed in the future so these therapies could be used in practice [39].

The blue light therapy uses blue light (wavelength 400–500 nm) in treatment of chronic wound biofilm. The mechanisms of acting are not well understood. One of the explanations is that microbial porphyrins are photodynamic activated and that leads to production of reactive oxygen species (ROS). This treatment is ease to conduct, has low potential for developing tolerance and acts against polymicrobials in biofilm but less effective against Gram positive pathogens [39].

Quorum sensing inhibitor is treatment based on inhibiting receptors and signal molecules which are used in process of biofilm formation as a way of interbacterial communication. That kind of communication is known as quorum sensing circuit. The problem for this inhibitor is their toxic effect on host cells [39].

Another treatment option are therapies that target the chronic wound biofilm microenvironment, indirectly affecting microbial growth and survival.

These therapies are based on modification of local pH, removal of exudate with negative pressure wound therapy and surfactants, formation of granulation tissue and angiogenesis with hyperbaric oxygen therapy and production of local ROS with bioelectric dressings. pH of the wound affects angiogenesis, collagen formation, activity of MMPs and immune cell function. pH in the chronic wound bed is in the alkaline range (7.15–8.9) [39], and is result of products of bacterial proliferation. This leads to reduced local oxygen release and growth of anaerobic bacteria. The treatment options include use of acid such as acetic acid, citric acid, boric acid, ascorbic acid and Manuka honey and the result is reduced pH of wound bed and treating biofilm.

Negative Pressure Wound Therapy is treating wounds with continuous or intermittent sub-atmospheric pressure applied to the surface of the wound. It affects exudates in the wound bed and decrease its accumulation, targets formation of granulation tissue and blood vessels, increase supply of oxygen and nutrients. This treatment has better effects when is used in combination with topical antiseptics [39]. Because chronic wounds have hypoxia, hyperbaric oxygen therapy or HBOT is one of choices of treatment. HBOT leads to high partial pressure of oxygen which increase production of reactive oxygen species and reactive nitrogen species. Local growth factors are activated, angiogenesis and extracellular matrix deposition are improved and that results in better healing process. This treatment also has antimicrobial effect. It can be used in combination with antibiotics and make their efficacy better [39].

Surfactants reduce surface tension between liquids and a surface. That make molecules having low potential to stick together. In wounds, surfactants reduce infection using the previously mentioned mechanism, so microbes have low ability to adhere to surface of the wound. There is surfactant based wound dressings as a therapeutic option [39]. Electrical and electrochemical treatment is with not well-known mechanism of acting. There are studies which show that it affects production of superoxide radicals, improves migration of keratinocytes and that results in death of pathogenic bacterial cells in biofilm. It is still in experimental phase [39]. There is also treatment option that targets bacteria and the chronic wound biofilm microenvironment, both directly and indirectly impacting microbial growth and survival. Probiotics act against pathogenic bacteria. They usually compete for nutrients, produce bacteriocins which have antimicrobial activity, have a key role in host inflammatory response lowering anti-inflammatory mediators, improve proliferation of cells and have impact on pH of wound bed. That results in better wound healing process [39]. Mesenchymal stem cells or stromal cells (MSCs) are multipotent cells with possibility to self-renew. These cells are found in bone marrow, adipose tissue, endometrium and placenta. Treatment with MSCs have antimicrobial effect, and responsible for that are secreted antimicrobial peptides cathelicid, defensins and lipocalins. Other mechanism of acting is by modulating function of host immune cells such as T cells, NK cells, monocytes and neutrophils leading to improved healing process [39].

CONCLUSION

Development of biofilm and overexpression of matrix metalloproteinases are responsible for delayed healing process. Because chronic wounds have impact on quality of patients' life and they are economic burden for every country worldwide, there is need for precise diagnosis of underlying causes of chronic wounds.

That will help in differentiation of biofilm from bacterial infection, responsible pathogenic bacteria and potential therapeutic options. Usually, biofilms are recognized based on wound recalcitrance, recurring infection, antibiotic resistance and increasing of wound fluid. Treatments should be addressed to every possible cause of delayed healing process. Problem with bacteria resistance induce more research studies for development of non-conventional therapies which are with promising results for future management options.

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