MICROBIOLOGICAL STUDIES OF SALIVA, GINGIVAL SULCUS AND GINGIVAL POCKETS CONTENT IN ALCOHOL PRODUCTION WORKERS WITH PERIODONTAL TISSUE DISEASES

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Abstract

Introduction. In the last decade, a number of studies conducted on the species and quantitative composition of the microflora of periodontal pockets of patients have shown the participation of complex, multicomponent associations of microorganisms in the development of inflammatory processes in periodontal tissues. Significant variability of microorganisms under the influence of the external environment, including the industrial one, the formation of new microbial associations, and increase in the number of resistant strains determine the need and constant relevance of identifying the microorganisms that are most commonly present in the periodontal pockets of certain groups of patients, taking into account the exogenous influences. **Aim of the study:** to investigate the species composition of periodontal pocket microflora and the frequency of isolation of certain types of microorganisms, for possible specificity of the microbial landscape under the influence of occupational pathogens in generalized periodontitis. **Materials and methods**. Saliva bacterioscopic examinations were performed on 62 alcohol production workers, of whom 28 were diagnosed with catarrhal gingivitis (CCG) and 34 with generalized periodontitis (GP) of I-III degrees. The control group consisted of 21 people without dental morbidity. Study of microorganisms was carried out using the Bergey classification scheme. Statistical processing of the obtained results was performed on a personal computer using the licensed programs «Microsoft Excel» and «Statistica 8».**Results and discussion.** The results of microbiological studies on alcohol production workers with catarrhal gingivitis indicated the highest percentage of spirochetes, fusobacteria and veilonellae (68.2%) while, in individuals with intact periodontium, the above species were not infected. The share of α-hemolytic streptococci accounted for 54.5% ($p<0.05$). In generalized periodontitis in patients of the main group, α-hemolytic streptococcus, spirochetes, fusobacteria, wayloneles and fungi of the Candida grnus were inoculated in 100% of cases. In 27 patients (50.9%) cryptococci were identified, which were not studied in patients with catarrhal gingivitis and in those with intact periodontium. The frequency of inoculation of β-hemolytic streptococci exceeded that of the comparison group by 61.37 % ($p<0.05$) and, in patients with catarrhal gingivitis – by 44.81 % ($p<0.05$). In distillery workers with generalized periodontitis from the main group, a significant increase in *Escherichia coli* was observed in the subjects, compared to the data of both previous groups (p; $p_1<0.05$). **Conclusions.** In patients with periodontal tissue diseases working in alcohol production, an increase in gram-negative microflora and, accordingly, an increase in the degree of oral dysbiosis were determined, in the presence of a wide range of pathogenic microflora and yeast-like fungi, the frequency of which progresses with the deepening of inflammatory and destructive changes in the periodontium.

Keywords: *chronic generalized periodontitis, chronic catarrhal gingivitis, saliva, periodontal pockets, oral microbiocinosis*.

1. INTRODUCTION

The pathogenesis of periodontal diseases is polypathogenic, consisting of numerous and different links: pathological processes at the level of the whole organism, its cells and environments; biochemically reactive substrates. Thus, today, generalized periodontitis is considered not only as periodontal inflammation, but also as a reaction of the body to the effects of bacterial infection due to the adverse effects of various nonspecific factors [1]. Among the latter, the most acute problem today is the problem of environmental disorders that can affect the development and course of periodontal diseases due to changes in local resistance, the development of autosensitizing mechanisms, and changes in the immune status of the person in general [2].

The significant impact of the unfavorable environmental factors on the state of human organs and systems is confirmed by the tendency to increase the incidence of periodontal disease in industrialized countries which, in different age

groups, reaches 80 to 100% [3]. In this regard, the risk group for periodontal diseases naturally includes workers of industrial enterprises who are exposed to occupational pathogens of different nature, intensity and duration of exposure in the course of work, which determines the peculiarities of periodontal diseases evolution and justifies approaches to their treatment and prevention in workers of different occupational groups [4].

An important role in the full functioning and maintenance of the integrity of the entire complex of periodontal tissues is played by the oral fluid, which contains factors of teeth and oral mucosa protection, promotes mechanical and chemical cleansing from bacterial and toxic effects, plays a role in the metabolism of the integumentary epithelium, and is "a means of combining periodontal structures with the environment" [5]. The oral microflora can become an etiopathogenic irritant if the protective factors of the periodontium are weakened, among which the quantitative and qualitative parameters of the oral fluid play an important role [6]. In cases of occupational contact with harmful factors of the production environment (exposure to high temperatures, toxic substances), the composition and properties of the oral fluid change, which is one of the reasons for the high incidence of dental diseases in workers of industrial enterprises. Experimentally, a change in the properties of the oral fluid (viscous saliva, dry mouth) and, accordingly, a dysfunction of the salivary glands, with a relatively short local exposure to pollutants, the mechanism of which may be associated with the depletion of the salivary glands as a result of massive irritation by an alimentary factor, has been established [7]. A decrease in saliva secretion and an increase in its viscosity impairs cleansing of the mucous membrane from microorganisms and their waste products, inhibits the formation and secretion of secretory immunoglobulin A, which prevents bacteria from attaching to the tooth surface, and also contributes to increased tartar deposition [8]. In situations of insufficient salivation, oral microorganisms easily overcome the reduced resistance of the mucous membrane, which leads to the development of an inflammatory process [9].

