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ORIGINAL ARTICLES

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Cytological and microbiological investigations of professional hygiene efficiency in patients with generalized periodontitis

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ABSTRACT

Aim: The purpose of this study is to assess the impact of occupational hygiene procedures for microbiological and cytological contents of periodontal pockets.

Material and Methods: Cytological and microbiological content of the periodontal pockets before treatment and after professional hygiene procedures including scaling with hand instruments and root cementum polishing have been investigated in patients with periodontitis.

Results: According to obtained data it can be resumed that in periodontitis patients with the depth of pockets 3-5,5 mm before professional hygiene all the pockets contain great number of *Cocci, Spirochetes, Candida Albicans, Flagellated* rods and *Protozoa* species. It was proved by revealing of small amount of Polymorphonuclear leukocytes with active phagocytosis. After scaling and planing of the roots, a decrease in the number of *Protozoa* and *Candida Albicans* was observed in 97% and 72% of the investigated cells, respectively.

Conclusions: Cytological and microbiological content of periodontal pockets before treatment and after professional hygiene procedures including scaling and root planning testify to the level of local protective mechanisms, especially process of phagocytosis and virulence of microbial species in periodontal pockets.

KEY WORDS: periodontitis, scaling, microbiological, cytological investigation

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INTRODUCTION

Professional oral hygiene is an important part of treatment of periodontitis patients. It includes the removal of dental calculus – scaling, as well as leveling and polishing the surface – root planning [1]. The procedure for leveling and grinding the enamel and planning of tooth root cement improves the treatment results, as pathologically altered layers are removed from the surface of the root of tooth, which contains the remains of the gram-negative microflora. The surface of the root of tooth is aligned and smoothed out after the manipulation procedures by dental scaling, which makes the plane of the dental root surface smooth up to the mirroring effect [2, 3]. Medical practice proves that after performing the instrumental procedure of dental scaling, the degree of smoothness of the root part of tooth is not as important criterion as the reduction of the critical number of gram-negative microflorae [4-6]. Main purpose of professional hygiene in these patients is reduction of total number and partial inactivation of microbiota in periodontal pockets.

Pathogenesis of periodontal diseases is usually associated with microorganisms, mostly anaerobes, of the subgingival dental plaque. In advanced periodontitis, gram-negative anaerobic flora is prevailing: bacteriodes, fusobacteria etc. Developed periodontitis is characterized by the presence of great numbers of spirochetes and different specific microorganisms. Microflora of the oral cavity may be of different kinds and variable localization [7, 8].

The presence of periodontal pathogens and their metabolic by-products in the mouth may in fact modulate the immune response beyond the oral cavity, thus promoting the development of systemic conditions.

The process of tissue destruction results from the elaboration of bacterial substances that directly or indirectly cause degradation of the periodontal tissues [9] (Fig. 1).

The production of immunoglobulin-degrading proteases by (*P. gingivalis, P.intermedia, P.melaninogenica, Capnocytophaga sp.*) may counteract these host defenses. Some bacteria produce substances suppressing the activity of polymorphonuclear leucocytes and lymphocytes. *A. actinomycetemcomitans* produces leukotoxin inhibiting the function of PMNs and killing of mature B and T cells, *P. gingivalis* inhibites of superoxide production by PMNs [9, 10].

Pathogenic bacteria also produce enzymes capable of degrading host tissues: collagenase, trypsin-like enzyme, keratinase, arylsulfatase, neuraminidase, fi-

