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CHRONIC RECURRENT APHTHOUS STOMATITIS IN GASTROINTESTINAL DISEASES: ORAL MICROFLORA AND IMMUNOLOGICAL STATUS

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Abstract
The conducted study is devoted to the study of the pathogenesis of recurrent aphthous stomatitis. The question of the etiopathogenesis of this disease still remains open. However, the prevalence of the disease is growing with all modern research methods. There were 50 patients examined in 2021 – 2023 years. All the patients were examined at the Hospital Therapeutic Dentistry Department of Tashkent State Dental Institute and diagnosed with resurrent aphthous stomatitis.10 patients without oral pathology were examined as control.The microflora in biomaterials was determined in the bacteriological laboratory of the second clinic of the Tashkent Medical Academy. The immunological analysis parameters of blood were determined in the laboratory of "IMMUNOGEN TEST" LLC. Determination of the cellular component of the immune system was carried out using venous blood taken from patients. The main subpopulation of lymphocytes in peripheral blood was studied.Obtained results showed natural imbalance in the microbiocenosis of the oral cavity. It has been shown that the origin of recurrent aphthous stomatitis may be the result of damage to the oral epithelium caused by a T-cell immune response.

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Keywords: Recurrent aphthous stomatitis, clinical course, microbiology, immunology.

Introduction

Chronic recurrent aphthous stomatitis (RAS) is a chronic inflammatory disease of the mucous membrane of the oral cavity, the disease is characterized by the appearance of aphthous ulcers, periodic remissions and frequent relapses. According to the World Health Organization, approximately 10-20% of the world's population is affected by this disease [Khasanova, Akhmedov, 2018]. In the pathogenesis of chronic inflammatory processes, much attention is paid to the state of the microbiocenosis of the oral mucosa [Bankvall et al., 2014]. The importance of gastrointestinal pathology and liver diseases in the pathogenesis of RAS has been shown on the basis of clinical and experimental studies [Al-Zahrani et al., 2021; Jajam et al., 2017; Lin et al., 2019; Shakeri et al., 2009]. At the same time, microecology of the gastrointestinal tract plays an important role in the development of oral cavity diseases. The normal microflora of the gastrointestinal tract performs protective, metabolic and immune-stimulating functions. If the change in the microflora of the gastrointestinal tract, that is, the development of dysbacteriosis, causes the disruption of various metabolic processes in the macroorganism, the lack of micronutrients, a decrease in the function of the immune system, and irreversible processes in organs and systems [shcherbakov and dr., 2001]. The development of dysbacteriosis causes saprophytic and conditionally pathogenic microflora to manifest virulence properties in the gastrointestinal tract. According to most clinicians, intestinal dysfunction is accompanied by oral mucosal disorders. This condition is the anatomical, physiological and humoral connection of different sections of the gastrointestinal tract [Karakov i dr., 2016; Robakidze, Shchukina, 2019]. 4 levels of dysbacteriosis are distinguished depending on the condition of patients' feces. The 1st degree of dysbacteriosis is the latent phase of microflora dysfunction. Dysbacteriosis level 2 is the starting phase, which is characterized by a deficiency of bifidobacteria based on a reduced amount of normal or lactobacilli or a decrease in their acid-forming function. At the same time, staphylococci that

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coagulate plasma, or proteins, or *Candida* fungi multiply in a transient state. At the 3rd level of dysbacteriosis, the phase of aggression of aerobic flora is considered. In this case, changes in 2 phases take a permanent form. At the same time, erythrocyte hemolysis and capsule formation occur. At the 4th level of dysbacteriosis, the associative phase of dysbiosis with functional disorders of the digestive system is observed [shcherbakov and dr., 2001]. Thus, chronic recurring aphthous stomatitis caused by gastrointestinal dysbacteriosis is one of the aggressive diseases.

