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THE STATE OF MICROBIOCENOSIS AND LOCAL PROTECTION FACTORS OF THE ORAL CAVITY IN PATIENTS WITH CHRONIC VIRAL HEPATITIS "C"

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ABSTRACT

The microbial flora composition of the oral cavity is heterogeneous. Both various quantitative and qualitative composition of organisms are determined in different parts of the oral cavity. Representatives of the unstable microflora of the oral cavity are found, as a rule, in very small quantities and in short periods of time. Long-term stay and vital activity in the oral cavity is prevented by local non-specific protective factors - saliva lysozyme, phagocytes, as well as lactobacilli and streptococci, which are constantly present in the oral cavity, which are antagonists of many non-permanent inhabitants of the oral cavity. In a healthy body, a constant microflora acts as a biological barrier. The article presents research date of the state of microflora and local immunity of the oral cavity in patients suffering from viral hepatitis C.

Key words: microflora, oral cavity, hepatitis C, protective factors.

VIRUSLI GEPATIT C AZOB CHEKAYOTGAN BEMORLARDA MIKROBIOSENOZ VA MAHALLIY OG‘IZ BO‘SHLIG‘INI HIMOYA QILISH OMILLARNING BUZILISHI

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ANNOTATSIYA

Og‘iz mikrobial florasining tarkibi bir xil emas. Turli sohalarda organizmlarning har xil miqdoriy va sifat tarkibi aniqlanadi. Doimiy bo‘lmagan og‘iz mikroflorasining vakillari, qoida tariqasida, juda oz miqdorda va qisqa vaqt ichida topiladi. Ularning og‘iz bo‘shlig‘ida uzoq vaqt turishi va hayotiy faoliyati mahalliy o‘ziga xos bo‘lmagan

himoya omillari - tupurik lizozimi, fagotsitlar, shuningdek og‘iz bo‘shlig‘ida doimiy ravishda mavjud bo‘lgan laktobakteriyalar va streptokokklar bilan to‘sqinlik qiladi, ular ko‘plab o‘zgaruvchan og‘iz aholisining antagonistlari hisoblanadi. Sog‘lom tanada doimiy mikrofloralar biologik to‘siq vazifasini bajaradi. Maqolada virusli gepatit C bilan og‘rigan bemorlarda mikrofloraning holati va og‘iz bo‘shlig‘ining mahalliy immuniteti bo‘yicha tadqiqotlar ma’lumotlari keltirilgan.

Kalit so‘zlar: mikroflora, og‘iz bo‘shlig‘i, gepatit C, himoya omillari.

СОСТОЯНИЕ МИКРОБИОЦЕНОЗА И МЕСТНЫХ ФАКТОРОВ ЗАЩИТЫ ПОЛОСТИ РТА У БОЛЬНЫХ ХРОНИЧЕСКИМ ВИРУСНЫМ ГЕПАТИТОМ «С»

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АННОТАЦИЯ

Состав микробной флоры полости рта неоднороден. В разных участках определяется различный как количественный, так и качественный состав организмов. Представители непостоянной микрофлоры полости рта обнаруживаются, как правило, в очень незначительных количествах и в короткие периоды времени. Длительному пребыванию и жизнедеятельности их в полости рта препятствуют местные неспецифические факторы защиты - лизоцим слюны, фагоциты, а также постоянно присутствующие в полости рта лактобациллы и стрептококки, которые являются антагонистами многих непостоянных обитателей полости рта. В здоровом организме постоянная микрофлора выполняет функцию биологического барьера. В статье приводятся данные исследований состояния микрофлоры и местного иммунитета полости рта у пациентов, страдающих вирусным гепатитом С.

Ключевые слова: микрофлора, полость рта, гепатит С, факторы защиты.

BACKGROUND

The current stage in the development of dentistry is characterized by the introduction of new effective preventive and diagnostic measures, which became possible thanks to the discoveries made in the study of the mechanisms of the formation of pathological conditions [5].

Many bacteria and fungi are able to effectively colonize the surface of the mucous membranes of the oral cavity and teeth. Understanding the form in which

microorganisms exist there fundamentally affects the effectiveness of the prevention and treatment of diseases, as well as the conditions for maintaining normal microflora. It has been established that in the human body, all bacteria and unicellular fungi during reproduction, including in the oral cavity, form communities of varying complexity, one example of which is the well-known colonies [5,7].

