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THE INFLUENCE OF SOCIO-ECONOMIC FACTORS ON THE NUMBER OF EXTRACTED TEETH AMONG THE POPULATION OF SKOPJE REGION IN THE REPUBLIC OF NORTH MACEDONIA

ВЛИЈАНИЕ НА СОЦИО-ЕКОНОМСКИТЕ ФАКТОРИ ВРЗ БРОЈОТ НА ЕКСТРАХИРАНИ ЗАБИ КАЈ НАСЕЛЕНИЕТО ОД СКОПСКИОТ РЕГИОН ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Abstract

There is numerous data about the impact of individual social status on personal health in social epidemiology. Socioeconomic factors with the greatest exogenous impact on individual health are as follows: sociocultural (family, school, workplace, media availability) and socioeconomic (education, material resources, allocation of funds). Also, everyone has their own health potential, which depends on immunity, physical activity, nutritional habits, and stress absorption and health behavior: diet, harmful habits, hygiene. The aim of this research is to trace dental health, as part of general health among the residents of the Skopje region, considering the number of extracted teeth in each individual as well as the current condition of the remaining teeth in correlation with the specified socioeconomic factors, in addition with differences in gender, age, and ethnicity. Based on the mentioned parameters, an epidemiological study was carried out on the territory of the city of Skopje which included 582 subjects from each municipality of Skopje, distributed in percentage terms according to the number of inhabitants in each municipality. The participants completed a questionnaire relating to the abovementioned risk factors and they underwent a clinical examination of the oral cavity. The extracted teeth as well as the number of teeth for extraction were noted in the examination. The obtained data showed that social class and education have a significant influence on the studied parameters. **Key words:** social status, education, extracted teeth, oral hygiene.

Апстракт

Социјалниот статус на една индивидуа влијае врз нејзиното здравје за што постојат бројни податоци во социјалната епидемиологија. Од социо-економските фактори коишто влијаат врз здравјето ќе ги издвоиме: социо-културните (семејство, училиште, работно место, медиумска достапност) и социјално-економски фактори (образование, материјални ресурси, распределба на средства) кои имаат најголемо егзогено влијание врз индивидуа со сопствен здравствен потенцијал (имунитет, физичка активност, нутритивни навики, стрес амортизација) и сопствено здравствено однесување (начин на исхрана, штетни навики, хигиена). Целата на трудот е да се проследи денталното здравје како дел од општото здравје кај жителите во скопскиот регион, преку бројот на екстрахирани заби кај една индивидуа, како и моменталната состојба на преостанатите заби во корелација со наведените социо-економски фактори, надополнето со ралики во пол, возраст и етничка припадност. Врз основа на сите наведени параметри беше спроведена епидемиолошка студија на територијата на град Скопје, која опфати вкупно 582 испитаници од сите скопски општини, процентуално распоредени според бројот на жители во секоја општина. Беше пополнет анонимен прашалник со сите прашања кои соодветствуваат на горенаведените фактори на ризик и клинички преглед на забалото. Во прегледот беа нотирани екстрахираните заби, како и бројот на преостанати заби за екстракција. Добиените податоци покажаа дека социјалната класа и образованието имаат значајно влијание врз испитуваните параметри. **Клучни зборови:** социјален статус, образование, екстрахирани заби, орална хигиена.

Introduction

The whole world population lives in organized societies that may have different characteristics and different levels of development.

According to numerous data in different countries, the individual social status has a great impact on individual health. It is known that biological factors are the ones which determine the occurrence of disease, but they are not the only ones and are complemented by the social

environment of the individual. Poverty is considered one of the most important determinants of health and disease, which is followed by the lifestyle of the individual: nutritional habits, physical activity, oral hygiene and professional dental follow-up. Even most developed societies face health status inequality due to different socioeconomic background.

According to researchers in different countries, there are various divisions of social categories which are determined by different parameters. Still, the basic parameters for determining social class are income, education and place of residence.

Despite the significant decline in the incidence of tooth loss in the last two decades[1], socioeconomic inequalities as factors persist over time, which are particularly emphasized in developing countries[2,3]. The correlation between the socioeconomic status and various health problems in the population is a constant subject of public debates[4].

Similar to general health, oral health follows social moments. Oral health can be affected by the individual's responsibility on the one hand, which includes oral hygiene and dental visits, and by dental availability and socioeconomic factors on the other hand[5].

However, there are studies from countries with higher standard, which have been investigating the correlation between socioeconomic factors, individual behavioral factors, and oral health. For example, insufficient access to dental services explains poor oral health and periodontal disease symptoms among poor Swedish adults[6]. Dental programs partly contribute to the increase in the number of healthy teeth in the lower social class in the United Kingdom (Donaldson). In contrast, lack of dental visits and oral hygiene were not associated with more extracted teeth in low-income areas of Australia[7].

Latin American countries still have a high prevalence of tooth loss[8]. But there is a significant difference between them. Tooth loss is an important dental health problem that affects physical and psychosocial health, as well as quality of life, due to reduced chewing ability and limitation of social interaction[9,10].

The socioeconomic status of an individual is defined on the basis of several factors which are grouped into two groups: sociocultural (family, school, workplace, media availability) and socioeconomic factors (education, material resources, allocation of funds). These factors have the greatest exogenous influence on an individual with their own health behavior (physical activity, nutritional habits, harmful habits, oral hygiene).

According to the study by Bayat, there are bigger possibilities for more tooth extractions rehabilitated by dental prosthesis in poorer populations with lower levels of education. Tooth extraction is the method of choice among the poor population because this service is cheaper than dental treatment and is covered by mandatory social Insurance. A study in Iran describes dental care system which is mostly private, and health insurance does not provide adequate coverage for dental services[11].

Similarly, other comparative studies indicate more extracted teeth[12]. not compensated by prosthetic fabrications[13] in low social classes in different countries.

Dental caries, as the most common reason for tooth extraction, is a worldwide problem, which is significantly greater in families with a low socioeconomic level, single parents or a low level of education. Low health levels are not simply a failure of the health services, but are associated with inadequate income, education and housing[14]. High income increase opportunities to utilize health services and prevent diseases. In contrast, low income influence the underutilization of health services due to reduced purchasing power for drugs and transportation costs. Families with lower economic capacity have a hard time fulfilling their basic needs. Because of that, they have a hard time providing health services[15,16]. Many studies show that the prevalence of caries is higher in children who come from families with low socioeconomic status. This is because children from these type of families consume a lot of cariogenic foods. Also, there is a lack of knowledge about dental and oral health, and they rarely make dental visits[17]. This is usually due to various factors such as family isolation, inadequate finances, parental indifference, lack of appreciation for the value of oral health, and even lack of understanding of the importance of oral health by the parents. Health education can be one of the solutions for reducing the lack of information as a factor of health problems. Health education may change an individual's habits (from harmful to beneficial) which will positively affect their dental health[18]. The level of education is a very influential factor on the individual's attitude towards healthy life. A person with higher level of education will have better knowledge about health, which will affect their habits for healthy life. In his research, Afrimelda states that a person with a higher level of education, pays more attention to their own dental health and vice versa, if someone has low or no education, then oral health care is also at a low level. The same opinion is conveyed by Silvia et al.[20] in 2014, according to whom, the higher level of education - the more the value of health is recognized, the individual is more easily employed and earns to meet their health needs. On the other hand, lack of education will hinder the development of one's attitude towards newly recognized values.

Tooth loss can have a negative impact on an individual's quality of life regardless of age. The prevalence of tooth loss has increased in the aging population. For this

reason, researchers are more focused on studies that include older individuals[21]. They analyze the quality of nutrition, phonetic and aesthetic function, as well as the psychological and social well-being of the individual[22]. Tooth loss indicates an individual's concern for dental disease treatment, which is considered one of the most useful indicators of oral health status[23]. The available data from the field of dental social epidemiology is very limited, therefore, the idea for conducting such studies in our country becomes significant. In addition, frequent changes in the healthcare system also affect health, even more so, as an increasing number of dental services are paid for[24].

The State Statistics Office of RNM determines the income indicator by quintiles according

to the total income in the family on an annual level[25]. The calculation of the poverty parameter is based on the income data for the family per member on a monthly level, using the data from Laeken Household Indicators (LHI). According to the poverty indicator, the population is divided in 5 social classes (from 5.000 to 30.000 Macedonian denars per family member monthly). Numerous studies point out problems with oral health in the population in rural areas, which leads to the extraction of teeth without the possibility of their conservative restoration. Insufficient health information, access to state dental services, fear of dentists, diet rich with sugars and poor oral hygiene are cited as reasons[26].

Aim

Guided by the available knowledge from social epidemiology, we determined the objectives of this research: to determine a correlation between socioeconomic and sociocultural factors as exogenous factors (income, education, place of residence, proximity to a dental facility) and health behavior (visiting a dentist, maintaining oral hygiene, diet, BMI) as an endogenous factor regarding the number of extracted teeth and teeth for extraction in the population, supplemented by differences in sex, age, ethnicity.

Material and method

For this research, we created an epidemiological study in which socioeconomic indicators (income, education, place of residence, proximity to a dental facility), hygiene and health habits, which are of interest to the study, (brushing teeth and regular visits to the dentist, nutritional habits, BMI) were observed, as well as their influence on the number of extracted teeth and the number of remaining teeth indicated for extraction.

The research sample consisted of a total of 582 respondents, proportionally distributed in each of the 17 municipalities in Skopje, according to the number of residents in the municipality. The research was conducted over a period of 2 years in various dental facilities (Health Center, Polyclinic, Dental office) on the territory of the respective municipality. The research team conducted a random survey of the respondents, which allowed us to obtain data for the survey questionnaire, which was completed with their consent. The questionnaire contained all the data necessary for the research:

1. Socioeconomic indicators (place of residence, education, income)
2. Sociocultural factors (infrastructural and media connection, proximity to a dental office)
3. Health potential (physical activity, nutritional habits, BMI)
4. Health behavior (harmful habits, maintenance of oral hygiene, regular visits to the dentist)

The clinical examination was performed by members of the research team with a single-use instruments in the Clinical Center, Health Center or dental office in the municipality that accepted cooperation with the team. Data on the number of missing teeth in the oral cavity, as well as remaining teeth diagnosed for extraction, were recorded in the questionnaire.

All patients with serious general health disorders (unregulated diabetes, unregulated blood pressure and serious cardiovascular disorders, liver diseases, hematological diseases, malignant processes in the body), that may affect the quality of life and teeth condition of the individual, were excluded from the research.

The obtained data was appropriately statistically processed, and the results are presented in a table.

Results

From 582 surveyed respondents, aged 18-86 years, 268 were male and 314 were female, which indicates the homogeneity of the sample in terms of gender. The average number of extracted teeth was 7.08 ± 7.1 and 7.06 ± 7.8, respectively in the group of male and female subjects, which indicates statistical insignificance in relation to the gender parameter. The average number of teeth for extraction was 1.17 ± 2.1 in the group of male subjects, 1.16 ± 2.5 in the group of female subjects which is also statistically insignificant.

The structure of the research sample in terms of nationality was Macedonians - 71.65%, Albanians - 18.045%, Turks - 2.23%, Gypsies - 4.98%, Serbs - 1.89% and Bosniaks - 1.2%. The average value of the number of extracted teeth is the highest among

The members of Turkish nationality (12), followed by Gypsies and Bosniaks with 8, Albanians with 6, Serbs with 5, and Macedonians with 4 extracted teeth on aver-

Table 1. Extracted teeth and teeth for extraction according to place of residence

Skopje region					
place of residence	Statistical parameters				p-level
	n	mean ± SD	min- max	median (IQR)	
extracted teeth					
urban	472	6.57 ± 7.3	0 – 32	4 (1.5 – 8.5)	Z=3.8 ***p=0.00015
rural	112	9.19 ± 7.9	0 – 32	7 (4 – 12)	
teeth for extraction					
urban	472	0.98 ± 2.02	0 – 14	0 (0 – 1)	Z=3.6 ***p=0.00035
rural	112	1.96 ± 3.2	0 – 20	1 (0 – 3)	

Z(Mann-Whitney test) ***p<0.0001

age. The comparison of the number of extracted teeth between different nationalities, made with post-hoc analysis, showed a significant difference in relation to the number of extracted teeth in the group of people of Turkish nationality with p=0.003. Regarding the remaining number of teeth for extraction, the difference is statistically significant between members of Turkish nationality and the other nationalities with p=0.0006, where the remaining number of teeth for extraction is 2 on average.

According to the place of residence, 470 (80.76%) were from urban areas, and 112 (19.24%) from rural areas. The place of residence had a significant effect on the number of extracted teeth and teeth to be extracted (p=0.00015, p=0.00035, respectively). A significantly higher number of extracted teeth and a significantly lower number of teeth for extraction was detected in the subjects from urban areas (table 1).

The level of education has a significant influence on the number of extracted teeth and teeth for extraction (p<0.0001). Respondents with lack of education and with primary education have a significantly higher number of extracted teeth and teeth for extraction than respondents with higher education (p<0.0001, p=0.0026, respectively).

The mean value of the number of extracted teeth in the group without education is 10, in the group with pri-

Table 2. Extracted teeth and teeth for extraction according to level of education

Skopje region					
Education	Statistical parameters				p-level
	n	mean ± SD	min - max	median (IQR)	
extracted teeth					
Without education	7	8.67 ± 4.9	0 – 14	10 (4 – 12)	H=37.6 ***p=0.0000 1vs4 *p=0.031 2vs 4 ***p=0.00000
primary	57	9.17 ± 9.7	0 – 32	8 (0 – 14)	
High school	295	8.29 ± 7.6	0 – 32	6 (2 – 11)	
faculty	225	4.88 ± 6.1	0 – 32	4 (1 – 6)	
teeth for extraction					
Without education	7	5.14 ± 4.1	0 – 10	4 (2 – 10)	H=39.8 ***p=0.00000 1vs4 **p=0.0026
primary	57	1.93 ± 3.7	0 – 20	0 (0 – 3)	
High school	259	1.42 ± 2.4	0 – 14	0 (0 – 2)	
faculty	225	0.52 ± 1.2	0 – 7	0 (0 – 0)	

H(Kruskal-Wallis test) *p<0.05, **p<0.1, ***p<0.0001

Table 1. Extracted teeth and teeth for extraction according to health education

Skopje region					
visit a dentist	Statistical parameters				p-level
	n	mean ± SD	min- max	median (IQR)	
extracted teeth					
no	113	13.69 ± 8.9	0 – 32	12 (7 – 20)	Z=9.4 ***p=0.000000
yes	471	5.48 ± 6.1	0 – 32	4 (1 – 8)	
teeth for extraction					
no	113	3.26 ± 3.8	0 – 20	2 (0 – 5)	Z=7.2 ***p=0.000000
yes	471	0.67 ± 1.3	0 – 10	0 (0 – 1)	

mary education - 8, in the group with high school education – 6, and in the group with higher education - 4.

Regarding the number of teeth for extraction, half of the group without education have more than 4 teeth for extraction, while half of the subjects in the other groups do not have teeth for extraction (table 2).

The income indicator determines the individual's connection to one of the social classes that differ from each other in terms of hygiene habits and the attitude towards taking care of their oral health. (table 4).

