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Biology of the oral cavity in children living in Chirchik city

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Abstract: The study of microbiological and immunological parameters of the oral cavity among children living in the industrial city of Chirchik, the total number of anaerobes, aerobes, phagocytic activity of neutrophils, the level of lysozyme and the immunoglobulin A secretion fraction of the secretory fraction (sIgA) has been studied.

To achieve this goal, we conducted microbiological and immunological studies in 57 children lived in Chirchik city.

All these children, depending on their age, were divided into 3 groups:

Group I included 17 children aged 6-7 years.

Group II consisted of 25 children aged 8-12 years.

The third group consisted of 15 children aged 13-15.

In children aged 6-7 years, it can be noted that the total number of the optional group of microbes is higher than in anaerobic. In this case, the quantitative indicators of the lactobacillus are especially affected among the anaerobes, so their number is Lg. 2.10 ± 0.1 cfu / ml. Alarming in this group of children is the sowing of pathogenic strains of staphylococcus. The remaining groups of microbes, although there are minor changes, but they are unreliable.

At children in the age of II and III group the flora of the oral cavity in many respects stands close to normal indices. Although it should be noted a significant increase in the number of streptococci, especially strains of Str.mutans and mitis.

In children in the age group 6-7 years, almost all indicators show immunodeficiency, so the lysozyme titer was 14 ± 0.41 mg /%, it was normally 19.180 ± 0.60 mg /%, the index of phagocytosis was 48.4 ± 1.45% significantly lower than the control indices, the level of secretory immunoglobulin of class A (sIgA) was 1.7 ± 0.1 g / l, which is much lower than normal values. It is interesting to note that in children older than 8-12 and 13-15 years, these indicators have improved significantly, although it should be noted that their immunodeficiency has worsened, only in terms of the fraction of secretory immunoglobulin A (sIgA).

From the above, it follows:

1. According to the sanitary and hygienic and microbiological studies carried out, it is possible to state that the city of Chirchik is an ecologically unfavorable region.

2. The study of oral micro-ecology in children in

Chirchik made it possible to establish that in children in the age group 6-7 years the most expressed are dysbiotic conditions, the main feature of which is the decrease in the amount of lactobacillus, but the increase in the number of staphylococci and fungi of the genus Candida

3. When studying the local factors of oral protection in children living in Chirchik in the age aspect, it was found that immunodeficiency states are most pronounced in children of the I group.

4. The study of the state of colonization resistance of microbes in children living in the city of Chirchik established that the most expressed diturbances in these issues were noted in the children of group I.

Keywords: anaerobes, aerobes, phagocytosis, secretory immunoglobulin A (sIgA), children, Str.mutans and mitis, lactobacilli, indices.

We live in the 21st century, it is a century of scientific and technical progress, based on the widespread introduction of new technologies into our lives. At the same time, it should be noted that scientific and technological progress is the decisive factor in the growth of social production. With the development of the scientific and technological revolution, man's influence on nature inevitably increases, which becomes more and more noticeable. Often it is associated with pollution of the air pool, reservoirs, a violation of the soil cover.

Modern medical research shows that the health of the human population, including dental, in the last decades, unfavorable tendencies persist, while the following factors determine the health consequences of the population: the way of life, the ecology of the natural environment, the genotype of the population and the level of medical care.

Adverse environmental factors primarily have a negative impact on the health of children, including dental status. At the same time, the state of dental health of children is one of the sensitive indicators reflecting the quality of the environment, as for any developing and actively growing tissues of the maxillofacial area of the child any concentrations and doses of harmful substances are potentially dangerous.

Based on the above stated issues and problems, we put before ourselves:

Purpose: to study the state of quantitative and qualitative indices of flora and indicators of local factors of oral cavity protection in children living in the city of Chirchik.

It is quite obvious that industrial emissions are among the important regional environmental factors that negatively affect the health of the population. One of the industrial cities in the Tashkent region is the city of Chirchik, where such industrial enterprises as the Chirchik chemical plant "Maxam-Chirhiq", the Uzbek enterprise of refractory and heat-resistant metals (UzERHRM), transformer and caprolactan factories are located. They throw their industrial waste into the atmosphere (dust, sulphurous gases, nitrogen dioxide and ammonia, etc.)

Material and methods of investigation.

To achieve this goal, we conducted microbiological and immunological studies in 57 children living in Chirchik city.