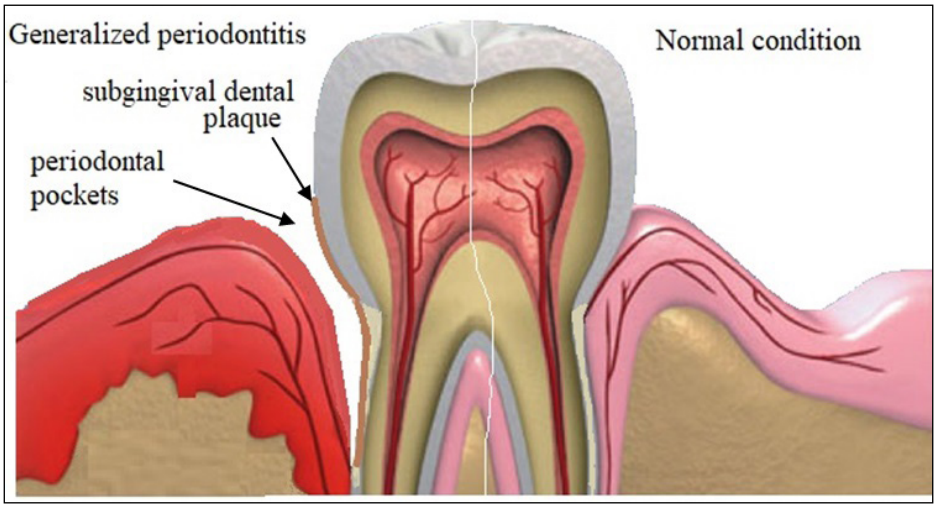

Fig. 1. Clinical situation before the operation teeth 1.4 and 1.5.

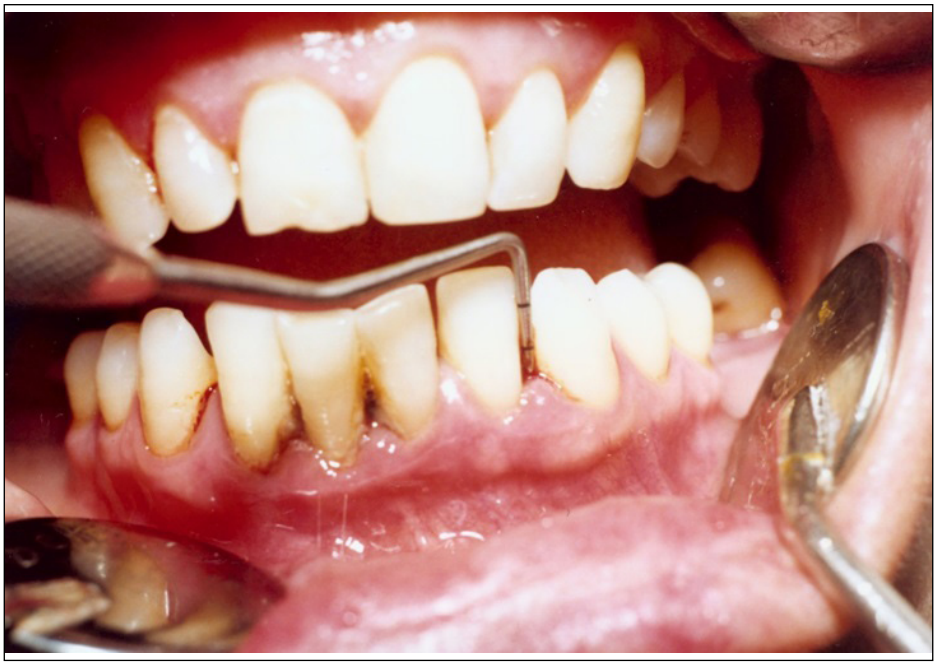

Fig. 2. Slowly progressing not active periodontitis, II-nd stage of heaviness in patient B., 43-year-old male. The depth of a periodontal pocket at the medial surface of 33 tooth is 6mm.

