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Posted Date: 13 October 2025

doi: 10.20944/preprints202510.0831.v1

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Article

Biological Alterations of the Oral Cavity in Individuals Affected by Pediculosis

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Abstract

It is known that an integral part of the oral microecosystem is normal microflora, which provides colonization resistance (Khabilov, N. L., F. K. Usmonov T. O. Mun. 2014). Oral microflora is considered as a primary target for any factor that directly or indirectly affects ad-hesion and colonization resistance of resident, transient and additional microfiora. At the same time, the most important environmental determinants that cause the habitat of microbes in this section of the digestive tract are the state of the dental and jaw system, food, redox potential, the degree of resistance of the mucous membranes of the oral cavity. According to I.G. Ponamareva (1993), studying the functioning of the mecha-nisms of the oral cavity, it is possible to obtain data on both adverse external influences and disorders of neurohumoral regulation, as a consequence of some disease.

Keywords: microbiocenosis; pathogenization; biochemical regulation

Background/Objectives:

It is known that the oral cavity, its mucous membrane and lymphoid apparatus of the maxillofacial area play a unique role in the interaction of the human body with the surrounding world of microbes.

However, the exact mechanism determining the causal role of opportunistic flora in inflammatory diseases of the maxillofacial area is still unknown. The main postulate is the provision on the determinating role of regulatory mechanisms in the balance of all components of microbiocenosis, the violation of which leads to the development of the pathological process. Confirmation of this is the polyetiology of opportunistic infections - associations of several species are usually separated from the inflammatory focus, which is confirmed by the dynamics of the level of antibodies to their antigens (Akbarov A. N.et al., 2007).

At the same time, the possibility of quantifying the properties of the microbes themselves, that is, regulatory influences within microbiocenosis - the pathogenization of residents - cannot be ignored. Most likely, the first and second factors are closely related at the molecular level of biochemical regulation of microecology.

True bubble is one of the most serious diseases in dermatological practice. In most patients with bubbles, the pathological process begins with a lesion of the mucous membrane of the mouth and lips. For more than one year, the oral cavity may be the only place where rashes are localized. They can occur in any area of the oral mucosa. The primary element of the bubble is the intraepidermal bladder located on an uninflamed base. Since the bubble coatings are very thin, only erosion is detected when examining the oral cavity, in some cases covered with scraps of the bubble tire. This is facilitated by maceration of the bladder with saliva, traumatization of teeth, food lump and other factors. At the same time, damage to the oral mucosa is painful and causes difficulties when talking and eating, and in some patient's erosion pain is noted even during sleep.

Methods

Along with dental studies in the same sick people suffering from oral bladders, we conducted microbiological and immunological studies.

To do this, all examined people collected oral fluid, 2 hours after eating in sterile dishes. From the received material in the problem laboratory for clinical microbiology, immunology and mycology (TMA - head, prof. Muhammedov I.M.), prepared serial breedings, in the subsequent of which a certain volume was sown on the surface of highly selective nutrient media: agar for anaerobes, Endo medium, milk-salt agar, Kalina medium, blood agar, MRS-4 medium, Saburo Wednesday and others.

Crops on blood agar, Endo, milk-salt agar, Saburo were cultivated under normal conditions for 18-24 hours at a temperature of 37°C, and cultivation of crops for the release of asporogenic anaerobes was carried out by the method of "sealed" polyethylene bags.

After these deadlines, the sown cups were removed from the thermostat, the grown colonies were counted, the group and species of isolated colonies of microorganisms were determined. Based on the data of microscopy of Gram-colored smears, the nature of growth on highly selective nutrient media.

Immunological studies. In parallel with microbiological studies in the same patients with oral bladder, the state of local protection factors was studied, namely the state of the lysozyme titer, the phagocytosis index and the level of class A secretory immunoglobulin (sIgA).

