wjpmr, 2018,4(11), 318-323

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

VIOLATIONS OF MICROECOLOGY AND LOCAL FACTORS OF ORAL CAVITY PROTECTION IN PATIENTS WITH HEPATOBILIARY PATHOLOGY

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Article Received on 18/09/2018 **Article Revised on 09/10/2018** **Article Accepted on 30/10/2018**

ABSTRACT

Quantitative and qualitative indices of the oral fluid microflora are presented in patients with hepatobiliary pathology suffering from chronic cholecystitis. It was found that the total number of the facultative group of microbes exceeds the amount of anaerobic microbes. Among gram-positive cocci, staphylococci predominate. The number of gram-negative microbes belonging to the genus Escherichia and Proteus has significantly increased. Among the anaerobic group of microbes, the number of lactobacilli decreased almost 2 times. In determining the colonization resistance of microbes in various biotopes of the oral cavity in patients with chronic cholecystitis, significant changes occurred, compared to healthy individuals, it was possible to establish a reliable increase in colonization in all biotopes of gram-negative flora and fungi of the genus Candida. Local protective factors were studied: lysozyme titer, phagocytosis index and level of secretory immunoglobulin A (sIgA), the results showed the development of immunodeficiency in all studied parameters.

KEYWORDS: dyskinesia of the gallbladder, oral cavity, dentogingival grooves, oral fluid microflora, chronic cholecystitis, sIgA.

INTRODUCTION

In recent decades, gallbladder and biliary tract diseases have increased, which is more than 11% of the total number of diseases of the digestive system.[10,11,12] Thus, dyskinesia of the gallbladder is isolated in 6% of cases. The frequency of dyskinesia is 170 per 1000 population. The disease is characterized by motor-tonic abnormalities of bile duct sphincters and a violation of the bile composition, mainly the content of cholesterol, phospholipids and lecithin changes.[7,8,9,14]

It is known that very often dyskinesia of the gallbladder passes into chronic cholecystitis, when the inflammatory process develops in the gall bladder itself.

It is proved that the pathogenesis of this disease is played by the role of infection and stagnation of bile, acting simultaneously. It is believed that the main causes of the disease are colibacillary and coccal infections.[3,4,13]

All of the foregoing allows us to believe that the violation of bile flow into the intestine has a pronounced effect on motor and suction processes, which in turn affects the state of the intestinal microflora. In addition, due to anatomical proximity and functional conjugation, the pathology occurring in one of the organs of the gastroduodenal zone undoubtedly causes shifts on the part of other organs of this zone.[6,7,16,18] This circumstance indicates the need to consider the problem of stomach and bile duct disease from the point of view of the integrity of the gastroduodenal complex.

It is interesting to note about the relationship between the oral cavity and the gastrointestinal tract, because it is a predisposing factor to the development of the disease. Thus, it is established that in patients with gastroesophageal disease, chronic pancreatitis, gastric ulcer disease of the oral cavity significantly increase, in comparison with healthy individuals. In addition, modern researchers have proved that after the course of treatment of dysbacteriosis in patients with generalized periodontitis, positive dynamics of local immunological parameters is observed, which is another confirmation of the close relationship between diseases of the oral cavity and gastrointestinal tract.[4,9,10,13,17,19]

The state of health of tissues and organs of the oral cavity: lips, teeth, tongue, cheeks, palate, etc. determine the level of human health within the framework of the

integrative concept. In this connection, the idea of II. Mechnikov about the leading role of the oral cavity in maintaining human health from the modern world outlook.

The microflora of the oral cavity is a highly sensitive microbiota indicator system that reacts both with qualitative and quantitative shifts in response to pathological changes in the organs and systems of the human body.

In the course of evolution with constant interactions of the human body of numerous populations of environmental microorganisms, adaptation accompanied by the improvement of the symbiosis of the human organism took place, as well as the selection of microorganisms capable of adhesion and colonization of the microbiota biotope of the oral cavity. As a result, there were stable symbiotic associations of microorganisms, as well as peculiar ecological niches of microbiota in the natural depressions of the crown parts of teeth, dentogingival grooves in fissures, dentogingival grooves on the back of the tongue.[17,19,20]

According to the latest literature, the total area of the skin and mucous membranes of an adult reaches 500 m^2. Of this area, 80.5% falls on the surface of the large intestine, 16% on the mucous membrane of the lungs, 0.5% (2.5 m^2) on the surface of the skin and only 0.01% (0.05 m^2) of the oral cavity.