In recent years, it has been shown that the colonization of certain niches in the human body, including the oral cavity, by microbes carrying dangerous toxins leads to the emergence of various diseases. In this case, the indirect action of bacteria plays a key role in the lesions that occur in the oral cavity and beyond. The oral cavity, due to its anatomical and physiological features, constantly encounters various microorganisms. At the same time, the composition of the normal microflora is determined by the presence of certain receptors on cells, the level of hormones, and other factors that affect the interaction of microbes in the human body [3].

Viral hepatitis C (HCV) is currently one of the urgent public health problems due to its prevalence in the population, the high incidence of liver cirrhosis and hepatocellular carcinoma, the development of extrahepatic manifestations that determine the difficulties in diagnosing the disease and its treatment [1].

The hepatitis C virus is responsible for 20% of all cases of acute hepatitis, and chronic HCV infection is responsible for the development of 70% of chronic hepatitis, 40% of all cases of end-stage cirrhosis, 60% of hepatocellular carcinoma, and in 30% of cases referral for liver transplantation.

Of interest is the fact that infection with hepatitis viruses, including HCV, is also possible through the oral mucosa, for example, when kissing. It has already been proven that hepatitis B and C viruses are transmitted by all body fluids - up to 30% hepatitis B virus and up to 5% hepatitis C virus.

It is known that half of patients with chronic hepatitis C have extrahepatic manifestations of the disease, including lesions of the oral cavity, they have a high incidence of symptoms of periodontal diseases, there was the presence of periodontitis and gingivitis, and in patients with chronic hepatitis C, these diseases are mainly represented by generalized forms of severe and moderate degree, lymphocytic sialadenitis [6].

With the development of dysbiotic processes in the oral cavity, as well as other pathological processes in the gastrointestinal tract, the entry of endotoxin into the systemic circulation increases, which may be the cause of the intoxication syndrome and the deepening of chronic inflammation in tissues [2,4].

At the same time, there have been few significant studies on the state of the oral mucosa in patients with chronic viral hepatitis C in the Republic of Uzbekistan so far. In this regard, the study of extrahepatic manifestations of viral hepatitis C in the oral

mucosa will contribute to the improvement of organizational, therapeutic and preventive measures aimed at improving the quality of life of patients.

AIMS: Based on the foregoing, we set ourselves the goal of studying in patients suffering from viral hepatitis C, quantitative and qualitative changes in the microflora of the oral cavity, as well as violations occurring in local oral protection factors, based on which it is necessary to develop an algorithm for diagnosis, treatment and prevention. identified disorders in patients, thereby improving the quality of life of patients.

MATERIALS AND METHODS

To achieve this goal, we conducted microbiological and immunological studies in 132 patients suffering from viral hepatitis C and being hospitalized at the Republican Specialized Scientific and Practical Medical Center of epidemiology, microbiology, infectious and parasitic diseases.In all these patients, as a rule, oral fluid was taken by flushing from the oral mucosa (by rinsing); for this, test tubes with 4.5 ml of sterile saline were prepared . The material obtained by this method was considered as the first dilution. A number of serial dilutions were prepared from this material in the laboratory, and then a certain volume of them was seeded onto the surface of highly selective nutrient media. For this, we used nutrient media produced by the Indian company «Hei Media», such as Endo medium, yolk-salt agar, Sabouraud-agar, MPC-4, Muller Hinton medium, etc. Inoculations on blood agar, Endo, milk-salt agar and Sabouraud's medium were cultivated under normal conditions for 18-24 hours at a temperature of 37^0C, and the cultivation of inoculations to isolate anaerobes was carried out in an anaerobic balloon by using gas generating cartridges.After the specified periods, all the inoculated dishes were removed from the thermostat, the grown colonies of microbes were counted, the group and species belonging of the isolated colonies were determined based on the microscopy data of Gram-stained smears, the nature of growth on selective nutrient media and biochemical properties.When working according to the modified method, the result was taken into account according to the last dilution in which the growth of bacteria was obtained, the number of microorganisms was calculated according to the following formula: K = 200 x P (CFU/ml), the number of microbes of each species was expressed in CFU/ml.