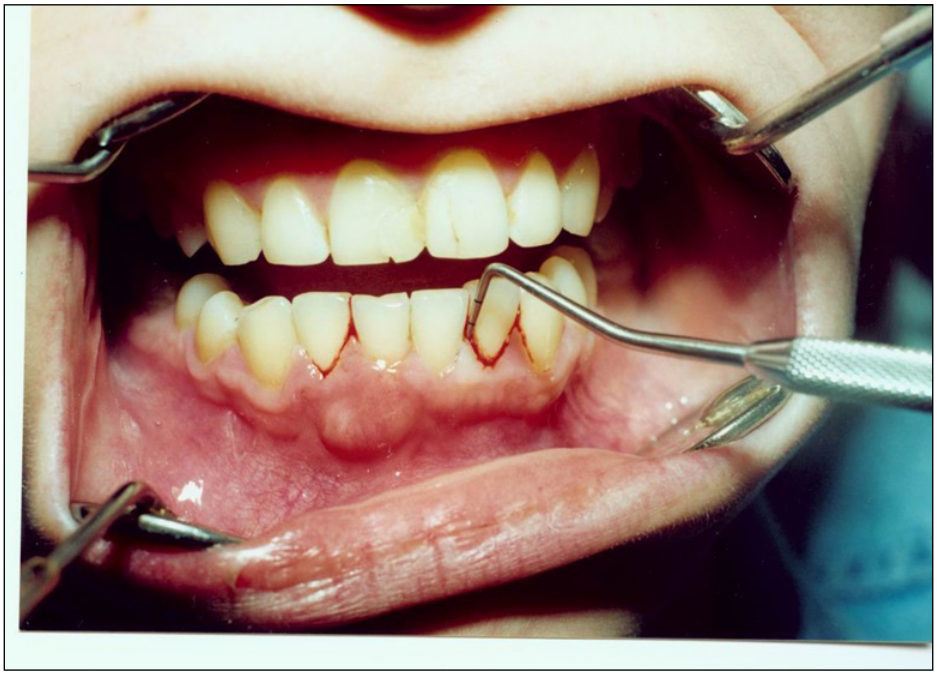

Fig. 3. Chronic generalized periodontitis, II-nd stage of heaviness, bleeding on probing of 1st degree, chronic periodontal abscess in the region of 41 and 42 teeth.

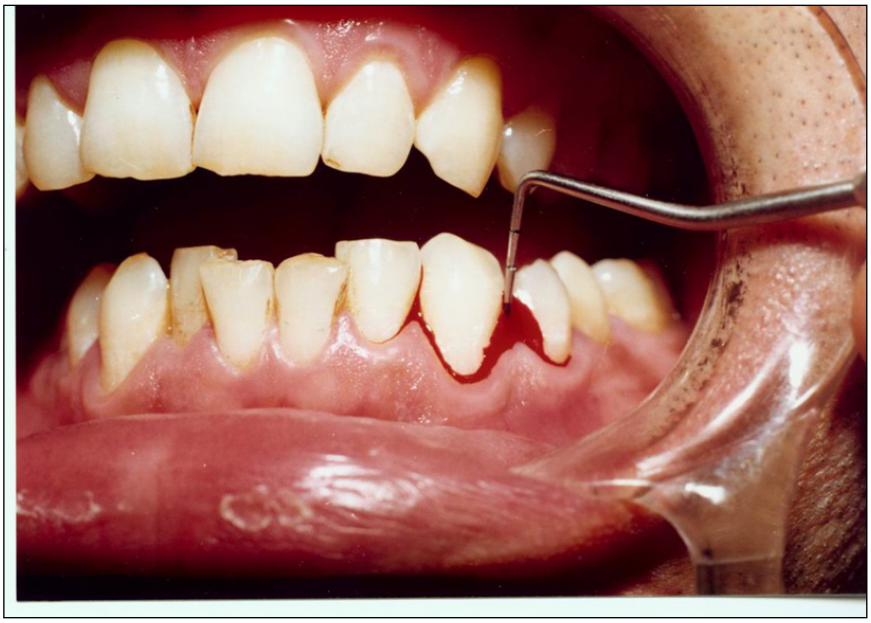

Fig. 4. Exacerbation of chronic generalized periodontitis, II-nd stage of heaviness, bleeding on probing of the 3-rd degree.