It is known that the human fetus has sterile biotopes of the oral cavity and nasopharynx. However, passing through the birth canal of the mother at birth, the oral cavity and nasopharynx are populated with microbial associations of the biocenosis, which, in turn, sets the algorithm for the development of the microbiota of the organism and immunity. In natural conditions, the microbiota of the protective biofilm of the oral cavity first of all serves as an antimicrobial filter preventing the colonization of the biotope by pathogenic microorganisms, translocation and penetration of toxins into the internal environment of the body, especially the respiratory and digestive ones.

It has been proved that by means of a microbiota of a biofilm the human body regulates immune responses of local and systemic levels. Protective biofilm is an active sorbent, which removes toxins from the body. It supports the energy and trophic metabolism, fulfills the role of a specific regulator that maintains harmony in the relationship of the organism with its own, indigenous microflora of the oral cavity of the biotope that are translocated into the biotope during ingestion and respiration, and also with microorganisms.

Proceeding from the above, we set ourselves the goal to study quantitative and qualitative shifts in microecology and local factors of oral cavity protection in patients with chronic cholecystitis.

MATERIAL AND METHODS

To achieve this goal, we conducted microbiological and immunological studies in 52 people, of whom 10 were healthy individuals, and 42 of them were sick with chronic cholecystitis. All these patients were taken oral fluid by flushing from the oral mucosa (by rinsing) for this pre-prepared tubes with 9 ml of sterile saline according to the method of Efimovich O.I., 2002.[5]

The resulting material in this way was considered as the first dilution (101). From this material, a number of serial dilutions were prepared in the laboratory, subsequently they were sowed sectorally on the surface of highly selective nutrient media. For this purpose, we used nutrient mediums produced by the Indian firm HeiMedia, such as Endo, Milk Salt Agar, Saburoagar, MPC-4, Blauroka, etc.

Seeds on bleeding powder, Endo milk molochnomagar, Saburo was cultivated under normal conditions for 18-24 hours at 370C, and the cultivation of crops for anaerobic release was carried out in anaerobic by using gas-generator cartridges in thermostatic conditions for 3-5 days. At the end of the indicated dates, all the sown Petri dishes 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 Gram staining microscopy data of the growth pattern on selective nutrient media.

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

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

When working on a modified procedure, the result was taken into account at the last dilution in which the growth of the bacterium was obtained, the number of microbes was calculated according to the following formula: K = A * P / CFU / ml /, the number of microbes of each species was expressed as LgKO / ml.

IMMUNOLOGICAL RESEARCH

In parallel with microbiological studies, local factors of oral protection such as phagocytic activity of neutrophils, lysozyme level and immunoglobulin A titer of the secretory fraction (sIgA) were studied in the same patients with chronic cholecystitis. To determine the phagocytic activity of neutrophils in the oral fluid, a

modified procedure was used by Antonova A.V., 1999.[2] To do this, the selected oral liquid was purified, washed with a buffered solution and centrifuged at 1000 rpm for 10 minutes, the packed liquid was poured off, and 0.5 ml of saline was added to the precipitate. After that, 0.1 ml of latex suspension (5 * 108 in 1 ml) with a diameter of 0.5 μm was added to 0.2 ml of the obtained suspension in a tube. The mixture was incubated in a wet chamber for 30 minutes at 370C. Subsequently, from this mixture, the mints were prepared and stained by Romanovsky-Giemsa. Smears were examined under a microscope, counted at least 100 neutrophils with latex and without it in each preparation the index of phagocytosis was determined and expressed in%.

The activity of lysozyme in the oral fluid was determined by the method of the proposed Sh.R. Aliev, 2004,[1] which included the use of paper discs (similar to antibiotic discs) and thoroughly impregnated them in the oral liquid. After these discs were placed on the surface of Müller Hinton's nutrient agar in Petri dishes previously seeded with lawn by the daily culture of M. Luteus strain No. 003596/126 national collection of human infection microorganisms of the Institute of Emission of the Ministry of Health of the Republic of Uzbekistan, the crops were incubated in a thermostat at 370C, the activity of lysozyme in the oral liquid was determined by the diffusion method in Agar.

Determination of the immunoglobulin class titer of the a secretory fraction (SIGA)

The method is based on the Mancini method, which is based on the measurement of the diameter of the precipitation ring formed when a carbonaceous liquid is introduced into wells cut out in an agar layer in which monospecific sera are pre-dispersed. Under standard experimental conditions, the diameter of the precipitation ring is directly proportional to the concentration of immunoglobulin.

We also conducted a study of the state of colonization resistance of microbes in the oral biotopes of patients with chronic cholecystitis, such as the gum, tongue, cheeks and palate (Levinson U., 2015).