In the last decade, a number of studies on the species and quantitative composition of the microflora of periodontal pockets of patients have been conducted, which have shown the participation of complex, multicomponent associations of microorganisms in the development of inflammatory processes in periodontal tissues [10]. Significant variability of microorganisms under the influence of the external environment, including the industrial environment, the formation of new microbial associations, and an increase in the number of resistant strains necessitate and constantly require the identification of microorganisms that are most commonly present in the periodontal pockets of certain groups of patients, taking into account the exogenous influences [11].

In the professional literature, in addition to occasional reports about a significant prevalence of dental diseases in alcohol production workers, we have not found materials on the study of the species composition of saliva microflora and periodontal pockets under the influence of harmful factors of this production, which led to further research in the context of studying the mechanisms of development of the dystrophic and inflammatory processes in the periodontium in workers of this industry.

The **aim** of the study was to investigate the species composition of the periodontal pocket microflora and the frequency of isolation of certain types of microorganisms, for establishing the possible specificity of the microbial landscape under the influence of occupational pathogens in generalized periodontitis.

2. MATERIALS AND METHODS

Saliva bacterioscopic examinations were performed on 62 alcohol production workers, of whom 28 were diagnosed with catarrhal gingivitis (CCG) and 34 with generalized periodontitis (GP) of I-III degrees. The control group consisted of 21 people without dental morbidity.

The material for the study was taken with a loop, placed in a tube with culture medium and transported to the laboratory, where it was seeded on solid selective media. The shape and grouping of cells were examined on live and stained cell preparations using an Ergaval microscope. Bacterial motility was observed in the "hanging drop" preparation of young bacterial cultures [12]. The

presence of spores was determined by Ozheshko staining [13]. To detect the content of microbes, the mixed unstimulated saliva was diluted 10 times with saline and mixed with May-Grunwald dye in a ratio of 1 volume of dye to 1.5 volumes of saliva [14]. At the same time, diluted saliva was stained by Gram stain using carbolic solution of genzian violet, Lugol's solution and basic fuchsin solution by Zyl [15]. The study of microorganisms was carried out using the Bergey classification scheme.

Statistical processing of the obtained results was performed on a personal computer using the licensed programs «Microsoft Excel» and «Statistica 8».

3. RESULTS

As a result of bacterioscopic examinations, we found that the number of gram-positive bacteria in the comparison group was 80.26% and gram-negative microorganisms – 10.74 %.

In alcohol production workers with catarrhal gingivitis, gram-positive bacteria accounted for 66.96 % of the total number of microorganisms detected in this disease, which was 1.2 times less than the percentage of the comparison group (p_1<0.001). At the same time, in patients with CCG, an increase in gram-negative bacteria was found to be 3 times higher than in the comparison group (p < 0.001).

In patients with generalized periodontitis, the number of gram-positive flora decreased to 45% which, in percentage terms, was 1.8 times less than in the comparison group (p<0.001) and 1.5 times less than in patients with CCG (p_1≤ 0.001). Gram-positive flora was detected in patients with GP in 4 times more than in the comparison group (p<0.001), and, accordingly, 3 times more than in patients with CCG (p_1<0.001).

Table 1. Saliva microorganisms' content in the study groups

Indicators	Comparative group, n = 21	Chronic catarrhal gingivitis, n = 28	Generalized periodontitis, n = 34
Total number of microorganisms, cl/ml	$3.26\times10^6\pm0.22\times10^6$	$3.45\times10^7\pm0.51\times10^7$ *	$6.84\times10^7\pm0.53\times10^7$ *, •
The number of gram-positive bacteria, cl/ml %.	$2.91\times10^6\pm0.18\times10^6$	$2.31\times10^7\pm0.56\times10^7$ *	$3.08\times10^7\pm0.53\times10^7$ *, •
The number of gram-negative bacteria, cl/ml %.	$0.35\times10^6\pm0.22\times10^6$	$1.14\times10^7\pm0.52\times10^7$ *	$3.76\times10^7\pm0.56\times10^7$ *, •
Ratio of gram-negative and gram-positive bacteria	0.12	0.49	1.23

