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SOME FEATURES OF THE MICROBIOTA COMPOSITION OF WICKHAM'S STRIAE SURFACE AND EROSIVE- ULCERATIVE ELEMENTS IN PATIENTS WITH ORAL LICHEN PLANUS

A comparative study of the microbiota profile of the Wickham mesh surfaces (a typical form of lichen planus (LP) (L43.80) and erosive-ulcerative elements in the erosive-ulcerative form (L43.82) of LP with localization on the buccal mucosa was carried out. In the studied microbiota of the surface of erosive and ulcerative elements, significant differences were revealed from the composition of the microbiota of the surface of the Wickham mesh, which is associated with clinical manifestations of the oral cavity mucous lining (LP OCML). The data on the quantitative and qualitative composition of microorganisms in the erosive-ulcerative form of LP were significantly different from the typical form of LP, which indicates the need for further studies of the microbiota in other forms of LP OCML. The revealed changes in the composition of the microbiota in the typical form of LP OCML can serve as a potential prognostic criterion for the conditional norm, and in the erosive-ulcerative form, as a criterion indicating the progression and aggravation of the clinical picture.

Keywords: lichen planus, typical form, erosive-ulcerative form, Wickham mesh, erosions, ulcers, microbiota, Candida spp.

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Introduction. The presence of a microbial factor in the development of lichen planus involves qualitative and quantitative changes in the composition of gram positive and negative bacilli and cocci depending on the form of oral mucosal lichen planus (OLP). According to numerous studies, the oral microbial community in patients with OLP OCML undergoes dysbiosis in a significant number of cases. Signaling pathways involved in cellular processes such as keratinization, inflammation, and T-cell responses are triggered in the pathogenesis of the development and aggravation of OLP erosive forms [1-5, 7-9, 12-16, 18 - 21].

Numerous foreign studies present data on the composition of the oral microbial flora in rubella mucosa, the presence of significant amounts of Capnocytophaga sputigena, Eikenella corrodens, Lactobacillus crispatus, Mobiluncus curtisii, Neisseria mucosa, Prevotella bivia, Prevotella intermedia and S. Agalactiae, Bacteroides ureolyticus, Dialister species, Staphylococcus haemolyticus and Streptococcus agalactiae [6]. An increase number of Neisseria, Haemophilus, Fusobacterium, Porphyromonas, Rothia, Actinomyces and Capnocytophaga in oral mucosal smears was observed in patients with OLP by 16S sequencing [9, 10], as well as evidence of changes in the overall structure of the salivary microbiome in OLP in the form of high values of Porphyromonas and Solobacterium and low Haemophilus, Corynebacterium, Cellulosimicrobium and Campylobacter, Streptococcus comprising in erosive-ulcerative form [16].

Oral microbiocenosis disorder in patients with OLP is characterized by high levels of yeast-like fungi of Candida and Aspergillus, Alternaria and Sclerotiniaceae unidentified, Bovista, Erysiphe, Psathyrella, etc. Aspergillus has been identified as a "OLP-associated" fungus because of its detection with higher frequency in the clinical course of OLP compared to healthy patients [17].

A number of researchers provide evidence of the relationship between the clinical OLP OCML manifestations and the persistence of infection in the oral tissues, while the role of fungal-bacterial interactions in the OLP pathogenesis remain virtually unstudied, which led to the purpose and relevance of our investigation.

The aim of this research is a comparative study of cheek mucosa microbiota profile in patients with the typical OLP form (L43.80) and erosive-ulcer OLP form (L43.82).

Material and methods. The comparative microbiological research of some representatives of microbiota surface of erosive-ulcerative elements and Wickham's striae was carried out during complex clinical investigation of patients (n=111) with ICD-10 diagnosis (L43.82) of erosive-ulcerative and typical forms of OLP (L43.80).

We examined smears obtained from the surface of Wickham's striae and erosive-ulcerative elements localized on the cheek mucosa. Swabs were taken before individual oral hygiene and food intake, delivered to the laboratory within 2 hours. The isolation of individual representatives of the microbiota was carried out by seeding on traditional, special and differential diagnostic media, followed by biochemical identification using HiCrome and Lachema media series. Due to a small number of species diversity, some representatives of microbiota were not identified to species.

An electronic database was created, which allowed statistical analysis of independent sample data in R Studio environment using nonparametric Mann-Whitney criterion. Differences in the frequency of occurrence or absence of a trait were determined using the χ2 criterion, including Yates' correction. The null hypothesis of no difference was rejected in favor of the alternative hypothesis if the p-value did not exceed 0.05 in all criteria.

