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Oral Microbiocenosis in Cases of Inflammatory Periodontal Diseases in Children with Tuberculosis

Objective — to examine the microbial spectrum of the oral cavity in cases of inflammatory periodontal diseases of children with tuberculosis.

Materials and methods. A study of the microbial habitat of oral biotopes and gingival surfaces was conducted in 97 children aged 6 to 17 years with inflammatory periodontal diseases and tuberculosis (treatment group). The control group consisted of 20 practically healthy children of the same age. The periodontal diseases were diagnosed according to M.F. Danilevsky's classification (2000). The study of oral microbiocenosis and gingival surfaces in the examined groups of children was performed in accordance with Order No. 234 of the Ministry of Health of Ukraine as of 2006.

Results and discussion. Aerobic, facultative anaerobic, and anaerobic bacteria persisted in the oral fluid and on the gingival surfaces of children with tuberculosis and inflammatory periodontal diseases. The most frequent colonies of bacteria were *Streptococcus*, in particular *S. mutans*, *S. salivarius*, *S. β-haemolyticus*, *Staphylococcus* (*S. aureus*, *S. epidermidis*).

The significant evidence of *Neisseria* (*N. catarrhalis*) and *Corynebacterium* (*C. xerosis*) was revealed in the oral cavity and gingival surfaces of the examined children. The microflora of children from the treatment group revealed mostly *Lactobacillus* genus. The microbial associations of the examined children consisted of *Candida*, *E. coli* fungi. Smear microscopy revealed viruses. *S. aureus* was higher in the children from the treatment group as compared to the healthy children. In addition, the concentration of microorganisms was higher on the gingival surface versus the oral fluid and was more evident in the children from the treatment group.

Conclusions. The obtained spectrum of the oral microbial contamination in the children with tuberculosis and inflammatory periodontal diseases revealed the persisting associations of aerobic, facultative anaerobic, and anaerobic bacteria. The inflammatory periodontal diseases in tuberculosis-infected children were accompanied by the evident increase in the frequency and concentration of microorganisms increasing with the higher severity of the specific process.

Keywords

Children, tuberculosis, periodontium, microbiocenosis, oral fluid, gingival surface.

The significant deterioration of people's health, including dental, observed in Ukraine over the last two decades has been caused by the evident polymorbidity. Periodontal disorders have been the most crucial issues in modern dentistry due to their widespread, the scope of lesions, pathological changes in the relevant tissues, other organs and systems, homeostasis disorders [2, 4, 18].

Based on the World Health Organization (WHO) reports, the recent epidemiological studies have shown a high incidence of periodontal diseases in children and adolescents both globally (80 %) and in Ukraine (60–70 %). The incidence and intensity of periodontal diseases increase with age: gingivitis is revealed in children as young as 5 years old, and by the age of 40, periodontal diseases of varying

severity affect 100 % of the population [4, 8, 19]. The high incidence of inflammatory periodontal diseases has been caused by the decrease in the quality of life, unfavorable environmental, climatic and geographical conditions, endemic areas [7, 11, 12]. The decrease in the health quality has significantly increased the spread of chronic diseases in adults and children. Among diseases with a tendency to develop into chronic processes, tuberculosis has been one of the most widely spread and crucial medical and social issues both in Ukraine and worldwide [15, 25].

The scientific research results prove that lesions of various organs and systems are accompanied by pathological changes in periodontal tissues [6, 7]. In 85 % cases, general somatic diseases are concomitant or activate pathological changes in periodontitis. Tuberculosis is one of the concomitant pathologies affecting the periodontium inflammation. The disease has caused epidemics among children. According to WHO data, 1.2 million children worldwide were affected by tuberculosis in 2019 [13, 16]. The periodontium pathology in patients with tuberculosis has become crucial for the last two decades. Harmful environmental pollution, the violation of water and soil microelement structure, advanced biotechnology products are factors affecting health. The immune system, being one of the major systems of homeostasis and playing a key role in developing pathological changes, is the most sensitive and immediately reacts to the adverse impact of the harmful factors [3, 14].