In parallel with microbiological studies in the same patients, local oral protection factors were studied, such as the phagocytic activity of leukocytes, the level of lysozyme, the titer of class A immunoglobulin, secretory fractions (s Ig A) of Mancini.

RESULTS

At the same time, we studied the quantitative and qualitative composition of the microflora, as well as the indicators of local protection factors in the oral cavity in patients with viral hepatitis C. We obtained the most interesting data when conducting

quantitative microbiological studies in the oral fluid of patients with viral hepatitis C. The data obtained from these studies are presented in table № 1. The table shows that in patients suffering from viral hepatitis C, dysbiotic changes in the microflora develop in the oral cavity.

Thus, in the flora of the oral fluid in patients with HCV upon admission to the clinic, it can be seen that the most significant changes are observed in the anaerobic flora. A characteristic trend of these changes is a significant decrease in the total number of anaerobes, which amounted to 3.30±0.2 colony forming unit (CFU) /ml at a rate of 7.70±0.2 CFU/ml.

The same trend towards a decrease in seeding is observed in cultures of lactobacilli when their number was 2.15±0.15 CFU/ml, at a rate of 4.30±0.15 CFU/ml. Although it should be noted that among the anaerobic flora, the number of peptostreptococci increased and amounted to 5.10±0.21 CFU/ml. Apparently, this is due to compensatory-adaptive processes in the oral cavity.

Table № 1.

The state of the microflora of the oral cavity in patients with viral hepatitis C
Lg (M±m) CFU/ml.

№	Groups of microbes	Number of microbes in 1 ml of saliva		
		Norm	In patients with HCV	P
1	Total number of anaerobes	7.70±0.2	3.30±0.2	
2	Lactobacilli	4.30±0.1	2.15±0.1	
3	Peptostreptococci	5.0±0.1	5.10±0.2	
4	Total number of aerobes	5.0±0.1	8.30±0.4	
5	Staphylococcus golden	0	2.30±0.1	
6	Staphylococcus epider	4.10±0.2	3.30±0.2	
7	Staphylococcus saprof.	2.30±0.1	4.30±0.2	
8	Streptococcus gr.A	0	3.0±0.1	
9	Enterococcus	4.30±0.2	3.65±0.1	
10	Escherichia LP	2.15±0.1	5.60±0.3	
11	Escherichia LN	2.30±0.1	6.10±0.2	
12	Fungi Candida	3.15±0.2	5.15±0.2	

However, the most pronounced quantitative changes in patients with HCV occurred in the facultative group of microbes. as can be seen from the table, most of the microbes of this group increased by 2 orders of magnitude, this especially affected such microbes as saprophytic staphylococci, Escherichia and fungi. And only epidermal staphylococci and enterococci decreased somewhat.

It should be noted that microbes appeared in the oral cavity in children with HCV, which were not sown in the control group, here we mean the detection of cultures of Staphylococcus aureus and lactose-negative Escherichia. It is appropriate to note

that these are nothing more than strains that have more pronounced aggressive properties, and treating dentists must take this fact into account when providing medical care to such sick patients (Fig. 1, 2, 3, 4, 5, 6).

Along with microbiological studies, immunological studies were carried out in the same patients suffering from HCV. At the same time, much attention was paid to the study of the state of local protection factors, such as lysozyme titer, phagocytic index of neutrophils, the level of the secretory fraction of immunoglobulin class A (Ig A) in the oral fluid. The data obtained from these studies are presented in table №. 2. The table shows that in patients suffering from HCV, according to all studied parameters, there is a secondary immunodeficiency.

It is known that most pathogenic viruses have an immunosuppressive effect on our body, in fact, in our studies, we found complete confirmation of these postulates, and also once again convinced ourselves of the unity of the body's homeostasis.

Table № 2

Indicators of local oral protection factors in patients with viral hepatitis C.

№	Indicators	Norm	In patients with HCV	P
1	Lysozyme titer mg/%	18.0±0.61	11.5±0.25	
2	Phagocytic index %	54.2±1.30	38.5±1.5	
3	Level of s Ig A g/l	2.10±0.11	0.61±0.12	

We obtained the most interesting data when studying the colonization resistance of microbes in oral cavity biotopes, such as the gums, the surface of the tongue, cheeks and palate in HCV patients.