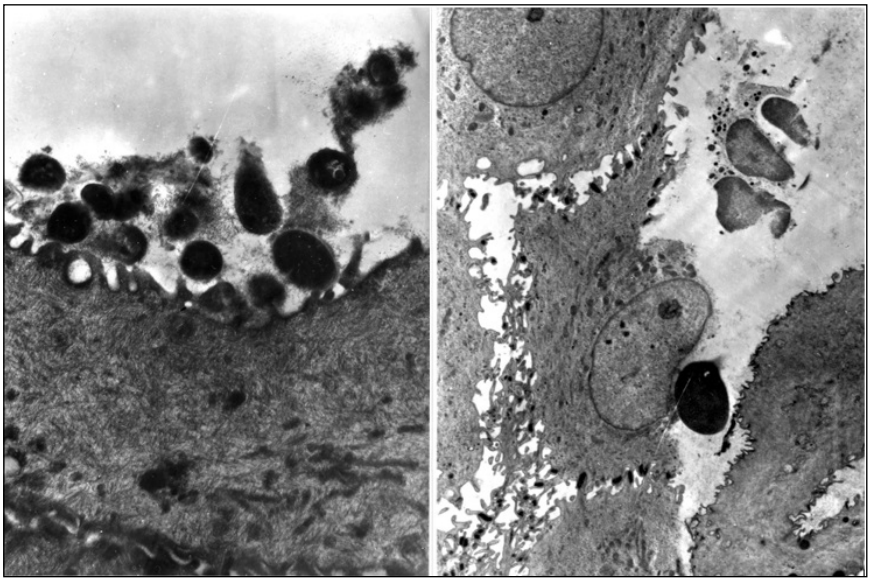

Fig. 5. Ultrastructural organization of the surface layers of human gums with dystrophic periodontal damage (periodontitis):
a) accumulation of bacterial bodies on the surface of the epithelium. Coll. x 5,000.
b) stratified cells of the granular layer, in the spaces between which elements of destroyed platelets and microbes were found. Coll. x 5.

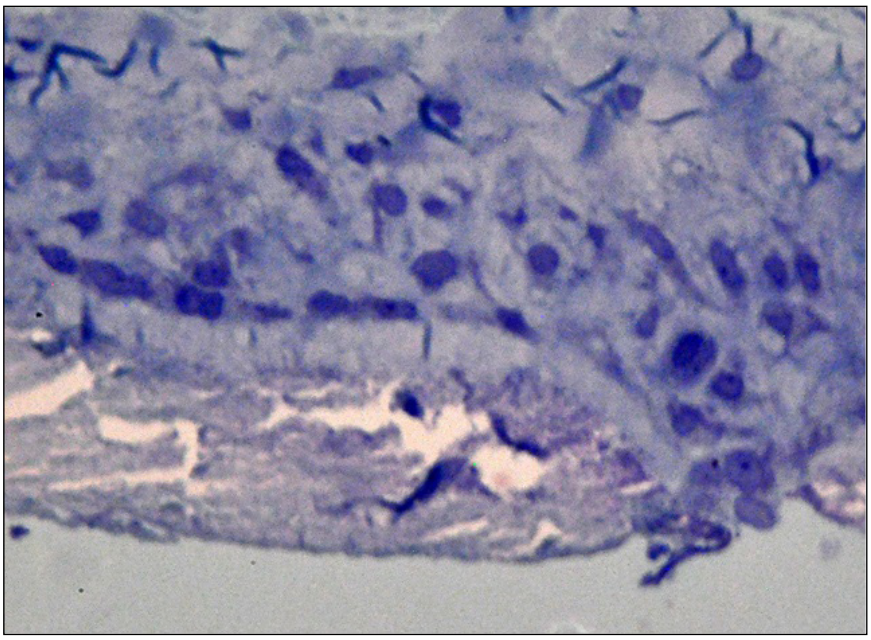

Fig. 6. A fragment of the multilayered squamous epithelium of the gums with moderately pronounced dystrophic changes. Semi-thin cut. Stained with toluidine blue. Magnification 400. (The photos were taken on a Nikon E200 microscope with a Nikon D5000 camera).

Table 1. Cytological content of periodontal pockets in patients with generalized periodontitis (periodontal pockets 3-5,5 mm)

Time of examination	Cells (M±m)					
	Polymorphonuclear leukocytes			Macrophages	Lymphocytes	Epithelial cells
	without changes	without changes	without changes			
Before the treatment (1)	27,7±0,7	11,7±0,5	12,3±0,5	0-1	0-1	1-2
After the treatment (2)	5,2±0,2	1,7±0,2	2,8±0,2	1-2	1-2	1-2
P 1,2	< 0,01	< 0,01	< 0,01	-	-	-

Table 2. Distribution of periodontal pockets according to the content of *Flagellated rods* and *Protozoa* in patients with Generalized periodontitis (periodontal pockets 3-5,5 mm)

Periodontal pockets being explored		Estimation of microbiota *			
		Flagellated rods		Protozoa	
		-; ±	+; ++	-; ±	+; ++
Before the treatment	absolute number	63	237	233	67
	%	21,0±2,3	79,0+2,3'	77,8±2,3	22,2±2,3
After the treatment	absolute number	152	108	251	9
	%	58,3±3,0	41,7±3,0	96,5±1,1	3,5±1,1
t		9,8		7,5	
P		< 0,01		< 0,01	

* - not fined, ± - small amount, + - moderate amount, ++ - many.

