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IMMUNOMICROBIOCENOSIS OF PERIODONTAL POCKETS OF PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS

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Abstract:

The data about quantitative and qualitative composition of periodontal pockets microflora and unspecific immune resistance factors of patients with chronic generalized periodontitis in comparison with crevicular fluid microflora of the people with intact periodontium were presented in this article.

Key words: generalized periodontitis, microflora, periodontal pockets, phagocytosis, lysozyme.

Background:

Oral cavity diseases, as any human diseases are mostly induced and determined by two groups of factors: external (microorganisms in part) and systemic internal ones, among which inheritance, immune and endocrine systems state play major role. The disease origin and result are detected by mentioned external and internal factors interaction. The dental diseases majority haven`t their specific causative agent and appear as oral cavity microbiocenosis changes result [1, 4, 7, 10]. Periodontal diseases belong to such diseases.

Unlike many medical infections, periodontal diseases ate not caused by pathogens that have their primary habitat outside the host. Instead, they are associated with a shift in the balance of the resident microflora, a similar situation to dental caries. The microorganisms may produce disease

directly, by invasion of the tissues, or indirectly via bacterial toxins [6, 8]. The host response to these challenges may be protective, for example phagocytosis of invading bacteria, or destructive, for example immune complex activation of osteoclasts. Frequently it is a combination of both and the interaction between these components which determines the wide spectrum of disease that is seen clinically [12].

The microflora of the healthy gingival crevice is relatively sparse and composed mainly of Gram-positive cocci, especially *Streptococcus* spp. The crevice has a lower redox potential than most sites within the oral cavity, which encourages initially the growth of *Actinomyces* spp. and an increase in capnophilic (carbon dioxide-requiting) bacteria such as *Actinobacillus actinomycetemcomitans*. Toxins released by these bacteria induce an inflammatory response in the gingival tissues with a resultant increase in gingival crevicular fluid. How and the provision of nutrients essential to the changing needs of the plaque flora. The environment ultimately changes to one that can support the growth of obligately anaerobic bacteria and spirochaetes. The inflammatory reaction in the gingiva progresses, with resultant chronic marginal gingivitis [1, 6].

Cross-sectional and longitudinal studies of the predominant cultivable microflora have revealed that of the 300-400 bacterial species that can inhabit the oral cavity, only a small number are regularly associated with periodontal diseases. According to the specific plaque hypothesis, particular species of microorganism are responsible for causing each type of periodontal disease. For example, early workers observed large numbers of a spirochaetes in sections of tissue from acute necrotizing ulceracive gingivitis, and believed this organism to play a major role [4, 8]. Later, studies on localized juvenile periodontitis implicated *Actinobacillus actinomyce-temcomitans* as a possible pathogen in this disease while, more recently, *Porphyromonas gingivalis* has been suggested as an important

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agent in adult periodontitis [6]. Owing to the difficulties in identifying aetiological organisms in periodontal disease, a modified set of criteria has been developed for a specific microorganism to be considered as a periodontal pathogen. The species that have been implicated in periodontal disease by various workers are *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Bacteroides forsythus* and *Prevotella intermedia* are currently viewed as the mainstream periodontal pathogens [1, 3, 5, 9].

Adult periodontitis may have its onset in adolescence and continue for the life of the individual, the severity increasing with age. Early work suggested that the course of the disease was a slow, constant and progressive destruction of the tissues [8]. However, more recently it has been proposed that the disease occurs in short periods (bursts) of destruction followed by periods of inactivity, these occurring randomly with respect to time and site within an individual [8]. There is currently much interest in developing methods to detect exactly when periodontal disease is active. The efficiency of periodontal disease prevention could be greatly increased and treatment better focused if the clinician or public health administrator were able to identify, in advance, those sites, subjects or groups who would experience periodontal disease activity. Periodontal disease activity refers specifically to the dynamic stage of the disease characterized clinically by loss of supporting bone and connective tissue attachment.

The search for the aetiology of periodontal disease must consider both host and microbial factors. The exact roles of each remain unclear but, as with most infections, it seems likely that the clinical outcome in periodontal disease is a result of the complex interactions between a wide range of host and microbial factors [12].

Microbiology latest successes provide the dependence of passionately inflammatory-dystrophic process in periodontal pocket homing with opportunistic

and pathogenic microorganisms [2, 3, 4]. There was determined a dependence between oral cavity hygiene state and of periodontal pockets disbiosis degree in the patients with periodontitis by most authors [1, 4, 6,11].

The present work aim was to study the changings character of periodontal pockets microbiocenosis as well as the local natural immunity in the patients with chronic generalized periodontitis.

