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Analysis of the bacterial flora of periodontal pockets and evaluation of the diagnostic value and the validity of using the PET test in the detection of Sockrasky's red complex pathogens at various stages and degrees of periodontitis - discussion of selected clinical cases.

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Abstract:

Introduction: In microbiological diagnostics, a significant role is played by the PET test based on a polymerase chain reaction developed thanks to medical engineering methods, which, by amplifying specific fragments of genetic material, enables the identification of microorganisms. As a consequence, it is possible to quantify and qualify a sample of biological material under study, which has found application in the diagnosis and treatment of periodontitis.

Aim:

The following paper shows the validity of carrying out the PET test in people with clinically diagnosed chronic periodontitis who also suffer from other loads, such as smoking or systemic diseases, as well as the physiological state of pregnancy. Another goal is also to confirm the presence of Sockransky's red complex bacteria responsible for the development of periodontitis.

Material and Methods:

The research was carried out at the Clinic of Conservative Dentistry and Periodontal Diseases, performing an examination of 4 patients, and then after determining the deepest periodontal pockets, biological material was collected from them and sent to a bacteriological laboratory, where it was analyzed.

Results:

The quantitative and qualitative determination of the samples of biological material collected from the pockets of the patients showed the presence of the red complex bacteria according to Sockransky, especially the species: *Treponema denticola* and *Porphyromonas gingivalis*.

Conclusions:

In the studied clinical cases, the PET test confirmed the presence of bacteria responsible for periodontitis. The legitimacy of its use was also confirmed, emphasizing that it is a tool that can be used by a dentist in implementing therapeutic decisions in the treatment of periodontitis.

Key words: periodontitis; diagnostic methods; PET microbiological test.

Introduction

Periodontitis is a chronic infectious disease that destroys the tissue surrounding the tooth, i. e. the gums, periodontal membrane, and bone. Microbiological, immunological, and genetic factors are the causes of the disease. An important etiopathological component of periodontitis is the microbiological factor. According to Sockransky's current theory of bacterial complexes, isolated microorganisms can be divided into several groups, e. g. due to their virulence and the severity of the provoked periodontitis. To the violet complex belongs *Aggregatibacter actinomycetemcomitans*, which is responsible for aggressive forms of periodontitis, such as localized juvenile periodontitis, or is isolated in refractory periodontitis.

The red complex includes the most pathogenic bacteria i.e. *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*. The orange complex has also been determined, which includes e. g. *Fusobacterium nucleatum* and *Prevotella intermedia*.

Age, gender, systemic diseases, medications and stimulants such as cigarettes are significant risk factors for periodontitis. Hormonal fluctuations, such as pregnancy, are also an important risk factor.

In our research, we selected a group of patients with systemic diseases such as hypertension, diabetes, nicotine use and also pregnant patients.

A lot of research has confirmed a strong correlation between diabetes, hypertension and periodontitis. In the course of hyperglycaemia, there are many structural changes in the blood vessels and activation of the immune system. These effects are manifested by inflammation and modification of natural barriers and immune defences i. e. the mucosa of the oral cavity. In hypertension, periodontitis causes systemic inflammation that affects vasodilatory function and leads to an increase in blood pressure. Therefore periodontitis is a factor that modifies systemic disease.

During pregnancy, the concentration of female sex hormones is high in the blood. These hormones are metabolized by periopathogens and that causes growth of their population and the development of periodontitis.

The important risk factor for periodontitis is also nicotine use, because nicotine and its metabolites contribute to the disruption of the specific immune response, increase the percentage of absolute anaerobic periopathogens and the tendency to deposit tartar.

These factors contribute to a more severe course of periodontitis, so non-surgical treatment as a 'gold standard' may be ineffective. In these cases, careful analysis of the etiological factors and individual assessment of the bacterial flora are required.

Unquestionably, treatment success depends very much on the diagnostic value of examination. There have been many diagnostic methods developed in clinical periodontology over the years, which differ in specificity and sensitivity.

With the development of biotechnological and engineering techniques, the microbiological test PET plays an important role in the diagnosis of etiological factors of periodontitis. Its functional principle is based on the polymerase chain reaction (PCR) and enables

qualitative and quantitative analysis of the bacterial niche of the subgingival region of the oral cavity. This method was introduced by Kary Mullis, who was awarded the Nobel Prize in Chemistry. The prize was awarded in 1993 for the introduction of the polymerase chain reaction mentioned above as a diagnostic method for medical, forensic, and judicial purposes.