Notes:
*p – significant difference in relation to the data of the comparison group
•p_1 – significant difference in the data of patients with chronic catarrhal gingivitis

Thus, in patients with periodontal tissue diseases, an increase in conditionally pathogenic microflora, which includes gram-negative microorganisms, is studied, so that an increase in their number causes the development of microbial intoxication, which contributes to deepening of the inflammatory and destructive changes in the tooth-retaining tissues. The ratio of the number of gram-negative and gram-positive microflora is an indicator of dysbiosis in the oral cavity. According to Table 1, in patients with CCG, this indicator is 4.1 times higher than in the comparison group (p≤0.001) while, in patients with GP, the ratio of microflora exceeds the data of healthy individuals by 10.2 times (p<0.001), and 2.5 times higher than in patients with catarrhal gingivitis.

Thus, in alcohol production workers with inflammatory diseases of periodontal tissues, the bacterioscopic method of examination revealed the predominance of gram-negative

microflora, the pathogenicity of which contributes to the emergence of a cascade of metabolic changes in the tooth-retaining tissues against the background of dysbiotic phenomena.

The results of microbiological studies (Table 2) in alcohol production workers with periodontal tissue diseases showed that the gingival sulci and gingival pockets of the oral cavity of the subjects were colonized by various types of microorganisms, the total colonization of which in patients with catarrhal gingivitis being 4.48+0.12 lg CFU/ml and 4.77+0.16 lg CFU/ml in patients with generalized periodontitis, which was by (13.99 %) and (21.37 %) more than the total colonization of gingival sulci of persons with intact periodontium (13.93+0.13 lg CFU/ml) ($p<0.05$).

Table 2. Bacteriological content of gingival sulci and periodontal pockets in alcohol production workers with periodontal tissue diseases

Populations of microorganisms	Comparative group, n=20			Main group					
				Chronic catarrhal gingivitis, n=20			Generalized periodontal disease, n=53		
	n	%	Degree of contamination lg CFU/ml	n	%	Degree of contamination lg CFU/ml	n	%	Degree of contamination lg CFU/ml
Coagulase-positive staphylococci	10	50.0	3.94±0.16	11	50.0	4.39±0.09*	19	35.8	4.51±0.12 *,•
Coagulase-negative staphylococci	8	40.0	4.02±0.17	11	50.0	4.51±0.11*	18	33.9	4.48±0.18 *,•
α-Hemolytic streptococci	10	50.0	6.75±0.11	12	54.5	6.84±0.11*	53	100.0	6.98±0.12 *,•
β-hemolytic streptococci	7	35.0	3.39±0.17	11	50.0	5.37±0.20*	48	90.6	5.42±0.19 *,•
Neisseria	12	10.0	2.65±0.11	10	45.5	6.52±0.16*	44	83.0	6.84±0.16 *,•
Lactobacillus	15	75.0	3.77±0.16	10	45.5	3.96±0.10*	12	22.6	3.25±0.15 *,•
Klebsiella	0	0	–	5	22.7	1.81±0.22*	36	67.9	2.94±0.18 *,•
Escherichia coli	5	25.0	2.14±0.08	16	72.2	1.83±0.10*	45	84.9	2.19±0.13 *,•
Corynebacterium	5	25.0	3.44±0.17	10	45.5	3.35±0.13*	36	67.9	4.24±0.18 *,•
Spirochetes	0	0	–	15	68.2	–	53	100.0	–
Fusobacteria	0	0	–	15	68.2	–	53	100.0	–
Wayloneles	0	0	–	15	68.2	–	53	100.0	–
Candida fungi	4	20.0	5.23±0.17	10	45.5	6.24±0.31*	53	100.0	6.85±0.18 *,•
Cryptococcus	0	0	–	0	0	–	27	50.9	–

Notes:
* $p<0.05$ – significant difference in relation to the comparison group
• $p_1<0.05$ – significant difference in the indicators of patients with chronic catarrhal gingivitis

In patients with intact periodontium, the microflora was represented by facultative and obligate anaerobic species: coagulase-positive staphylococci (50.0%), α-hemolytic streptococci (50.0%), coagulase-negative staphylococci (40.0%) and β-hemolytic streptococci (35.0%). The largest percentage (75.0%) was accounted for by lactobacilli, whose physiological importance determines the environment favorable for the development of bifidus- and other microflora, which play a significant role in the regulation of normobiocenosis and its stability. By creating an acidic environment, *Lactobacillus* prevents the development of pathogenic, putrefactive and gas-forming microflora.

In alcohol production workers with catarrhal gingivitis, the highest percentage was accounted for by spirochetes, fusobacteria and wayloneles (68.2%) while, in patients with intact periodontium, the above species were not infected. The share of α-hemolytic streptococci accounted for 54.5%, $p<0.05$.