Results and their discussion. The results of the microbiological study showed that representatives of Gram-positive and Gram-negative facultative anaerobic cocci and bacilli, as well as Candida yeast-like fungi in the average quantity from 2 to 6 Lg CFU/unit were found in almost all examined patients, regardless of the OLP form, in the microbiota of the Wickham's striae surface or erosion surface.

Nevertheless, a comparative analysis of microbiota representatives showed a significant difference in their quantitative and qualitative content. The preponderance of representatives of genera Fusobacterium ($P < 0.01$) and Candida ($P < 0.001$) was marked in patients with erosive-ulcer OLP form (L43.82), that gave the grounds to define them in the basic clinical group (n=86), other patients with typical OLP form (L43.80) formed a comparison group (n=25) (Fig. 1).

Representatives of 10 genera were studied of all the variety of qualitative and quantitative composition of Wickham's striae microbiota and erosive-ulcerative elements. A significant predominance of Candida spp. ($P < 0{,}001$) was observed in erosive-ulcerative form L43.82, this contributed to the possibility of further division of the main clinical group into two clinical subgroups - I main clinical group with erosive-ulcerative OLP form L43.82 (n=46) with 2nd degree dysbiosis manifestations, and II main clinical group with erosive-ulcerative OLP form L43.82 (n=40) with 3rd degree dysbiosis manifestations.

The detection rate of Gram-positive and Gram-negative facultatively anaerobic cocci Streptococcus spp. and Staphylococcus spp. was 84.0% and 76.0% of cases, Gram-positive and Gram-negative facultatively anaerobic bacilli respectively in 77.3% and 39.9% cases, yeast-like fungi of the Candida genus in 4% cases in the typical OLP form (L43.80).

The ratio between Gram-positive and Gram-negative cocci was 1:0.9, between streptococci to staphylococci 1:1.10, Gram-positive bacilli and facultatively - anaerobic cocci Lactobacillus spp.and Streptococcus spp.was 0.81:1. in the microbiota studied.

Lactobacillus spp. was detected in 68.0% of the microbiological investigations with a quantitative value of 6.5 Lg CFU/unit, in 4.0% of the cases Candida spp. constituted the limit of normal values from 2.0 to 2.9 Lg CFU/unit. The content of individual representatives of Gram-positive and Gram-negative facultative anaerobic cocci and bacilli, Staphylococcus spp., Neisseria spp., Streptococcus spp. and Lactobacillus spp. ranged from 3.4 to 6.2 lg CFU/mL (Table 1).

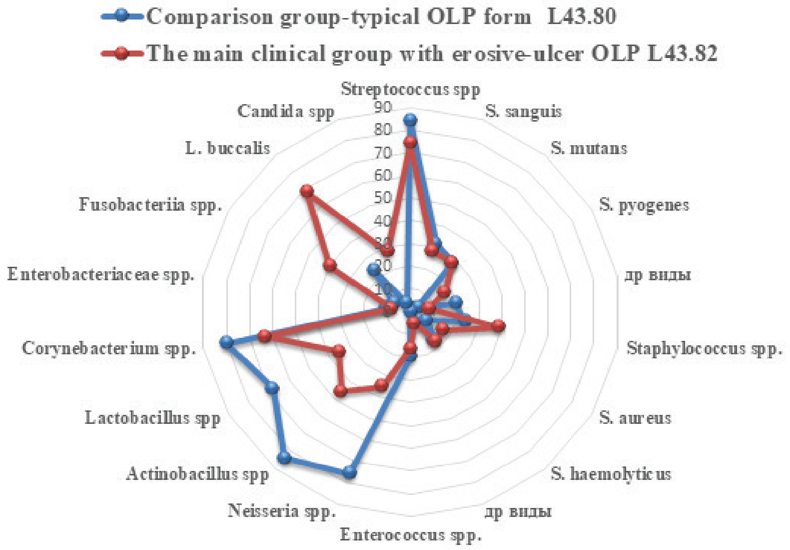
The frequency of detection of Gram-positive facultatively anaerobic cocci (Streptococcus spp.) amounted to 97.83% and 92.5% of cases respectively; Gram-positive and Gram-negative facultative-anaerobic bacilli - 47.82% and 47.5% of cases, in the main clinical groups with erosive-ulcerative OLP forms (L43.82) in the surface microbiota of erosive-ulcerative elements. The detection rate of Gram-positive and Gram-negative facultative-anaerobic bacilli was 59, 41%, 65.05%, 92.75%, 49.17% of cases, respectively, yeast-like fungi in 26.08% from 3 to 5 Lg CFU/unit and in 47.5% at 5 to 7 Lg CFU/unit, which was statistically different at $p<0.001$ (Table 1).