Table 3. Distribution of periodontal pockets according to the content of *Candida Albicans, Cocci* and *Spirochetes* in patients with Generalized periodontitis (periodontal pockets 3-5,5mm)

Periodontal pockets being explored		Estimation of microbiota *					
		Candida		Cocci		Spirochetes	
		±	+;++	-;±	+;++	-;±	+;++
Before the treatment	absolute number	103	197	69	231	90	210
	%	34,3±2,7	65,7±2,7	23,1 ±2,4	76,9±2,4	30,1±2,6	69,9±2,6
After the treatment	absolute number	189	71	141	119	125	135
	%	72,7±2,8	27,3±2,8	54,4±3,1	45,6±3,1	48,1±3,1	51,9±3,1
t		9,8		8,0		4,5	
P		<0,01		<0,01		<0,01	

* - not fined, + - small amount, + - moderate amount, ++ - many.

bronectin- degrading enzyme, phospholipase A [10]. Some bacterial products inhibit the growth or alter the metabolism of host tissue cells, these are: ammonia, volatile sulfur compounds, fatty acids, peptides, indole.

Thus, virulent properties can be broadly categorized into factors that enable a bacterial species to colonize and invade host tissues, and factors that enable a bacterial species to directly or indirectly cause host damage [11, 12]. Bacterial species that have been identified as capable of tissue invasion are strongly associated with diseased sites. The ability to invade has been proposed by Loesche W.J. [11, 13] as a key factor distinguishing pathogenic from non-pathogenic gram-negative species. For bacteria to survive in the periodontal environment, they must evade the host mechanisms involved in bacterial clearing.

Macrophages, monocytes exposed to bacterial endotoxin (lipopolysaccharide) release interleukin-1, tumor necrosis factor and prostaglandins. These host- derived cytokines stimulate a bone resorption, activate or inhibit other host immune cells [12, 13].

Neutrophils can be seen in relatively high numbers in both the gingival connective tissue and the sulcus. It is common to see them migrating through the sulcular and junctional epithelium. These cells perform a protective role by phagocytizing bacteria and other foreign substances. They contain lysosomes which in turn contain a variety of hydrolytic enzymes that kill bacteria after phagocytosis. When neutrophils die these enzymes are released and may contribute to tissue destruction.

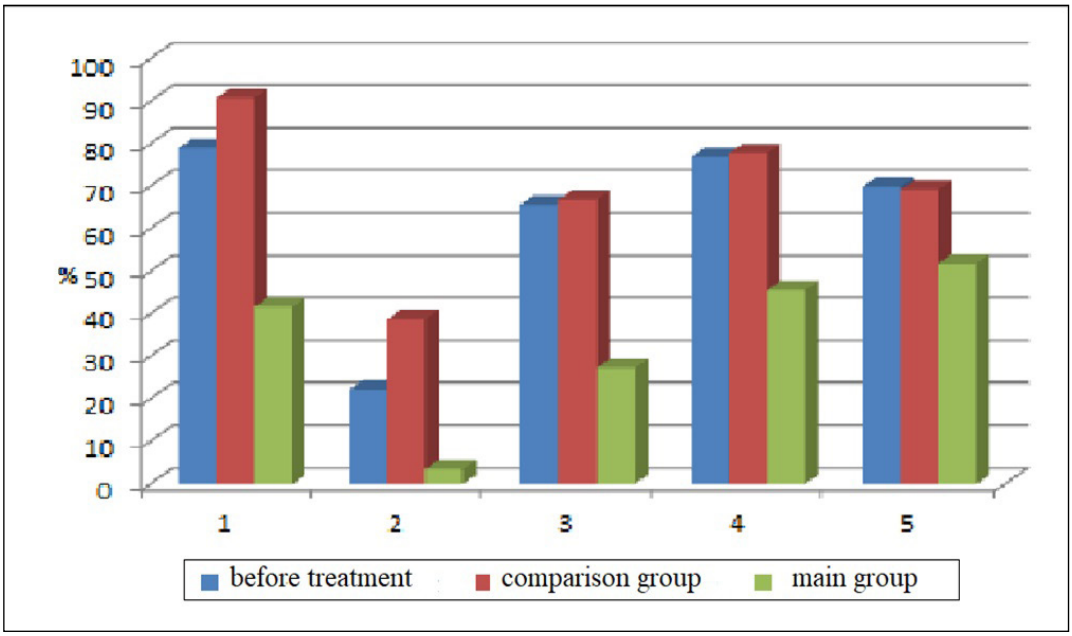

Fig. 7. Characteristics of the microflora of periodontal pockets in mild periodontitis before and after treatment. Horizontally: 1 – *Flagellated rods*; 2 – *Protozoa*; 3 – *Candida*; 4 – *Cocci*; 5 - *Spirochetes*. Vertically: the number of periodontal areas (%).