To determine the phagocytic activity of neutrophils in saliva, the collection and treatment of saliva was carried out according to the method of Habilov N (1998) in modification by Irsaliyev, Kh. I., R. H. Nigmatov, and N. L. Habilov (2006). To do this, the selected saliva was cleaned, washed with a buffer solution and centrifuged at 1000 rpm for 10 minutes, the additive liquid was drained, and 0.5 ml of physiological solution was added to the sediment. To 0.2 ml of the resulting mixture, 0.1 ml of latex particles suspension was added to the test tube. The mixture was incubated in a wet chamber for 30 minutes, at 37°C, constantly shaking. Subsequently, smears were prepared from this mixture according to the type of blood smear, painted according to Romanovsky-Gimza. At least 100 neutrophils with latex and without latex in each drug were counted under a microscope.

The activity of lysozyme in the oral fluid was determined by us using the method of Aliyev Sh.R. (1994), which included the use of sterile paper discs. For this purpose, saliva was taken on an empty stomach in sterile dishes, then paper discs (similar to antibiotic ones) were taken with tweezers and carefully impregnated in saliva, then these discs were placed on the surface of the nutritious agar, in Petri dishes sown with a daily *Micr. lysodenticus*, Determination of class A immunoglobulin - secretory fraction (sIgA), the method is based on the Mancini method (1984), which is based on the measurement of the diameter of the precipitation ring formed by applying oral fluid into the holes cut into the agar layer in which the monospecific antiserum is predisposed. Under standard experience conditions, the diameter of the precipitation ring is directly proportional to the concentration of immunoglobulin.

Results

It is known that an integral part of the oral microecosystem is normal microflora, which provides colonization resistance (Khabilov, N. L., F. K. Usmonov T. O. Mun. 2014). Oral microflora is considered as a primary target for any factor that directly or indirectly affects adhesion and colonization resistance of resident, transient and additional microflora. At the same time, the most important environmental determinants that cause the habitat of microbes in this section of the digestive tract are the state of the dental and jaw system, food, redox potential, the degree of resistance of the mucous membranes of the oral cavity. According to I.G. Ponamareva (1993), studying the functioning of the mechanisms of the oral cavity, it is possible to obtain data on both adverse external influences and disorders of neurohumoral regulation, as a consequence of some disease.

Taking into account the above, we also studied the state of colonization resistance of various areas of the oral cavity: gums, tongue, cheeks and palates, both in the control group and in sick people with bubbles. To do this, we used special stainless steel sleeves with a certain depth and surface (cm²), which after thorough sterilization were filled with highly selective nutrient media, then placed in sterile Petri dishes and stored in the refrigerator. When receiving patients, they were sown with prints, for this purpose these sleeves were applied to the surface of the mucous membranes: gums, tongue, cheeks and pails for 2-3 seconds, then these sleeves were again placed in petri dishes and transferred to a thermostat at a temperature of 37oC for 24-48 hours. After these terms, the cups were removed from the thermostat, sleeves were taken away and the grown colonies were counted (KOE/cm²), after which morphology, tinctorial, cultural and biochemical properties were studied from grown crops, thereby establishing the appearance of the grown microbe. The data obtained from these studies are presented in tables 1, 2.

Studies of bacterial colonization of the oral cavity in healthy people have led to argue that, despite the significant range of variation, the microflora of adults living in Uzbekistan is characterized by stable constancy. Thus, in the process of studying the microflora of the oral cavity, more than 1000 cultures of microorganisms belonging to 8 families, 10 genera and 16 species were isolated and studied.

According to our studies, it has been established that the density of the microbial population in the oral cavity is a fundamental characteristic of communities and largely depends on the topography of ecological food. Its greatest value was observed in the gums (4.9 0.3 CFU/cm²), the minimum on the mucous membranes of the sky (1.470.1 FU/cm²). At the same time, the predominant number and visible composition in biocenosis was gram-positive flora, which colonized 100% of the subjects. It is interesting to note that the main part of the oral microflora in healthy people was representatives of Streptococcus, while the dominant species was Str.salivarius (Table 1).

Among the gram-positive cocal flora, staphylococci occupied a significant place in colonization, with their number prevailing on the surface of the tongue and gums (4.60\$0.3, KOE/cm² and 3.59±0.5 KOE/cm²). Mostly among the staphylococci was St.epidermidis. Among other studied groups of microorganisms, in matters of colonization of the oral cavity, this property was very poorly possessed by gram-negative sticks (esherichia and klebsiella), and fungi of the genus Candida had the ability to colonize only the mucous membranes of the tongue and gums.