Aim

The aim of the clinical tests was to analyze the bacterial flora in patients with diseases, such as diabetes, arterial hypertension and in a patient in a physiological state of pregnancy and in a patient suffering from nicotine addiction and also the assessment of the diagnostic value and the validity of using the PET test in the above-mentioned clinical situations.

Materials and methods

The research material was made up of four patients selected from among patients from Conservative Dentistry and Periodontal Clinic in the Academic Centre of Dentistry in Zabrze. Patients were admitted during clinical classes by students of dentistry at the Faculty of Medical Sciences in Zabrze. Each of the patients suffered from clinically diagnosed periodontitis of various stages and degrees, they did not use antibacterial mouthwashes as well as did not take antibiotics for the last 6 months.

First, the patients were interviewed about their health condition, with particular emphasis on chronic diseases, medications taken and stimulants used, as well as symptoms that caused the patients to visit the clinic.

Then, a physical examination was performed, during which the API oral hygiene index and the index informing about the number of bleeding places after probing, which is BoP, were assessed, and periodontal pockets depth (PD) and clinical attachment loss (CAL) were measured. The results were written down on the periodontal examination card. The patients also had an orthopantomographic image taken, which was used for the initial evaluation of the periodontium and to determine the degree of bone loss. Based on the obtained information the stage and degree of periodontal disease were determined in accordance with the 2017 guidelines.

To assess the stage that informs us of the severity and complexity of the disease, CAL was chosen as the primary determining factor. In case of difficulty in determining CAL, the clinician may be guided by the percentage of radiological bone loss and this was also done in the discussed studies.

The grades allow us to assess the rate of disease progression. This assessment is influenced by direct and indirect evidence as well as age and modifiable risk factors, the presence of which, regardless of the basic diagnosis, increases the severity of the disease.

Direct evidence includes analysis of older X-rays while indirect evidence includes the assessment of bone loss at the most affected tooth, taking into account the patient's age

For each patient qualified for the study due to the course of periodontitis, a PET diagnostic kit made by MIP Pharma was prepared. The kit contains four sterile vials, five sterile paper points for drawing biological material, an individual questionnaire with patient data information that may be clinically relevant, and an envelope.

In our clinical trials, the test was used in the Standard version that allows the detection of Bacteria of the Purple Sockransky's complex which includes *Aggregatibacter actinomycetemcomitans* and the red complex, which contains the following species of bacteria: *Porphyromonas gingivalis*, *Tannerella forsythia* i *Treponema denticola*.

The PET test is based on a Real-Time PCR technique (polymerase chain reaction), it is a reflection of the natural process of replication of genetic material and enables the amplification of precisely defined DNA fragments in an enzymatic reaction that takes place due to Taq polymerase isolated from the *Thermus aquaticus* strain and Pfu polymerase isolated from the *Pyrococcus furiosus* strain.

The place of these reactions is not a living organism, but a device called a thermocycler which serves as a heating tube with an electronic temperature regulation system.

In the process of the polymerase chain reaction, three stages can be distinguished, which differ in the temperature of the process:

-phase I consisting in the denaturation of the double-stranded DNA template; takes place at a temperature of 90-95°C

-phase II, which consists in annealing of the primers to the single-stranded DNA template, takes place at 70-65°C

-phase III consisting in the synthesis of the complementary strand; takes place at a temperature of about 72°C

In order to create a complete diagnostic picture, a number of the following activities were undertaken. All patients had abundant deposits of dental calculus which were removed by

supragingival scaling. The hygienization treatment was necessary to gain access to pathological periodontal pockets and to measure their depth, as well as to draw biological material. The information obtained on the basis of the specialist examination was used to determine the deepest pathological pockets, over 4 mm, and then biological material was collected from these places using sterile paper points, which were kept in the pocket for about 20 seconds.

The criterion that had to be observed to maintain the diagnostic value of the PET test was the necessity to preserve the material not contaminated with blood or pathological exudate. In the next stage, the paper points were placed in a vial and, after completing the application form, sent to the laboratory.

After a period of 2-3 weeks from sending the sample, quantitative and qualitative results were obtained as well as the indication of the best therapeutic direction compatible with the sensitivity of the microbes of a given complex based on the literature.

Bacteria marked with the symbol: *, in the coexistence of another species, were the main periopathogen in a specific clinical situation.