All these children, depending on their age, were divided into 3 groups:

Group I included 17 children aged 6-7 years.

Group II consisted of 25 children aged 8-12 years.

The third group consisted of 15 children aged 13-15.

In all these children, the oral liquid was taken away by flushing from the mucous membrane of the oral cavity (by rinsing) for this purpose tubes were prepared with 9 ml of sterile physiological solution.

(Efimovich OI, 2002) The obtained material was considered as the first dilution in this way (101), a number of serial dilutions were prepared from this material in the laboratory, later a certain volume was sown on the surface of differential diagnostic nutrient media. For this purpose, we used highly selective nutrient media produced by the Uzbek-Indian firm Hei-Media, such as Endo, Milk-Salt Agar, Saburo Agar, Bifido Agar, MPC-4 (milk-inducing medium).

Crops on blood agar, Endo, milk-salt agar and Saburo were cultured under ordinary conditions for 18-24 hours at 37 ° C, and cultivation of the crops for the isolation of anaerostatic method was carried out in anaerobic by the use of gas-generator chucks.

Crops in anaerostat with media of MRS-4, CAB, and Blourook were placed into a thermostat at 37 °C for 3-5 days. After the indicated expiration, all the sown plates were removed from the thermostat, the grown colonies were counted, the group and species belonging to the isolated colony of microbes were determined on the basis of the smear microscopy data of the Gram stained, growth pattern on selective nutrient media.

The generic affiliation of staphylococci and micrococci was determined by the following tests: pigment presence, microscopy data, glucose fermentation under anaerobic conditions. To differentiate the staphylococcus species there were used: the ability to produce hemolysin, plasmacoagulase, lecithinase, to ferment mannitol under anaerobic conditions. In the presence of all these properties, the cultures we studied were classified as Staphylococcus aureus. Epidermal staphylococci did not have similar properties.

To Streptococcus group D we referred strains fermenting mannitol giving growth in 40% bile, 6.5% Na chloride reducing in milk 1% blue.

When working on a modified procedure, the result was taken into account with the last dilution, in which the growth of the bacterium was obtained, the number of microorganisms was calculated according to the following formula: k = A * 200 * P (CFU / ml). The number of microbes of each species was expressed in Lg. CFU / ml

In parallel with microbiological studies, local factors of oral protection, such as phagocytic activity of neutrophils, lysozyme level and immunoglobulin A secretory fraction (sIgA), were studied in the same age groups in all age groups.

Definitions of the phagocytic activity of neutrophils in the oral fluid were carried out by a modified method of Antonov A.V. (1996). To do this, the selected oral liquid was purified, washed with a buffered solution, and centrifuged at 1000 r/m for 10 minutes, the supernatant fluid was poured out and 0.5 ml of saline was added to the sediment.

To 0.2 ml of the obtained suspension in a test tube, add 0.1 ml of a suspension

of latex particles (5* 108 1 ml) with a diameter of 0.8 mkm. The mixture was incubated in a moist chamber for 30 minutes at 37°C. Subsequently, from this mixture the smears were stained by Romanovsky Giemsa. Counted at least 100 neutrophils with and without latex in each preparation, the index of phagocytosis was determined and expressed in%.

The activity of lysozyme in the oral fluid was determined by us using the method proposed by Sh.R.Aliyev (2004), which included the use of sterile paper discs. For this purpose, they took with tweezers paper discs (similar to antibiotic discs) and thoroughly impregnated them in the oral liquid. Then these discs were placed on the surface of M?ller Hinton's nutrient agar in Petri dishes, M. Luteus daily culture strain No. 003596/126 / the national collection of human infection microorganisms of the Research Institute of Emission MHRUZ, the crops were incubated in a thermostat at a temperature of 37 °C , the activity of lysozyme in the oral fluids was determined by the diffusion method of agar.

Definitions of the class A immunoglobulin titer of the secretory fraction (sIgA). The method is based on the method of Manchani (1994) which is based on measuring the diameter of the precipitate ring formed when oral fluid is applied to the alveolus cut in the agar layer in which monospecific sera are pre-dispersed.

Under standard test conditions, the diameter of the precipitation ring is directly proportional to the concentration of immunoglobulin.

It seemed to us interesting to study the state of colonization resistance of various oral biotopes in the examined children, such as: the gum surface of the tongue, cheeks and palate.