Not only the immune but also microbial factors play a significant role in the chain of periodontium pathologies. The course of periodontal diseases depend on the nonspecific resistance of the entire body, the tissues involved in the pathological process, and the oral microflora [10, 17, 23]. Most periodontal diseases, like tuberculosis, are of an inflammatory nature and might be affected by local and general factors caused by the body resistance disorders. The periodontal inflammatory processes developed in childhood cause not only tooth loss but also increase the risk of severe diseases [1, 20, 22, 24].

The oral microbial ecology consists of 500 different microorganisms. The discovery of periodontal pathogens among them allowed to define a relatively small group of pathogens causing inflammatory and destructive periodontal disorders. The microflora structure and the ratio of its representatives depend on numerous factors in the oral cavity, including the saliva antimicrobial factor, gingival fluid, oral hygiene, the evidence of a somatic pathology, nutrition, environment, biosphere pollution, etc. [9, 10, 21].

The study of combined diseases, like periodontium pathologies in children with tuberculosis, has

become important in the conditions of health deterioration and tuberculosis epidemics in Ukraine. The solution will allow to diagnose periodontal inflammatory diseases in time and develop efficient treatment and prevention methods.

Objective – to examine the microbial spectrum of the oral cavity in cases of inflammatory periodontal diseases of children with tuberculosis.

Materials and methods

97 children affected with tuberculosis aged 6–17 were examined and referred to the treatment group: 21 (20.6 %) children had a high risk of tuberculosis, 29 (30.0 %) children had clinical forms of primary tuberculosis, 47 (49.4 %) adolescents with the secondary pulmonary tuberculosis. The control group consisted of 20 almost healthy children.

Periodontal diseases were classified according to the M.F. Danilevsky classification (2000).

To study the microbiocenosis of oral fluid and gingival surface in the children under study, the samples were collected with a sterile swab to be further dipped into a glucose broth medium. After 24 hours of incubation in the thermostat, the collected samples were inoculated in the differential diagnostic nutrient media to comply with Decree No. 234 of the Ministry of Health of Ukraine as of 2006. The microscopy of the samples from the glucose broth was performed. The growth in the nutrient media was assessed in 24 hours. The microorganisms were identified based on their morphotinctorial and cultural properties. The bacterial flora spectrum was identified by studying the colonies in the primary inoculations of the pathological samples on Petri dishes with 5 % blood agar used to identify the microflora based on the evidence of typical growth. White or golden convex colonies with hemolysis were evident for *Staphylococcus*, while α and β -hemolysis were observed in cases of *Streptococcus*. The evidence of coagulase strains was used to finally identify *S. aureus*. The growth in the Sabouraud medium was assessed in 48–72 hours. The evidence of white convex colonies with a characteristic smell of dairy products confirmed the evidence of *Candida* fungi. In the thioglycolate medium the growth of Lactobacilli was revealed and identified during microscopy. Catalase tests were applied to identify non-pathogenic *Neisseria*. The cultural and biochemical properties in the nutrient media were examined to identify cytochrome oxidase.

The statistical processing of the results was made by applying the variation statistics techniques implemented with the help of the standard software package Statistica for Windows 6.0.

Table 1. The quantitative structure of oral microbiocenosis in the examined children with tuberculosis and inflammatory periodontal diseases (CFU/ml)

Types of microorganisms	The concentration of microflora in biotopes			
	Oral fluid		Gingival surface	
	Control group	Treatment group	Control group	Treatment group
<i>Str. mutans</i>	3.38 ± 0.32	5.45 ± 0.22*	3.59 ± 0.23	5.98 ± 0.18*
<i>Str. salivarius</i>	4.82 ± 0.28	3.21 ± 0.16*	4.25 ± 0.05	—
<i>Str. β-haemolyticus</i>	4.42 ± 0.27	3.34 ± 0.11*	4.29 ± 0.25	3.08 ± 0.23*
<i>S. aureus</i>	1.70 ± 0.54	4.05 ± 0.35*	1.32 ± 0.35	4.72 ± 0.21*
<i>S. epidermidis</i>	2.45 ± 0.15	5.08 ± 0.37*	2.85 ± 0.13	5.18 ± 0.26*
<i>N. catarrhalis</i>	2.15 ± 0.03	4.28 ± 0.13*	2.34 ± 0.18	4.86 ± 0.19*
<i>E. coli</i>	2.17 ± 0.06	5.28 ± 0.35*	2.20 ± 0.09	5.47 ± 0.26*
<i>C. xerosis</i>	4.12 ± 0.18	3.98 ± 0.23	3.52 ± 0.20	3.01 ± 0.32*
<i>Lactobacillus</i>	3.52 ± 0.28	4.96 ± 0.23*	3.60 ± 0.17	5.38 ± 0.27*
<i>Candida fungi</i>	2.32 ± 0.16	3.52 ± 0.16*	2.05 ± 0.09	4.80 ± 0.35*

Note. *p < 0.05 versus the control group.

