ORIGINAL ARTICLE

CONTENTS

Results of microbiological study of dental biofilm in generalized periodontitis against the background of different body reactivity

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ABSTRACT

Aim: To study the spectrum, frequency ofisolation and level of colonization of dental biofilm with microorganisms in generalized periodontitis against the background of different body reactivity.

Materials and Methods: 216 people with the diagnosis of generalized periodontitis. Depending on the state of reactivity of the organism, the patients were divided into 3 groups: with normo-, hyper- and with hyporeaction. The patients underwent patch surgery. After the surgery, dental biofilm was taken. Microbiological studies included the isolation and species identification of dental biofilm microorganisms, the results of quantitative studies of microflora: the level of colonization was expressed in colony-forming units per 1 ml (CFU/ml); the frequency of microorganisms isolation was expressed in absolute numbers. Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0.

Results: The studies have shown that in different states of the body's reactivityin patients with generalized periodontitis after flap surgery, different quantitative and qualitative composition of the microflora of the dental biofilm is determined. In case of normal body reactivity, there are predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora; in case of impaired (hyper- and hypo-) reactivity, there are predominantly aerobic-anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

Conclusions: The identified differences in the quantitative and qualitative composition of the microflora of the dental biofilm indicate the key role of the body's reactivity in the studied processes.

KEY WORDS: periodontitis, reactivity of the organism, microflora, dental biofilm

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INTRODUCTION

Among the topical problems of modern dentistry, generalized periodontitis is one of the leading ones [1-3]. The key factor in the development of generalized periodontitis is the influence of conditionally pathogenic and specific periodontopathogenic microflora of the dental biofilm, as it initiates the development of inflammation and immunopathological reactions in the periodontium [4-6]. Microorganisms of the dental biofilm are combined into microbial associations, which ensures their coexistence with each other [7]. At the same time, they can even enhance the pathogenic effect of each other (synergism of action) and inhibit the growth of competing microbial species [8,9]. Periodontal pathogens are highly aggressive towards periodontal tissues due to their ability to adhere to the gingival epithelium and invade deep into the tissues (up to the cementum of the tooth root), due to the secretion of destruction enzymes [10,11]. Under the influence of prolonged microbial persistence, the pathological process in periodontal tissues acquires signs of chronic inflammation, which ultimately leads to irreversible destruction of the periodontium and alveolar bone [12,13]. At the same time, complex multicomponent processes of inflammation and destruction occur simultaneously and continuously in periodontal tissues [14]. A complex of microcirculatory, hematological, immunological and connective tissue reactions to damage develops [15-17]. Microcirculatory disorders initiate tissue hypoxia, activation of free radical oxidation, disorganization of biological membranes with the release of physiologically active proinflammatory substances (prostaglandins, cytokines). The outcome of the disease is largely determined by the compensatory abilities of the protective mechanisms of the periodontium and the body as a whole, which are determined by the body's reactivity [18-20].

AIM

The aim of this study was to investigate the spectrum (genus and species of microorganisms), frequency of isolation and level of colonization of dental biofilm with microorganisms after flap surgery in patients with generalized periodontitis of II, III severity degrees against the background of normal, hyper- and hyporeactivity of the body.

MATERIALS AND METHODS

We examined 216 patients (82 men and 134 women) aged 45 to 55 years with a diagnosis of generalized periodontitis II, III severity, chronic course. The diagnosis was made on the basis of clinical examination, radiography, and periodontal samples in accordance with the International Classification of Diseases ICD-10. Depending on the state of the body's reactivity, patients were divided into three groups: the first - normoreaction (132 patients, 61%); the second - hyperreaction (46 patients, 21%); the third - hyporeaction (38 patients, 18%). Patients were divided into groups depending on the state of the body's reactivity based on the identified clinical and laboratory differences. All patients underwent flap surgery as indicated. After surgery (on the 1st day), dental biofilm was taken. The dental biofilm was collected using sterile curettes from the cervical region of the vestibular surfaces of the upper teeth (canines, premolars and first molars), followed by rapid application to a standard sterile swab of the Sarstedt transport system (Germany), which allows to increase the time of transportation to the bacteriological laboratory up to 72 hours.

Microbiological studies included the isolation and species identification of dental biofilm microorganisms using aerobic and anaerobic cultivation techniques by inoculating clinical material from a transport swab onto special nutrient media. Cultivation of the material on the nutrient media was carried out in a thermostat at a temperature of 37 degrees C for 3-5 days. Identification of the isolated pure cultures was carried out by morphological, cultural and biochemical characteristics, as well as by using bioMerieux identification test strips: ARI Staph., ARI 20 Strep., ARI 20 E, ARI 20 A. The results of the quantitative study of microflora: the level of colonization was expressed in colony-forming units per 1 ml (CFU/ml); the frequency of isolation of microorganisms was expressed in absolute numbers.

Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0 (STA862D175437Q).

RESULTS

The results of the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis and normal body reactivity are presented in table (Table 1).

As can be seen from the table, the most common aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus*, respectively, were found in 77.2 and 87.1% of cases. Their spectrum is represented by the following species: *Str. mitis, Str. mutans, Str. intermedius, S. haemolyticus, S. hominis. In isolated cases, S. auricularis, S. warneri, S. aureus and S. schleiferi* were found. It should be noted that such species of *Streptococcus* and *Staphylococcus* as *Str. pyogenes, Str. faecium, Str.equinus, S. cohnii, S. simulans, S. lentus* were not detected in patients with AP of this group. When assessing the level of colonization of the dental biofilm with aerobic cocci, it was found that the number of CFU of bacteria in 1 ml of clinical material on average, is 10^6. Facultative gram-negative bacilli *Escherichia coli* and *Enterobacter* were found, respectively, in 77.7 and 8.3% of cases. At the same time, such representatives of this group of bacilli as *Klebsiella*, *Citrobacter* and *Capnocytophaga* were not detected in the dental biofilm. In 24.2% of cases, bacteria of the genus *Peptostreptococcus* (*species P. anaerobius, P. prevotii*) and the genus *Porphyromonas* (*species P. gingivalis*) were isolated, in 15.9% of cases - bacteria of the genus *Prevotella* (*species P. oralis*). In addition, gram-negative anaerobic bacteria of the genus *Fusobacterium (species F. Nucleatum* and *F. necrophorum)* were detected in 65.27% of patients. The average number of CFU of these anaerobes in 1 ml of clinical material was $10 - 10^{57}$. Anaerobic bacteria of the general *Actinomyces* and *Bacteroidas*, as well as aerobic bacteria of the genus *Pseudomonas* were not detected. Thus, the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis with normal body reactivity on the 1st day after surgery indicates the presence of predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora.

The results of the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis against the background of hyperreactivity of the body indicate a number of differences from such parameters in patients with normal reaction. As can be seen from the table, aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus* were found in the dental biofilm of persons of this group in 56.5% of cases, which is significantly less frequent compared to the first group. The spectrum of detected aerobes was significantly narrowed in patients of the second group compared to the normal reaction. Thus, the number of streptococci species decreased by 3 species: *Str. mitis*, *Str. mutans*, *Str. intermedius*. The assessment of the level of dental biofilm colonization with streptococci showed that it was commensurate with the values in the first group - the number of CFU of bacteria in 1 ml of clinical material was on average 10^6 . At the same time, the level of staphylococci colonization, on average, reached 10^5 , which is 10 times

Table 1. The frequency of microorganisms isolation and the level of dental biofilm colonization in normal, hyper- and hyporeactivity of the body on the 1st day after surgical treatment (abs. / CFU / ml)

Genus and species of isolated microorganisms		norm-reaction (n = 132)	hyper- reaction (n = 23)	hypo- reaction (n = 19)
Aerobic and facultative coccigenus Staphylococcus	*Staphylococcus*	115 / 10 -10^{48}	13 / 10 -10^{28}	10 / 10 -10^{28}
	S. haemolyticus	21/10^{8}	6 / 10 -10^{24}	7 / 10 -10^{24}
	S. auricularis	18/10^{5}	4/10^{6}	3/10^{6}
	S. capitis	12/10^{7}	4/10^{4}	3/10^{4}
	S. hominis	10/10^{4}	3/10^{4}	3/10^{4}
	S. warneri	9/10^{4}	3/10^{4}	2/10^{4}
	S. aureus	-	4/10^{5}	2/10^{5}
	S. lentus	-	-	-
	S. schleiferi	-	3/10^{8}	2/10^{8}
	S. cohnii	-	-	-
	S. simulans	-	-	-
Aerobic and facultative cocci genus Streptococcus	*Streptococcusspp.*	102 / 10 -10^{58}	13 / 10 -10^{57}	9 / 10 -10^{57}
	Str.sanguis	-	7 / 10 -10^{57}	6 / 10 -10^{57}
	Str.mitis	42 / 10 -10^{58}	-	-
	Str.mutans	30/10^{7}	-	-
	Str. pyogenes	-	-	-
	Str. Faecium	-	5/10^{5}	4/10^{7}
	Str.intermedius	22 / 10 -10^{58}	-	-
	Str.equinus	-	5/10^{8}	4/10^{8}

less than in patients with a normal reaction. In addition to aerobic cocci, the appearance of aerobic gram-negative bacilli of the genus *Pseudomonas* was noted in the dental biofilm. It should be noted that the spectrum of facultative gram-negative bacilli has also expanded, the frequency of detection and the level of colonization have increased. Thus, representatives of the genera *Klebsiella* and *Capnocytophaga* appeared. In addition, the number of cases of isolation of *Escherichia coli* and *Enterobacter* from the bacillus has increased. The expansion of the spectrum, the increase in the frequency of isolation and the level of colonization of the dental biofilm with gram-positive and gram-negative anaerobes is noteworthy. Thus, anaerobic bacteria of the genera *Actinomyces* and *Bacteroidas* were detected; the frequency of isolation of *Peptostreptococcus*, *Porphyromonas*, *Prevotella* and *Fusobacterium* increased. At the same time, the level of colonization of the dental biofilm with *Peptostreptococcus, Porphyromonas and Fusobacterium* increased 5-10 times. Thus, the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis against the background of increased body reactivity indicates the predominance of aerobic- anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