Clinical case 1

A 22-year-old patient reports in an anamnesis that she suffers from type I diabetes and celiac disease.

She has been treated at the Conservative Dentistry and Periodontal Clinic in the Academic Centre of Dentistry since 2019. She is under the constant care of a diabetologist. She takes insulin permanently. During the visit on 23.11.2021, the value of the API index was determined, which was 87% and BoP 25%. Based on the clinical and radiological examination, the patient was diagnosed with a localized form of periodontitis in stage II, grade B.

The pantomographic image, which was ordered during the visit, showed horizontal atrophy of the alveolar processes, and a widened periodontal ligament space at tooth 13.

At the visit, biological material was drawn from pockets in the area of teeth 21 (PD 4 mm), 27 (PD 5 mm), and 37 (PD 5 mm) in order to perform the PET test.

The obtained results showed the presence of periopathogens of the red complex - *Treponema denticola* (the dominant pathogen) in the amount of 1.4×10^4 and *Porphyromonas gingivalis* in the amount of 4.0×10^3 .

The recommended treatment was clindamycin 1200 mg / day for 7 days or metronidazole 750-1500 mg / day for 7 days as an alternative treatment.

Clinical case 2

In 2021, a 36-year-old patient came to the Conservative Dentistry and Periodontal Clinic in the Academic Centre of Dentistry. The anamnesis shows that the patient is in the third trimester of pregnancy, during which she was diagnosed with anaemia of unknown etiology. The woman does not report any other diseases and does not take any medications.

Clinical examination showed the presence of extensive deposits of dental calculus, gingivitis and periodontal pockets from 4 to 6 mm, at tooth 48 buccal 7 mm:

The API index value was determined as 52% and the BoP index value-42%. The OPG image provided by the patient shows generalized horizontal bone loss.

Based on the clinical and radiological examination, the patient was diagnosed with a generalized form of periodontitis in stage II, grade B.

The patient was qualified for the PET test and material was drawn from pockets around teeth 15 and 48.

The test results showed the presence of red complex pathogens with a particular indication of *Treponema denticola* in the amount of 4.5×10^4 .

A treatment with clindamycin 1,200 mg / day for 7 days was recommended.

Clinical case 3

A 62-year-old patient reports in an anamnesis that she suffers from hypertension, a condition after thyroidectomy/strumectomy. The patient regularly takes medicines: angiotensin converting enzyme inhibitors, bisoprolol, and evothyroxine.

The API index value was determined as 100% and the BoP index value-32%. The OPG image shows advanced horizontal bone loss.

Based on the clinical and radiological examination, the patient was diagnosed with a localized form of periodontitis in stage III, grade B.

For the PET test, biological material was drawn from tooth pockets: 42 (PD 5 mm) and 21 (PD 7 mm). Other pockets were excluded from the test because of the heavy bleeding and the risk of misrepresentation of the result.

The test results showed the presence of red complex pathogens - *Porphyromonas gingivalis* in the amount of $1,6 \times 10^5$ and *Treponema denticola* in the amount of $3,5 \times 10^5$.

According to the test results, the recommended treatment is clindamycin 1200 mg / day for 7 days or metronidazole 750-1500 mg / day for 7 days as an alternative treatment.

Clinical case 4

A 61 years old patient came for a periodontal consultation before making a lower skeleton prosthesis.

The patient has a history of diseases of the eye, thyroid gland, arterial hypertension, and therefore takes the following drugs: an angiotensin II receptor antagonist in combination with a diuretic and a calcium antagonist, and levothyroxine due to hypothyroidism.

In addition, he reports compulsive smoking (15 pcs / day).

The clinical examination revealed poor hygiene, abundant supragingival and subgingival deposits, teeth with varying degrees of mobility: 31 and 41 in the 1st degree and 34, 35 and 38 in the 2nd degree of mobility and the periodontal pockets were measured.

The API value was determined, which was 100%, while the percentage of bleeding sites after probing according to the BoP index was 23%.

The orthopantomographic image provided by the patient shows mixed, vertical and horizontal bone defects, tooth 26 in the Godon phenomenon and teeth 24, 25, 17 and 15 endodontically treated (including tooth 15 with an incomplete canal and changes in periapical tissues).

On the basis of clinical and imaging examination, the patient was diagnosed with a localized form of periodontitis in stage III, grade C.