Table 1. Features of colonization resistance of microbes in various areas of the oral cavity in healthy.

№	Groups of microbes	Areas of the oral cavity			
		gum	tongue	cheek	palate
1.	Lactobacteria	2,00±0,1	1,80±0,1	1,20±0,1	1,00±0,1
2.	Streptococcus salivarius (%)	4,90±0,2	2,95±0,2	1,70±0,2	1,35±0,1
3.	Streptococcus mutans	2,15±0,1	2,40±0,4	1,25±0,1	1,10±0,01
4.	Streptococcus mitis	3,35±0,2	2,20±0,2	1,35±0,1	1,49±0,1
5.	Staphylococci	4,60±0,3	3,59±0,5	3,90±0,2	1,45±0,1
6.	Escherichia	0	1,20±0,1	0	0
7.	Klebsiella	0	0	0	0
8.	Mushrooms of the genus Candida	2,10±0,2	3,15±0,1	0	0

The development of microecology, which considers the oral cavity as a habitat, and its microflora as an open self-regulating system with a variety of interacting individuals and populations, determines changes in its functioning under the influence of various destabilizing factors (including endogenous ones). To perform these tasks, it was interesting to consider the microflora of the oral cavity in patients suffering from oral bubbles. Of the 1,120 crops from various ecological oral niches in bubble patients, 980 aerobic, 110 anaerobic representatives of flora were isolated, of which 800 were gram-positive microorganisms, 115 gram-negative representatives and 105 yeast-like fungi.

Conditionally pathogenic microorganisms in the microflora of patients were represented by 10 species of microbes, of which 2 belonged to the family Enterobacteriaceae, 4 to the family Micrococcaceae, 2 belonged to yeast-like fungi. Attention is drawn to the significant increase in the frequency and density of colonization of oral mucosa with yeast-like fungi. At the same time, characteristically, this process affected all the studied anatomical biotopes of the oral cavity: gums, cheeks and sky, with the incidence of fungi on the mucous membrane increased by 3-5 times, and the colonization density by 2-3 times depending on the biotope (Table 2). Analyzing the species composition of fungi, it should be said that *C.albicans*, *C.pseudotuberculosis*, *c.kruset*, *C.tropicalis* were most often found in descending order. The increase in the frequency of occurrence led to an increase in the dominance of the latter on all the biotopes studied.

Assessing the study of colonization by yeast-like fungi in the oral cavity of bubble patients, we can say that the colonization density of these microorganism’s increases hundreds of times. With the greatest intensity, fungi colonized the mucous membranes of the cheek, the sky, where their number exceeded the content on the surface of the tongue, which is the main habitat of this microorganism in healthy people. In the species structure of fungi, there was a growth of species unusual for microbiocenosis in healthy individuals.

These changes occurring in the oral cavity in patients with bubbles, regarding the appearance of candidiasis dysbacteriosis, in our opinion, can be explained with the development of acquired secondary immunodeficiency in these patients in the oral cavity.

Among the gram-positive oral microorganisms in patients suffering from bladders, there is a pronounced tendency to colonize the mucous membranes, while staphylococci dominate these issues, while various strains of streptococci fade into the background and their ability to colonize significantly decreases.

Table 2 Characteristics of colonization resistance of microbes in various areas of the oral cavity in patients with bubbles (Mim, KOE/cm2)

The table shows that normally in healthy people, the microflora of the oral cavity is quite diverse. At the same time, lactobacteria prevail in the anaerobic group of microbes, so their number is 4.60 ± 0.14 CFU/ml. It interesting to note that the total number of anaerobes and an optional group of microbes in healthy individuals is almost the same. The optional group of microbes is dominated by staphylococci and streptococci. Among the gram-positive coccal flora, the largest number consists of strains: streptococcus salivarium and epidermal staphylococcus, so their number is: 4.40 ± 0.20 CFU/mi and 4.20 ± 0.17 CFU/ml, respectively.

Table 3. The state of oral microfiora in patients suffering from bubbles.