The results of the analysis of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis against the background of hyporeactivity of the body on the 1st day after surgery indicate their similarity to those in hyperreactivity of the body and a number of differences from such parameters in normal body reactivity. As can be seen from the table, aerobic facultative gram-positive bacteria of the genus *Streptococcus* and *Staphylococcus* were found in the dental biofilm, respectively, in 47.4 and 52.6% of cases, which is comparable to the values in hyperreactivity and much less frequent compared to the values in normoreactivity. At the same time, the spectrum of detected aerobes was similar to that in the second group and significantly narrowed compared to that in the first group. Assessment of the level of colonization of the dental biofilm with streptococci showed that it was commensurate with the values in the normal body reactivity - the number of CFU of bacteria in 1 ml of clinical material, on average, was 10^{6}. At the same time, the level of staphylococci colonization, on average, reached 10^{5}, which is 10 times less than that of patients in the first group. In addition to aerobic cocci, the appearance of aerobic gram-negative bacilli of the genus *Pseudomonas* was noted in the dental biofilm. It should be noted that the composition of the dental biofilm also expanded the spectrum, increased the frequency of detection and the level of colonization of facultative gram-negative bacilli compared to that of the normoreactivity of the organism. Thus, representatives of the genera *Klebsiella* and *Capnocytophaga* appeared. In addition, the number of cases of isolation of

Escherichia coli and *Enterobacter* bacilli has increased. As well as against the background of increased reactivity of the organism, the expansion of the spectrum, increase in the frequency of isolation and the level of colonization of dental biofilm with gram-positive and gram-negative anaerobes is noteworthy. Thus, anaerobic bacteria of the genera *Actinomyces* and *Bacteroidas* were found in the content of the dental biofilm; the frequency of isolation of *Peptostreptococcus*, *Porphyromonas*, *Prevotella* and *Fusobacterium* increased. At the same time, the level of colonization of the dental biofilm by *Peptostreptococcus*, *Porphyromonas* and *Fusobacterium* increased 5-10 times. Thus, the analysis of the quantitative and qualitative composition of the dental biofilm microflora in patients with generalized periodontitis against the background of reduced body reactivity on the 1st day after surgery, as well as in case of increased body reactivity, indicates the presence of predominantly aerobic- anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes.

DISCUSSION

Generalized periodontitis has all phases of inflammation: alteration, exudation, and proliferation. Wound healing is also an inflammatory reaction characterized by a cascade of neuro-regulatory, biochemical, neuro-trophic, immunological and functional disorders, microcirculatory and metabolic disorders [2]. The healing of the postoperative wound after flap surgery in patients with generalized periodontitis II, III degrees of severity also represents an inflammatory reaction with the appropriate ratio of the phases of necrotic and reparative processes. It is known that the intensity and duration of the inflammatory reaction during the healing of a myocardial infarction is determined by the form of the initial reactivity of the body and determines the complicated and uncomplicated consequences [21]. In the conducted study, we showed that in different states of the body's reactivity in patients with generalized periodontitis after flap surgery, different quantitative and qualitative composition of the microflora of the dental biofilm is determined. In case of normoreactivity of the organism, there are predominantly aerobic-anaerobic associations with a wide range and quantitative predominance of aerobic microflora; in case of impaired (hyper- and hypo-) reactivity, there are predominantly aerobic-anaerobic associations with an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes. The revealed differences in the quantitative and qualitative composition of the dental biofilm microflora indicate the key role of the organism's reactivity in the studied processes.

CONCLUSIONS

In the dental biofilm of patients with generalized periodontitis with impaired (hyper- and hypo-) reactivity of the body after surgery, an expansion of the spectrum, frequency of isolation and level of colonization of facultative and obligate anaerobes was found compared to that in the normal reaction. We consider the correction of the quantitative and qualitative composition of the microflora of the dental biofilm in patients with generalized periodontitis with impaired body reactivity with bringing it to the values of normal reactivity as a condition for optimizing postoperative wound healing and further stabilization of the process in periodontal tissues. Therefore, we consider it promising to develop methods of targeted drug correction that will bring microbiological parameters to those corresponding to the normoreactivity of the body.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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