To solve this problem, we used stainless steel liners with definite depth and surface which after thoroughful sterilization under aseptic conditions were poured by highselective nutrient media, after which were placed into cups of Petry and kept in frigo. While examining children crop with unprints was produced, for this purpose these liners from the surface side with nutrient media were added to the surface of mucuous membrane: gum, tongue, cheeks and palate for 2-3 sec., then these liners again were placed into the cups of Petry and put in thermostat at temperature 37 °C for 24-48 hours. After the incubation expiration cups were removed from the thermostat, liners with crops were taken from them and account of grown colonies was produced, after which morphology, cultural, tinctorial and biochemical properties were studied at grown culture, at the same time type of grown microbe was established.

Results of the study

The received data of researches are presented in the table №1. As can be seen from the table in children aged 6-7 years, it can be noted that the total number of the optional group of microbes is higher than in anaerobic. In this case, the quantitative indicators of the lactobacillus are especially affected among the anaerobes, so their number is Lg. 2.10 ± 0.1 cfu / ml. Alarming in this group of children is the sowing of pathogenic strains of staphylococcus in the remaining groups of microbes, although there are minor changes, but they are unreliable.

At children in the age of II and III group the flora of the oral cavity in many respects stands close to normal indices. Although it should be noted a significant increase in the number of streptococci, especially strains of Str.mutans and mitis

Table 1

Characteristics of oral fluid microflora in children living in Chirchik age aspect Eg M ± m CFU / ml

№	Groups of microbes	Norm	Age groups		
			6-7 years	8- 12 years	13-15 years
1	General. quant. anerob	5,8±0,4	4,60±0,2	5,60±0,3	6,10±0,4
2	Lactobacillus	4,7±0,3	2,10±0,1	2,60±0,2	2,10±0,2
3	Peptostreptococcus	3,85±0,3	3,30±0,2	5,0±0,3	5,30±0,3
4	General. quant. aerobic	5,60±0,4	5,30±0,4	5,10±0,3	5,10±0,3
5	Staf zolotis	0	2,10±0,1	0	0
6	Staph epidermis	4,40±0,3	3,30±0,1	5,0±0,3	5,30±0,3
7	Strep Salivar	4,70±0,2	2,10±0,3	3,0±0,2	4,0±0,2
8	Strep mutans	2,40±0,2	4,0±0,2	4,0±0,3	5,0±0,3
9	Strep mitis	2,60±0,2	3,0±0,2	5,0±0,3	5,0±0,3
10	Esherichia LP	1,40±0,1	1,0±0,1	0	0
11	Escherichia LN	0	1,30±0,1	1,30±0,1	1,0±0,1
12	Protey	1,40±0,1	1,60±0,1	2,30±0,1	0
13	Mushrooms gr Candida	2,15±0,1	2,60±0,1	3,10±0,2	1,60±0,1

Immunological data of lysozyme titer, phagocytosis index and level of secretory immunoglobulin of class A (sIgA) are given in table №2. It can be seen from the table that in children aged 6-7 years almost all indices show immunodeficiency, so the lysozyme titer was 14 ± 0.41 mg /%, it was normally 19.180 ± 0.60 mg /%, the index of phagocytosis was 48.4 ± 1,45% that is significantly lower than the control indices, the level of secretory immunoglobulin of class A (sIgA) was 1.7 ± 0.1 g / l, which is much lower than normal indices. It is interesting to note that in children older than 8-12 and 13-15 years, these indicators have improved significantly, although it should be noted that their immunodeficiency has worsened, only in terms of the fraction of secretory immunoglobulin A (sIgA).

Table 2

The condition of local factors of oral cavity protection in children living in Chirchik age aspect

№	Indicators	Norm	Age groups		
			6-7 years	8- 12 years	12- 15 years
1	Titer of lysozyme mg /%	19,8±0,60	14,0±0,41	16,0±0,31	15,0±0,30
2	Display. Phagocytosis %	59,1±1,60	48,4±1,45	51,0±2,0	50,0±2,5
3	Level of the secretory IgA (sIgA) g / l	2,0±0,30	1,7±0,1	1,3±0,1	1,4±0,1

Apparently, this is quite obvious at the age of 12 and 15 years, this is the period of the onset of puberty, which is visible and contributes to the improvement of the indices of local factors of oral cavity protection in children and is aimed at

protecting the oral mucosa from the development of pathological processes. It is interesting to note that these positive changes in the indicators of local factors of oral cavity protection in children are completely correlated with the state of microecological processes.