Therefore, the relationship between the microflora of the oral cavity and immunological changes in the course of chronic recurrent aphthous stomatitis in gastrointestinal diseases was studied in the article.

Materials and methods: All patients included in the study signed an informed consent to participate in the research. As a control, people of the same age without significant background pathology, who did not suffer from chronic recurrent aphthous stomatitis, participated in the study.

Exclusion criteria. A mandatory criterion for non-participation in the selection of volunteers was the presence of severe somatic pathology, including patients suffering from cancer and blood diseases, diseases of the cardiovascular system, liver and kidneys. Women: pregnant, lactating and menopausal.

Exclusion criteria. Occurrence of severe somatic pathologies and complications in the volunteer during the study. Refusal of the patient from the study.

All the patients were examined at the Hospital Therapeutic Dentistry Department of Tashkent State Dental Institute and diagnosed with resurrent aphthous stomatitis.

The microflora in biomaterials was determined in the bacteriological laboratory of the second clinic of the Tashkent Medical Academy. A series of serial dilutions was prepared and then a certain volume of them was spread on the surface of the highly selective nutrient medium. For this we used the nutrient medium like Endo medium, Yellow-salt agar, Sabouraud agar, MPC-4, Muller Hinton medium etc. manufactured by Hei Media, India. Blood agar, Endo, milk-salt agar and Sabouraud's medium were cultured under normal conditions at 37 ^{0} C for 18-24 hours, and cultivation of crops to isolate anaerobes was carried out in an

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anaerostat using gas generator cartridges. After the specified period, all inoculated dishes were removed from the thermostat, the grown microbial colonies were counted, and the group and species of the isolated colonies were determined based on the microscopy data of Gram-stained smears, the nature of growth in the selective nutrient. Environment and biochemical properties. When working according to the modified method, the result of bacterial growth was calculated according to the final dilution obtained, the number of microorganisms was calculated according to the following formula: K = 200 x P (CFU / ml), the number of each type of microbes was expressed in CFU / ml.

The immunological analysis parameters of blood were determined in the laboratory of "IMMUNOGEN TEST" LLC. Determination of the cellular component of the immune system was carried out using venous blood taken from patients. Studies were performed on a BD Accuri C6 flow cytometer. To determine the phenotype of immunocompetent cells in whole blood, a panel of monoclonal antibodies was used to determine the total number of T-lymphocytes (CD-3), T-helper cells (CD-4). T-suppressors (CD-8), B-lymphocytes (CD-20), natural killer cells (CD-16). The analysis process is carried out in several steps, including adding the required volume of monoclonal antibodies corresponding to the Eppendorf tube, adding the blood of the patient being tested, incubation in the dark at room temperature, adding lysis solution, and then incubation at room temperature. The results are recorded automatically in the flow cytometer using a special program.

Statistical analysis was performed using the statistical software «Statistica» (v13.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); non-normal variables were reported as median (M). A probability value of $P<0.05$ was considered statistically significant.

Results of microbiological examination of the mucous membrane of the oral cavity

Oral mucosal diseases, including RAS, are one of the diseases whose etimology remains abstract, and changes in the composition of the oral mucosa and resident

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salivary microbiome have been revealed in recent years. This state of dysbiosis affects the barrier function of the oral epithelium and the balance of the immune response, leading to the development and exacerbation of diseases [Lin et al., 2021]. Also, the non-constancy of the composition of the oral microbiome depends on many factors, in particular, various gastrointestinal diseases [Cui et al., 2016], physical and mental stress [Stoy et al., 2023], the effects of medications [Makins, Ballinger, 2003] - all this can affect the proportion of microorganisms in the microflora. The change of oral microflora in RAS patients and after treatment is presented in Figure 1, which consists of the following bacteria - bifidobacteria, lactobacteria, *E. coli* , represented by propionobacteria, streptococci, enterococci, eubacteria and bacteroids. Microflora is a collection of microorganisms living in a certain area of the body.