Results and discussion

The study of the oral microflora of the examined children revealed that the oral fluid and gingival surfaces of children with tuberculosis (Table 1) were most frequently colonized by *Streptococcus* bacteria, namely by *S. mutans*, *S. salivarius*, *S. β-haemolyticus*, genus *Staphylococcus* (*S. aureus*, *S. epidermidis*). The colonization of *S. mutans* was (5.45 ± 0.22) CFU/ml in the oral cavity and (5.98 ± 0.18) CFU/ml on the gingival surfaces being higher in the treatment group as compared to the healthy children (3.38 ± 0.32) CFU/ml and (3.59 ± 0.23) CFU/ml, (p < 0.05) accordingly. The colonization of *S. salivarius* was lower in the oral cavity of children from the treatment group as compared to the healthy children: (3.21 ± 0.16) CFU/ml vs (4.82 ± 0.28) CFU/ml, (p < 0.05). The microbial colonization of *S. salivarius* on the gingival surfaces of children from the treatment group was not revealed. The revealed colonization of *S. β-haemolyticus* was lower in children from the treatment group as compared to the healthy children: (3.34 ± 0.11) CFU/mL vs (4.42 ± 0.27) CFU/mL (p < 0.05) in oral fluid and (3.08 ± 0.23) CFU/mL vs (4.29 ± 0.25) CFU/mL (p < 0.05) on the gingival surfaces.

The significant evidence of *Neisseria* (*N. catarrhalis*) and *Corynebacterium* (*C. xerosis*) was revealed in the oral fluid and gingival surfaces of the examined children. *Lactobacillus* prevailed in the microflora spectrum of children from the treatment group. The microbial associations in the examined children included *Candida* and *E. coli* fungi. Viruses were revealed during the smear microscopy. The colonization of *S. aureus* was higher in the treatment group as compared to the healthy children. The concentration of microorganisms was higher on the

gingival surfaces versus the oral fluid, and more evident in the treatment group: (4.72 ± 0.21) CFU/mL versus (4.05 ± 0.35) CFU/mL (p < 0.05) correspondingly.

The similar tendency was observed for *S. epidermidis*, which colonization predominated in the oral fluid (5.08 ± 0.37) CFU/mL (p < 0.05) and on the gingival surfaces (5.18 ± 0.26) CFU/mL (p < 0.05) in the treatment group. In addition, the colonization of *N. catarrhalis* and *Escherichia coli* predominated in the treatment group as compared to the healthy children.

N. catarrhalis prevailed on the gingival surfaces (4.86 ± 0.19) CFU/mL and in oral fluid (4.28 ± 0.13) CFU/mL vs the control group (2.34 ± 0.18) CFU/mL and (2.15 ± 0.03) CFU/mL, respectively (p < 0.05). The same was observed for *E. coli* (5.47 ± 0.26) CFU/mL and (5.28 ± 0.35) CFU/mL as compared to (2.20 ± 0.09) CFU/mL and (2.17 ± 0.06) CFU/mL, respectively (p < 0.05). The similar was for the concentration of *Lactobacillus* and *Candida* fungi.

The frequency and concentration of microorganisms was studied in the children with tuberculosis and inflammatory periodontal diseases (Table 2).

The frequency and concentration of microorganisms on the gingival surface apparently increased being higher in cases of localized periodontitis as compared to catarrhal gingivitis. This particularly applied to *S. aureus* revealed in 84.62 % cases of localized periodontitis versus 66.67 % in cases of catarrhal gingivitis and 15 % in the control group (p > 0.05).