The State Statistics Office determines the social economic status by quintiles according to the total income in the family on an annual level. The calculation of the poverty parameter is based on the income data for the family per member on a monthly level, using the data from Laeken Household Indicators (LHI). According to the poverty indicator, the population is divided in 5 social classes (class 1 - 0-5000; class 2 - 5001-10 000; class 3 - 10001-15 000; class 4 – 15.001-20.000; class 5 over 20 000 Macedonian denars per family member)

Table 4. Extracted teeth and teeth for extraction (correlations with different variables)

Skopje region		
correlations		
variable	Spearman R	p-level
extracted teeth		
age	0.742	***0.00000
Social class	-0.046	0.27
BMI	0.233	***0.00000
oral hygiene	-0.465	***0.00000
proximity of dental office	0.065	0.11
teeth for extraction		
age	0.208	***0.00000
Social class	-0.176	***0.00002
BMI	0.211	***0.00000
oral hygiene	-0.3315	***0.00000
proximity of dental office	0.222	***0.00000

The highest percentage of respondents who do not visit a dentist (due to lack of habit or financial means) is observed among the members of social class 1, and according to that, they have, on average, the highest number of extracted teeth and present teeth indicated for extraction. On the other hand, lack of funds leads to worry and loss of motivation to maintain oral hygiene.

Subjects who regularly visit a dentist compared to subjects who do not visit a dentist had a significantly lower number of extracted teeth ($p<0.0001$), and a significantly higher number of respondents who visit a dentist have an average of 5.48 ± 6.1 extracted teeth, with a median of 4 teeth; respondents who do not visit a dentist have an average of 13.69 ± 8.9 extracted teeth, with a median of 12 teeth. Respondents who visit a dentist have an average of 0.67 ± 1.3 teeth for extraction, while respondents who do not practice visiting a dentist have an average of 3.26 ± 3.8 teeth for extraction (table 3).

The number of extracted teeth significantly correlates with age, body mass index and oral hygiene ($p<0.0001$). According to the value of Spearman's coefficient, the correlation between the number of extracted teeth with age and BMI was positive, direct ($R=0.742$, $R=0.233$), while the correlation between the number of extracted teeth and oral hygiene was negative, indirect ($R = -0.486$). It shows that the number of extracted teeth increases with increasing age and body mass index and decreases with increasing frequency of daily oral

hygiene. The number of teeth for extraction significantly correlates with age, social class, body mass index, oral hygiene, and proximity to dental office ($p<0.0001$). According to the value of Spearman's coefficient, the correlation between the number of extracted teeth and age, BMI and the distance to dental office was positive, direct (R=0.208, R=0.211 and R=0.222, respectively), while the correlation between the number of teeth for extraction and social status and oral hygiene was negative, indirect (R= -0.176 and R= -0.3315). It shows that the number of teeth for extraction increases with increasing age, body mass index and distance to dental office and decreases with higher degree of social status and with increasing frequency of daily oral hygiene.

Discussion

Social epidemiology is a relatively new branch of epidemiology whose task is to evaluate the manner in which the social status of an individual affects their own health[27].

Numerous factors affect oral health, as part of an individual's overall health. If we note that the most common reason for tooth extraction is caries and its complications, then the promotion of health habits and daily oral hygiene should be an imperative in every society. Our research was carried out for each social economic indicator individually and, on the other hand, extracted teeth and the presence of teeth for extraction were tracked as leading parameters for the state of oral health, analogous to the study by Gilbert GH, Duncan RP, Shelton BJ[28].

The income indicator determines the individual's connection to one of the social classes which differ from each other in terms of hygiene habits and the attitude towards taking care of their oral health (table 3 and 4).

Highest percentage of respondents who do not visit a dentist (due to lack of habit or financial means) is observed among the members of social class 1, and according to that, they have, on average, the highest number of extracted teeth and present teeth with an indication for extraction. On the other hand, lack of funds leads to loss of motivation to maintain oral hygiene. We will compare our results with the research of Neto JM, Nadanovsky P.[29] who conducted a research in one company which provided dental care for its employees where, for a period of 2 years, most teeth were extracted among the lowest social classes. These findings are consistent with the findings obtained in our research. The inequality of social classes causes health inequalities, which of course affects the oral health of an individual (respondents with the lowest education have the worst oral health) and, on average, the highest number of extracted teeth.

From all socioeconomic indicators, education is one of the most stable because it is acquired in early life and has a long-term effect on the individual's way of life[30].

Higher education is associated with regular teeth brushing and regular visits to the dentist, which, in turn, leads to a decrease in the number of extracted teeth and the number of teeth for extraction among respondents with higher education (table 2). When we talk about education, we should also mention the data from the research by Teodora T. 31 where it is emphasized that among younger respondents, the key is educating their parents who impose the health culture in the family.

The place of residence also affects our two examined parameters. The population from urban areas has a larger number of extracted teeth, and fewer teeth for extraction, which indicates that they do not visit the dental office for the purpose of treatment and prevention, but for tooth extraction. The population from rural areas has a significantly higher number of teeth for extraction, which indicates that all therapeutic possibilities have already been exhausted, that all past stages of the tooth have been missed in relation to the possibility of a wider therapeutic approach. In that regard, regardless of the place of residence, the number of lost teeth is the same for both groups[32].

The number of extracted teeth and the number of teeth for extraction significantly correlate with age, body mass index, oral hygiene, and proximity to a dental office. The older the patients - the more teeth they have extracted. What is worrying is the excess body weight that correlates with our parameters, which can be interpreted with inattentiveness to health in general and insecurity in social contacts according to the findings of Kabat W[33].

Lack of oral hygiene is the most significant factor for tooth loss, and it is associated with low education, insufficient health awareness or lack of funds for health care.

Conclusion

From the presented data it can be concluded that:

- The level of education is a direct participant in the formation of habits for regular dental check-ups and regular dental hygiene, which indirectly leads to a negative correlation with the number of extracted teeth and teeth indicated for extraction
- The lowest social categories have the highest number of extracted teeth and teeth indicated for extraction

These conclusions should be used in order to act on the risk factors, while seeking corrections in the health policy for dental protection of the population and raising

the awareness of dental health for all relevant factors which are responsible for this problem on the entire territory of the Republic of North Macedonia.

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DETERMINING THE KNOWLEDGE ABOUT PHARMACOVIGILANCE OF DENTISTS FROM THE REPUBLIC OF NORTH MACEDONIA

УТВРДУВАЊЕ НА ЗНАЕЊЕТО ЗА ФАРМАКОВИГИЛАНЦА НА СТОМАТОЛОЗИТЕ ОД РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Abstract

Introduction: Pharmacovigilance is defined as the science of detection, assessment, understanding, and prevention of the adverse effects of drugs or other related problems. Clinicians play a crucial role in preventing ADRs by recognizing, managing, and reporting ADRs to the national pharmacovigilance centers (NPCs). The majority of dentists are not aware, nor do they participate in the FDA's MedWatch program directed at drug safety. Given the fact that dentists play a crucial role in preventing and reporting adverse drug reactions, as well as the broad spectrum of pharmacotherapy agents being used in dentistry, the aim of our study was to determine the knowledge of dental professionals regarding pharmacovigilance in the Republic of North Macedonia. Material and Methods: This study included 100 doctors of dental medicine, employed in public and private healthcare institutions in the city of Skopje. The research was conducted using an anonymous survey questionnaire intended for healthcare professionals. For this purpose, a modified version of the questionnaire, according to Gupta et al., was used. The collected data were statistically processed using SPSS Statistica v23 for Windows, with tests adequate to the sample characteristics. **Results:** Less than half of the dentists (12-45%) correctly answered the questions concerning dentists' knowledge of pharmacovigilance systems (Q1-Q4, Q7, Q11-Q13). More than half of the dentists (58-70%) answered correctly to only two questions (Q5 and Q6) concerning dentists' knowledge of pharmacovigilance systems. **Conclusion**: Dentists have insufficient knowledge regarding pharmacovigilance. Taking into account the fact that healthcare professionals, including doctors of dental medicine, have a key role in reporting adverse drug reactions, their education and more active involvement in pharmacovigilance processes is essential for an ideal functioning of the healthcare system. **Key words:** dentists, pharmacovigilance, adverse drug reactions.

Апстракт

Вовед: Фармаковигиланцата е дефинирана како наука за откривање, проценка, разбирање и спречување на несаканите реакции на лековите (НРЛ) или други поврзани проблеми. Лекарите играат клучна улога во спречувањето на НРЛ преку препознавање, управување и известување за НРЛ до националните центри за фармаковигиланца (НЦФ). Мнозинството стоматолози не се свесни, ниту пак учествуваат во програмата MedWatch на FDA насочена кон безбедноста на лековите. Со оглед на фактот дека стоматолозите играат клучна улога во спречувањето и пријавувањето на несаканите реакции на лекот, како и широкиот спектар на фармакотераписки агенси кои се користат во стоматологијата, целта на нашата студија беше да го утврдиме знаењето за фармаковигиланцата на стоматолозите во Република Северна Македонија. **Материјал и методи:** Оваа студија опфати 100 доктори по дентална медицина, вработени во јавни и приватни здравствени установи во Скопје. Истражувањето беше спроведено со помош на анонимен анкетен прашалник наменет за здравствените работници. За таа цел, се користеше модифицирана верзија на прашалникот според Gupta и сор.. Собраните податоци беа статистички обработени со помош на SPSS Statistica v23 за Windows, со тестови соодветни на карактеристиките на примерокот. **Резултати:** Помалку од половина од стоматолозите (12-45 %) одговорија точно на прашањата кои се однесуваат на знаењето на стоматолозите за системите на фармаковигиланца (Q1-Q4, Q7, Q11-Q13). Повеќе од половина од стоматолозите (58-70 %) одговорија точно на само две прашања (Q5 и Q6) кои се однесуваат на знаењето на стоматолозите за системите на фармаковигиланца. **Заклучок:** Стоматолозите имаат недоволно знаење за фармаковигиланца. Имајќи го предвид фактот дека здравствените работници, вклучително и докторите по дентална медицина, имаат клучна улога во пријавувањето на несаканите реакции на лековите, нивната едукација и поактивно вклучување во процесите на фармаковигиланца е од суштинско значење за идеално функционирање на здравствениот систем. **Клучни зборови:** стоматолози, фармаковигиланца, несакани реакции од лекови.

Introduction

Pharmacovigilance is defined as the science of detection, assessment, understanding, and prevention of adverse effects of drugs or other related problems[1]. The importance of pharmacovigilance was first highlighted in 1848, when a girl named Hannah Greener from England passed away after being administered chloroform for anesthesia to remove an infected toenail. Due to concerns around the safety of using anesthetics, the Lancet set up a commission to tackle this issue, encouraging doctors to report deaths caused by anesthesia[2].

Clinicians play a crucial role in preventing ADRs by recognizing, managing, and reporting ADRs to the national pharmacovigilance centers (NPCs). Safe and rational prescription of drugs require therapeutic reasoning and appropriate selection of drugs for each patient[3]. The Food and Drug Administration (FDA) constantly tries to balance the promotion of greater drug safety with a quicker drug-review process[4]. The director of the Center for Drug Evaluation and Research, which now includes the Center for Biologics Evaluation and Research, oversees the balance of drug safety versus innovation through science. Dentists have traditionally not been included in this process. Drug utilization by dentists has not been determined by the pharmaceutical industry. However, the recent FDA opioid drug-safety initiative program[5] has shown that dentists contribute to the overprescribing of opioids, which led to stricter prescription patterns already in place in some states. The majority of dentists are not aware, nor do they participate in the FDA's MedWatch program[6] directed at drug safety. As more targeted drugs, aimed at reducing drug-adverse effects are developed, the US drug safety net would require the participation of all prescribers, especially for the completeness of all electronic medical records. One example of dentists participating in this process was the reporting of osteonecrosis of the jaw[7-9].

The medical histories dentists keep are, for the most part, isolated and remain in their offices. Electronic dental records, as part of the patient's electronic health record or electronic medical record under the broader banner of the electronic medical home, will forever change how dentists record medical histories[4]. Pharmacotherapy is playing an important role in the treatment and therapy for the management of different oral and dental diseases, such as periodontal disease[10], diseases of the dental pulp[11-12], aphthous ulcerations[13-14] as well as immune mediated diseases affecting the oral mucosa[15-17].

Given the fact that dentists play a crucial role in preventing and reporting adverse drug reactions, as well as the broad spectrum of pharmacotherapy agents being used in dentistry, the aim of our study was to determine the knowledge about pharmacovigilance of dental professionals in the Republic of North Macedonia.

Material and method

This study included 100 doctors of dental medicine, employed in public and private healthcare institutions in the city of Skopje.

The research was conducted using an anonymous survey questionnaire intended for healthcare professionals. For this purpose, a modified version of the questionnaire, according to Gupta et al. [18], was used.

Limitations of the study (possible risks and errors)

Measures were taken for two common limitations in this type of study:

- Selective bias. Doctors were selected from health institutions from different municipalities in the territory of the city of Skopje, in order to obtain a representative sample.
- Incomplete and involuntary disclosure of data – when filling in the anonymous questionnaire by the subjects, there is a risk of inadequate response.

The collected data were statistically processed using SPSS Statistica v23 for Windows, with tests adequate to the sample characteristics.

Results

This study included 100 doctors of dental medicine. The average age of the respondents in the whole group was 48.3±13 years. The majority of respondents were female 87.0%, while only 13.0% were men.

Questions about dentists' knowledge of pharmacovigilance:

1.To the first question (Q1) "Define the term pharmacovigilance", the correct answer was given by 12.0% of the surveyed doctors of dental medicine (table 1).

2. To the second question (Q2) "The most important goal of pharmacovigilance is:", the correct answer was given by 27.0% doctors of dental medicine (table 1).

3. To the third question (Q3) "Is there a mandatory obligation to report adverse drug reactions", the correct answer was given by 25.0% of the doctors of dental medicine (table 2).

4. To the fourth question (Q4) "Which of the listed health professionals has the obligation to report the adverse reactions of a drug that is put on the market", the correct answer was given by 22.0% of the doctors of dental medicine (table 2).

Table 1. Questions 1 and 2 of the questionnaire

answers Q1	Doctors of dental medicine		answers Q2	Doctors of dental medicine	
	N	%		N	%
A	24	24.0	**A**	27	27.0
B	48	48.0	**B**	30	30.0
C	16	16.0	**C**	15	15.0
D	12	12.0	**D**	27	27.0
Total	100	100.0	**Total**	100	100.0
*correct answer: D. A system for detecting, collecting, monitoring, evaluating and ensuring the appropriateness of new data on drug safety and the risk-benefit ratio related to the use of the drug or its interaction with other drugs			*correct answer: D. Determination the hitherto unknown adverse drug reactions		

Table 2. Questions 3 and 4 of the questionnaire

answers Q3	Doctors of dental medicine		answers Q4	Doctors of dental medicine	
	N	%		N	%
A	25	25.0	**A**	62	62.0
B	16	16.0	**B**	0	0.0
C	59	59.0	**C**	16	16.0
/	/	/	**D**	22	22.0
Total	100	100.0	**Total**	100	100.0
*correct answer: A. yes			*correct answer: D. All of the listed		

5. On the fifth question (Q5) "Does the Republic of Macedonia have an established system of pharmacovigilance", the correct answer was given by 70.0% of the doctors of dental medicine (table 3).

6. To the sixth question (Q6) "If you consider that the system of pharmacovigilance has been established, which regulatory body is responsible for monitoring adverse drug reactions", the correct answer was given by 58.0% of the doctors of dental medicine (table 3).

7. To the seventh question (Q7) "Where is the international center for monitoring adverse drug reactions", the correct answer was given by 19.0% of the doctors of dental medicine (table 4).

8. To the question (Q8) "Has any of your patients experienced an adverse reaction from a drug", 45.0% of the surveyed doctors of dental medicine answered in the affirmatively (table 4).