According to our studies (Tables № 3, 4), it was found that the density of the microbial population in the oral cavity in the control group is a fundamental characteristic of communities and largely depends on the topography of the ecological niche.

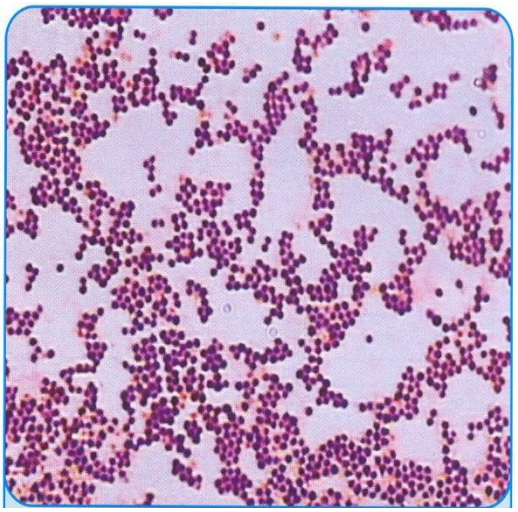
Table №3

The state of colonization resistance of microbes of biotopes of the oral cavity in the control group (M±m) CFU/cm^2

№	Groups of microbes	Biotopes of the oral cavity				
		gum	tongue	cheek	palate	P
1	Lactobacilli	2.90±0.1	2.30±0.1	2.10±0.1	1.0±0.1	
2	Streptococcus salivarius	5.30±0.2	4.15±0.2	1.30±0.1	1.10±0.1	
3	Streptococcus mutans	4.10±0.2	3.60±0.1	2.30±0.1	1.0±0.1	
4	Streptococcus mitis	3.60±0.2	3.45±0.1	2.5±0.1	1.0±0.1	
5	Staphylococcus	3.40±0.2	4.0±0.2	3.0±0.1	1.30±0.1	
6	Escherichia	2.15±0.1	2.0±0.1	0	0	
7	Klebsiella	0	0	1.15±0.1	0	
8	Fungi	3.0±0.1	2.10±0.1	0	0	

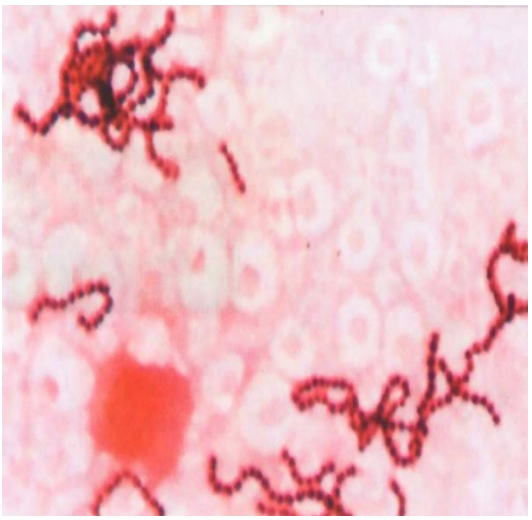
At the same time, Gram-positive flora prevailed in terms of abundance and species composition in the biocenosis, which was colonized in 100% of the subjects. It is interesting to note that the main part of the microflora of the oral cavity in the control group consisted of representatives of the genus Streptococcus, among which the dominant strains were Streptococcus salivarius. These data are in good agreement with the literature data.