Materials and methods:

Clinical, laboratory and microbiological examination have been performed correspondingly to the putted aim in the 36 patients with chronic generalized periodontitis (CGP). The patients age was fluctuated from 45 till 65 years. 75% of the people comprised women while 25% were men. Corresponding 20 people on age and gender with an intact periodontium comprised control group. Clinical and laboratory-instrumental examination consisted of the general-clinical methods of diagnostics with studying the patients complaints, anamnesis and objective state as well as major gingival indexes and probes using [5,9].

The material taken from periodontal pockets of patients with periodontitis or from gingival crevice in the control group was performed with a sterile paper pin 1 sm long placed into sterile physiological solution and wash carefully. Microbial colonization as well as opportunistic and pathogenic microorganisms release was performed by the material inoculation to the special, selective and differentially diagnostic media: bloody agar, yolk-salt agar, Sabouraud`s agar and Endo media. Samples were incubated during 24-48 h at the temperature 37^0 C. The microbial colonization was determined by microorganisms calculation in 1 ml of the material (colony forming units per milliliter - CFU/ml).

Oral cavity natural immunity state was assessed by salivary lysozyme activity determining as well as gingival blood leucocytes phagocytic activity detective

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method and their ability to capture latex particles. Phagocytic index and percent of phagocytosis were calculated by general methods [12].

Five major periodontal pathogenic bacteria content peculiarities microbiological examination was performed by polymerase chain reaction (PCR) method with a help of special reagents of „Gentex" company.

The examined results statistic processing was made on the PC by means Microsoft Excel Office 2007. The received results valuability was analyzed by the Student`s criterium.

Results discussion:

Studying the periodontal pockets microflora of quantitative and qualitive content in the patients with periodontitis and the people`s control group as well as the microbiological examinations performed analysis exhibited a significant difference in periodontal pockets colonization in these mentioned groups (Tab. 1). Periodontal pockets general microbial colonization in the patients with periodontitis comprised $1,95x10^{10} \pm 0,84x10^{10}$ CFU/ml, while the one of the control group was $1,55x10^{9} \pm 0,50x10^{9}$ CFU/ml so, it was reliably increased in 12 times ($p < 0,001$).

It has been established as a result of the microbiological examinations performed that the patients with CGP had dominant opportunistic and pathogenic microorganisms associations comparatively to the people with the intact periodontium with prevalent saprophytic and opportunistic microflora (*Lactobacilli, Streptococci*) (Tab. 1). *Staphylococci, Spirochaetes, Bacteroides* and *Candida* fungi were dominant in the periodontal pockets microslides content in the patients with CGP.

Thus, microbiocenosis qualitative content the of patients with periodontitis was characterized by the staphylococci highest percentage comprising 91,7% but reliable differences comparatively to the same index in healthy people weren`t determined.

Periodontitis patients quantity whith bacteroides in their periodontal pockets was

higher than the control group same index in 2,5 times ($p<0,05$) while *Spirochaetes* were revealed of were revealed in 1,7 times ($p<0,05$) more often, than in the control group.

Candida fungi released frequency was reliable increased in 2,7 times ($p<0,01$) in the patients with periodontitis that was also characteristic for them. At the same time saprophytic microflora quantity significant lowering was determined in the periodontal pockets of patients with periodontitis. So, the patients percentage with *Streptococci* in periodontal pockets got decreased in 1,7 times ($p<0,05$) and comprised 35% (Tab. 1).

Table 1. Composition of periodontal pockets and gingival crevice microflora (M±m, %)

Microorganisms	Frequency of microorganisms colonization			
	The intact n=20 %	Absolute values	Patients with CGP, n=36 %	Absolute values
Streptococcus spp.	100	20	58,3	19
Staphylococcus spp.	85	17	91,7	35
Lactobacillus spp.	100	20	50	18
Actinomyces spp.	35	7	41,7	15
Leptotrichia spp.	65	13	25	9
Bacteroides spp.	35	7	75	27
Veilonella spp.	20	4	16,7	6
Spirochaetaceae	50	10	83,3	30
Corynebacterium spp.	35	7	33,3	12
Candida spp.	25	5	66,7	24

It should be mentioned that dysbiosis disorders were visibly expressed in the patients with CGP. *Lactobacilli* release frequency from periodontal pockets in the patients sick in CGP was reduced in 2 times ($p<0,01$) comparatively to the intact group. These microorganisms were revealed in 20% ($p<0,001$) of cases against 100% in the hosts with an intact periodontium. CGP patients amount with *Leptotrichia* release from periodontal pockets was reliably diminished in 3 times ($p<0,01$).

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The percent of people whose microslides from periodontal pockets contained *Actinomycetes, Veilonella* and diphtheroides didn`t have reliable differences between sick people and the ones with no changes in periodontal tissue.