9. To the question (Q9) "Have you ever submitted a report for an adverse reaction to a drug", only 16% of the doctors of dental medicine answered affirmatively (table 5).

10. To the question (Q10) "Have you seen the application form for an adverse reaction to a drug", 21.0% of the doctors of dental medicine answered affirmatively (table 5).

11. To the question (Q11) "Serious adverse reactions and events (without fatal outcome) from the use of med-

Table 3. Questions 5 and 6 of the questionnaire

answers Q5	Doctors of dental medicine		answers Q6	Doctors of dental medicine	
	N	%		N	%
A	70	70.0	**A**	58	58.0
B	6	6.0	**B**	2	2.0
C	23	23.0	**C**	10	10.0
/	/	/	**did not answer because of lack of accurate information**	30	30.0
Total	100	100.0	**Total**	100	100.0
*correct answer: A. yes			*correct answer: a. MALMED		

Table 4. Questions 7 and 8 of the questionnaire

answers Q7	Doctors of dental medicine		answers Q8	Doctors of dental medicine	
	N	%		N	%
Did not answer	29	29.0	**Yes**	45	45.0
A	20	20.0	**No**	25	25.0
B	20	20.0	**I do not know**	30	30.0
C	12	12.0	**/**	/	/
D	19	19.0	**/**	/	/
Total	100	100.0	**Total**	100	100.0
*correct answer: D. Sweden			No correct answer (opinion/statement)		

Table 5. Questions 9 and 10 of the questionnaire

answers Q9	Doctors of dental medicine		answers Q10	Doctors of dental medicine	
	N	%		N	%
Yes	16	16.0	**Yes**	21	21.0
No	42	42.0	**No**	61	61.0
I do not know where to submit this report	40	40.0	**I do not know**	18	18.0
I do now know how to fill this report	2	2.0	**/**	/	/
Total	100	100.0	**Total**	100	100.0
No correct answer (opinion/statement)			No correct answer (opinion/statement)		

Table 6. Questions 11 and 12 of the questionnaire

answers Q11	Doctors of dental medicine		answers Q12	Doctors of dental medicine	
	N	%		N	%
A	20	20.0	**A**	0	0
B	18	18.0	**B**	8	8.0
C	35	35.0	**C**	50	50.0
D	27	27.0	**D**	42	42.0
Total	100	100.0	**Total**	100	100.0
*correct answer: D. 15 days			*correct answer: D. During Phase 4 of the clinical trial		

Table 7. Questions 13 and 14 of the questionnaire

answers Q13	Doctors of dental medicine		answers Q14	Doctors of dental medicine	
	N	%		N	%
A	15	15.0	**Yes**	17	17.0
B	45	45.0	**No**	37	37.0
C	32	32.0	**I do not know**	46	46.0
D	8	8.0	**/**	/	/
Total	100	100.0	**Total**	100	100.0
*correct answer: B. Spontaneous reporting			No correct answer (opinion/statement)		

icine are reported within", 27.0% of the surveyed doctors of dental medicine gave the correct answer (table 6).

12. To the question (Q12) "Rare adverse drug reactions can be detected in the next stages of a clinical trial", the correct answer was given by 42.0% of the surveyed doctors of dental medicine (table 6).

13. To the question (Q13) "Which of the following methods is most often used to monitor adverse reactions to new drugs placed on the market", 45.0% of the surveyed doctors of dental medicine gave the correct answer (table 7).

14. To the question (Q14) "Does your facility have a person/committee who/which is monitoring adverse drug reactions", 17.0% of the doctors of dental medicine answered affirmatively (table 7).

Discussion

The system of pharmacovigilance in the Republic of North Macedonia is regulated by the Law on Medicines and Medical Devices (Official Gazette of the Republic of Macedonia No. 106/07, 88/10, 36/11, 53/11, 136/11, 11/12, 147/13, 164/13, 27/14, 43/14 and 88/15) and the Rulebook on the method of reporting, the content of the form for reporting adverse drug reactions, and the method of organization of the pharmacovigilance system[19].

Organization and monitoring of the collection and assessment of adverse drug reactions, processing and assessment of the obtained data on drug safety is carried out by the Agency for Drugs and Medical Devices, through the National Center for Monitoring Adverse

Drug Reactions[19]. The Agency for Medicines and Medical Devices of the Republic of Macedonia (MALMED) was established on September 16, 2014, based on the Law on Medicines and Medical Devices, as an independent body of the state administration. The founder of MALMED is the Government of the Republic of Macedonia.

Spontaneous reporting of adverse drug events in the post-marketing phase is crucial for registering adverse drug reactions[20]. However, clinical studies make it possible to determine the frequency of adverse reactions reliably, as well as to assess the toxic potential of the drug. It is necessary for pharmacovigilance to be planned in detail, to be systematically implemented and to have an equally important role both in clinical trials, before the drug is put on the market, and after the drug is put on the market, after it has been approved. The importance of drug safety should be the same as the importance of drug efficacy[21]. For this reason, regulatory bodies are of crucial importance. However, the functioning of the system requires the active participation of both health professionals and patients. Therefore, our research examined the knowledge of dental medicine doctors related to pharmacovigilance.

The results obtained from the survey on question no. 1 and no. 2, and which refer to the assessment of the knowledge of healthcare workers about the concept and purpose of pharmacovigilance indicate that the knowledge of doctors of dental medicine is not sufficient (table 1). The obtained results for the knowledge of dentists in our research, compared to other researches[22,23], indicate that dentists do not know enough about the concept and purpose of pharmacovigilance.

Our survey showed that 25.0% of the doctors of dental medicine know that there is a mandatory obligation to report adverse drug reactions (table 2). In the available literature, a large number of health professionals declare that the reporting of adverse drug reactions is a professional obligation and they recognize it as such[24-29]. In order to obtain more detailed information about the knowledge of doctors of dental medicine, regarding their obligation to report adverse drug reactions, the survey questionnaire also contained a question on the exact determination of healthcare professionals who have the obligation to report adverse drug reactions. The results showed that only 22.0% of the doctors of dental medicine answered positively - that all health professionals have an obligation to report an adverse reaction to a drug (table 2).

The results obtained in our research indicate that the largest number of doctors of dental medicine know that a system of pharmacovigilance has been established in the Republic of North Macedonia, and more than half (58%) know that the Agency for Medicines and Medical Devices (MALMED) is a regulatory body responsible for monitoring adverse reactions to drugs (table 3). However, a small number of respondents in our survey correctly answered the question that the international center for monitoring adverse drug reactions is located in Uppsala, Sweden (table 4). Similar results were obtained in the study conducted by Nisa et al.[30].

From Table 4 we can observe that doctors of dental medicine, in their daily clinical practice, face the problem of "adverse drug reactions". Then again, what was significant for us was whether the health workers submitted a report on an adverse reaction to a drug, for which the question was also incorporated in the survey questionnaire. Only 16% of the surveyed doctors of dental medicine answered positively (table 5). However, a large percentage of those surveyed do not know where to submit and how to fill out the reports for an adverse reaction to a drug (table 5). The obtained results indicate that a large part of the adverse drug reactions that have occurred remain unreported, which is actually indicated by the available literature[31-35].

When asked if they have seen the report form for an adverse drug reaction, 21.0% of the doctors of dental medicine answered positively to this question (table 5). This form is part of the legal regulations of our country and is provided as a mandatory form of reporting adverse drug reactions in accordance with the Regulation on the method of reporting, the content of the form for reporting adverse drug reactions and the manner of organizing the pharmacovigilance system[36].

Adverse drug reactions can occur both in clinical trials of the drug and after the drug has been put on the market. In order to assess the knowledge of dental professionals, we surveyed them regarding the deadline for reporting serious adverse reactions and events (without fatal outcome) from the use of a drug. To this question, 27.0% of the doctors of dental medicine gave the correct answer, that is, that these adverse reactions and events should be reported within 15 days (table 6). We also asked them at what stage of clinical trials can rare adverse drug reactions be detected. 42.0% of the doctors of dental medicine gave the correct answer to this question, i.e. that during phase 4 of the clinical trial, the rare adverse reactions to the drug can be detected (table 6).

Conclusions

Based on the results from our research and the data from the available literature, we can conclude that dentists have insufficient knowledge regarding pharmacovigilance. Taking into account the fact that healthcare professionals, including doctors of dental medicine,

have a key role in reporting adverse drug reactions, their education and their more active involvement in pharmacovigilance processes is essential for an ideal functioning of the healthcare system.

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SALIVARY CALCIUM AND PHOSPHATE LEVELS IN CORRELATION WITH DENTAL CARIES INTENSITY

КОНЦЕНТРАЦИЈА НА САЛИВАРНИ КАЛЦИУМ И ФОСФАТИ ВО КОРЕЛАЦИЈА СО ИНТЕНЗИТЕТОТ НА ДЕНТАЛЕН КАРИЕС

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Abstract

Objective: The objective of our study was to determine and compare calcium and phosphate levels in unstimulated saliva in patients with varying caries intensity. **Material and methods:** The study involved 109 subjects from both sexes, from 12 to 15 years of age, divided into 3 groups according to their caries intensity: group with very low and low caries intensity, involving 31 (28.44%) subjects; group with moderate caries intensity involving 30 (27.52%) subjects; and a group with high and very high caries intensity, involving 48 (44.04%) subjects. Saliva samples were collected with the spitting method, according to the recommendations by Navazesh. Salivary calcium and phosphate levels were determined using the spectrophotometric methods CALCIUM ARSENAZO and PHOSPHOMOLYBDATE/UV accordingly. **Results:** The average concentration of salivary phosphates was 7.86 ± 3.3 mmol/L in subjects with very low and low caries intensity, 5.96±2.9 mmol/L in subjects with moderate caries intensity, and 5.08 ± 2.6 mmol/L in subjects with high and very high caries intensity. Post-hoc analysis for inter-group comparisons confirmed the statistically significant difference between groups with very low and low caries intensity and those with high and very high caries intensity (p=0.0005). The statistically significant difference was observed in calcium concentration as per caries intensity (p<0.0001). Subjects from the group with high and very high caries intensity showed a significantly lower level of calcium compared to the subjects from the group with very low and low caries intensity (p=0.000001), and a significantly lower level of calcium compared to the subjects with moderate caries intensity (p=0.0014). There is a correlation between the salivary calcium and phosphate levels and caries intensity (R=0.353, R=0.51 respectively). **Conclusion:** Salivary calcium and phosphate levels were significantly lower in the subject group with high and very high caries intensity, compared to the subject group with very low and low caries intensity. The intensity of caries significantly correlates with the concentration of salivary phosphates and salivary calcium. **Key words:** Caries intensity, saliva, salivary calcium, saliva phosphates.

Апстракт

Цел на трудот: Целта на нашето истражување беше да се утврди ис пореди нивото на калциум и фосфати во нестимулирана плунка кај пациенти со различен интензитет на кариес. **Материјал и методи:** Истражувањето вклучи 109 испитаници од двата пола, на возраст од 12 до 15 години, поделени во 3 групи според интензитетот на кариес: група со многу низок и низок интензитет на кариес, вклучувајќи 31 испитаник (28.44%), група со среден интензитет на кариес, вклучувајќи 30 испитаници (27.52%) и група си висок и многу висок интензитет на кариес, вклучувајќи 48 испитаници (44.04%). Примероците од плунка беа колекционирани со методот на исплукување, според порепораките на Navazesh. Нивоата на саливарен калциум и саливарни фосфати беа определени со спектрофотометриските методи CALCIUM ARSENAZO и PHOSPHOMOLYBDATE/UV соодветно. **Резултати:** Просечната концентрација на саливарните фосфати беше 7.86 ± 3.3 mmol/L кај испитаниците со многу низок и низок интензитет на кариес, 5.96±2.9 mmol/L кај испитаниците со среден интензитет на кариес, и 5.08 ± 2.6 mmol/L кај испитаниците со висок и многу висок интензитет на кариес. Post-hoc анализата за интергрупна споредба потврди статистички значајна разлика помеѓу групите со многу низок и низок интензитет на кариес и оние со висок и многу висок интензитет на кариес (p=0.0005). Статистички сигнификантната разлика беше потврдена и за концентрациите на калциум во однос на интензитетот на кариес (p<0.0001). Испитаниците од групата со висок и многу висок интензитет на кариес презентираше сигнификантно пониски нивоа на калциум во споредба со испитаниците од групата со многу низок и низок интензитет на кариес (p=0.000001), и сигнификантно пониски нивоа на калциум во споредба со испитаниците од групата со среден интензитет на кариес (p=0.0014). Постои корелација помеѓу нивоата на саливарен калциум и фосфати и интензитетот на кариес (R=0.353, R=0.51 соодветно). **Заклучок:** Нивоата на саливарен калциум и саливарни фосфати беа сигнификантно пониски кај испитуваната група со висок и многу висок интензитет на кариес, во споредба со испитуваната група со многу низок и низок интензитет на кариес. Интензитетот на кариес сигнификантно корелира со концентрацијата на саливарните фосфати и саливарниот калциум. **Клучни зборови:** Интензитет на кариес, плунка, саливарен калциум, саливарни фосфати.

Introduction

Dental caries is considered the most common disease in people, following the common flu, and is without a doubt a public health problem which is among the most widely spread global diseases connected with dental biofilm[1]. It is a multi-factor chronic disease of the hard dental tissues, characterized with demineralization of the non-organic part and destruction of the organic content of teeth. The World Health Organization (WHO) has defined caries as a localized post-eruptive process of external origin, which involves softening of the hard dental tissue and consequently creation of a cavity[2].

The risk of occurrence of caries includes physical, biological, ecological factors, factors connected to behavior and lifestyle, cultural and hygiene-dietary habits[3,4].

Effective preventive measures against occurrence of caries include inhibiting cariogenic microorganisms, mechanical control of the dental plaque and controlled intake of sugars, in order to reduce the amount of biofilm and the levels of specific pathogens.5 The World Health Organization (WHO) has reported that 60% to 90% of school children and almost 100% of adults worldwide suffer from caries. Therefore, prevention against caries plays an indispensable role in the promotion of public health[6].

Many authors have emphasized the importance of the relation between dental biofilm and dietary sugar, as the primary etiological factors for the incidence of dental caries, whereas, if one of these factors is absent, caries cannot occur[7-10].

Literature presents convincing reasons for using saliva as a diagnostic fluid. As a clinical tool, it offers numerous advantages compared to serum, mainly because it is easy to collect, store and deliver in sufficient amounts for further analysis[11]. Due to the non-invasiveness of the saliva collection procedure, patients are less anxious and uncomfortable, which makes it easy to repeat the sampling and monitor the disease over time[12]. Saliva is also easier to handle during diagnostic procedures because it does not clot, reducing the need of additional manipulation. It greatly affects initiation, maturing and metabolism of the dental plaques[13].

Calcium and trivalent phosphate ions play an important role in the protection of teeth against development of caries and erosions which, together with hydroxyl ions, maintain the mineral saturation of the saliva compared to the minerals in the dental tissues, a fact that is highlighted by numerous authors[14-22]. The structure of the dental enamel is predominantly composed of hydroxyapatite which involves ions of calcium and phosphates[15]. The high concentrations of calcium and phosphates in the saliva enable ionic exchange with the surface of the dental tissues, which starts with eruption of teeth and lasts until their maturity. This provides for remineralization of the initial carious process, before tooth cavity occurs, mainly due to the presence of ions of calcium and phosphates in the saliva[23].