The ratio between Gram-positive and Gram-negative cocci was practically equal - 1:2,04 and 1:1,95, the ratio of Streptococci to Staphylococci respectively 1:1,87 and 1:1,94, the ratio of Lactobacillus spp. to Streptococcus spp. on the average was 0,47:1 and 0,49:1 in the composition of the studied microbiota of the surface of erosive-ulcerative elements. In 45.33% of cases, lactic acid species forming the family Lactobacillus spp. were detected at a quantitative value of 5.0 Lg CFU/unit. (lower than the comparison group at 1.5 Lg CFU/unit), differences at $p<0.05$ were determined only in the quantitative value of yeast-like fungi of Candida spp. genus (Table 1).

Yeast-like fungi of the genus Candida spp in the amount from 3,0 to 5,0 Lg CFU/ed (3,0-4,0 times more than in the comparison group),were revealed in the microbiota of the erosive-ulcerative surface elements of the patients of the first main clinical group, in the second main clinical group the quantity of yeast-like fungi from 4,0-6,0 Lg CFU/ed was 26,08% (2,0-3,0 times more than in the comparison group) (Table 1).

Depending on the form of OLP OCML and the place of obtaining material for microbiological study, the species diversity among streptococci and staphylococci was represented by more than 3 species of microorganisms.

As follows from Table 1, the main significant microorganisms detected from the surface of Wickham's striae and erosive-ulcerative elements were representatives of opportunistic pathogens in the form of yeast-like fungi of the genus Candida (26.08%, 47.5%), and Fusobacteri-



Degree of incidence (%) of some microbiota representatives of the Wickham striae surface and erosive ulcerative elements in patients with typical and erosive ulcerative forms of the Lichen Planus in the oral cavity mucous lining (LP OCML) ia spp among Gram-negative facultative anaerobic bacilli (52.17%, 50.0%).

The quantitative content of the main inhabitants of the microbiota in the form of oral streptococci, regardless of the form of lichen planus and place of collection of material for microbiological study, was within the limit of 105 to 109 Lg CFU/unit.

When analyzing the composition of the microbiota from the Wickham striae surface (comparison group), representatives of the Streptococcus spp were found in the amount of 4.9 Lg CFU/ed in 84.0% of studies, the species ratio of Str. sanguis:Str. mutans was 1:1.14, with the presence of β-hemolytic streptococcus found in 4.0% of cases.

Streptococcus spp. was detected in the examined microbiota from the surface of erosive-ulcerative elements of the cheek mucosa in 97.83 and 92.5% of cases in the amount of 3.6 lg CFU/unit and 3.2 lg CFU/unit, respectively in the I and II main clinical groups of patients with erosive-ulcerative OLP (L43.82) (1.36 and 1.53 times lower than in the comparison group). The species ratio of S. sanguis:S. mutans was 1:1.38 and 1:1.3, S. Pyogenes was detected in 21.73% and 22.5% of microbiological studies, which was significantly different from the comparison group L43.80 at p<0.05 according to χ^2 - criterion.

The patients of the comparison group (L43.80) had a staphylococcal prevalence of 24.0% at 4.7 lg CFU/unit in smears obtained from the surface of the cheek mucosa at the Wickham striae location, the species ratio of S. aureus:S. haemolyticus was 0.5:1.

The clinical groups I and II with erosive-ulcerative OLP (L43.82) had the prevalence of staphylococci 2.17 and 1.97 times higher than in the comparison group in the studied microbiota from the surface of erosive-ulcerative elements, the number varied from 3.4 to 3.6 lg CFU/unit, the species ratio of S. aureus:S. haemolyticus 0.8:1 and 1.12:1, which was distinguishable from the comparison group L43.80 (values are significant at p<0.05 for the main clinical group I). (Table 1).

We revealed differences in the composition of some representatives forming the microbiota of the Wickham's striae surface and the surface of erosive and ulcerative elements, and also presented heterogeneity of their distribution among patients with the erosive and ulcerative form of oral lichen planus (L43.82).

Conclusion. There are changes of associative intermicrobial relationships accompanied by a significant decrease

Performance indicators of the species composition for microbiota of the reticular grid surface and erosive ulcerative elements in patients with typical and erosive ulcerative forms of the Lichen Planus in the oral cavity mucous lining (LP OCML)