Groups of microbes	The number of microbes in 1 ml of saliva	
	Normal	In patients with pemphigus
Total number of anaerobes	5,69±0,15	3,60±0,13
lactobacilli	4,60±0,14	2,20±0,15
Peptostreptococci	3,77±0,11	4,49±0,21
Total number of aerobes	5,30±0,17	7,59±0,30
Staphylococcus aureus	0	3,00±0,12
Staphylococcus epidermalis	4,20±0,17	4,30±0,21
Streptococcus salivarius	4,40±0,20	3,00±0,15
Streptococcus mutans	2,30±0,10	4,15±0,17
Streptococcus mitis	2,70±0,15	4,00±0,19
Escherichia LP	1,40±0,01	0
Escherichia LN	0	2,11±0,10
Protea	1,50±0,01	3,00±0,10
Klebsiella	1,00±0,01	0,71±0,01
Mushrooms of the genus Candida	2,11±0,18	4,29±0,22

Conclusions

The gram-negative flora of the oral cavity in these patients is represented by escherichs, proteins and klebsiellas and their quantitative parameters are insignificant. However, Candida fungi in the same patients are 12.11 ± 0.18 . It should be noted that the quantitative parameters of oral microorganisms in healthy people obtained by us are quite correlated with the data available in literary sources. In these same healthy people, in parallel with the quantitative characteristics of the flora of the oral cavity, we have considered the spectrum and frequency of occurrence of microorganisms. The data obtained are presented in Figure. The figure shows that the flora in the oral fluid is represented by several types of microbes. At the same time, the frequency of occurrence is dominated by such microbes as: streptococci, namely *Str.salivarius* (100%), followed by *Str.mitis* (80%) and followed by *Str.mutans* (60%) and *Staphylococcus* culture (45%). The lowest rates incidence is occupied by gram-negative microbes such as: *escherichia* (10%), proteins (8%), *klebsiella* (5%). The average parameters in terms of occurrence were yeast-like mushrooms (20%). However, with the appearance of pathological processes in the oral cavity, namely the development of the bladder, significant changes occur in the flora of the oral cavity. The materials of our microbiological studies in these patients are presented in Table 3. The table shows that these patients have significant changes in the total number of anaerobes and in the same indicators of the optional group of microbes, so their number was: 3.60 ± 0.13 and 7.59 ± 0.30 FU/ml, respectively. At the same time, the number of lactobacteria in the anaerobic group has been reduced. The most significant shifts occurred in the optional group of microbes, so in the gram-positive coccal flora there is a decrease in the amount of *streptococcus salivarius*, but against this background the sowing rate of streptococci mutans and mitis has increased significantly. At the same time, another thing is alarming that microbes that are not typical of this biotope began to be sown in the oral cavity. This refers to the emergence of growth of pathogenic *staphylococcus* (*st. aureus*) and *escherichia*. It's no secret that it is these microbes that have a set of pathogenicity enzymes that will determine the state of oral monitoring in these patients.

Another negative indicator is a significant increase in the number of fungi of the genus *Candida* in these patients, so their number was 4.29 ± 0.22 CFU/ml, at the norm the indicator was 12.11 ± 0.18 CFU/ml, that is, almost doubled, this can undoubtedly lead to the development of the picture. It is quite appropriate to note that all these quantitative and qualitative changes in the microflora of the oral cavity in patients suffering from bladders should be the basis of therapeutic care in these people. And only taking into account these changes and the direction of therapeutic care to eliminate developed oral dysbiosis can the expected positive results be achieved. In the same sick people with oral bladders, we considered the state of the spectrum and frequency of occurrence of microorganisms. The data obtained in these studies are presented in Figure 1b. The figure shows that the dominant position in terms of frequency of occurrence belongs to such microbes as: fungi of the genus *Candida* (80%), *Staphylococcus aureus* (50%). It is known that in healthy people, the main flora of the oral cavity is occupied by streptococci, as can be seen from the figure, almost all strains of streptococci have lost their dominant ability and especially decreased the frequency of *streptococci salivarius* (30%), normally it was equal (100%). In fact, these data fully confirm the data we received from the same patients during quantitative studies of oral flora.

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