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Determination of the Microbial Landscape of the Oral Cavity in Patients with Parasitic Invasion, In the Treatment of Chronic Recurrent Aphthous Stomatitis

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Abstract: The article describes studies on the state of nonspecific resistance of the oral cavity, which was evaluated by studying the indicator of secretory immunoglobulin A (sIgA) and lysozyme activity (Liz). Quantitative determination of sIgA in oral fluid was performed by radial immunodiffusion.

Keywords: Nonspecific resistance of the oral cavity, secretory immunoglobulin A, oral fluid, oral mucosa.

Relevance. Chronic recurrent aphthous stomatitis (HRAS) is an infectious disease caused by a combination of environmental, micro- and macro-organisms. HRAS is characterized by frequent recurrence of AFT on the oral mucosa (SOPR) and with a violation of the integrity of the epithelium, local inflammation and severe pain.

Chronic recurrent diseases of the oral cavity organs include: HRAS, desquamative glossitis, candidiasis, multiform exudative erythema, and chronic recurrent cracks of the lips and corners of the mouth, Behcet's disease, Stevens - Johnson syndrome, desquamative gingivitis. In the practice of a dentist, HRAS is most often found [1.3.5.7.9].

A.I. Rybakov (1978) believed that HRAS occupies 5% of all pathologies of the SOPR. In the XXI century, scientists call the figure of 20% of the world's population suffers from aphthae at some point in life, most often from 20 to 40 years. In the prepubescent period, the incidence is not gender-specific, and in the pubertal period, women are more likely to get sick.

The pathogenesis of HRAS is based on changes in humoral and cellular immunity – a decrease in reactivity and suppression of nonspecific immunity due to the presence of foci of chronic infection, often chronic tonsillitis, gastrointestinal pathology, and the effects of adverse exo- and endogenous factors such as environmental conditions and chronic stress [2.4.6.8.10].

Complex therapy of HRAS is necessarily aimed at a variety of clinical manifestations of pathology, treatment of comorbid diseases and the age of the patient, and monitoring of the effectiveness of therapy should be carried out based on the results of changes in the microbial landscape of saliva and the concentration of secretory IgA.

Despite the large number of proposed treatments, HRAS continues to be a complex and largely unsolved problem. There is an urgent need for new, more effective ways to treat this disease; therefore, the search for new, optimal methods is absolutely justified.

Material and methods. On the basis of the Department of Therapeutic Dentistry of the Bukhara State Medical Institute, for the period 2019-2021, we examined 97 patients with HRA aged 23-46 years (average age 36.4±3.3 years), of which there were 43 men, 54 women. The average age of the observed men was 34.7±3.1 years, women 39.2±3.2 years.

The initial diagnosis of HRAS was made according to generally accepted criteria based on complaints, a carefully collected anamnesis, the prescription of the process and an objective examination. Information about the effectiveness and methods of previous therapy was taken into account. The final diagnosis was made after studying these dental diagnostic methods.

Due to the lack of reference values and inconsistency of literature data, a control group was formed to determine the indicators in healthy individuals, which included 22 practically healthy individuals with intact oral mucosa, whose average age was determined as 32.1 ± 1.4 years, without violation of the dental arch and formula, and changes in the oral cavity and gums.

The quantitative distribution of patients in all groups is presented in Table 1.

Distribution of patients by groups

Patient groups	Absolute quantity	Share in %	Middle age	Paul	
				Men Abs/ %	Women Abs/ %
Ia	25	25,77	34,5±1,8	10/23,26	13/24,07
Ib	24	24,74	35,2±2,1	11/25,58	15/27,78
IIa	24	24,74	35,2±1,7	11/25,58	12/22,2
IIb	24	24,74	36,1±1,9	11/25,58	14/25,93
py	22		37,5±2,7	10/45,45	12/54,55
Total	97	100	36,4±3,3	43/44	54/56

In patients of all groups, the severity of the inflammatory process in the tissues of the SOPR was the same, the therapeutic measures carried out were different.

Patients of group I (49 people, 50.51%) underwent pathogenetic treatment of helminthiasis, against the background of standard treatment of HRAS. 48 patients (49.49%) of group II received standard treatment for HRA.

Both groups were separated during treatment, so in the Ia subgroup the preparation "Garlic Oil extract" was applied topically and inside "Mekretin", in the IIa – the preparation "Garlic Oil extract" was applied topically against the background of standard therapy.In our study, clinical, dental, laboratory and statistical research methods were used

Nonspecific resistance of the oral cavity. The state of nonspecific resistance of the oral cavity was assessed by studying the indicator of secretory immunoglobulin A (sIgA) and lysozyme activity (Liz). Quantitative determination of sIgA in oral fluid was performed by radial immunodiffusion (RID), the result was expressed in g/l [11.13.15.17.19.21.23.25.27.29.31.33].

The principle of the Mancini REED method is as follows:

1. A layer of agar with a monospecific antiserum to the bottom of the classes of immunoglobulins (antibodies) is applied to a solid base (glass, polystyrene plate or Petri dish);
2. wells are made in this layer of agar, into which the test serum from patients (antigen) is introduced;
3. the diffusion of serum immunoglobulins (antigen) from wells is accompanied by an interaction reaction with the antiserum contained in the agar.

As a result of the interaction, a turbidity disk is formed in the agar (precipitate). The area of this disk is directly proportional to the amount of antigen introduced into the wells (i.e., the amount of immunoglobulin of a certain class).