Neutrophils are a major component of the innate host response against bacterial challenge, and under homeostatic conditions, their microbicidal functions typically protect the host against periodontitis. However, a number of periodontal pathogens developed survival strategies to evade neutrophil microbicidal functions while promoting inflammation, which provides a source of nutrients for bacterial growth [13-15].

Neutrophils play a key role in periodontal health and disease. In their absence or when they are functionally inferior, as is the case with some congenital disorders, patients develop severe forms of periodontitis at an early age. These observations make it possible to state that the presence of immunocompetent neutrophils is important for homeostasis. However, the presence of excess or hypersensitive neutrophils due to systemic priming or innate immune conditioning results in an imbalance of the host-microbe interaction in the periodontium, leading to dysbacteriosis and inflammatory tissue breakdown [16-18].

In a rat model of periodontal disease using bacterial species incriminated in the pathology of human periodontitis (*Aggregatibacter actinomycetemcomitans (A.a), Fusobacterium nucleatum (F.n) and Streptococcus oralis (S.o)*) neutrophilic infiltrate in the periodontal tissue, and periodontal lysis was identified [19, 20].

That is why it is important to estimate not only microbiota but also cytological spectrum of periodontal pockets to evaluate host cells respond to bacteria invasion.

AIM

The aim of present study is estimation of the influence of professional hygiene procedures on the microbiological and cytological content of periodontal pockets.

MATERIALS AND METHODS

During the study, we examined 47 patients (20-40 years old) with generalized periodontitis. All the patients were thoroughly motivated for the prophylactic dental examination and the following treatment in the case of necessity. Periodontal condition of all teeth has been conducted (including the estimation of pockets depth and presence of bleeding on probing) during the examination) (Fig. 2, Fig. 3).

In each patient with generalized periodontitis minimum 20 teeth were preserved and not less than 10-12 true periodontal pockets (3-5,5 mm) and radiographic symptoms of bone destruction have been revealed. All the patients were not reported serious general pathology.

Mechanical plaque and calculus cleaning and tooth surface polishing was conducted in each periodontal pocket being combined with antiseptic – 0,2% Solution of Chlorhexidinum bigluconat.

The level of inflammation in periodontal tissues have been estimated with bleeding index [2, 6]. Gingival bleeding varies in severity, duration and the ease with which it is provoked. The severity of the bleeding depends upon the intensity of the inflammation (Fig.4).

Smears for research were taken from periodontal pockets after rinsing the oral cavity with physiological solution and saliva secretions. When making smears, Romanovsky staining was used.

RESULTS

Conducted investigations, considering clinical data, allow to some extent the estimation of pathological process in periodontal pockets.

According to cytological analyses (Table 1), before professional hygiene all smears show great number of unchanged Polymorphonuclear leukocytes (PMN), in average 27,7±0,7; relatively small amount of PMN with active phagocytosis and great number of destroyed PMN.

Almost all samples show some Lymphocytes and Epithelial cells, but macrophages are absent. Great number of destroyed Polymorphonuclear leukocytes should be noted (12,3±0,5). This type of cytological picture testifies to insufficient functional activity of local protective factors, Polymorphonuclear leukocytes.

All sections were prepared from the obtained tissue blocks using a UMTP-3M ultramicrotome and contrasted in solutions of uranyl acetate and lead citrate. Contrasted ultrathin sections were studied and photographed using an electron microscope UEMV - 100K (accelerating voltage 75 kV) (Fig. 5, Fig. 6).

After the debridement and root planing procedures analyzed smears from periodontal pockets show substantial decrease in number of Polymorphonuclear leukocytes (5,2±0,2). Considerable lowering of Polymorphonuclear leukocytes with active phagocytosis (1,7±0,2) has been noted. Especially important and positive change after professional hygiene tends to be substantial decrease (2,8±0,2) in number of destroyed PMN. Almost all samples show solitary Lymphocytes and Epithelial cells, with some macrophages, being completely absent before the treatment. These changes testify to positive course of pathological process in the periodontium.