In order to perform the PET test, material was collected from pockets at teeth 34 (PD 6mm), 35 (PD 7 mm) and 27 (PD 7 mm), the result of which proved the presence of red complex bacteria: *Porphyromonas gingivalis* in the amount of $2,1 \times 10^5$ and *Treponema denticola* in the amount of $1,1 \times 10^5$.

According to the test results, the recommended therapy is clindamycin at a dose of 1,200mg/d for 7 days, and as an alternative treatment, metronidazole 750-1500 mg/d for 7 days.

Discussion

Numerous studies show that hypertension and periodontitis have a pathogenetic connecting component. One of the main mechanisms by which blood pressure increases is damage to the vascular endothelium in response to the systemic inflammatory process. Periodontitis can be the cause of systemic inflammation, in which they are involved, inter alia, C-reactive protein or interleukin 1b and 6, which in turn may reduce vasodilator function by reducing the production of nitric oxide. Based on the previously proven relationship between periodontal diseases and cardiovascular diseases, early detection and eradication of red complex bacteria in the course of periodontitis should be a way to increase the effectiveness of the treatment of hypertension [10]. In our patient suffering from arterial hypertension, an increased number of *Porphyromonas gingivalis* was detected in the PET test, which pathogen may cause an inflammatory reaction, directly causing an increase in blood pressure [4, 10].

Another interdependence that needs to be discussed is the effect of smoking on the course of periodontal disease. As shown by numerous studies, the components of tobacco smoke are not indifferent to periodontal tissues. Nicotine, as its main component, changes the conditions in the oral cavity (especially in the gingival fissures) due to its metabolic profile [7, 21]. It modifies the composition of the bacterial microflora of these areas in favor of the colonization of anaerobic bacteria of the red complex, among others, detected in the PET test: *Porphyromonas gingivalis* and *Treponema denticola* [16, 18, 19, 20]. In addition, it disturbs the blood flow in the gums by stimulating the release of catecholamines: adrenaline, and noradrenaline, which results in shrinkage of gingival capillaries, what is more, due to lowering the level of prostacyclin PGI₂ - increases the aggregation of platelets and causes the formation of blood clots in these vessels and closure of their lumen [7,21]. The effect of these changes is a lower percentage of bleeding sites when probing periodontal pockets despite the presence of active inflammation, as evidenced by clinical case 4. It is also worth mentioning that nicotine metabolites, including cotinine, and trans-3'-hydroxycotinin, contribute to the disturbance of the mechanisms of the specific immune response, both cellular and humoral. They impair chemotaxis and phagocytosis of phagocytosis and reduce the production of IgA and IgG antibodies, thus weakening the immune system's response to plaque antigens. [7.21]. A particularly important effect of heavy smoking is the predisposition to an easy and

accelerated deposition of tartar, which results from the increased concentration of calcium and phosphorus ions and a decreased pH of the plaque [7.21]. The result of this dependence, especially in combination with poor oral hygiene, is visible hygiene negligence in the form of abundant supra- and subgingival deposits.

Conclusions

1. In the studied clinical cases, the PET test confirmed the presence of bacteria responsible for periodontitis.
2. In a diabetic patient, the test confirmed the presence of *Treponema denticola*. A high percentage of these microorganisms in causing periodontitis in diabetics is confirmed in the literature.
3. In the pregnant patient qualified for the study, the pathogen *Treponema denticola* was isolated to the greatest extent. The detection of specific periopathogens in pregnant women enables the improvement of diagnosis, the implementation of appropriate treatment, and thus the increase in care for pregnant women, prevention of premature birth and low birth weight of newborns.
4. Based on the previously proven relationship between periodontal diseases, and diseases of the cardiovascular system, early detection of red complex bacteria and antibiotic therapy based on the antibiogram as a part of periodontitis therapy should be a way to increase the effectiveness of the treatment of hypertension.
5. By changing the composition of the oral bacterial microflora and modulating the immune system, nicotine promotes the development of red complex bacteria such as *Porphyromonas gingivalis* and *Treponema denticola*, which was confirmed by the results of our research.
6. The drug resistance indicated in the PET test, although it is determined on the basis of data from the literature, indicates therapeutic possibilities supporting scaling and root planning in cases resistant to treatment.
7. The PET microbiology test is a tool that can be used by a dentist practicing in primary care in motivating the patient to proper hygienisation and regularity, as well as in therapeutic decisions.

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