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 PERIODON-TAL 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) Cl 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). Co

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 tissues⁶. 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 methods¹⁻⁷.

However, despite the high prevalence of these diseases, adequate screening programs are lacking. Several papers⁵⁻¹² 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 smoking¹³, 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 professionals¹⁴. 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 examination¹⁵. 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 diseases¹⁵. 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 periodontists¹⁶⁻¹⁸.

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 cavity¹⁹⁻²². 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 μ l 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 μ l 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 correctly²⁸. 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.

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

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

| Table 2 A | nalveie | of homoglobin | in caliva | (Hb) | according | o diagnostic | aroune |
|------------|----------|---------------|------------|-------|-----------|--------------|--------|
| Table Z. P | liarysis | or nemoglobin | ili saliva | (10) | according | o ulagnostic | groups |

| Categories | | Healthy (N=42) | Gingivitis (N=46) | Periodontal disease (N=112) | Total (N=200) | р | |
|--|---|-------------------|----------------------|-----------------------------------|------------------|---------------------------------|--|
| Hb_0 | N | 36 | 3 | 2 | 41 | | |
| 115-0 | % | 85,71% | 6,52% | 1.79% | 20,50% | Gingivitis/ | |
| ⊔b_1 | N | 6 | 32 | 44 | 82 | Periodontal | |
| | % | 14,29% | 69,57% | 39,29% | 41% | disease | |
| ⊔h_2 | N | 0 | 7 | 42 | 49 | $(110 - 0 \rightarrow 110 - 3)$ | |
| 110-2 | % | 0% | 15,11% | 37,50% | 24,50% | X ² =16,730; df=2; | |
| Hh-3 | N | 0 | 4 | 24 | 28 | p=0,0008* | |
| 110-5 | % | 0% | 8,70% | 21,43% | 14% | | |
| Hb-0 – no bleeding; Hb-1 – weakly positive; Hb-2 – moderately positive; Hb-3 – strongly positive X²= 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 \rightarrow 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) | | | | | | р | | | |
|---|--|------------|---------|------------------------------|-----|----|--|--|--|--|
| | N | Mean±SD | Min/Max | Percentiles (25th/50th/75th) | | | | | | |
| Healthy | 42 | 4,23±1,44 | 2/9 | 3 | 4 | 5 | V ² -111 100. | | | |
| Gingivitis | 46 | 8,15±2,94 | 4/15 | 6 | 7.5 | 10 | X ⁻ (2) ⁼ 111,182; | | | |
| Periodontal disease | 112 | 12,02±3,15 | 4/20 | 10 | 12 | 14 | p=0,0001* | | | |
| 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 sco | n | | | |
|---|---|---|--------------------|--|--|
| | Non-adjusted ¹ | Adjusted ¹ | р Р | | |
| Hemoglobin (Hb) | r ₍₂₀₀₎ =0,702; p=0,0001* | r ₍₂₀₀₎ =0,662; p=0,0001* | Z=0,7430; p=0,4574 | | |
| $\begin{array}{c c} \textbf{Diagnostic group} \\ \textbf{(Dg)} \end{array} \begin{array}{c} r_{(200)} = 0,744; & r_{(200)} \\ p = 0,0001^{*} \end{array} \begin{array}{c} p = 0 \\ p = 0 \end{array}$ | | r ₍₂₀₀₎ =0,686; p=0,0001* | Z=1,1812; p=0,2375 | | |
| ¹ Pearsons moment order correlations *significant for p<0,05 | | | | | |

 $0\rightarrow 2$ 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 PERIODON-TAL 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 \rightarrow periodontal disease) (table 4).

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

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

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

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 DIS-EASE 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 disease²⁹⁻³¹. 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
- 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.

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 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 PERIODON-TAL 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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