Fig#1 Growth of a culture of staphylococcus on blood agar



Fig#2 A smear from a pure culture of staphylococcus, Gram-stained

Fig#3. Streptococcus culture growth on blood agar



Fig#4. Gram-stained smear from a pure culture of streptococcus

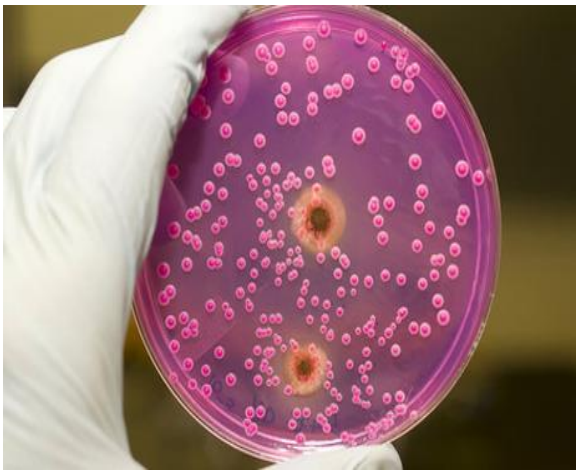


Fig #5. Growth of fungal culture on Sabouraud's medium

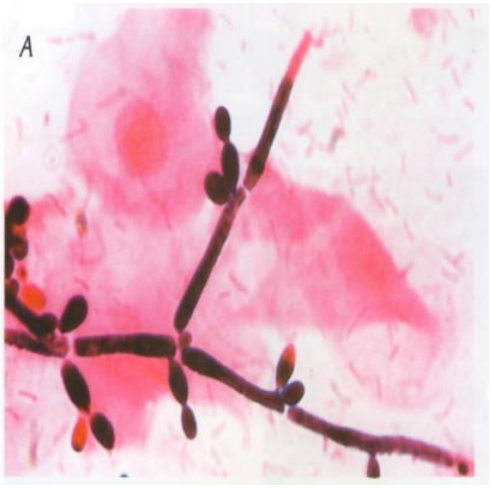


Figure #6. A smear from a pure fungal culture

Although it should be noted that among the Gram-positive flora, staphylococci occupy a significant place in colonization, while their number prevailed on the surface of the gums and tongue. Among other studied groups of microbes, gram-negative rods weakly possessed this property in matters of colonization of the oral cavity, and fungi of the genus Candida had the ability to colonize only the mucous membrane of the tongue and gums.

It is quite obvious that the study of the ability of microbes to colonize various objects of the oral cavity, apparently, is undoubtedly associated with the state of the oral fluid, as well as the presence of special receptors in our cells with which the relationship occurs.

The next group of our studies on the colonization resistance of microbes in various biotopes of the oral cavity consisted of patients suffering from HCV. The materials of these studies are presented in Table № 4.

Table № 4

Characteristics of colonization resistance of microbes according to biotopes of the oral cavity in patients with viral hepatitis C (M±m) CFU/cm2

№	Groups of microbes	Biotopes of the oral cavity				
		gum	tongue	cheek	palate	P
1	Lactobacilli	1.80±0.1	1.45±0.1	0	1.0±0.1	
2	Streptococcus salivarius	2.10±0.1	2.60±0.1	1.0±0.1	1.0±0.1	
3	Streptococcus mutans	1.85±0.1	1.30±0.1	1.20±0.1	1.0±0.1	
4	Streptococcus mitis	1.35±0.1	1.20±0.1	1.10±0.1	1.10±0.1	
5	Staphylococci	2.40±0.1	3.20±0.2	2.30±0.2	1.30±0.1	
6	Escherichia	2.20±0.2	1.30±0.1	1.30±0.1	1.10±0.1	
7	Klebsiella	1.0±.1	1.10±0.1	1.0±0.1	1.0±0.1	
8	Fungi	2.15±0.2	2.0±0.1	1.30±0.1	1.80±0.1	

The table shows that in these patients there were significant changes in the issues of colonization in almost all biotopes.

DISCUSSION

At the same time, it is interesting to note that in almost all biotopes there are the following shifts:

- a significant decrease in the ability of colonization in strains of lactobacilli;
- a sharp increase in the ability to colonize the culture of staphylococci and fungi of the genus Candida;
- among the gram-negative flora, one can state a stable position for colonization in cultures of Escherichia and Klebsiella.

CONCLUSIONS

Thus, based on the conducted microbiological studies, the following conclusions can be drawn:

1. In sick people suffering from viral hepatitis C, dysbiosis develops in the oral cavity, a characteristic feature of which is a significant decrease in the number of anaerobes, but an increase in the number of facultative flora.
2. Viral hepatitis C has an immunosuppressive effect on the body of patients, as a result of which, first of all, there is a pronounced decrease in local protective factors in the oral cavity, among which the lowest values for immunoglobulins A - the secretory fraction and phagocytosis indicators.
3. Among all the studied parameters, namely dysbiosis, immunodeficiency, colonization resistance and the clinical course of HCV, there is a positive correlation, which indicates the unity of the homeostasis of the human body.

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