Bifidobacteria live mainly in the large intestine and help the body get rid of undigested food residues. Bifidobacteria are also necessary to improve the absorption and hydrolysis of fats, protein and mineral metabolism, synthesis of biologically active substances and vitamins. The microflora of the stomach is represented by lactobacilli and yeasts . It is, for example, a little less than in the intestines, because hydrochloric acid acts here. Lactobacilli live in all parts of the gastrointestinal tract and their number is as high as bifidobacteria. These microorganisms are invaluable for humans. They prevent the growth and reproduction of fast-adapting saprophytic-pathogenic and pathogenic microorganisms, stimulate the immune system and participate in digestion.

E.coli , as well as lactobacilli, live in the child's body in the first days after birth. *E.coli* forms a membrane in the large intestine and adheres to the epithelium. Due to such a film, it is complicated for pathogenic microbes to establish themselves in the body. Propionobacteria have active antagonistic properties against fast-adapting conditional-pathogenic and pathogenic bacteria, therefore, they participate in immune processes. Peptostreptococci break down milk proteins and are also involved in carbohydrate fermentation. Enterococci are classified as fast-adapting conditionally pathogenic microbes, but at the same time, they perform an important function in the human body. *Helicobacter pylori* is one of the

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bacteria that causes inflammation and ulcers in the mucous membrane of the stomach.

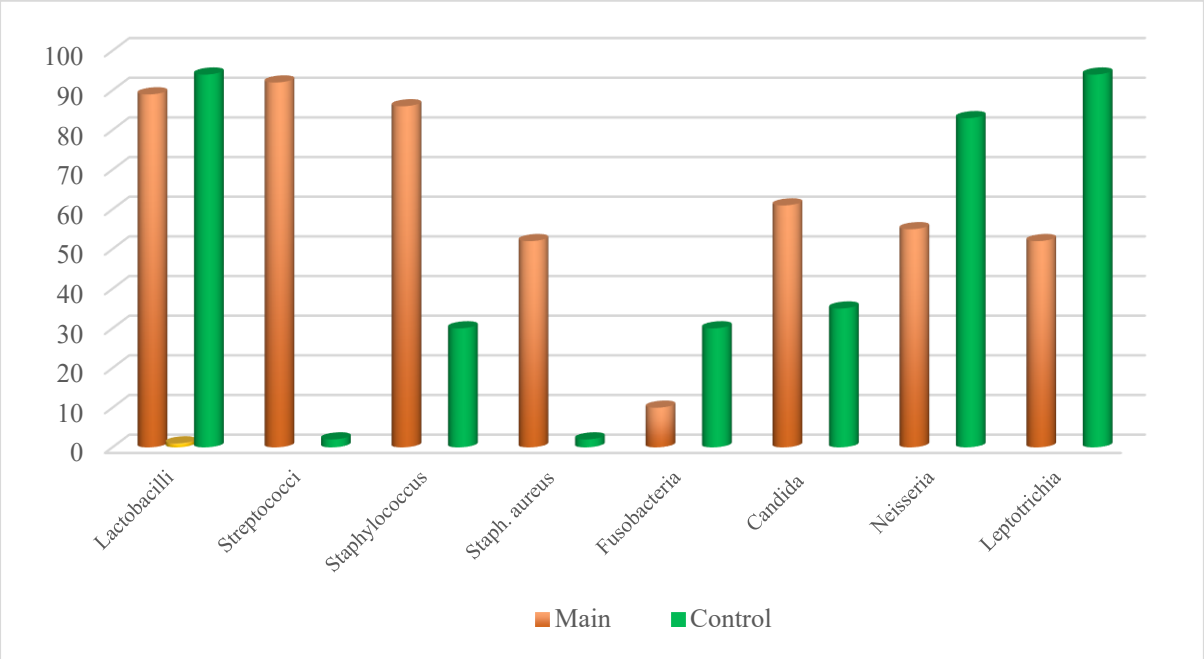

Figure 1. Changes in oral microflora in RAS patients and after treatment.