To accomplish this task, we used stainless steel liners with a certain depth and surface, which after thorough sterilization under aseptic conditions were filled with highly selective nutrient media, after which Petri dishes were placed and stored in a refrigerator. In the examination of patients with chronic cholecystitis, the cultures were seeded with prints, for this purpose these cartridges were applied to the surface of the mucous membranes by the nutrient media: the gum, tongue, cheeks and sky for 2-3 seconds, then these sleeves were again introduced into Petri dishes and placed in a thermostat at temperature 370Snah 24-48 hours, after the incubation of the cup was removed from the thermostat was taken from them sleeve with crops and counted the grown colony CFU/cm2. After that, morphology, cultural, tinctorial and biochemical properties were studied in the grown up colonies, thereby establishing the microbial species.

RESULTS

In all examined patients with chronic cholecystitis, we conducted a study of quantitative and qualitative research of oral fluid microflora. The data obtained in these studies are presented in Table 1. As can be seen from the table, in healthy people the microflora of oral fluid is quite diverse and is characterized by the following features: the quantitative indices of anaerobic microbes are significantly higher than aerobic, the predominant flora of the oral cavity is Gram-positive cocci, among which parity belongs to streptococci. Gram-negative flora, as a rule, is insignificant. Among the anaerobic microbes the bulk of the lactobacilli.

At the same time, the study of the quantitative parameters of oral fluid flora in patients with chronic cholecystitis, we see a completely different picture. Thus, the total number of the facultative group of microbes exceeds the amount of anaerobic microbes. Among gram-positive cocci, staphylococci predominate.

The number of gram-negative microbes belonging to the genus Escherichia and Proteus has significantly increased. Among the anaerobic group of microbes, the number of lactobacilli was almost halved.

Table 1: Characteristics of oral fluid microflora in patients with chronic cholecystitis Eg M ± m CFU / ml.

№	Groups of germs	Norm	Number of microbes in 1 ml of saliva
			In patients
1	Total number of anaerobes	6,30±0,5	4,60±0,2
2	Lactobacilli	5,70±0,4	3,10±0,4
3	Peptostreptococci	4,85±0,3	5,0±0,3
4	Total number of aerobes	4,60±0,2	6,30±0,5
5	Staphylococcus aureus	0	5,60±0,3
6	Staphylococcus epidermis	3,40±0,2	6,10±0,4
7	Streptococcus salivarius	3,70±0,3	4,15±0,3
8	Streptococcus mutant	2, 0±0,2	5,10±0,4
9	Streptococcus mitis	2,10±0,1	4,60±0,3
10	Esherichia LP	1,30±0,1	2,30±0,2

11	Escherichia LN	0	3,10±0,2
12	Proteus	1,10±0,1	4,10±0,2
13	Mushrooms of the genus Candida	2,0±0,1	3,15±0,1

In the same patients, along with conducting a microbiological study, we also carried out immunological studies to study the state of local factors of oral protection. In this case, mainly studied such indicators as: lysozyme titer, phagocytosis index and level of secretory immunoglobulin A (sIgA). The data obtained in these studies are presented in Table 2. From the data given, it can be seen that in patients with chronic cholecystitis, immunodeficiency is noted for all parameters studied. Thus, the lysozyme titer was 14.0 ± 0.3 mg /%, the phagocytosis index was 40.1 ± 2.0 mg /%, the level of secretory immunoglobulin A was 1.4 ± 0.1 g / l.

Naturally, the question arises as to what the microbiological and immunological changes revealed by us in patients with chronic cholecystitis are related. Apparently, and it is quite obvious that a decrease in the intake of bile in the 12th colon affects indirectly the processes of translocation of microbes from the intestine to the oral cavity, which in turn affects the state of local factors of oral protection. In addition, it is known that bile itself is an antibacterial substance, ie it destroys and lyses many microbes.

Obviously, all these issues cause the syndrome of excessive growth of microbes in the oral cavity; which is characterized by the development of dysbiosis.

Table 2: The state of local factors of oral cavity protection in patients with chronic cholecystitis.

№	Indicators	The number of microbes in 1 ml of saliva	
		Norm	In patients
1	Titer of lysozyme mg /%	19,60±0,5	14,0±0,3
2	The index of phagocytosis%	56,0±2,5	40,1±2,0
3	The level of secretory IgA (sIgA) g / l	2,10±0,1	1,4±0,1

Interesting data we obtained in the study of colonization resistance of microbes in the biotopes of the oral cavity, such as; gum, tongue, cheek and palate in patients with chronic cholecystitis.