The most interesting data were obtained in studying the colonization resistance of microbes of the biotopes of the oral cavity, such as the gum, the surface of the tongue, cheek and palate, in the children of the Chirchik age group.

According to our study (Table 3), it is established that the density of the microbial population in the oral cavity in healthy children is a fundamental characteristic of communities and largely depends on the topography of the ecological niche. Its greatest value was noted in the gum (4.01 ± 0.3 CFU / cm2), minimal on the mucous membranes of the palate (1.20 ± 0.1 cfu / cm2). In this biocenosis, the predominant in number and species composition was gram-positive flora which colonized 100% of the surveyed. It is interesting to note that the main part of the microflora of the oral cavity in healthy children was composed by representatives of the genus Streptococcus, in this dominant species being Str. salivarius.

Among gram-positive flora, a significant place in colonization was occupied by staphylococci, while their quantity prevailed on the surface of the tongue and gum. Among other studied groups of microbes in matters of colonization of the oral cavity, these properties were very poorly possessed by gram-negative rods (Escherichia, Klebsiella) and the fungi of the genus Candida had the ability to colonize only the mucous membranes of the gum and tongue.

Table 3

The condition of colonization resistance of microbes of oral biotopes in healthy children

M ± m CFU / sm2

№	Microbe groups	Biotopes of the oral cavity			
		Gums	tongue	cheek	palate
1	Lactobacillus	**2,15±0,1**	**1,80±0,1**	**1,15±0,1**	**1,15±0,1**
2	Strep Salivar	**4,11±0,3**	**2,75±0,1**	**1,30±0,1**	**1,0±0,1**
3	Strep mutation	**1,7±0,1**	**2,1 0±0,1**	**1,10±0,1**	**1,10±0,1**
4	Strep mitis	**2,45±0,2**	**2,30±0,1**	**1,30±0,1**	**1,20±0,1**
5	Staphylococcus aureus	**3,75±0,2**	**2, 0±0,1**	**1,10±0,1**	**1,0±0,1**
6	Esherichia	**0**	**1,15±0,1**	**0**	**0**
7	Klebsiella	**0**	**0**	**0**	**0**
8	Mushrooms gr Candida	**1,30±0,1**	**2,15±0,1**	**0**	**0**

It is quite obvious that the study of microbes ability to colonization of various oral biotope allows to understand that intimate processes which occur in oral cavity apparently undoubtedly connected with the condition of PH oral fluid, as well as from the presence of special receptors in our cells.

Table 4

The characteristics of colonization resistance of oral biotopes in children living in Chirchik city with the age of 6-7 years

№	Microbe groups	Biotopes of the oral cavity			
		Gums	**tongue**	**cheek**	**palate**
	Lactobacillus	**1,0±0,1**	**0,60±0,1**	**0**	**0**
	Strep Salivar	**2,80±0,1**	**2,65±0,1**	**1,30±0,1**	**1,10±0,1**
	Strep mutation	**2,60±0,1**	**2,80±0,2**	**2,10±0,1**	**2,0±0,1**
	Strep mitis	**2,40±0,2**	**1,80±0,1**	**1,60±0,1**	**1,0±0,1**
	Staphylococcus aureus	**4,60±0,3**	**3,80±0,2**	**3,10±0,1**	**2,20±0,1**
	Esherichia	**2,10±0,1**	**2,15±0,1**	**2,30±0,1**	**2,0±0,1**
	Klebsiella	**2,10±0,1**	**1,90±0,1**	**1,70±0,1**	**1,30±0,1**
	Mushrooms gr Candida	**4,80±0,3**	**3,80±0,2**	**3,20±0,2**	**2,60±0,1**

The next group of our studies on the study of the colonization resistance of microbes of various biotopes of the oral cavity consisted of children living in the city of Chirchik for 6-7 years. The data obtained in these studies are presented in Table 4. It can be seen from the table that these children experienced significant changes in colonization issues in almost all biotopes. It is interesting to note that almost all biotopes noted the following shifts to them can be related to: in all biotopes there is a significant decrease in the ability of colonization in all types of streptococci, while the prevailing place in these issues has passed to staphylococci and fungi of the genus Candida. Secondly, it is alarming that in all biotopes the colonization ability of lactobacillus cultures was reliably reduced, and in some biotopes such as cheeks and palate they generally eliminated. Among the gram-negative flora, it is possible to cancel the stable state of colonization of Escherichia and Klebsiella.