Proteus mirabilis, *E. coli, Klebsiella pneumoniae* are usually identified in inflammatory diseases of the skin, gastrointestinal tract, genitourinary tract, nasopharynx. Also, in patients with chronic recurrent aphthous stomatitis, enterococci (*Enterococcus spp* - in 30% of cases), staphylococci (*Staphylococcus spp* - in 50% of cases), streptococci (*Streptococcus spp* - in 40% of cases) were recorded.

The manifestation of chronic recurrent aphthous stomatitis is a diagnostic sign of intestinal dysbacteriosis: *S. aureus with hemolytic properties* and *S. epidermidis* isolation indicates dysbacteriosis of the mucous membranes of the oral cavity and intestines. A decrease in the content *of Streptococcus* and *Lactobacillus* with an increase in the number of *Candida* indicates that the dysbiotic conditions of these bacteria have worsened.

It has been shown that anaerobic microflora, mainly bacteroids and spirochetes predominate with stomatitis, as a result of which they are called "fuso-

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spirochetosis". At the same time, bacteriological examination often showed the presence of other microorganisms, for example, representatives of other anaerobic microflora (veillonella, peptostreptococci, vibrios, actinomycetes), as well as streptococci and staphylococci.

The study of the microflora of the mucous membrane of the affected area was carried out before and after treatment, and the following results were found. The range of detected microorganisms is diverse and indicates the development of conditionally pathogenic microflora, which also indicates the occurrence of dysbiocenosis in the oral cavity with chronic recurrent aphthous stomatitis.

Among patients of the main group, *Staphylococcus* spp · amount was recorded in 30% of cases (5 patients), *St. aureus was found in 2% of cases at a titer of* 10^{3} KHB/ml, *Streptococcus spp*. At 10^{5} KHB/ml - in 25% of cases, and *Streptococcus pneumoniae among streptococci* - in 83% of cases. *Neisseria was detected in 59% of cases at a titer of* 10^{10} KHB/ml and Leptobacteria at a titer of 10^{3}-10^{4} KHB/ml in 94% of cases. It was shown that *Leptotrichia* buccalis was detected at a titer of 10^{3}-10^{4} KHB/ml, and in 64.7% of cases - *C. albicans at a level of* 10^{4}-10^{5} KHB/ml. In the comparison group, *C. albicans* 10^{3}-10^{4} KHB/ml was found in 35.41% cases.

In patients of the main group, mainly cocci microflora was detected in 40.5% of cases, in which the average percentage of strains was 82.3 ± 6.3% . 39.2% of this coccal microflora was *Streptococcus* , 85.9% *Staphylococcus* , 84.4% *Peptostreptococcus* , 34.3% *Enterococcus* . Gram- negative bacilli were found in 25.0% of patients, including enterobacteria in 20.3%, *Pseudomonas in 4.7%, actinomycetes in 20.7%, and Corynebacterium* in 17.2% .

In the comparison group, the percentage of coccal microflora was on average 71.4±5.4% of strains, in which *Staphylococcus was detected* in 67.2% of cases, *Streptococcus* in 67.2% of cases, *Peptostreptococcus* in 64.1% of cases, and *Enterococcus in 21.5% of cases*. Gram-negative bacilli were found in 24.0% of patients, including enterobacteria in 18.0%, *Pseudomonas in 5.1%*, actinomycetes in 20.7%, and *Corynebacterium in 16.2%* .

Based on the obtained data, the following conclusion can be made, for example, with the frequent detection of conditionally pathogenic flora, in particular

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Candida fungi, in patients with chronic recurrent stomatitis against the background of chronic gastroduodenitis, it was shown that there is a natural imbalance in the microbiocenosis of the oral cavity.

Results of immunological studies in patients.