The demineralization of hard dental tissues occurs when there is a non-proportional content of calcium and phosphate minerals between the tooth and the oral environment. In such cases, hydroxyapatite crystals of the enamel dissolve as a result of the acids produced by microorganisms, which leads to demineralization of the tooth. The low level of calcium and phosphates in the saliva affects the balance between the process of demineralization and remineralization, and the results in dental caries. This also explains the importance of salivary concentrations of calcium and phosphate ions in the maintenance and preservation of tooth integrity against the process of demineralization[24].

Since literature data indicate a connection between the concentration of salivary calcium and salivary phosphates and carious processes, the objective of our research was to determine and compare calcium and phosphate levels in unstimulated saliva in patients with varying caries intensity.

Material and method

This study included 109 subjects from both sexes, from 12 to 15 years of age, with maintained general and oral health. The subjects were:

- pupils in the 6th and 7th grade from the primary schools „Petar Pop Arsov“ and „Dimitar Pop-Georgiev Berovski“,
- pupils in the first year of the Medical high school „Dr.Panche Karagjozov“ and
- patients from the PHI University Dental Clinical Center „St. Panteleimon“ - Clinic for Pediatric and Preventive Dentistry and the Clinic for Orthodontics.

We obtained permits from the school authorities, as well as written consent from the parents for the implementation of the research.

The criteria for inclusion of the subjects were as follows:

- Children with permanent dentition, from 12 to 15 years of age (we avoided mixed dentition because caries in primary teeth may compromise the results),
- Children without localized or systemic disease affecting saliva secretion,
- Permanent residents of the city of Skopje who regularly consume local water.

Children who couldn't cooperate during the examination and collection of material were excluded from this study.

The subjects were divided in three groups according to the caries intensity, i.e. according to the DMFT index values (WHO, Geneve, 2000) in the following manner:

- Subjects with DMFT index values between 0.0-2.4 were categorized in the group with very low and low caries intensity
- Subjects with DMFT index values between 2.5-3.8 were categorized in the group with moderate caries intensity, and
- Subjects with DMFT index values between 3.9 and over 5.6 were categorized in the group with high and very high caries intensity.

All subjects underwent clinical, laboratory examinations, survey and statistical analysis of the obtained results.

Clinical trials

The clinical research was conducted in the aforementioned institutions using a probe and dental mirror, and we have determined the DMFT index value using the Klein-Palmer system for every subject.

Establishment of the DMFT index, using Klein-Palmer system[25], was achieved by summarizing the total number of decayed, extracted and restored-filled permanent teeth.

DMFT = Decayed teeth + missing due to caries + filled teeth

Saliva sample collection, processing and laboratory analyses:

The saliva sample collection, processing and laboratory analyses were conducted in the Biochemistry laboratory at the Ss. Cyril and Methodius University in Skopje, Faculty of Dentistry Skopje, Department of Oral and Periodontal Diseases. According to the recommendations by Navazesh[26], unstimulated saliva from all subjects was being collected for 10 minutes. Subjects were instructed not to eat or drink liquids except water for 90 minutes before saliva collection.

The collected saliva was first mixed using a vortex device (DRAGONLAB MX-S), at highest speed-2, after which, it was centrifuged for 10 minutes, at 4000 RCF (xg) in centrifuge (BIOBASE – High Speed Refrigerated Centrifuge). After centrifuging, we collected 500-1000µl of the supernatant of the centrifuged saliva, using a micropipette, and distributed the samples into small plastic single use tubes (Eppendorf tubes). The processed saliva samples were frozen at -20^{O}C, and the analysis of samples was conducted 15 days as of the date of freezing at the latest.

The next phase of our study was to determine the salivary levels of calcium and phosphates. The analyses were made upon fast defrosting of the samples.

Salivary calcium and phosphate levels were determined spectrophotometrically using BioSystem reagents, with the following methods accordingly: CALCIUM ARSENAZO (650 nm) and PHOSPHOMOLYBDATE/UV (340nm).

All the materials and reagents we used had the relevant degree of purity necessary for analysis.

The data were statistically processed using Statistica SPSS v23.0 for Windows.

Results

This study included 109 subjects distributed in 3 groups according to caries intensity: a group with very low and low caries intensity, involving 31 (28.44%) subjects; a group with moderate caries intensity, involving 30 (27.52%) subjects; and a group with high and very high caries intensity, involving 48 (44.94%) subjects. (table 1)

Table 1. Distribution of samples according to caries intensity

Caries intensity	n (%)
P	31 (28.44)
Y	30 (27.52)
A	48 (44.04)

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a (group with high and very high caries intensity)

Table 2 shows the descriptive parameters of the analyzed salivary biomarkers (phosphates and calcium).

The average concentration of salivary phosphate levels was 7.86 ± 3.3 mmol/L, 5.96 ± 2.9 mmol/L and 5.08 ± 2.6 mmol/L in the subject group with very low and low caries intensity, the group with moderate intensity, and the group with high and very high caries intensity, respectively. The median value of the salivary levels was 7.98 mmol/L, 5.96 mmol/L and 4.3 mmol/L in the subject group with very low and low caries intensity, the group with moderate intensity, and the group with high and very high caries intensity, respectively (table 3).

Salivary calcium showed average and median values of 1.37 ± 0.3 µmol/L and 1.47 µmol/L in the group with

Table 2. Statistical parameters of salivary markers

Parameter	Descriptive statistics		
	mean ± SD	min - max	median (IQR)
Phosphates (mmol/L)	6.11 ± 3.1	1.08 – 14.05	5.22 (3.74 – 7.98)
Calcium (mmol/L)	1.22 ± 0.4	0.64 – 3.46	1.21 (0.98 – 1.42)

Table 3. Values of saliva phosphates – groups according to caries intensity

Groups	Descriptive statistics - **Phosphates (mmol/L)**		p-value
	mean ± SD	**median (IQR)**	
p	7.86 ± 3.3	7.98 (4.95 – 10.88)	H=14.0 ***p=0.0009 post-hoc p vs a ***p=0.0005
y	5.96 ± 2.9	5.96 (3.53 – 7.35)	
a	5.08 ± 2.6	4.3 (3.405 – 5.675)	

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a (group with high and very high caries intensity)
H (Kruskal-Wallis test), post-hoc (Mann-Whitney test); ***p<0.0001

Table 4. Values of salivary calcium – groups according to caries intensity

Groups	Descriptive statistics - **Calcium (mmol/L)**		p-value
	mean ± SD	**median (IQR)**	
p	1.37 ± 0.3	1.47 (1.15 – 1.52)	H=28.6 ***p=0.00000 post-hoc p vs a ***p=0.000001 y vs a **p=0.0014
y	1.28 ± 0.2	1.355 (1.21 – 1.41)	
a	1.09 ± 0.4	1.055 (0.89 – 1.14)	

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a(group with high and very high caries intensity)
H(Kruskal-Wallis test), post-hoc (Mann-Whitney test); ***p<0.0001

Table 5. Values of salivary calcium – groups according to caries intensity

Correlation			
Caries intensity	**Spearman R**	t	p-value
&Phosphates (mmol/L)	0.353	3.898	***0.00017
&Calcium (mmol/L)	0.510	6.136	***0.00000

*p<0.05, ***p<0.0001

very low and low caries intensity, respectively, 1.28 ± 0.2 µmol/L, and 1.355 µmol/L in the group with moderate caries intensity, respectively, 1.09 ± 0.4µmol/L, and 1.055 µmol/L in the group with high and very high caries intensity, respectively (table 4).

Table 5 shows the correlation between salivary biomarkers and caries intensity. The analysis showed that the intensity of caries significantly correlates with the concentration of salivary phosphates (p=0.00017) and salivary calcium (p<0.0001) (table 5).

Discussion

Dental caries is a chronic disease which affects teeth and is considered globally most widely spread disease in humans. Caries occurs as a result of a complex interaction between cariogenic bacteria which produce acids and fermented carbohydrates, including many other factors of the host, such as teeth and saliva, clearly within a particular time interval. The risk of occurrence of dental caries involves various factors, including the high number of cariogenic bacteria, reduced saliva flow, insufficient exposure to fluorides and other remineralizing substances, as well as insufficient oral hygiene, inadequate diet and bad socio-economic conditions[3].

Our study included 109 subjects from both sexes at the age from 12 to 15 years with maintained general and oral health, distributed in three groups according to caries intensity, i.e. a group with very low and low caries intensity - 31 subjects (28.44%), a group with moderate caries intensity - 30 subjects (27.52%), and a group with high and very high caries intensity - 48 subjects (44.04%) (table 1). From the provided data, we can observe that the group with high and very high caries intensity has the highest number of subjects.

From our data, we can observe that the mean salivary phosphate levels were 6.11±3.1mmol/L, with a minimum of 1.08mmol/L and a maximum of 14.05 mmol/L. The calcium salivary levels in our study were 1.22±0.4mmol/L, with a minimum of 0.64mmol/L and a maximum of 3.46 mmol/L (table 2).

Table 3 shows the average values of salivary phosphates, according to the three studied groups. We can notice that the highest level of phosphates was in the group with very low and low caries intensity. The statistical analysis results showed that caries intensity significantly affects phosphate concentration in the saliva ($p=0.0009$). The post-hoc analysis for inter-group comparison confirmed a statistically significant difference in salivary phosphate levels between the group with very low and low caries intensity and the group with high and very high caries intensity ($p=0.0005$). Subjects with very low and low caries intensity showed significantly higher levels of phosphates compared to the subject group with high and very high caries intensity (table 3). Our results are consistent with the results of Stanton et al.[27] The statistical analysis of the results obtained from the concentrations of phosphates in the saliva in subjects from 12 to 15 years of age does not correspond to the study by a group of authors who reported increased level of phosphates in the saliva of caries active children.19,20

From Table 4 we can conclude that the salivary calcium levels are highest in the group with very low and low caries intensity. A statistically significant difference was confirmed in the salivary calcium levels as per caries intensity ($p<0.0001$). Subjects from the group with high and very high caries intensity showed a significantly lower level of calcium compared to the subjects from the group with very low and low caries intensity ($p=0.000001$), and a significantly lower level of calcium compared to the subjects with moderate caries intensity ($p=0.0014$) (table 4). The statistical analysis of our results regarding the calcium ion concentrations in the saliva of our subjects corresponds to the study by Aruna et al1[8], which reported increased levels of calcium in the saliva of caries resistant children and another study by a group of authors who discovered that persons with higher concentrations of calcium in the saliva have more intact teeth and are less prone to dental caries[21]. However, our results do not correspond to the study by Turtola et al.[16], and Elizarova and Petrovich[28], who reported increased concentration of calcium ions in the saliva in children with increased caries activity.

The study conducted by Gayathri R[15] indicates that the more severe the form of caries in children, the higher the concentration of calcium and phosphate ions in the saliva, which does not correspond to our results.

Considering the correlation between salivary calcium and phosphate ions, according to the value of Spearman's coefficient of correlation, there is a correlation between all the groups and salivary levels of calcium and phosphates in our study, which indicates that a change of caries intensity leads to a change in the concentrations of phosphates and calcium in the saliva, and vice versa ($R=0.353$, $R=0.51$ respectively) (table 5).

Jolly et al.[29] evaluated salivary calcium and phosphorus and found an increase in salivary calcium levels in caries-free children and no difference in salivary phosphorus between ECC (early childhood caries) and caries-free children. Similarly, Gandhy and Damle[30] reported an increase in inorganic phosphate level in children with rampant caries. The increase in salivary calcium levels in caries-active children could be due to the release of calcium from demineralized tooth, thereby increasing salivary calcium levels[31]. On the other hand, few studies insisted that there was no difference in salivary calcium and phosphate level in caries-free and caries-active children[32-35]. One of the possible explanations for no difference in calcium in both the groups could be due to the fact that saliva is a blood filtrate and the unaltered level of calcium in children with ECC might be due to the regulatory role of the parathyroid hormone (PTH), maintaining its level homogeneously in both ECC and caries-free children[36,37].

We should emphasize that dental caries and complications resulting thereof may cause serious problems not only for oral health, but also for the whole organism and

general health, and the quality of life of children and their families. Dental caries as a disease causes pain, psychological issues, problems with speech and consumption of food, and is a common reason for children's absences from school.

The use and analysis of saliva is not only important for dental diseases, it also has an increased importance in the field of medicine. Modern medicine uses salivary samples as an alternative for bodily fluids since it is easy to collect and has the same clinical-biochemical, pharmacological and toxicological parameters as blood. Recent studies have investigated the possibility to use saliva as a potential diagnostic fluid, to determine many components as they are determined in the blood, such as hormones, medicines, drug, and other toxic matters[38].

On that account, by using saliva as a diagnostic medium we may predict predisposition to dental caries and recommend its application in formulating preventive programs in everyday dental practice.

Conclusions

The analysis of the data obtained as a result of the established objectives of our study led us to the following conclusions:

The concentration of Calcium ions had significantly lower values in the subject group with high and very high caries intensity, compared to the subject group with moderate caries intensity and the subject group with very low and low caries intensity. There was also significant correlation of the DMFT index with the concentration of calcium ions in the saliva, which refers to the fact that DMFT index values increased with the reduction of calcium concentration, and vice versa, subjects with higher caries intensity showed lower calcium values.

The significant difference was confirmed in the phosphate concentration as per caries intensity. Subjects with high and very high caries intensity had significantly lower level of phosphates compared to the subjects with low and very low caries intensity. There was also significant correlation of the DMFT index with the phosphates in the saliva, which refers to the fact that DMFT index values increased with the reduction of phosphate concentration, and vice versa, subjects with higher caries intensity showed lower phosphate values, and vice versa.

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UNSTIMULATED AND STIMULATED SALIVATION IN PATIENTS BEFORE AND AFTER COMPLETE DENTURE REHABILITATION

НЕСТИМУЛИРАНА И СТИМУЛИРАНА САЛИВАЦИЈА КАЈ ПАЦИЕНТИ ПРЕД И ПО ТОТАЛНО ПРОТЕЗИРАЊЕ

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Abstract

Aim of the study: To determine the influence of new complete dentures placement on the amount of secreted unstimulated and stimulated saliva. **Material and Methods:** This study included 50 subjects of both sexes, aged 30-70 years. The subjects were divided into two groups: experimental - consisting of 25 edentulous subjects who came for fabrication of complete dentures; control group - consisting of 25 subjects with at least 20 teeth present in their mouth. We collected total unstimulated and stimulated saliva (spitting method) from all subjects from both groups, control and examined group, according to the recommendations by Navazesh. The collection of saliva in the control group was performed only once, and in the examined group on three occasions: before taking an anatomical impression for the fabrication of complete dentures, immediately after the placement of the complete dentures, and one month after dentures placement. The data were statistically processed using descriptive statistics, ANOVA, Mann Whitney U test, and Post-hoc Tukey HSD test. **Results:** There is a statistically significant difference in the amount of unstimulated and stimulated saliva between the patients from the control group and the patients from the examined group before the prosthodontic rehabilitation (p=0.000002; p=0.000005), as well as between the control group and the examined group immediately after the prosthodontic rehabilitation (p=0.000089; p=0.005206). There was not a statistically significant difference in the amount of unstimulated and stimulated saliva between the patients from the control group and the patients from the examined group 1 month after the prosthodontic rehabilitation (p=0.466855; p=0.748857). **Conclusions:** In edentulous patients, complete dentures have a significant impact on the salivary flow. Additionally, the amount of unstimulated and stimulated saliva increases significantly immediately after placing the dentures. After a certain period of adaptation, the salivary flow normalizes. There is no significant difference in the amount of secreted unstimulated and stimulated saliva between the patients, one month after wearing the dentures, and the subjects from the control group. **Key words:** Saliva, complete dentures, salivary flow.