Investigation of microbial spectrum followed cytological analysis of periodontal pockets (Table 2, Table 3). *Cocci, Spirochetes, Candida Albicans, Flagellated rods and Protozoa* have been revealed. Bacteria were in different stages of phagocytosis. In 79% of investigated smears from periodontal pockets before treatment prevailed flagellated rods, in 22% Protozoa were in great amounts. *Candida Albicans* was present in big quantities in 66% of examined smears, *Cocci* - in 77% of examined samples and Spirochetes - in 70% of cases.

All investigated pockets were much less contaminated after scaling and root planing. 58% of investigated samples showed small amount or absence of *Flagellated rods* and there were almost 97% of periodontal pockets with very small amounts or not revealed Protozoa species. It should be mentioned that in 42% of pockets *Flagellated rods* were still in big quantities (Table 2).

In 72% of pockets after treatment *Candida* was not defined, while *Cocci* and *Spirochets* were in big quantities in 45% and 52% of pockets accordingly (Table 3).

According to obtained data it can be resumed that in periodontitis patients with the depth of pockets 3-5,5 mm before professional hygiene all the pockets contain great number of *Cocci, Spirochetes, Candida Albicans, Flagellated rods and Protozoa* species. Functional activity of local defence mechanisms in examined samples, especially Polymorphonuclear leukocytes, was insufficient before the treatment. It was proved by revealing of small amount of Polymorphonuclear leukocytes with active phagocytosis, great number of destroyed olymorphonuclear leukocytes and absence of macrophages in examined smears.

After scaling and root planing positive changes in the number of microbial species and cytological spectrum of periodontal pockets have been observed. Considerable decrease in number of *Protozoa* and *Candida Albicans* was present in 97% and 72% of investigated pockets accordingly (Fig.7).

Almost half of investigated samples were characterized by low level of *Cocci* and *Spirochets*. These changes were accompanied by positive modification of periodontal pockets cytology: considerable lowering of Polymorphonuclear leukocytes with active phagocytosis substantial decrease in number of destroyed PMN.

DISCUSSION

Almost all specimens show single lymphocytes and epithelial cells with some macrophages present. It can be assumed that phagocytosis, as a reaction to the presence of pathogenic microbes in periodontal pockets, can be expressed to a certain extent. In a good or satisfactory condition of the organism phagocytosis leads to the total elimination of microbes, while in lowered resistance of the organism or very high virulence of microbes in smears were revealed neutrophils filled with great number of microbes. The presence in smears from periodontal pockets of great numbers of microbes in combination with absence or weak phagocytic reaction of neutrophils is considered as a bad prognostic sign for periodontal pocket [5, 6].

Superficial and intermediate cell values were significantly greater in patients with AP than in patients with CP or the control group. Histiocyte number was higher in patients with CP than in those with AP and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with CP, and there were differences between patients with periodontitis and the control group [10, 12].

A significant decrease in clinical and biochemical parameters after two months of treatment in the curcumin group ($P<0.05$). Almost the same pattern for the chlorhexidine group ($P<0.05$), with minor differences from

baseline for albumin (P>0.05). A reduction in clinical parameters (P<0.05) and an increase in CRP, ALP and TP levels were observed after scaling and root planing in the SRP group. There were no significant differences between the three main groups in terms of clinical parameters (P>0.05), except for gingival index and biochemical parameters (P<0.05) [8, 11, 13].

Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of this study was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis (AP) and chronic periodontitis (CP). Superficial and intermediate cell values were significantly greater in patients with AP than in patients with CP or the control group. Histiocyte number was higher in patients with CP than in those with AP, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with CP, and there were differences between patients with periodontitis and the control group [12, 14-20].

CONCLUSIONS

Cytological and microbiological content of periodontal pockets before treatment and after professional hygiene procedures including scaling and root planning testify to the level of local protective mechanisms, especially process of phagocytosis and virulence of microbial species in periodontal pockets.

The presence in smears from periodontal pockets of great numbers of microbes in combination with absence or weak phagocytic reaction of neutrophils is considered as a bad prognostic sign for periodontal pocket.

Cytological and microbiological investigations of periodontal pockets help in estimation of treatment efficiency and prognosis in patients with periodontitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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