The similar tendency was observed for the concentration of microorganisms with (4.75 ± 0.21) CFU/mL in cases of localized periodontitis as compared to (4.08 ± 0.12) CFU/mL in cases of catarrhal gingivitis (p < 0.05). The same dynamics was

Table 2. The frequency and concentration of microorganisms revealed on the gingival surfaces in children with tuberculosis depending on the form of periodontal pathology

Microorganisms	Control group (healthy children) (n = 20)		Treatment group			
	Frequency (%)	Concentration CFU/ml	Catarrhal gingivitis (n = 54)		Localized periodontitis (n = 43)	
			Frequency (%)	Concentration CFU/ml	Frequency (%)	Concentration CFU/ml
<i>Str. mutans</i>	85.0	4.56 ± 0.13	92.59	5.24 ± 0.12	100.0	5.97 ± 0.23
<i>Str. β-haemolyticus</i>	15.0	4.30 ± 0.16	62.96	3.47 ± 0.13	82.25	3.72 ± 0.16
<i>S. aureus</i>	15.0	1.75 ± 0.12	66.67*	4.03 ± 0.29	84.62*	4.75 ± 0.21
<i>S. epidermidis</i>	0	0	48.15*	4.16 ± 0.31	69.23*	5.14 ± 0.23
<i>N. catarrhalis</i>	20.0	2.15 ± 0.14	37.04*	4.26 ± 0.16	46.15*	4.90 ± 0.23
<i>Lactobacillus</i>	100.0	4.28 ± 0.15	100.0	4.75 ± 0.13	100.0	5.42 ± 0.28
<i>Candida</i>	20.0	2.68 ± 0.11	55.56	3.28 ± 0.12	76.92*	4.86 ± 0.29

Note. *p < 0.05 versus the control group.

evident for *S. epidermidis*, *N. catarrhalis* and *Candida* fungi. We also found that inflammatory periodontal diseases and tuberculosis increased the frequency and concentration of microorganisms in the examined children. The more severe the specific process was, the higher the frequency and concentration of the microorganisms were. In cases of secondary tuberculosis, the frequency of *Str. mutans* increased from (90.48 ± 2.7) % up to 100.0 % in the group with a higher risk of disease; *S. β-haemolyticus* increased from 19.05 % to 70.0 %; *S. aureus* increased from 28.57 to 76.67 %; *N. catarrhalis* increased from 23.81 to 40 %; *Candida* fungi increased from 33.33 % in the group with a higher risk of tuberculosis to 76.67 % in cases of secondary tuberculosis (p < 0.05). The frequency of microorganisms revealed in the oral fluid and on the gingival surfaces was moderate (10⁴ CFU/ml) and high (10⁶ CFU/ml). The rate of *S. mutans* 10⁴ CFU/ml and 10⁶ CFU/ml in the oral fluid and on the gingival surfaces respectively was evidently higher in the children with tuberculosis as compared to the healthy ones.

The rate of children with high titers (10⁶ CFU/ml) of *S. mutans* both in the oral fluid (60.0 ± 2.0) % and on the gingival surfaces (90.0 ± 2.6) %, p < 0.05 prevailed in the treatment group. Oral hygiene proved to affect the titer of *S. mutans* in the oral fluid and on the gingival surfaces. (45.0 ± 1.6) % of the examined children from the treatment and control groups with high titers did not brush their teeth at all. *S. β-haemolyticus* was also revealed in the children with tuberculosis playing a crucial role in the development of a respiratory pathology. The rate of children with moderate and high titers of *S. β-haemolyticus* revealed in the oral fluid and on the gingival surfaces was apparently higher as compared to the healthy children from the treatment group.

The amount of children with moderate and high titers of *S. aureus* revealed in their oral fluid and on their gingival surfaces was higher in the treatment group as compared to the healthy children. The similar dynamics was observed for moderate and high titers of hemolytic *S. epidermidis* both in the oral fluid and on the gingival surfaces of the treatment group children.

The evidence of hemolytic *S. epidermidis* points at the significant colonization resistance disorders of the oral cavity in children with inflammatory periodontal disease and pulmonary tuberculosis. The moderate titers of *Lactobacillus* were by 1.5 times more frequently revealed in the oral fluid and on the gingival surfaces of the treatment group children, 60.0 and 50.0 % respectively (p < 0.05).