The materials of these studies are presented in Table 3.4, as can be seen from Table 3 in healthy people, the indices of colonization resistance depend on the topography of the etiological niche. It was noted that its greatest significance was noted in the gum and tongue, minimal on the mucous membranes of the sky. In this case, the predominant in number and species composition in the biocenosis was the gram-positive flora which colonized 100% of the surveyed biotopes. In this case, parity belongs in all matters of microbes belonging to various types of streptococci. Although it should be noted that among the gram-positive cocci flora, a significant place in colonization is 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, this property was very poorly possessed by gram-negative rods: Escherichia and Klebsiella, and fungi of the genus Candida have the ability to colonize only the mucous membranes of the gum and tongue.

Table 3: The indices of colonization resistance of microbes of biotopes of the oral cavity in healthy M ± m CFU / cm^2.

№	Groups of germs	Biotopes of the oral cavity			
		Gums	tongue	cheek	palate
1	Lactobacilli	3,15±0,3	2,30±0,1	1,30±0,1	1,0±0,1
2	Strepsalivar	4,30±0,3	3,75±0,3	1,45±0,1	1,0±0,1
3	Strepmutans	2,70±0,2	3,10±0,2	1,10±0,1	1,10±0,1
4	Stremmitis	3,45±0,2	3,30±0,2	1,30±0,1	1,20±0,1
5	Staphylococcus aureus	3,0±0,2	3, 0±0,2	1,0±0,1	1,0±0,1
6	Escherichia	0	0	0	0
7	Klebsiella	1,10±0,1	0	0	0
8	Mushrooms grandida	1,30±0,1	2,15±0,1	0	0

It is quite obvious that the study of the ability of microbes to colonize different biotopes of the oral cavity makes it possible to understand those processes that occur in the oral cavity and apparently are undoubtedly related to the state of ph of the oral fluid, as well as the presence of specific receptors in our cells.

The next group of our studies on the study of colonization resistance of microbes in various biotopes

of the oral cavity was made by patients with chronic cholecystitis. The data obtained in these studies are presented in Table 4. It can be seen from the table that patients with chronic cholecystitis experienced significant changes in colonization in almost all biotopes. It is interesting to note that the following changes occur in almost all biotopes: they include:

Firstly, colonization by biotopes of lactobacilli was reliably reduced, and in the biotope of the sky it was generally eliminated.

Second, there is a decrease in the ability to colonize biotopes of microbes related to streptococci.

Third, there is a significant increase in colonization, almost all biotopes in the oral cavity, of gram-negative microbes, such as Escherichia and Klebsiella. Mushrooms of the genus Candida have the same tendency.

Table 4: Characterization of colonization resistance of oral biotopes in chronic holicystitis M±m CFU / cm^2.

№	Groups of germs	Biotopes of the oral cavity			
		Gums	tongue	cheek	palate
1	Lactobacilli	2,0±0,1	1,30±0, 1	2,10±0, 1	0
2	Strepsalivar	3,0±0,2	3,10±0,2	1,30±0,1	1,0±0,1
3	Strepmutans	2,10±0,1	3,0±0,2	1,30±0,1	1,10±0,1
4	Stremmitis	2,0±0,2	2,10±0,1	1,15±0,1	1, 0±0,1
5	Staphylococcus aureus	3,60±0,2	4,0±0,3	3,0±0,2	2,60±0,1
6	Escherichia	2,0±0,1	2,30±0,1	2,10±0,1	1,60±0,1
7	Klebsiella	1,60±0,1	2,0±0,1	2,0±0,1	1,30±0,1
8	Mushrooms grandida	3,15±0,3	4,10±0,3	4,0±0,3	3,10±0,3

CONCLUSION

Thus, based on the microbiological and immunological studies of the oral fluid in patients with chronic cholecystitis, the following conclusions can be drawn:

1. Microbiological studies of the oral fluid in patients with chronic cholecystitis showed that they have dysbiosis in the oral cavity, a characteristic feature of which is a significant increase in the number of staphylococci against a background of a decrease in streptococci.
2. Immunological studies, according to the state of local factors of oral protection, such as lysozyme titer, the phagocytosis index and the level of immunoglobulin A secretory fraction showed the development of immunodeficiency in all studied parameters.
3. Study of colonization resistance of microbes in various biotopes of the oral cavity such as: gum, tongue, cheek and palate, in patients with chronic cholecystitis allowed to establish a significant decrease in lactobacilli and streptococci, but increased colonization in all biotopes of gram-negative flora and fungi of the genus Candida.

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