Microorganism	Clinical group n=111						Comparative analysis of intergroup differences between the main group and the comparison groups Statistics χ^2	
	Comparison group Typical OLP form L43.80 (n=25)		I clinical main group with erosive-ulcerative OLP L43.82 (n=46)		II clinical main group with erosive-ulcerative OLP L43.82 (n=40)		p-level	
	abs	%	abs	%	abs	%	**I**	**II**
Gram-positive facultative anaerobic cocci								
Streptococcus spp.	21	84.0	45	97.83	37	92.5	**χ2=2.854.** *p*=0.092	**χ2=0.441.** *p*=0.507
			χ2 =0.431. *p*=0.512					
S. sanguis	8	32.0	18	39.13	13	32.5	χ2 =0.355. p=0.552	χ2 =0.002. p=0.967
			χ2 =0.408. *p*=0.523					
S. mutans	7	28.0	13	28.26	10	25.0	χ2 =0.001. p=0.982	χ2 =0.072. p=0.789
			χ2 =0.116. *p*=0.734					
S. pyogenes	1	4.0	10	21.73	9	22.5	χ2 =3.893*. p=0.049	χ2 =4.205*. p=0.041
			χ2 =0.007. *p*=0.933					
Other types	5	20.0	4	8.69	5	12.5	χ2 =1.870. p=0.172	χ2 =0.665. p=0.416
			χ2 =0.330. *p*=0.566					
Staphylococcus spp.	6	24.0	24	52.17	19	47.5	χ2 =5.269*. p=0.022	χ2 =3.590. p=0.059
			χ2 =0.187. *p*=0.666					
S. aureus	2	8.0	9	19.57	9	22.5	**χ2 =0.889**. p=0.346	**χ2 =1.385**. p=0.240
			χ2 =0.111. *p*=0.739					
S. haemolyticus	4	16.0	11	23.91	8	20.0	χ2 =0.663. p=0.436	χ2 =0.164. p=0.686
			χ2 ^{2}=0.190. *p*=0.663					
Other types	0	0	4	8.69	2	5.0	**χ2 =0.958**; p=0.328	**χ2 =0.158**. p=0.692
			χ2 =0.061. *p*=0.806					
Enterococcus spp.	5	20.0	10	19.56	8	20.0	χ2 =0.029. p=0.864	χ2 =0.000. p=1.000
			χ2 =0.039. *p*=0.844					
Gram-negative facultative anaerobic cocci								
Neisseria spp.	19	76.0	22	47.82	17	45.5	χ2 =5.269*. p=0.022	χ2 =6.987**. p=0.009
			χ2 =0.245. *p*=0.621					
Gram-positive facultative anaerobic bacilli								
Actinobacillus spp	21	84.0	24	52.17	27	67.5	χ2 =7.069**. p=0.008	χ2 =2.169. p=0.141
			χ2 =2.082. *p*=0.150					
Lactobacillus spp.	17	68.0	21	45.65	18	45.0	χ2 =3.252. p=0.072	χ2 =3.275. p=0.071
			χ2 =0.004. *p*=0.952					
Corynebacterium spp.	20	80.0	37	80.43	33	82.5	χ2 =1.749. p=0.186	χ2 =1.220. p=0.270
			χ2 =0.060. *p*=0.807					
Gram-negative facultative anaerobic bacilli								
Enterobacteriaceae spp.	3	10.87	5	10.87	4	10.0	χ2 =0.021. p=0.886	χ2 =0.064. p=0.801
			χ2 =0.017. *p*=0.896					
Fusobacteriia spp.	2	8.0	24	52.17	20	50.0	χ2 =11.781***. p<0.001	χ2 =10.317**. p=0.002
			χ2 =0.348. *p*=0.555					
L. buccalis	23	92.0	41	89.13	35	87.5	χ2 =0.150. p=0.699	χ2 =0.324. p=0.570
			χ2 =0.055. *p*=0.815					
Дрожжеподобные грибы								
Candida spp.	1	4.0	12	26.08	19	47.5	**χ2 =3.909***. *p*=0.049	**χ2 =11.700*****. *p*<0.001
			χ2 =4.255*. *p*=0.040					

Note: Bold typeface indicates χ2 statistics calculated with Yates correction.
*. **. *** - differences are statistically significant at p<0.05. p<0.01 and p<0.001. respectively

in the total number of representatives of lactic acid microorganisms Lactobacillus spp., strict anaerobes Actinobacillus spp., against some increase in titers of Gram-negative facultative-anaerobic bacilli Fusobacteriia spp. and L. Buccalis, as well as conditionally pathogenic yeast-like fungi Candida spp. in the composition of the studied microbiota obtained from the surface of erosive ulcerative elements

There was significant prevalence of opportunistic yeast-like fungus Candida spp. which on the average exceeded parameters of comparison group with typical OLP form (L43.80) almost 6,52 and 11,9 times (p<0,05) in the composition of investigated microbiota of erosive-ulcerous surface elements in patients of I and II main clinical groups with erosive-ulcerous OLP forms (L43.82). At the same time, the reliability of differences between the second main clinical group and the first main clinical group was p<0.001.

Thus, the identified features of the composition of the studied microbiota of the surface of erosive-ulcerative elements and Wickham's striae make it possible to substantiate the risks of aggravation of the pathological process on the oral mucosa depending on the form of oral mucosal lichen planus.

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