Different species opportunistic and pathogenic bacteria release rate was significantly varied in the patients` examined groups. So, periodontal pocket microorganisms separate groups characteristics analysis allowed determining that CGP patients possessed valuable in 3,3 times ($p<0,001$) lowering in *Streptococci* exhibition. These microorganisms belong to the symbiotic flora (Tab. 1).

Periodontal pockets microbial content molecular genetic study have established that 5 examined patients had DNA of one periodontal pathogenic bacteria type that comprised 13,9%. 10 patients (27,8%) were revealed to have two types of periodontal pathogenic bacteria. Three microorganisms species were revealed by us in 12 patients that comprised 33,3%. *Treponema denticola* and *Porphyromonas gingivalis* were the mostly often met in the associations. Four types of periodontal pathogenic bacteria were observed in 9 patients (25%). Besides mentioned microorganisms one could see also *Tanerella forsythensis* and *Prevotella intermedia*.

Periodontal pathogenic bacteria were placed in a proper order in the people sick in CGP by DNA release rate: *Tanerella forsythensis* (72,2%), *Prevotella intermedia* (58,3 %), *Porphyromonas gingivalis* (52,8 %), *Treponema denticola* (50%), *Actinobacillus actinomycetemcomitans* (36,1 %).

Thus, periodontal pockets microbiocenosis analysis performed in the patients with periodontitis showed valuable changes both in qualitative content and microorganisms' general amount which were the mostly expressed in the patients with CGP. The experiments made testify to microbial factor essential role in periodontal inflammatory diseases development at CGP.

Oral cavity natural immunity state study has demonstrated that CGP patients had phagocytic defensive link inhibition. So, the patients of examined group was characterized by phagocytosis percent equal to 41,8±3,14%, phagocytic index 3,9±0,43 while the control one - correspondingly 68,8±3,39% and 6,81±0,53. It was less to 39,3% ($p<0,05$) and 42,7% ($p<0,05$) correspondingly (Tab. 2).

Table 2. Natural immunity indexes in the patients with chronic generalized periodontitis

Examined indexes	Statistic indexes	Control group	Patients with generalized periodontitis
Percent of phagocytosis (%)	M±m p	68,8±3,39	41,8±3,14 0,05
Phagocytosis index	M±m p	6,8±0,53	3,97±0,43 0,05
Activity of lysozyme (mcg/ml)	M±m p	9,57±2,17	2,45±1,09 0,005

Phagocytosis as the natural immunity index plays rather important role in the organism reactive ability assessment. Neutrophils phagocytosis ability analysis allowed establishing blood polymorphs phagocytic insufficiency in the patients with chronic periodontitis. Antimicrobial potential weakening represents one of "functional exhaustion" signs under durable chronic process conditions coming after neutrophils previous stimulation.

As the given data show, resulted information, the oral saliva lysozyme activity in the patients with periodontitis gets decreased in 3,9 times ($p<0,05$) in comparison to the control group (Tab. 2). Thus, the investigations performed prove the fact that both microbial factor and weakening

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the host immune response in oral cavity have a crucial role in periodont inflammatory diseases development. Periodontal pathogenic bacteria aggressiveness is dealt to their capability to produce such toxic substances as endotoxins (lipopolysachharide), proteolytic enzymes, antigens, mitogens, chemotaxis factors and the other destructing periodontium tissue directly. The induction of an inflammatory response and IL-1 production in response to plaque antigens may cause indirect activation of host collagenases and stromolysins which degrade connective tissues [1,12]. Periodontal pathogenic microbes have an essential importance in fastly-progressing periodontitis forms development mainly in the young. Elder people have oral hygiene unsatisfactory state. That`s why mostly it`s impossible to separate a definite causative agent dealing to inflammatory process in periodontium. Probably, inflectional agent or agents represent non-single aetiological factors realizing their action through complicated interrelations to the host organism immune system autoreactive cells.

Periodontal inflammatory diseases can be described as an equilibrium disorder result between bacterial symbiosis and oral cavity tissues. Marginal infecting and active inflammatory reaction with immune response caused by it at the initial stages transforming then in the pathological one represent one major factor in periodontal diseases development.

Conclusions:

1. Periodontal pockets general microbial colonization in the patients with periodontitis is 12 times more ($p < 0,001$) than in the people with intact periodontium.
2. Periodontal pockets microbiocenosis in the patients with periodontitis is defined with aggressive differed opportunistic microflora colonization rate increase while symbiotic

stabilizing microflora expression rate is lowered.

3. Lowering the leucocytes phagocytosis activity is observed in the patients with periodontitis that is testified by phagocytic index lowering in 1,6 times ($p < 0,05$).
4. Lysozyme activity in saliva in the patients with periodontitis is lowered in 3,9 times ($p < 0, 005$) comparatively to norm.

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