Апстракт

Цел на трудот: Да се утврди влијанието на поставувањето на нови тотални протези врз количеството на излачена нестимулирана и стимулирана плунка. **Материјал и методи:** Истражувањето вклучи 50 испитаници од двата пола, на возраст од 30 до 70 години. Испитаниците беа поделени во две групи. Испитувана група – се состоеше од 25 беззаби испитаници кои имаа потреба од изработка на тотални протези; контролна група – се состоеше од 25 испитаници кои имаа најмалку 20 заби присутни во усната празнина. Колекционираме вкупна нестимулирана и стимулирана плунка (со методот на исплукување) од сите испитаници, од контролната група, како и од испитуваната група, според препораките од Navazesh. Колекционирањето на плунка од контролната група беше изведено само еднаш, а кај испитуваната група во три наврати: пред земањето на анатомски отпечаток, по изработката и аплицирањето на тоталните протези и 1 месец по носењето на тоталните протези. Податоците беа статистички обработени, користејќи: дескриптивна статистика, ANOVA, Mann Whitney U test и Post-hoc Tukey HSD test. **Резултати:** Постои статистички сигнификантна разлика во количеството на излачена нестимулирана и стимулирана плунка помеѓу пациентите од контролната група и пациентите од испитуваната група пред протетската рехабилитација (p=0.000002; p=0.000005), како и помеѓу пациентите од контролната група и пациентите од испитуваната група веднаш по тоталното протезирање (p=0.000089; p=0.005206). Не постои статистички сигнификантна разлика помеѓу количеството на излачена нестимулирана и стимулирана плунка помеѓу пациентите од контролната група и пациентите од испитуваната група 1 месец по протетската рехабилитација (p=0.466855; p=0.748857). **Заклучок:** Тоталното протезирање на беззабите пациенти има сигнификантно влијание врз количеството на излачена плунка. Дополнително, количеството на излачена нестимулирана и стимулирана плунка сигнификантно се зголемува по предавањето на тоталните протези. По одреден период на адаптација, количеството на излачена плунка се нормализира. Не постои статистички сигнификантна разлика во количеството на излачена нестимулирана и стимулирана плунка помеѓу пациентите 1 месец по носењето на тотална протеза и пациентите од контролната група. **Клучни зборови:** Плунка, тотални протези, количество на излачена плунка.

Introduction

The dramatic increase in the elderly population (over 60 years old) is associated with a period of life when people lose their teeth and wear complete dentures. As the length of life and the number and proportion of older people increase, in most industrialized and many developing nations, a central question is posed - whether the aging of this population will be accompanied by sustained or improved health, an improving quality of life, and sufficient social and economic resources[1]. The demographic data on population aging show that the need for rehabilitation of edentulous patients will remain considerable for many more decades. Conventional complete dentures are still a preferred treatment for edentulous patients, and this treatment modality improves oral health-related quality of life[2]. Edentulous patients have a higher risk of developing diseases of the oral mucosa, as well as a higher risk of developing: cardiovascular, gastrointestinal, endocrine, renal and other systemic diseases[3-9]. These problems are all the more pronounced as the age of the patient is more advanced[10].

The biological, physical and mechanical factors that improve the functional and aesthetic characteristics of complete dentures also have an impact on the retention and stabilization of the dentures. Good retention of complete dentures is obtained with the help of the valve effect, adhesion force, mechanical retention that depends on the anatomical characteristics of the jaws and the correct placement of the teeth. In doing so, the rules for occlusal and articulatory relations should be satisfied, space for the tongue should be provided, and aesthetics and phonation should be satisfied[11-16].

In older patients, it is not easy to ensure good retention of the dentures, due to uneven and rapid resorption of the alveolar ridge[17]. The retention of dentures not only depends on physical factors but is also related to the flow of saliva. The composition of saliva varies greatly in different individuals and in the same individual under different circumstances and stimulations[18]. Often, the reason for poor retention of complete dentures is insufficient amount of saliva in the mouth in adult individuals. The forces on which the retention of the dentures depends are divided into physical and physiological. Physiological forces, on the other hand, are related to the functions of the muscles of the face, lips and tongue[19]. From a functional point of view, the retention of dentures is determined by the balance between these two types of forces that change during speaking, chewing and swallowing[20].

The presence of an optimal amount of saliva in the mouth, with an appropriate consistency and quality, is especially important for edentulous patients, who require the fabrication of complete dentures. Prosthodontists should pay particular attention to the qualitative and quantitative characteristics of saliva before placing the dentures, during their production, and after placing the dentures[21].

Due to the fact that the literature indicates the importance, but also the association, between dental prosthetic rehabilitation and saliva, the aim of this study was to determine the influence of the placement of new complete dentures on the amount of secreted unstimulated and stimulated saliva.

Material and method

To realize the established goal, 50 subjects of both sexes, aged 30-70 years, were included in the study. The sample of subjects was divided into two groups:

- The first group, the experimental group, consisted of 25 edentulous subjects who came to the Clinic for Dental Prosthetics, at the PHI University Dental Clinical Centre "St. Panteleimon" in Skopje, for fabrication of complete dentures.
- The second group, the control group, consisted of 25 subjects who had at least 20 teeth present in their mouth. These subjects were also recruited at the PHI University Dental Clinical Centre "St. Panteleimon" in Skopje.

This study included patients who were in need of complete denture rehabilitation and patients who collaborated during the sample collection process.

Patients who smoke and/or drink alcohol, pregnant women, patients who have had surgical interventions of the salivary glands, patients who have had received head and neck irradiation, patients with Sjogren's syndrome, diabetes, rheumatoid arthritis, systemic lupus erythematosus and patients taking medications that affect the secretion of saliva, were excluded from this study.

The saliva collection was performed in the Biochemistry laboratory at the Ss. Cyril and Methodius University in Skopje, Faculty of Dentistry – Skopje, Department of Oral and Periodontal Diseases. We were collecting unstimulated and stimulated saliva from all subjects, both from the control and from the examined group, according to the recommendations by Navazesh[22], for 10 minutes. Subjects were advised not to eat, smoke, drink coffee, tea, coke, and brush their teeth one hour before saliva collection. Saliva collection was performed in the same period of the day (10-11h) for all subjects.

Collection of unstimulated saliva was performed by using the spitting method. Saliva accumulates on the floor of the oral cavity, and then the subject spits into a graduated tube every 60 seconds or whenever they get the urge to swallow the saliva accumulated on the floor

of the oral cavity. A funnel was also placed on the tube to facilitate the collection of saliva. For the collection of stimulated saliva, the gustatory stimulation method was used, 1-2 drops of lemon were dripped on the tip of the subject's tongue. At the moment when a sufficient amount of saliva had accumulated in the mouth, the subjects spat into a graduated test tube with a funnel.

We expressed the amount of saliva in milliliters for 1 minute. In this way, we obtained the values for the amount of saliva produced on average in one minute.

The collection of saliva in the control group was performed only once, and in the examined group on three occasions: before taking an anatomical impression for the fabrication of the complete dentures, immediately after the placement of the complete dentures, and one month after dentures placement.

The data were statistically processed using SPSS Statistica v20 for Windows, using the tests appropriate for the characteristics of the sample.

Results

This study included 50 subjects who were divided into two groups. The examined group (EG) consisted of edentulous patients, for whom acrylate complete dentures were made. The second group was the control group (CG), which consisted of 25 subjects who had at least 20 teeth in their mouths. Both groups were almost identical in terms of gender representation (48.0% and 52.0%) (table 1).

Table 1: Distribution of subjects according to gend

EG*/sex	Number	%
Male	12	48.0
Female	13	52.0
Total	25	100.0
CG**/sex		
Male	12	48.0
Female	13	52.0
Total	25	100.0

*EG – examined group; **CG – control group

The average age of the subjects in the examined group was 58.4±5.6 years, and in the control group it was 58.1±9.4 years (table 2).

The observed difference in age between the two groups is statistically not significant for p>0.05 (table 3).

Table 2: An overview of the average age of the subjects in the control group and the examined group

Age	Number	Mean	Minimum	Maximum	St.Dev.
EG*	25	58.4	49.0	67.0	5.551877
CG**	25	58.1	35.0	69.0	9.360021

Table 3. Mann Whitney U Test for age

	Rank Sum	Rank Sum	U	Z	p-level
Age	603.5000	671.5000	278.5000	-0.659697	0.509449

Table 4. Descriptive statistics for unstimulated saliva samples

Unstimulated saliva(ml/min)	n	Mean	Minimum	Maximum	St.Dev.
Before dentures placement – EG	25	0.35	0.2	0.6	0.100499
After dentures placement – EG	25	0.74	0.4	1.0	0.155134
1 month after dentures placement - EG	25	0.52	0.3	0.7	0.124766
Control group	25	0.56	0.3	0.8	0.122066

Table 5. ANOVA test for unstimulated saliva in the examined group

Examined group	SS	df	MS	SS	df	MS	F	P
Unstimulated saliva	1.893067	2	0.94653	1.19360	72	0.0165	57.096	0.000000

Table 6. Post-hoc Tukey HSD test for unstimulated saliva in the examined group

Examined group Unstimulated saliva	Before dentures placement	After dentures placement	1 month after dentures placement
Before dentures placement		0.000111	0.000155
After dentures placement	0.000111		0.000111
1 month after dentures placement	0.000155	0.000111	

Table 7. Mann Whitney U test for unstimulated saliva between the examined group and the control group

Unstimulated saliva	Rank Sum	Rank Sum	U	Z	p-level
Before dentures and CG*	881.0000	394.0000	69.00000	4.724594	0.000002
After dentures and CG*	435.5000	839.5000	110.5000	-3.91938	0.000089
1 month after dentures and CG*	675.0000	600.0000	275.0000	0.727607	0.466855

Both groups were homogeneous in terms of gender and age.

The average value of the amount of unstimulated saliva in the examined group before dentures placement was 0.35±0.1 (ml/min), after dentures placement it was 0.74±0.2 (ml/min), while 1 month after dentures placement it was 0.52±0.1 (ml/min), while in the control group it was 0.56±0.1 (ml/min) (table 4).

The observed difference in the average amounts of unstimulated saliva in the examined group before prosthetic rehabilitation, immediately after dentures placement and one month after dentures placement is statistically significant for $p<0.000000$ (table 5).

Post hoc Tukey HSD test is significant between the amount of unstimulated saliva before the prosthodontic rehabilitation and the amount of unstimulated saliva immediately after the prosthodontic rehabilitation. The difference is significant between the amount of unstimulated saliva before the prosthodontic rehabilitation and the amount of unstimulated saliva 1 month after the prosthodontic rehabilitation. According to the Post hoc Tukey HSD test, the difference between the amount of unstimulated saliva immediately after placing the dentures and the amount of unstimulated saliva after 1 month of wearing of the dentures is significant for $p<0.05$ (table 6).

The observed difference between the average amounts of unstimulated saliva in the studied group before placing the prostheses and the control group is statistically significant for $p<0.000002$. The observed difference between the average amounts of unstimulated saliva in the studied group after placing the prostheses and the control group is statistically significant for $p<0.000089$. The observed difference between the average amounts of unstimulated saliva in the studied group one month after placing the prostheses and the control group is statistically insignificant for $p>0.05$ (table 7).

The average value of the amount of stimulated saliva in the examined group before dentures placement is 0.7±0.1 (ml/min), after dentures placement it is 1.1±0.2 (ml/min), 1 month after dentures placement it is 0.9±0.1 (ml/min), while in the control group it is 0.9±0.1 (ml/min) (table 8).

Table 8. Descriptive statistics for the stimulated saliva samples

Stimulated saliva (ml/min)	n	Mean	Minimum	Maximum	St.Dev.
Before dentures placement – EG	25	0.7	0.5	1.0	0.115036
After dentures placement – EG	25	1.1	0.7	1.3	0.160000
1 month after dentures placement - EG	25	0.9	0.8	1.2	0.118040
Control group	25	0.9	0.7	1.3	0.135401

Table 9. ANOVA test for unstimulated saliva in the examined group

Examined group	SS	df	MS	SS	df	MS	F	P
Stimulated saliva	1.392800	2	0.696400	1.266400	72	0.01758	39.59318	0.000000

Table 10. Post-hoc Tukey HSD test for the stimulated saliva in the examined group

Examined group Unstimulated saliva	Before dentures placement	After dentures placement	1 month after dentures placement
Before dentures placement		0.000111	0.000114
After dentures placement	0.000111		0.001629
1 month after dentures placement	0.000114	0.001629	

Table 11. Mann Whitney U test for stimulated saliva between the examined group and the control group

Stimulated saliva	Rank Sum	Rank Sum	U	Z	p-level
Before dentures and CG*	873.0000	402.0000	77.00000	4.569371	0.000005
After dentures and CG*	493.5000	781.5000	168.5000	-2.79401	0.005206
1 month after dentures and CG*	654.0000	621.0000	296.0000	0.320147	0.748857

The observed difference between the average amounts of stimulated saliva in the examined group before dentures placement, immediately after dentures placement and one month after dentures placement is statistically significant for $p<0.000000$ (table 9).

Post hoc Tukey HSD test is significant between the amount of stimulated saliva before dentures placement, versus the amount of stimulated saliva immediately after dentures placement. The difference is significant between the amount of stimulated saliva before dentures placement, versus the amount of stimulated saliva 1 month after dentures placement. According to the Post hoc Tukey HSD test, the difference between the amount of stimulated saliva immediately after dentures placement and the amount of stimulated saliva after 1 month of wearing of the dentures is significant for $p<0.05$ (table 10).

The observed difference between the average amounts of stimulated saliva in the examined group before placing the dentures and the control group is statistically significant for $p<0.000005$. The observed difference between the average amounts of stimulated saliva in the examined group after placing the dentures and

the control group is statistically significant for p<0.005206. The observed difference between the average amounts of stimulated saliva in the examined group one month after placing the dentures and the control group is statistically insignificant for p>0.05 (table 11).

Discussion

If we take into account that complete dentures are made for patients at an advanced age, a period of life when it is difficult to accept new things, we can easily understand the expected problems that the dentist may encounter when making and placing the complete dentures. The problem becomes even more complicated if the patients have a systemic disease and/or receive a certain therapy, which negatively affects the secretion of saliva. Namely, xerostomia is present in a large number of adult patients, which makes it difficult to accept dentures. Beside the psychological characteristics of the patient and the adequacy of the fabrication, the presence of a sufficient amount of saliva, of adequate quality, is a very significant factor for accepting complete dentures.

The average value of the amount of unstimulated saliva in the examined group before placing of the dentures was 0.35±0.1(ml/min), immediately after placing the dentures was 0.74±0.2(ml/min), while 1 month after wearing the denture was 0.52±0.1(ml/min). In subjects from the control group, the amount of unstimulated saliva was 0.56±0.1 (ml/min) (table 4).

The observed difference between the average amounts of unstimulated saliva in the examined group before prosthodontic rehabilitation, immediately after placement, and one month after dentures placement is statistically significant for p<0.000000 (table 5).

According to the Post hoc Tukey HSD test, a statistically significant difference p<0.05 was also observed (table 6) between the amount of unstimulated saliva in all three periods of determining the amount of unstimulated saliva.

Compared to the control group, we observed a significant difference in the amount of unstimulated saliva in the examined group before dentures placement (where the secretion is reduced) and one month after wearing the dentures (where the secretion is increased) (table 7).