The frequency of detection of *Escherichia coli* in patients with catarrhal gingivitis was 72.7 %, which was 2.9 times higher than in the comparison group (25.0 %) ($p<0.05$). Attention was drawn to the increase in the frequency of *Candida* fungi inoculation in patients with catarrhalgingivitis (45.5 %) compared to (20.0 %) in the comparison group ($p<0.05$). At the same time, a significant decrease in the frequency of lactobacilli (1.6 times) was studied compared to the same indicator in the comparison group ($p<0.05$).

In patients of the main group (generalized periodontitis), α-hemolytic streptococcus, spirochetes, fusobacteria, wayloneles and *Candida* fungi were inoculated in 100% of cases. In 27 patients (50.9%), cryptococci were identified, which were not studied in patients with catarrhal gingivitis and in those with intact periodontium. The frequency of inoculation of β-hemolytic streptococci exceeded that of the comparison group by 61.37 % ($p<0.05$) and of patients with catarrhal gingivitis by 44.81 % ($p<0.05$). In distillery workers with generalized periodontitis, a significant decrease in *Escherichia coli* was studied in relation to the data of both previous groups (p, $p_1<0.05$).

4. DISCUSSION

Summarizing the above data, it is highly likely that alcohol production workers with periodontal disease have a disruption of the normal biocenosis of the oral cavity, which in this case is due to a decrease in lactobacilli and an increase in the concentration of periodontogenic microorganisms and fungi of the *Candida* genus (Fig. 1).

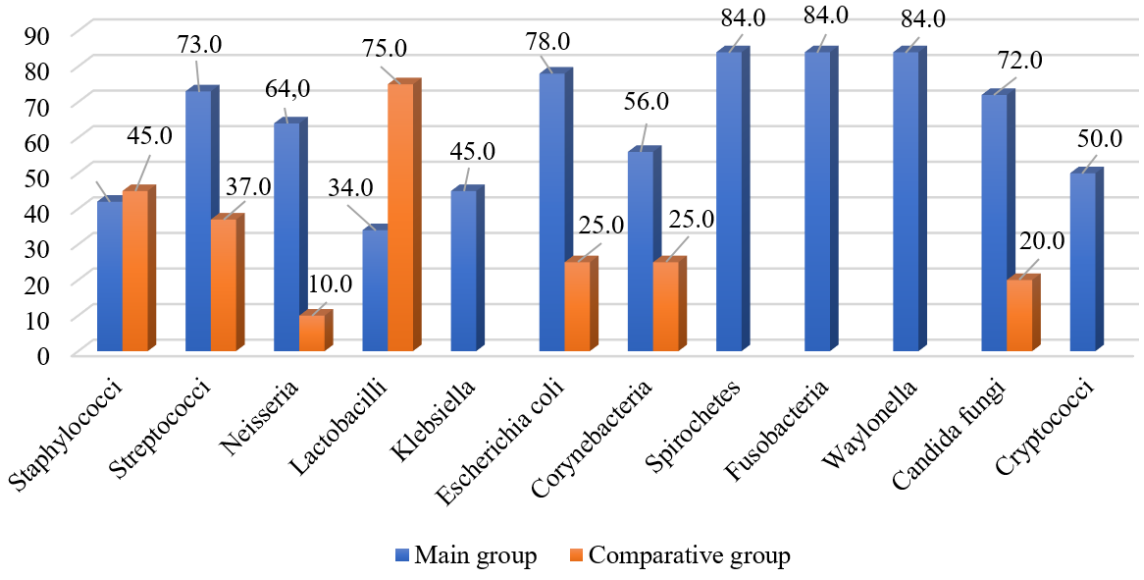

Fig. 1. Average frequency of microorganisms inoculated in the study groups

In terms of prognosis, a decrease in the number of lactobacilli inhibits the synthesis of amino acids, immunoglobulins, promotes a decrease in the activity of lysozyme and causes an increase in the permeability of vascular and tissue barriers to toxic products of pathogenic microorganisms, which are immunological adjuvants of bone resorption.

The increase in the number of *Escherichia coli* cultures can be assessed as a signal of a decrease in the immunological reactivity of the body in a selected contingent of patients with dysbiosis, which is further emphasized by the increase in *Candida* fungi. It is well known that fungi of *Candida* and *Cryptococus neoformus* are markers of immunodeficiency states, which is an indication for the inclusion of immunomodulatory drugs in the complex therapy of generalized periodontitis.

5. CONCLUSIONS

Thus, in patients with periodontal tissue diseases working in alcohol production, an increase in gram-negative microflora and, accordingly, an increase in the degree of oral dysbiosis was determined, in the presence of a wide range of pathogenic microflora and yeast-like fungi, the frequency of which progresses with the deepening of inflammatory and destructive changes in the periodontium.

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