These changes taking place in the colonization of microbes in the oral biotopes in children aged 6-7 years should alert the children's dentists, the possibility of developing a pathological condition in this group of children. Apparently, these children need to improve oral hygiene and conduct preventive interventions to normalize the identified disturbances.

The condition of colonization processes in the oral cavity, we considered, also in children living in the city of Chirchik with the age of 8-12 years (table number 5). It can be seen from the table that in this group of children there have been significant changes in relation to the quantitative parameters of the colonization processes of microbes in most biotopes of lactobacilli and streptococci. Although there is still a tendency to prevail in the colonization of microbes such as the fungi of the genus Candida

Microbiological studies of the colonization processes of microbes of various biotopes in the oral cavity in children living in the city of Chirchik with the age of 13-15 years are presented in Table 6. The table shows that in this age group, positive processes are even more expressed.

Table 5

Features of colonization resistance of oral biotopes in children living in Chirchik city with the age of 8-12 years

№	Microbe groups	Biotopes of the oral cavity			
		Gums	tongue	cheek	palate
	Lactobacillus	**16,0±0,1**	**1,30±0, 1**	**1,0±0,1**	**1,10±0,1**
	Strep Salivar	**3,15±0,2**	**2,30±0,1**	**1,15±0,1**	**1,00±0,1**
	Strep mutation	**2,10±0,1**	**2,15±0,1**	**2,0±0,1**	**1,20±0,1**
	Strep mitis	**3,0±0,2**	**2,30±0,1**	**2,15±0,1**	**2, 0±0,1**
	Staphylococcus aureus	**3,45±0,2**	**2,60±0,1**	**2,10±0,1**	**2,00±0,1**
	Esherichia	**2,10±0,1**	**2,0±0,1**	**1,60±0,1**	**1,20±0,1**
	Klebsiella	**1,10±0,1**	**1,15±0,1**	**1,30±0,1**	**2,0±0,1**
	Mushrooms gr Candida	**3,60±0,3**	**2,0±0,1**	**2,0±0,1**	**3,0±0,1**

Table 6

Indicators of colonization resistance of oral biotopes in children living in Chirchik city with the age of 13-15 years

№	Microbe group	Biotopes of the oral cavity			
		gums	tongue	cheek	palate
1	Lactobacillus	**17,0±0,1**	**1,15±0, 1**	**1,0±0,1**	**1, 0±0,1**
2	Strep Salivar	**3,30±0,2**	**2,15±0,1**	**1,30±0,1**	**1,15±0,1**
3	Strep mutation	**2,00±0,1**	**2,0±0,1**	**2,10±0,1**	**1,10±0,1**
4	Strep mitis	**2,60±0,1**	**2,60±0,1**	**2,30±0,1**	**1,60±0,1**
5	Staphylococcus aureus	**2,0±0,2**	**2,10±0,1**	**2, 0±0,1**	**2,10±0,1**
6	Esherichia	**2, 0±0,1**	**1,60±0,1**	**1,85±0,1**	**1,60±0,1**
7	Klebsiella	**1, 0±0,1**	**1,30±0,1**	**1, 0±0,1**	**1,0±0,1**
8	Mushrooms gr Candida	**3,30±0,2**	**2,0±0,1**	**2,10±0,1**	**2,60±0,1**

Thus, almost in all the studied biotopes it is noted show dominance of streptococcal cultures. In it the quantitative parameters of staphylococci decreased somewhat. However, it is alerting another concern is the preservation of high rates of colonization ability in all biotopes of microbes of the genus Candida.

Thus, on the basis of conducted microbiological and immunological studies of the oral cavity in children living in Chirchik in the age aspect. We can draw the following conclusions:

1. According to sanitary and hygienic and microbiological studies, it is possible to state that Chirchik is an ecologically unfavorable region.

2. The study of oral fluid micro-ecology in children in.

Chirchik made it possible to establish that in children in the age group 6-7 years the most expressed are dysbiotic conditions, the main feature of which is the decrease in the amount of lactobacillus, but the increase in the number of staphylococci and fungi of the genus Candida.

3. When studying local factors of oral cavity protection in children living in Chirchik in the age aspect, it was revealed that immunodeficiency states are most expressed in children of the I group.

4. The study of the state of colonization resistance of microbes in children living in the city of Chirchik established that the most expressed disturbances in these issues were noted in the children of group I.

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