The mean values of the amount of stimulated saliva were understandably greater compared to the amount of unstimulated saliva. In the examined group, those values were as follows: 0.7±0.1(ml/min) before dentures placement, 1.1±0.2(ml/min) immediately after dentures placement, and 0.9±0.1(ml/min) 1 month after wearing the dentures. In subjects from the control group, the amount of stimulated saliva was 0.9±0.1 (ml/min) (table 8).

The differences in the amount of stimulated saliva (between the three test periods in the examined group, as well as between the control group and the examined group) were identical, as well as the differences in the amount of unstimulated saliva (table 9, 10 and 11).

The obtained results about the changes that occur in the secretion of saliva after dentures placement are in accordance with the studies done by Maheshwari[23], Jansen[24], Gabay[25] and Streckfus et al.[26]. The authors in their research, observed an increase in the amount of saliva after the placement of the dentures. This was especially accurate regarding the amount of stimulated saliva.

In our edentulous subjects, we observed a significantly lower amount of saliva compared to the subjects from the control group. This is due to the absence of stimuli in edentulous patients, which would cause adequate salivation. What is characteristic is the significant increase in the secretion of saliva immediately after placing the dentures. During that period, the secretion of saliva was significantly higher compared to the secretion of saliva in patients from the control group. We believe that the significant increase in saliva secretion immediately after placing the dentures is due to the fact that patients perceive the complete dentures as a foreign body in the mouth during that period. After a certain period of adaptation, the salivary flow normalizes. This is indicated by the fact that there is no significant difference in the amount of unstimulated and stimulated saliva between the patients one month after wearing the dentures, and subjects from the control group. We believe that such changes in the secretion of saliva are positive for the patients' adaptation to the new situation in the mouth. Starting from the positive effects that salivary secretion possesses, the increased secretion of saliva has a positive effect not only on the adaptation to the dentures, but also on the prevention of the occurrence of candida infections and the preservation of the overall oral health[27-32].

Conclusions

Based on the data from literature and the results obtained in our research, we can conclude that complete dentures have a significant impact on the salivary flow in edentulous patients. Additionally, the amount of unstimulated and stimulated saliva increases significantly immediately after placing the dentures. After a certain period of adaptation, the salivary flow normalizes. There is no significant difference in the amount of unstimulated and stimulated secretion of saliva between the patients one month after wearing the dentures and the subjects from the control group.

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FOBT AS A SCREENING TOOL FOR PERIODONTAL DISEASE

FOBT КАКО АЛАТКА ЗА СКРИНИНГ НА ПАРОДОНТАЛНАТА БОЛЕСТ

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Abstract

Introduction: The detection or determination of certain biomarkers in saliva and gingival fluid is becoming an important part of laboratory diagnostics, not only for periodontal diseases but also for other diseases of tissues and organs in the oral cavity. Given the fact that bleeding from the gingiva is one of the first clinical signs of periodontal diseases, the determination of salivary hemoglobin levels can be used as a marker to assess the periodontal condition. **Aim of the study:** To determine the efficiency of the FOBT (Fecal Occult Blood Test) for the detection of hemoglobin in saliva by correlating its values with values from the Periodontal Health Self-Assessment Questionnaire (VSS) in healthy individuals, in patients with gingivitis and in patients with periodontal disease, as well as to see the possibility of using FOBT for screening periodontal disease. **Material and methods:** 200 randomly selected individuals who visited the University Dental Clinical Center "St. Panteleimon" in the period from January 2021 to January 2022 were included in the study. All subjects filled out a questionnaire for self-assessment of periodontal health (VSS). To determine the level of hemoglobin in saliva, we used a ready-made commercial FOBT (Fecal Occult Blood Test). Based on the clinical examination, the subjects were classified into 3 groups: healthy, gingivitis and periodontal disease. **Results:** ROC analysis of AUC values for diagnostic efficiency of FOBT in saliva for PERIODONTAL DISEASE indicated that the Fecal Occult Blood Test - FOBT in saliva, according to the obtained AUC value, has good diagnostic efficiency for periodontal disease [AUC=0.815 (0.755-0.875) CI 95%, p=0.000]. ROC analysis for the diagnostic efficiency of salivary Hb for periodontal disease indicated Cut off=2; Sensitivity=85.7% (0.75 – 0.92); Specificity=62.6% (0.53–0.71) and Jouden index=0.39. Both, positive predictive value (PPV) and negative predictive value (NPV) were determined: for PPV=0.59 (0.49-0.68) and for NPV=0.67 (0.78-0.93). **Conclusion:** FOBT as a method for detecting hemoglobin, i.e., subtle bleeding in the saliva, could be used as a screening test for early detection of periodontal diseases. **Key words:** FOBT, periodontal disease, screening.

Апстракт

Вовед: Откривањето или определувањето на одредени биомаркери во плунката и гингивалниот флуид станува важен дел од лабораториската дијагностика, не само на пародонталните заболувања туку и на другите заболувања на ткивата и органите во усната шуплина. Со оглед на фактот дека крвавењето од гингивата е еден од првите клинички знаци на пародонталните заболувања, определувањето на нивото на саливарниот хемоглобин може да се користи како маркер за проценка на пародонталната состојба. **Цел на студијата:** Да се одреди ефикасноста на FOBT (Fecal Occult Blood Test) за откривање на хемоглобин во плунката преку корелација на неговите вредности со вредностите од Прашалникот за самопроценка на пародонтално здравје (VSS) кај здрави индивидуи, кај пациенти со гингивитис. и кај пациенти со пародонтална болест, како и да се види можноста за користење на FOBT за скрининг на пародонталната болест. Материјал и методи: Во студијата беа опфатени 200 испитаници кои го посетија Универзитетскиот стоматолошки клинички центар „Свети Пантелејмон“ во периодот од јануари 2021 до јануари 2022 година. Сите испитаници пополнија прашалник за самопроценка на пародонталното здравје (VSS). За да го одредиме нивото на хемоглобин во плунката, користевме готов комерцијален FOBT (Fecal Occult Blood Test). Врз основа на клиничкиот преглед, испитаниците беа класифицирани во 3 групи: здрави, гингивит и пародонтална болест. **Резултати:** ROC анализата на AUC вредностите за дијагностичка ефикасност на FOBT во плунката за ПАРОДОНТАЛНА БОЛЕСТ укажа дека FOBT во плунка, според добиената AUC вредност, има добра дијагностичка ефикасност за пародонтална болест [AUC=0,755- 0,875) CI 95%, p=0,000]. ROC анализа за дијагностичка ефикасност на плунковниот Hb за пародонтална болест укажа на Cut off=2; Сензитивност=85,7% (0,75 – 0,92); Специфичност=62,6% (0,53–0,71) и Jouden index=0,39. Утврдени беа и позитивна предиктивна вредност (PPV) и негативна предиктивна вредност (NPV): за PPV=0,59 (0,49-0,68) и за NPV=0,67 (0,78-0,93). **Заклучок:** FOBT како метод за откривање на хемоглобинот, односно суптилно крварење во плунката, може да се користи како скрининг тест за рано откривање на пародонтални заболувања.
Клучни зборови: FOBT, пародонтална болест, скрининг.

Introduction

Periodontal diseases are still widespread in both developed and developing countries. These are considered a public health problem due to their widespread and high prevalence of these diseases in all age groups.

Gingivorrhagia or bleeding from the gingiva is one of the first clinical signs that indicates inflammatory changes of the periodontal tissues[6]. It is an objective and easily assessed sign, but unfortunately patients often neglect it, thinking that the bleeding is something common and transient, so considering the scarce symptomatology in terms of discomfort or pain in the oral cavity, patients visit their dentists too late, usually when the disease is already advanced, and thus the therapeutic possibilities are limited. That is why it is necessary to diagnose these diseases early, in the initial stage of their development, certainly using easy, safe, affordable and efficient methods[1-7].

However, despite the high prevalence of these diseases, adequate screening programs are lacking. Several papers[5-12] have proposed and described different methods for periodontal disease screening, which include questionnaires for self-assessment of periodontal health, with an emphasis on conventional risk indicators, age, gender, some harmful habits such as smoking[13], dietary habits, obesity, stress then mobility of the teeth, recession (retraction of the gingival tissue), bleeding from the gingiva, etc.

The diagnosis of periodontal diseases is established on the basis of a clinical examination and X-ray examinations performed by dentists and professionals[14]. In modern periodontology, numerous parameters are used to assess the condition of the gingiva and the deeper structures of the periodontal complex during the clinical examination[15]. However, all these studies are laborious and extensive and are not recommended for larger epidemiological studies. One of the indexes developed and recommended by the World Health Organization is the CPI (Community Periodontal index), which can be used to determine the presence and severity, that is, the degree of progress of periodontal diseases[15]. This index is widely used in public health as a screening index that can also be used for more extensive epidemiological studies. The biggest drawback is that this index can be used exclusively by trained dentists and specialist periodontists[16-18].

The detection or determination of certain biomarkers in saliva and gingival fluid is becoming an important part of laboratory diagnostics, not only of periodontal diseases but also of other diseases of tissues and organs in the oral cavity[19-22]. A number of promising biomarkers identified in saliva correlate with clinical parameters of periodontal disease.

Given the fact that bleeding from the gingiva is one of the first clinical signs of periodontal diseases, the determination of salivary hemoglobin levels can be used as a marker to assess the periodontal condition. In this way, hidden, subtle bleeding from the gingival tissue can be visualized, which can contribute to the early detection of periodontal diseases. Salivary tests could also be included in systematic examinations in a wider population with the aim of early diagnosis of periodontal diseases.

In several works, the level of salivary hemoglobin has been examined, in correlation with the degree of inflammation of the gingival tissue[23,24]. With all its advantages, primarily as a simple and relatively cheap method, the determination of the level of hemoglobin in saliva can be used as an alternative to a periodontal examination by non-specialized medical personnel.

There is evidence that the inclusion of two or more screening methods leads to more reliable data on the presence or risk of disease. With a combination of objective methods such as determining the level of certain biomarkers in saliva and subjective methods such as questionnaires for self-assessment of periodontal health, the results would be most appropriate[25,26].

Based on available literature, as well as the need to gain our own knowledge about the methods which would be best for screening periodontal disease, we determined the goal of our paper: to determine the efficiency of the FOBT (Fecal Occult Blood Test) for the detection of hemoglobin in saliva by correlating its values with values from the Periodontal Health Self-Assessment Questionnaire (VSS) in healthy individuals, in patients with gingivitis and in patients with periodontal disease, as well as to see the possibility of using FOBT for screening periodontal disease.

Material and method

To achieve the set goal, we conducted a study which included 200 randomly selected individuals who visited the University Dental Clinical Center "St. Panteleimon" in the period from January 2021 to January 2022. Regarding age, we included all eligible individuals between the ages of 25 and 75 years.

Inclusion criteria:

- The respondents should have more than 15 natural teeth in their mouth
- To be aged between 25 and 75 years

Exclusion criteria:

- Edentulous individuals
- Individuals with multiple prosthodontic appliances

- Individuals who have serious chronic diseases that can affect the periodontal status (uncontrolled diabetes, blood disorders)
- Patients on anticoagulant therapy
- Individuals gingival injuries or injuries on the rest of the oral mucosa.

All subjects filled out a questionnaire for self-assessment of periodontal health (VSS)[2], which consisted of 16 questions considering: demographic data (gender, age group, education), Body Mass Index (BMI), dietary habits, current chronic disease, family history of periodontal disease, stress, smoking status, frequency of alcohol consumption, visits to the dentist, and state of the dentition (bleeding gums, root exposure, luxation, prosthetic device, etc.).

To determine the level of hemoglobin in saliva, we used a ready-made commercial FOBT (Fecal Occult Blood Test)[27] which was carried out in the Biochemical Laboratory of the Department of Oral Diseases and the periodontist at the Faculty of Dentistry - Skopje, part of the University of St. Cyril and Methodius" in Skopje. Fecal Occult Blood Test is an immunochemical test intended for the qualitative detection of fecal occult (hidden) bleeding. It is designed to detect low levels of hemoglobin in the stool. Biotek's OnSite FOB-Hi Rapid Test that we used in our study consists of a plastic bottle closed with a cap that continues with a knurled collection stick filled with buffer liquid and a plastic cassette with a test strip (a dark red conjugate pad containing monoclonal antibodies - anti Hb antibodies, conjugated with colloidal gold and a nitrocellulose strip marked with C-control and T-test lines). When an adequate volume of the sample, in our case saliva mixed with buffer liquid, is placed on the precisely defined spot on the cassette, the sample begins to migrate by capillary movement through the cassette. If hemoglobin is present in the sample, it will bind to the anti-hemoglobin antibodies forming immunocomplexes which give the red coloring of the lines.

From the graduated plastic tubes, 500 µl of the saliva was then taken and transferred to other small plastic tubes. Then we dipped the collector or FOBT studded stick into the saliva from the small plastic tubes. We took 100 µl of the saliva in small plastic tubes with a micropipette, and then transferred it to the FOBT buffer liquid, followed by vigorous shaking and mixing on the vortex apparatus in order to mix the buffer liquid and saliva. Then, from the pre-mixed solution of buffer fluid and saliva, we dripped 2 drops onto a precisely determined spot on the tape cassette of the FOBT. We read the test results after 10 minutes. A red C line should first appear on the strip of the plastic cassette, i.e. the control line that will indicate that the test was performed correctly[28].

The positivity of the test is read on the T line (test line) which can be colored with different intensity of red color. Based on the absence and/or intensity of color, we grade the test as:

0 - Negative (absence of T line)

1 - Weakly positive (T line is discreetly red-colored)

2 - Moderately positive (T line is moderately red colored)

3 - Strongly positive (T line is intensely red)

For the clinical examination of the subjects, we used a periodontal probe and appropriate dental equipment. Based on the clinical examination, the subjects were classified into 3 groups:

1. Healthy subjects: without inflammatory changes of the periodontium and with discrete inflammatory changes at the edge of the gingiva
2. Subjects with gingivitis: with evident inflammatory changes of the gingival tissue
3. Subjects with periodontal disease: with clinically manifest periodontal disease, i.e. second stage of periodontal disease

The criterion for determining the presence of periodontal disease was the presence of clinical attachment loss of 4 mm on at least two surfaces of two different teeth in the mouth.

The collected data were statistically processed with SPSS Statistica v23 for Windows, with tests appropriate to the characteristics of the sample, which are necessary for meeting the set goals.

Results

To achieve the set goal, 200 patients who visited the University Dental Clinical Center "St. Panteleimon" in Skopje were included in this study. Respondents for the sample of the research were selected according to the method of simple random selection (random sampling) and respecting the set inclusion and exclusion criteria. Data of interest for the study were collected through: a) periodontal health self-assessment questionnaire (VSS); and b) FOBT (Fecal Occult Blood Test) for the detection of hemoglobin in saliva, as a biomarker for periodontal disease screening.

According to the clinical examination of the dentition, the subjects were classified into three groups: 1) healthy (without periodontal changes); 2) with gingivitis (inflammatory changes of the gingival tissue); and c) with periodontal disease (clinically manifest periodontal disease, i.e. second stage of periodontal disease). The frequencies of patients according to diagnostic groups and gender are shown in Table 1.