A high titer of *Lactobacillus* was more frequently revealed on the gingival surfaces of the children from the treatment group. The oral mucosa was frequently colonized by *Candida* fungi. Among factors causing *Candida* proliferation, is the decrease in the oral protection due to local and systemic factors caused by tuberculosis, in particular, by a 6 months' treatment with the use of antimycobacterial drugs for newly diagnosed tuberculosis patients following the 2HRZE4HR regimen, as well as the deterioration of oral hygiene of the majority of the examined children shifting the oral microflora balance in cases of tuberculosis.

Candida fungi revealed in the oral fluid with up to 10³ CFU/ml point at the fungi hostship, while the titer of above 10³ CFU/ml is the symptom of oral candidiasis. Therefore, the study on the frequency of the microorganisms in different oral biotopes of the examined children was performed.

Candida fungi were more frequently revealed in the oral fluid and on the gingival surfaces of children with pulmonary tuberculosis as compared to the

healthy children. This is partially due to the acid-producing activity of *S. mutans* contributing to *Candida*, especially on the gingival surfaces of children with localized periodontitis on the background of pulmonary tuberculosis.

Conclusions

The frequency and concentration of *S. mutans*, *S. β-haemolyticus*, representatives of the *Staphylococcus* such as *S. aureus* and *S. epidermidis* with hemolytic properties, as well as representatives of the *N. catarrhalis*, *Lactobacillus*, and *Candida* fungi were higher in the children with inflammatory peri-

odontal diseases on the background of pulmonary tuberculosis.

The evidence of *Candida* diagnostic titers in the oral cavity is not yet a reason for diagnosing candidiasis, but indicates the risk of its occurrence and the evidence of changes in the colonization resistance of the oral cavity of children with tuberculosis and inflammatory periodontal diseases.

Tuberculosis mycobacteria had a negative impact on the obtained spectrum of the microbial contamination of oral cavity in the examined children causing the general immune suppression and immune disorders of the oral cavity affecting the oral microbiocenosis.

No conflict of interests.

References

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Мікробіоценоз ротової порожнини при запальних захворюваннях пародонта в дітей, хворих на туберкульоз

Мета роботи — вивчити мікробний спектр ротової порожнини при запальних захворюваннях пародонта в дітей, хворих на туберкульоз.

Матеріали та методи. Проведено дослідження мікробного пейзажу біотопів ротової рідини та поверхні ясен у 97 хворих на туберкульоз дітей із запальними захворюваннями пародонта віком від 6 до 17 років (основна група). Контрольну групу утворено з 20 практично здорових дітей аналогічного віку. Діагностику захворювань пародонта проводили за класифікацією М.Ф. Данилевського (2000). Вивчення показників мікробіоценозу ротової рідини та поверхні ясен — відповідно до наказу МОЗ України № 234 від 2006 р.

Результати та обговорення. Установлено, що в ротовій рідині та на поверхні ясен у хворих на туберкульоз дітей із запальними захворюваннями пародонта персистують асоціації аеробних, факультативно анаеробних і анаеробних бактерій, з яких найчастіше визначали колонії бактерій роду *Streptococcus*, а саме *S. mutans*, *S. salivarius*, *S. β-haemolyticus*, *Staphylococcus* (*S. aureus*, *S. epidermidis*).

У дітей виявлено суттєве обмінення ротової рідини та поверхні ясен бактеріями родів *Neisseria* (*N. catarrhalis*) та *Corynebacterium* (*C. xerosis*). У спектрі мікрофлори в дітей основної групи переважали бактерії роду *Lactobacillus*. До складу мікробних асоціацій входили гриби роду *Candida* та *E. coli*. При мікроскопії мазків виявляли також вірусні включення. Колонізація *S. aureus* була вищою в дітей основної групи порівняно зі здоровими дітьми. Концентрація мікроорганізмів була більшою на поверхні ясен, ніж у ротовій рідині, найбільш виразно — у дітей основної групи.

Висновки. Спектр мікробної контамінації ротової порожнини у хворих на туберкульоз дітей із запальними захворюваннями пародонта характеризувався асоціаціями, що персистують, аеробних, факультативно анаеробних і анаеробних бактерій. Запальні захворювання пародонта у хворих на туберкульоз дітей супроводжувалися вірогідним зростанням частоти виявлення та концентрації мікроорганізмів, причому зі збільшенням тяжкості перебігу специфічного процесу це зростання було виразнішим.

Ключові слова: діти, туберкульоз, пародонт, мікробіоценоз, ротова рідина, поверхня ясен.

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