Quantitative or qualitative changes in the production of immunoglobulins in the blood serum are called dysgammaglobulinemia (gammapathies) and, as a rule, are accompanied by violations of the immune state of the body.

In this regard, the Mancini REED can be used to quantify classes of serum immunoglobulins in congenital and acquired (AIDS) immunodeficiency conditions, autoimmune diseases (primary myxedema, thyrotoxicosis, pernicious anemia, etc.), rheumatoid arthritis, lupus erythematosus [5].

Determination of the activity of the lysozyme of oral fluid was carried out by the photonephelometric method: "... due to the ability of lysozyme to lyse mucopolysaccharides of the cell walls of the reference strain Micrococus lysodeificus, the suspension of the test culture was standardized on FEK-56m using a green light filter, the initial light transmission was adjusted to 20%, oral fluid was added to the prepared microbial suspension, thermostated at 37 ° C for 60 minutes and nephelometry was performed under the same conditions as when standardizing the initial suspension, lysozyme activity in % was determined by the difference in the percentage of light transmission before and after incubation" [12.14.16.18.20.22.24.26.28.30.32].

Statistical processing of the results of the study was carried out using variational statistics using Microsoft Office Excel-2019 programs with the calculation of the mean square deviation and the arithmetic mean error by the method of moments (M ±m), mean square deviation (□), median, mode and interquartile intervals. To determine the statistical significance of the measurements obtained, the criteria for the reliability of Student differences (t) and the degree of reliability (P) were used for data with a normal distribution, the differences were assumed to be reliable at a 95% confidence interval (P 0.05).

Results and discussions. The parameters of nonspecific immunity of oral fluid correlated with the clinical picture of helminthic invasion, clinically intact helminthiasis reduced the content of lysozyme by 48.1 ±3.4% ($p<0.05$) and the tendency to decrease the concentration of sIdA by 18.2± 2.8% ($p>0.05$) in group I patients.

The decrease in lysozyme activity in saliva in patients of group II with relatively intact mucosa occurred by 20.02± 2.43% ($P<0.01$); and in group I - by 30.53±4.72% ($P<0.01$). Reduction of phagocytosis by 15.5±3.41% ($P<0.01$) and 31.26±4.69% ($P<0.01$), respectively (Table 2).

In 85.7% of our patients with HRA, the immunological parameters of the oral fluid were significantly reduced ($P<0.05$): IgA by 2.2 times and sIdA by 2 times, lysozyme activity by 1.9 times.

Table 2. Levels of biochemical and immunological parameters of oral fluid in patients with HRA of groups I and II of HRA and KG(M±δ)

Indicators of oral fluid	Group I (n=49)	II Group (n=48)	KG
Alkaline phosphatase, nmol/min×ml	1,92±0,08*	1,84±0,08*	1,29±0,07
Acid phosphatase, nmol/min×ml	1,94±0,15*	1,82±0,16*	1,52±0,09
Sialic acids, units.	8,62 ±0,14*	8,5±0,16*	6,8 ±0,18
Igg, g/l	0,19±0,006*	0,16±0,005*	0,07±0,004
IgG, g/l	0,14±0,005*	0,17±0,005*	0,09±0,003
Lysozyme, %	23,9±1,4*	28,4±2,1*	45,1±2,4
SIg A, g/l	0,14±0,04*	0,17±0,04*	0,31±0,08
Csb.	5,71±0,22*	5,41 ±0,23*	1,51±0,19

Note: *- statistically significant differences relative to the control ($p<0.05$).

It has been shown that the level of S-IgA correlates with the degree of dysbiosis: with I and II degrees of dysbiosis, sIgA and IgA in the oral fluid increases compared to the control. With dysbiosis of the III degree, there is a decrease in sIgA and IgA. The concentration of IgG increases as the degree of dysbiosis increases.

In patients with HRAS, a significant ($p<0.05$) average direct correlation was revealed between such indicators as S-IgA ($r=0.60$) and IgA ($r=0.34$) with alkaline phosphatase, with acid phosphatase ($r=0.52$) and ($r=0.46$), respectively. A significant ($p<0.05$) medium-strength inverse correlation between IgG and the level of lysozyme ($r= -0.53$) and sialic acids ($r= -0.48$) was established.

In comparison with the control, the levels of sialic acids, the activity of alkaline phosphatase, lysozyme and sIgA in the oral fluid were reduced in patients with group I, the Csb index was increased.

Conclusions.

In patients with HRAS, a significant ($p<0.05$) average direct correlation was revealed between such indicators as S-IgA ($r=0.60$) and IgA ($r=0.34$) with alkaline phosphatase, with acid phosphatase ($r=0.52$) and ($r=0.46$), respectively. A significant ($p<0.05$) average inverse correlation of IgG with the level of lysozyme ($r= -0.53$) and sialic acids ($r= -0.48$) was established.

In comparison with the control in patients with group I, the levels of sialic acids, the activity of alkaline phosphatase, lysozyme and SIDA in the oral fluid were reduced, the Csb index was increased. The Csb index is significantly increased, which tells us about active inflammation in the oral cavity. The lysozyme level decreased, which indicates a decrease in local immunity, which is confirmed by a decrease in S-IgA and IgA as the duration of the disease increases. Moreover, it is worth noting that all these processes proceeded more strongly in patients with helminthiasis.

In the control group, the levels of sialic acids, SIDA, lysozyme, and alkaline phosphatase activity were increased in the oral fluid relative to patients with HRA.

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