Table 1. Analysis of the sample according to diagnostic groups and gender

Diagnostic groups		Sex		Total	p
		Male	Female		
Healthy	N	16	26	42	
	%	38,10%	61,90%	21%	
Gingivitis	N	14	32	46	X^2=1,329; df=2; p=0,5145
	%	30,43%	69,57%	23%	
Periodontal disease	N	45	67	112	
	%	40,18%	59,82%	56%	
Total	N	75	125	200	
	%	37,59%	62,50%	100%	
X^2= Pearson Chi square test;				*significant for p<0,05	

Table 2. Analysis of hemoglobin in saliva (Hb) according to diagnostic groups

Categories		Diagnostic groups				p
		Healthy (N=42)	Gingivitis (N=46)	Periodontal disease (N=112)	Total (N=200)	
Hb-0	N	36	3	2	41	Gingivitis/ Periodontal disease (Hb-0 → Hb-3) X^2=16,730; df=2; p=0,0008*
	%	85,71%	6,52%	1.79%	20,50%	
Hb-1	N	6	32	44	82	
	%	14,29%	69,57%	39,29%	41%	
Hb-2	N	0	7	42	49	
	%	0%	15,11%	37,50%	24,50%	
Hb-3	N	0	4	24	28	
	%	0%	8,70%	21,43%	14%	
Hb-0 – no bleeding; Hb-1 – weakly positive; Hb-2 – moderately positive; Hb-3 – strongly positive X^2= Pearson Chi square test; *significant for p<0,05						

The analysis of hemoglobin in saliva (Hb) was carried out with the help of FOBT (Fecal Occult Blood Test) and it was analyzed in relation to 4 categories:

a) No bleeding (Hb-0);
b) Weak positive bleeding (Hb-1);
c) Moderately positive bleeding (Hb-2); and
d) Strong positive bleeding (Hb-3) (table 2).

In the entire sample, 82 (41%) of the subjects had weak positive (Hb-1) in saliva, followed by 49 (24.5%) in whom it was moderately positive (Hb-2) and 28 (14%) with strongly positive (Hb-3). Only 41 (20.5%) of the subjects were not determined (Hb-0) in saliva (table 2). For p>0.05, there was no significant association of the gender of the respondents from the entire sample with the category of presence of Hb in saliva (Pearson Chi square test: X2=1.554; df=3; p=0.6697).

For p<0.05, there was a significant association of the diagnosis of gingivitis/periodontal disease with belonging to Hb categories for bleeding in saliva (Hb-0 → Hb-4) for Pearson Chi square test: X2=16,730; df=2; p=0.0008. A significantly higher association of Hb-2 (moderate positive bleeding) and Hb-3 (strong positive bleeding) in saliva with periodontal disease was determined (table 2).

For the purposes of the study, a periodontal health self-assessment questionnaire (VSS) consisting of 16 questions was applied to the research patients. The answers to each question were coded with a score of

	Total score for the periodontal health self-assessment questionnaire (VSS)						p
	N	Mean±SD	Min/Max	Percentiles (25th/50th/75th)			
Healthy	42	4,23±1,44	2/9	3	4	5	$X^2_{(2)}$=111,182; p=0,0001*
Gingivitis	46	8,15±2,94	4/15	6	7.5	10	
Periodontal disease	112	12,02±3,15	4/20	10	12	14	
Total	200	9,49±4,21	2/20	6	10	13	

[1]X^2=Kruskal-Wallis H test; Z=Mann-Whitney U Test; *significant for p=0,00001*
healthy/gingivitis: Z=-6,391; p=0,00001*;
healthy/ periodontal disease: Z=-9,227; p=0,00001*;
gingivitis/ periodontal disease: Z=-6,168; p=0,00001*

Table 4. Correlation between Total Periodontal Health Self-Assessment Score and FOBT (Hb)

Paramter	Total score for VSS		p
	Non-adjusted[1]	Adjusted[1]	
Hemoglobin (Hb)	$r_{(200)}$=0,702; p=0,0001*	$r_{(200)}$=0,662; p=0,0001*	Z=0,7430; p=0,4574
Diagnostic group (Dg)	$r_{(200)}$=0,744; p=0,0001*	$r_{(200)}$=0,686; p=0,0001*	Z=1,1812; p=0,2375

[1]Pearsons moment order correlations
*significant for p<0,05

0→2 according to the potential risk for periodontal disease, where score 0=no risk and score 2=high risk. The minimum and maximum total score of the periodontal health self-assessment questionnaire was 0/26. The total periodontal health self-report score – VSS was calculated as the sum of the individual scores for the responses of each respondent in the sample.

The mean value of the TOTAL score from the periodontal health self-assessment questionnaire for the entire sample of respondents was 9.49±4.21 with a min/max value of 2/30 and 50% of respondents with a score <10 for Median IQR = 10 (6-13) (table 3).

For p<0.05, a significant difference was observed between the three diagnostic groups regarding the total score for self-assessment of periodontal health for Kruskal-Wallis test= X2(2)=111.182; p=0.0001. For p<0.05, this significance was due to (table 3): Significantly higher total score of self-reported periodontal health in GINGIVITIS compared to HEALTHY for Mann Whitney U test: Z=-6.391; p=0.00001; Significantly higher total score for self-assessment of periodontal health in PERIODONTAL DISEASE compared to HEALTHY for Mann Whitney U test: Z=-9.227; p=0.00001; Significantly higher total score for self-assessment of periodontal health in PERIODONTAL DISEASE compared GINGIVITIS with for Mann Whitney U test: Z=-6.168; p=0.00001.

With the correlation analysis, for p<0.05, the presence of:

1) a significant linear positive very strong correlation between VSS and Hb was detected (r(200)=0.702; p=0.0001) – with increasing VSS, the presence of Hb in saliva significantly increased (table 4);

2) a significant linear positive very strong correlation between VSS and Dg was detected (r(200)=0.744; p=0.0001) – with increasing VSS, the condition with Dg significantly worsened (healthy → periodontal disease) (table 4).

ROC analysis was performed and AUC values were calculated for the diagnostic efficiency of the Fecal

Table 5. ROC analysis of AUC value for diagnostic efficacy of salivary FOBT for periodontal disease

Area Under the Curve – Diagnostic efficacy for PERIODONTAL DISEASE					
Variable	Area	Std. Error[a]	Asymptotic Sig.	Asymptotic 95% C.I.	
				Lower Bound	Upper Bound
FOBT saliva	0,815	0,031	0,000	0,755	0,875
FOBT saliva = Fecal Occult Blood Test in saliva					

Occult Blood Test - FOBT in saliva for PERIODONTAL DISEASE (table 5).

ROC analysis of AUC values for diagnostic efficiency of FOBT in saliva for PERIODONTAL DISEASE indicated that the Fecal Occult Blood Test - FOBT in saliva, according to the obtained AUC value, has good diagnostic efficiency for periodontal disease [AUC=0.815 (0.755-0.875) CI 95%, p=0.000] (table 5).

In the following analysis, the cut off value for FOBT in saliva for the diagnosis of PERIODONTAL DISEASE was defined as a sensitivity of at least 80% with the highest possible specificity in order to fulfill the requirements of a screening test. ROC analysis for the diagnostic efficiency of salivary Hb for periodontal disease indicated Cut off=2; Sensitivity=85.7% (0.75 – 0.92); Specificity=62.6% (0.53–0.71) and Jouden index=0.39. Both positive predictive value (PPV) and negative predictive value (NPV) were determined: for PPV=0.59 (0.49-0.68) and for NPV=0.67 (0.78-0.93).

Discussion

IScreening is a process or procedure in medicine, which involves the use of simple methods in an apparently healthy population in order to identify individuals who are at risk of developing a certain disease or early detection of the disease[29-31]. Without screening, the diagnosis of diseases would be made only after symptoms appear Screening is a key component of modern health care. The rationale is simple and attractive: early detection of diseases in asymptomatic individuals as well as their timely treatment in order to reduce morbidity, mortality and treatment costs.

In order for a screening test to be used and be valid, certain criteria should be met, namely: it is relatively cheap, easy to use, acceptable to the patient, reliable and accurate. Validity of tests used in screening is the ability of the test to accurately identify diseased and healthy individuals. The ideal screening test should be exceptionally sensitive and extremely specific[32,33].

Screening of periodontal diseases is of great importance for their early and timely detection, with the aim of successful treatment of patients as well as improvement and control of systemic diseases related to periodontal diseases.

Still, in our country, there are no unified protocols and appropriate screening tests for early detection of periodontal diseases. As a reversible condition, if detected on time, gingival disease can be successfully cured without leaving consequences. Otherwise, inflammation from the gingiva can spread to deeper periodontal tissues and transform into periodontal disease, a disease that results in anatomical and functional disintegration of the periodontium. Hence, we can conclude that screening is of great importance for early and timely detection of periodontal diseases.

200 subjects of both sexes were included in the research, which were classified into 3 diagnostic groups according to clinical characteristics, namely:

1) HEALTHY subjects – 42 (21.3%);
2) subjects with GINGIVITIS – 46 (18.7%) and
3) subjects with PERIODONTAL DISEASE – 112 (60%) (table 1).

Salivary hemoglobin levels were determined using FOBT. Hemoglobin in saliva was determined in total for the entire sample of subjects as well as individually for each of the diagnostic groups. Hemoglobin in saliva was analyzed according to 4 categories and that is

a) no bleeding (Hb-0);
b) Weak positive bleeding (Hb-1);
c) Moderate positive bleeding (Hb-2) and
d) Strong positive bleeding (Hb-3).

Table 2 shows the results of the analysis of hemoglobin in saliva for the entire sample and individually by diagnostic group. From the obtained results, we conclude that in 86% of the total number of subjects, a certain level of hemoglobin was detected in saliva, which is a serious number and certainly confirms the existence of bleeding from the gingiva, that is, a certain degree of inflammation of the gingival tissue. Considering the fact

that bleeding from the gingiva is one of the first clinical signs indicating inflammatory changes of the gingival tissue, the determination of hemoglobin levels in saliva, as a screening test, would be useful for selecting subjects for whom further investigations are necessary for defining the diagnosis. It is also very significant that this test could reveal latent bleedings that the patient may not even notice. By determining the level of hemoglobin in saliva, as a screening test, periodontal diseases could be detected early and treated promptly. Periodontal disease begins with gingivitis, i.e. inflammation of the gingiva, but of course not all gingivitis ends with periodontal disease.

Also, from Table 2 and the results it can be observed that there is a greater association of moderate and strong positive bleeding in the saliva in patients with periodontal disease. The obtained results are logical, because it is expected that in a more advanced stage of the disease there will be more pronounced bleeding from the tissues. The results we obtained in our research on hemoglobin levels in saliva are in agreement with the results of Maeng et al.[34], with the results of Sarabathinam et al.[35] and Ramenzoni et al.[36], who, in their studies, determined significantly higher levels of Hb in saliva in subjects with periodontal disease compared to healthy subjects.

Table 3 shows the total scores for self-assessment of periodontal health according to diagnostic groups. From the table we can notice that there is a statistically significant difference in the total scores, determined by the Mann Whitney – U test, between the three studied groups, namely: healthy subjects and patients with gingivitis, healthy subjects and patients with periodontal disease, as well as between patients with gingivitis and patients with periodontal disease. The fact that higher scores indicate a higher risk for periodontal disease explains the determined difference between the three studied groups (lowest total score in healthy respondents, and the highest total score in patients with periodontal disease).

An analysis of the association of the total score of the Periodontal Health Self-Assessment Questionnaire (VSS) and the values obtained by the FOBT was also performed (table 4). We determined a significant linear positive very strong correlation between VSS and FOBT(Hb)(r(200)=0.702; p=0.0001) – with increasing VSS, the presence of Hb in saliva significantly increased. Gingival bleeding is an objective sign of inflammation in the gingival connective tissues. Bleeding occurs because of gingival inflammation and frequent microulcerations in the epithelium that lines the soft-tissue wall of a periodontal pocket. Bleeding from the gingiva is greater with greater tissue destruction and this is why Hb levels in patients with periodontal disease are higher compared to those with gingivitis. We also determined a significant linear positive very strong correlation between VSS and Dg (r(200)=0.744; p=0.0001), that is, with increasing values of VSS, the stage of the disease worsens. Since a higher score on the questionnaire indicates a higher risk of developing periodontal disease, the results of our study indicate the effectiveness of the VSS for self-assessment of periodontal health. The results of our research are not consistent with the results obtained by Cyrino et al.[37] and Gilbert et al.[38] regarding the efficacy of periodontal health self-report questionnaires. These authors believe that self-report questionnaires are not effective as screening methods because many individuals with periodontal health problems are unaware of their condition, do not notice, and ignore symptoms.

From Table 5 we can see that FOBT in saliva has good diagnostic efficiency for periodontal disease. The analysis of the results indicated the sensitivity and specificity on FOBT in saliva: Sensitivity=85.7% (0.75 – 0.92); Specificity=62.6% (0.53–0.71). Both, positive predictive value (PPV) and negative predictive value (NPV) were determined, and from the results we can see that the proportion of patients with PERIODONTAL DISEASE confirmed by Hb was 59%, while the proportion of patients with a negative finding for PERIODONTAL DISEASE confirmed by Hb was 67%. Our results regarding the possibility of using Hb in saliva as a screening marker for periodontal disease are in agreement with the results of Maeng et al.[34] as well as the results of Shimazaki et al.[39] who, in their research, came to the conclusion that determining the level of hemoglobin in saliva is a reliable method and can be used for periodontal disease screening. Regarding the determined sensitivity and specificity of the test, our results are in accordance with the results of Taniguchi-Tabata et al.[40] who in their study determined a sensitivity of the test of 75.9% and a specificity of 76.3%.

Conclusions

Based on the results of our research, as well as data from available literature, we came to the following conclusions:

- There is a positive linear correlation between hemoglobin levels in saliva and belonging to a certain diagnostic group of the respondents (healthy/gingivitis/periodontal disease). Hemoglobin levels in saliva increase as the disease progresses from healthy to periodontal disease.
- FOBT, as a method for detecting hemoglobin, i.e., subtle bleeding in the saliva, could be used as a screening test for early detection of periodontal

diseases. However, the values we obtained for its specificity make it insufficiently efficient. We believe that additional research is needed to prove its effectiveness.

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THE EFFICACY OF OZONIZED OLIVE OIL IN THE TREATMENT OF ORAL LESIONS: A CLINICAL STUDY

ЕФИКАСНОСТА НА ОЗОНИРАНОТО МАСЛИНОВО МАСЛО ВО ТРЕТМАНОТ НА ОРАЛНИТЕ ЛЕЗИИ: КЛИНИЧКА СТУДИЈА

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Abstract

The oral cavity functions as a dynamic ecosystem, maintaining a delicate balance between the entry of microorganisms, colonization modalities, nutritional equilibrium, and the host's defenses against their removal. Common entities frequently encountered in clinical practice include aphthous ulcerations, herpes and traumatic lesions, oral candidiasis, oral lichen planus and angular cheilitis. Numerous treatment modalities are available for these lesions. Topical ozone therapy, being an effective non-drug method, offers a minimally invasive technique that can be used for these conditions without any causing any side effects. **The aim of** the study is to evaluate the efficacy of ozonized olive oil in the treatment of recurrent aphthous stomatitis (RAS), herpetic and traumatic lesions. **Materials and methods:** A clinical study was conducted involving 35 patients, comprising 20 cases of aphthous ulcerations, 13 cases of herpetic lesions and 2 cases of traumatic lesions. Ozonized olive oil was topically applied twice daily until the lesion regresses. The size of the lesions and the intensity of the illness, evaluated using VAS scale, were recorded during the first visit and every other day until complete epithelization occurred. The lesions were photographed and documented. **Results:** All types of lesions, including aphthous ulcerations, herpetic lesions and traumatic lesions exhibited rapid regression and reduction of pain within a few days. None of the patients experienced any toxicity or side effects. **Conclusion:** Ozonized olive oil proves to be an effective topical agent for accelerating the healing process and alleviating pain associated with oral lesions, such as recurrent aphthous stomatitis, herpes, and traumatic lesions. **Key words:** Oral lesion, epithelization, ozone olive oil.