Immunity is the main development mechanism of RAS disease. Although a high titer of antibodies circulating in the blood was detected in patients using direct immunofluorescence microscopy, the results obtained in injured tissues using direct immunofluorescence microscopy showed negative results. According to the scientists, an increase in the factor of lymphokines and macrophages was found in the cellular immune response signalling. It has been shown that T-helper T-lymphocytes (CD4+) are mainly detected in the dermal cell infiltrate, and cytotoxic T-lymphocytes (CD8+) are mainly detected in the epidermis. Immune complexes are also involved in the pathogenesis of the disease and indicate damage to skin blood vessels. Some researchers have shown that CD4(+) cells are decreased and CD8(+) cells are increased, but CD4/CD8 may be decreased or normal in RAS [Sistig et al., 2002]. Additionally, CD8(+) cell counts and CD4/CD8 ratios have been shown to be higher in aphthous stomatitis when comparing primary and secondary outcomes [Lewkowicz et al., 2008].

CD4(+)-helper T-cells have been shown to be abundant in the pre-wound state of the disease, but CD8(+)-suppressor T-cells have been observed to decrease, and their ratio has been found to be 2/1. During the active ulceration of the disease, CD8(+)-suppressor T-cells increased and CD4(+)-helper T-cells decreased, and the ratio was 1/10. An increase in T-helpers was observed during the recovery period. These indicators indicate lymphocytotoxicity in RAS and report an imbalance in local immunoregulation [Jurge et al., 2006].

Studies were conducted on 40 RAS patients and 10 healthy donors. The main subpopulation of lymphocytes in peripheral blood was studied. The following results were obtained. The table shows that CD3+ CD19- CD45+ (T-lymphocytes) are decreased in patients with aphthous stomatitis compared to healthy people. On the other hand, the amount of CD3+ CD4+ CD8+ CD45+ (T-helpers/inducers) was found to be increased in patients with aphthous stomatitis compared to healthy controls.

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Table 1 Indicators of detection of lymphocyte phenotypic monoclonal antibodies in RAS patients

Indicators (%)	in RAS patients n=40	Control (healthy) n=10
CD 3+ CD 19- CD 45+ (T-lymphocytes)	73.45±1.48*	78.28±1.84
CD 3+ CD 4+ CD 8 + CD 45+ (T-helpers / inducers)	64.09±2.02*	55.09±2.02
IRI (index of immunoregulation CD 4+/ CD 8+)	1.63±0.12*	2.2±0.15
CD 3- CD 16+ CD 56+ CD 45+ (natural killers)	10.9±1.3*	15.0±1.4
CD - CD 19+ CD 45+ (V-lymphocytes)	4.1±1.26*	6.1±0.56
CD 38+ (predecessor T-V-lymphocytes)	18.6±1.68*	29.2±1.75

*- reliability compared to healthy donors, r< 0.05

IRI (immunoregulatory index CD4+/CD8+) and CD3-CD16+CD56+CD45+ (natural killers) were also found to be decreased in patients compared to healthy individuals. At the same time, CD- CD19+ CD45+ (V-lymphocytes) and CD38+ (precursor T-V-lymphocytes) indicators were also shown to decrease in patients compared to healthy people.

It has been shown that the origin of recurrent aphthous stomatitis may be the result of damage to the oral epithelium caused by a T-cell immune response. CD4(+)CD25(+) T-regulatory (Treg) cells can reduce the proliferation and effector function of other immune cells and thus play an important role in the control of the immune response. Treg cells from patients with aphthous stomatitis have been shown to inhibit the production of CD4(+) cytokines by T-effector cells compared to Treg cells from healthy individuals. In addition, it was found that Treg-cells obtained from patients have a 2-fold weaker effect on the inhibition of CD4(+)CD25(-) T-cell proliferation. However, the percentage of CD4(+)CD25(+)FOXP3(+) Treg cells in the peripheral blood of patients is low [Lewkowicz et al., 2008].

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