Апстракт

Оралната празнина претставува динамична еко-средина, со одржлив баланс помеѓу влезот на микроорганизмите, колонизациските модалитети, нутритивниот внес и одбраната на домаќинот. Некои од најчестите клинички ентитети во усната празнина ги вклучуваат афтозните улцерации, херпетичните и трауматските лезии, оралната кандидијаза, оралниот лихен планус и ангуларниот хеилитис. За овие лезии и состојби се достапни повеќе третмански модалитети. Топичната озон-терапија, како ефективен немедикаментозен метод претставува минимално инвазивна техника, која може да се применува кај овие состојби без никакви несакани ефекти. **Целта на студијата** е да ја проследиме ефикасноста на озонираното маслиново масло во третманот на рекурентниот афтозен стоматит (РАС), херпетичните и трауматските лезии. **Материјал и метод:** Клиничката студија беше спроведена кај 35 пациенти (20 афтозни, 13 херпетични и 2 трауматски лезии). Озонираното маслиново масло беше аплицирано 2 пати дневно, се до комплетна регресија на промените. Големината на лезиите и степенот на болка изразен по ВАС скалата, беа нотирани на првата посета и секој втор ден, до денот на комплетната епителизација. Лезиите беа фотографирани и документирани. **Резултати:** Сите лезии (афтозни улцерации, херпетични и травматски лезии) покажаа брза регресија и намалување на болката за неколку дена. Кај ниту еден пациент не беа регистрирани токсичност и несакани ефекти од препаратот. **Заклучок:** Озонираното маслиново масло претставува ефективен топичен агенс во процесот на заздравување и периодот на исчезнување на болката кај оралните лезии (рекурентниот афтозен стоматит, херпетичните и трауматските лезии). **Клучни зборови:** орални лезии, епителизација, озонирано маслиново масло

Introduction

Ozone, a triatomic gaseous molecule composed of three oxygen atoms, has demonstrated its efficacy in managing various pathologies in the field of medicine and dentistry[1]. Medical-grade-ozone used for medical purposes is a gas mixture consisting of 95–99.95% oxygen and 0.05–5% pure ozone[2].

Ozone therapy has gained a significant attention in the fields of medicine and dentistry. It possesses powerful oxidizing properties and exhibits strong antimicrobial activity against bacteria, viruses, yeasts and protozoa.

Additionally, it can stimulate blood circulation and immune responses, and has analgesic effects[3,4].

The first medical application of ozone dates back to 1870 when Dr. C. Lender employed it for purifying blood in test tubes[5].

Clinical literature reveals several studies highlighting the use of medical-grade ozone for the treatment of oral lesions and conditions[6]. Most of these studies focus on the use of the gaseous ozone, generated by specialized "Ozone Generators" which can be very expensive and require direct application to the lesion[7]. Due to the rigidity of this treatment, an alternative method of ozone application has been developed using an ozone solution. Olive oil, a revolutionary component in the solution, ensures better shelf life of the medication due to its chemical properties[8].

The treatment of erosive and ulcerative lesions in the oral cavity poses a significant challenge for dentists across all various specialties due to the chronic nature of the condition. Effective treatment approaches should consider the underlying etiopathogenesis of these lesions.

The oral cavity, due to its anatomical features, is constantly exposed to more than 300 types of microbes, making any erosion in this area susceptible to microbial contact. Many types of treatments have been used to manage oral ulcers, including mouthwashes (such as chlorhexidine), topical corticosteroids, topical tetracycline, immunoregulators, TNF inhibitors, systemic zinc sulphate, monoamine-oxidase inhibitors, low-energy laser, herbs, and others.

However, many of these therapies come with potential consequences such as dysbiosis, fungal infection, the development of microbial resistance and sensitization of the body[9,10]. In contrast, medical ozone has gained popularity as an antibacterial agent in multiple medical areas. Ozone exhibits broad-spectrum antimicrobial properties, effectively killing all kinds of bacteria, viruses, fungi and protozoa. Notably, the antiseptic effect of pure ozone is three hundred times stronger than that of chlorine, while being non-destructive and non-irritating to tissues. Therapeutic doses of ozone also poses antihypoxic and immunoreactive effects, potentiate the action of antibiotics, improve the rheological properties of blood and enhance microcirculation[11-15].

Ozone has been used in the treatment of ulcers affecting other parts of the gastrointestinal tract[16-18] blood vessels[19] and skin[20,21]. However, there is limited research available on the role of ozone in treating recurrent aphthous ulcers in the oral cavity.

This prompted the undertaking of the present study, which aimed to assess the effects of ozone olive oil in the treatment of oral lesions, including aphthous, herpetic and traumatic ulcers.

Material and method

A total of 35 patients participated in this clinical study, with 20 having aphthous lesions, 13 having herpetic lesions and 2 having traumatic erosions. The average age of the patients with aphthous lesions was 26 years, with 11 males and 9 females. The patients with herpetic lesions had an average age of 48 years, with 8 females and 5 males. The patients with traumatic erosions were both male, with one being 45 years old, and the other 8 years old, averaging 26 years (table 1). The study was conducted during the period from 1st of January 2021 until January 2023.

This study was conducted in accordance with ethical principles, specifically those outlined in the World Medical Association Declaration of Helsinki. The study was ethically approved by the Ethics Committee at the Faculty of Dentistry - Skopje, Ss. Cyril and Methodius University in Skopje, Macedonia.

All participants were fully informed about the study, and their informed consent was obtained prior to their participation in the study. All clinical examinations were performed on a dental chair. The diagnosis of all the lesions was based on the clinical presentation and symptoms.

Table 1. Oral lesions included in the study

	NUMBER OF PATIENTS	AVERAGE AGE	MALE	FEMALE
APHTHOUS LESIONS	20	26 years	11	9
HEPRETIC LESIONS	13	48 years	5	8
TRAUMATIC LESIONS	2	26 years	2	/

The main outcome measures of the study were pain levels, lesion size and duration. At the beginning of the study, ulcer size was measured using a periodontal probe which has an accuracy of up to 1 mm. Also, pain assessment was conducted using a VAS score (Visual Analogue Score), ranging from 0 to 10; with 0 indicating no pain and 10 indicating the most severe pain. The participants were instructed not to use any systemic or local drugs for the treatment of the ulcers or symptom relief throughout the period of the study. After establishing the diagnosis for each participant in the study, the size of the lesion was recorded in millimeters and photographed. Pain levels were also recorded using the VAS score. All of the patients were instructed to apply ozone olive oil, as explained in detail, massaging the oil into the affected area of the oral mucosa, twice a day without rinsing or moisturizing the area for 30 minutes after application. Control appointments were scheduled on the 3rd, 5th, 7th day, and so on for the purpose of monitoring the epithelization process of the lesions (as indicated by size reduction) and the reduction of pain levels until complete recovery. The entire process and changes were documented through photographs.

Results

As a result of the insufficient number of participants needed for statistical representation of data, we were able to report average values for certain parameters, including the initial size of the lesions (measured in mm), average pain levels, average epithelialization time and time of pain disappearance. These average values are presented in table 2.

For aphthous lesions, the average size was 3.35 mm, the average initial pain level was 4.16, the average epithelialization time was 4.3 days and the average time of pain disappearance was 3 days (figure 1-6).

Aphtae minor

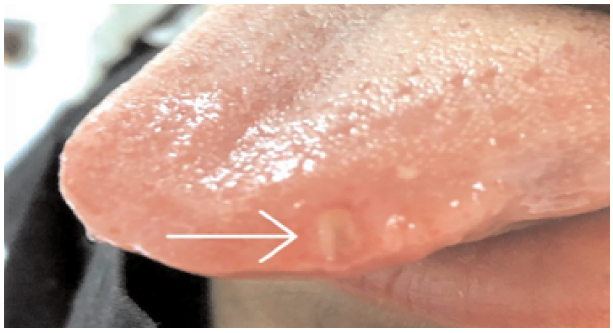

Figure 1. *First day (6 mm; VAS = 7)*

Figure 2. *Third day (2 mm VAS = 0)*

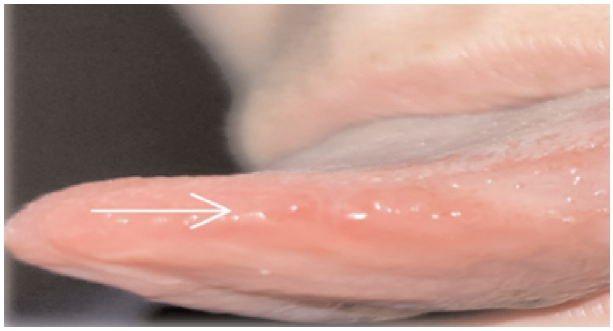

Figure 2. *Fifth day (0 mm VAS =0)*

Table 2. Average size of lesions, average pain levels, epithelization time and disappearance of pain numbered in days

	NUMBER OF PATIENTS	INITIAL AVERAGE SIZE OF LESIONS (мм)	INITIAL AVERAGE PAIN LEVELS VAS (1 -10)	AVERAGE EPITELIZATION TIME (days)	AVERAGE TIME OF DISAPPEARANCE OF PAIN (days)
APHTHOUS LESIONS	20	3,35	4,16	4,3	3
HEPRETIC LESIONS	13	8,87	4,37	4,25	3,75
TRAUMATIC LESIONS	2	5,5	4	2	3

Aphtae major

Figure 4. *First day (25 mm VAS =8)*

Figure 5. *Third day (15 mm VAS = 1)*

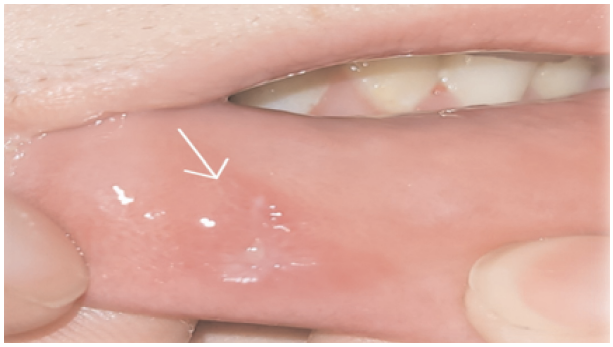

Figure 6. *Fifth day (0 mm VAS = 0)*

Herpes simplex recidivans

Figure 7. *First day (8mm VAS=5)*

Figure 8. *Third day (4mm VAS =0)*

Figure 9. *Fifth day (0mm, VAS =0))*

Herpes simplex recidivans

Figure 10. *First day (10 mm, VAS=3)*

Figure 11. *Third day (4mm, VAS=0)*

Figure 12. *Fifth day (0 mm, VAS=0)*

Erosio traumatica

Figure 13. *First day (5mm, VAS=5)*

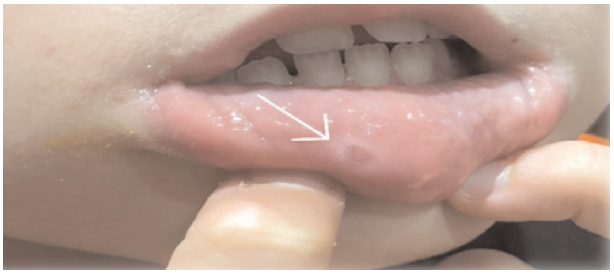

Figure 14. *Third day (2mm, VAS=0)*

Figure 15. *Fifth day (0mm, VAS=0)*

In the case of herpetic lesions, the average size was 8.87 mm, the average pain levels were 4.37, the epithelialization time was 4.25 days and the average time of pain disappearance was 3.75 days (figure 7-12).

Regarding traumatic lesions, the average size was 5.5 mm, the average pain level was 4 , the average epithelialization time was 2 days, and the average time of pain disappearance was 3 days (figure 13-15).

Some of the photos were taken by the patients, at home, so they are without protective gloves.

Discussion

Ozone is an inactivate form of oxygen (O_2) that exists as a trivalent molecule (O^3). Ozone naturally breaks down into two atoms of regular oxygen by releasing an atom of singlet oxygen over a period of approximately 20–30 minutes[22,23]. Despite being recognized as one of the most powerful oxidants in nature, the precise mechanisms underlying its therapeutic effects are unclear. Several theories have been proposed based on clinical observations. These theories include the generation of peroxides by ozonolysis of unsaturated fatty acids in cell membranes, activation of reactive oxygen species that act as physiological enhancers of various biological processes (including increased production of adenosine triphosphate), and increased expression of intracellular enzymes with antioxidant activity. It has been reported that exposure to ozone results in changes in the levels of many biological substances, such as cytokines (Interferon C, TNFs, TGFβ and IL-8), acute phase reactants and adhesion molecules from the integrin family such as CD11b.

Other reports have indicated increased motility and adhesion of peripheral blood polymorphonuclear cells to

epithelial cells after exposure to ozone. Similarly, major autohemotherapy-induced leukocytosis and enhanced phagocytic activity of polymorphonuclear cells have been reported.

Ozone therapy has proven to be useful in various ways for treating different diseases, thanks to its unique properties, including antimicrobial, immunostimulant, analgesic, antihypnotic, detoxicating, bioenergetic and biosynthetic actions.

Ozone causes inactivation of bacteria, viruses, fungi, yeast and protozoa. It disrupts the integrity of the bacterial cell membrane through the oxidation of phospholipids and lipoproteins. Even at low concentration of 0.1 ppm, ozone is sufficient to inactivate bacterial cells, including their spores[24]. In fungi, ozone inhibits cell growth at certain stages, with budding cells being the most sensitive[25]. In the case of viruses, ozone damages the viral capsid and disrupts the virus-to-cell contact though peroxidation, thereby disrupting the reproductive cycle[26].

Ozone therapy leads to an increase in the rate of red blood cell glycolysis. This stimulation results in the production of 2,3-diphosphoglycerate, leading to an increased release of oxygen in the tissues. Ozone activates the Krebs cycle by enhancing the oxidative carboxylation of pyruvate, stimulating production of ATP. It also causes a significant reduction in NADH and aids in the oxidation of cytochrome C. Moreover, it stimulates the production of enzymes that act as free radical scavengers and cell-wall protectors, such as glutathione peroxidase, catalase and superoxide dismutase and prostacyclin, which is a vasodilator[27].

When administered at a concentration between 30 and 55 µg /cc, ozone causes the greatest increase in the production of interferon and the greatest output of tumor necrosis factor and interleukin-2, triggering a cascade of subsequent immunological reactions[28].

Conclusions

The results obtained from this study indicate the following:

- A relatively fast epithelialization process of recurrent aphthous lesions, herpetic lesions and traumatic erosions, accompanied by a reduction in pain over a short period of time after treatment was observed.
- None of the patients experienced adverse effects or toxicity resulting from the topical agent, further confirming its safety.
- The application of ozone therapy was atraumatic, painless and noninvasive and the patients generally responded positively to the topical agent.

The current clinical literature lacks sufficient data on the utility of ozone therapy as a treatment option for certain oral condition, despite numerous studies available for conventional treatment modalities.

However, despite the study's limitations due to the small number of participants, the results were clear. The quick and effective healing of the lesions and reduction in pain levels over a short period of time are just two of the many benefits of localized ozone therapy that justify its long-term use in treating different types of periodontal conditions.

The main takeaway from this study is that ozone therapy is poised to revolutionize the field of dentistry in the near future.

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