Importance of antimicrobial factors for microbiocenosis and local immunity of the oral cavity in children with mucoviscidosis

TETYANA KHMIZ[1,B,E], MARYNA TKACHENKO[1,A,C], NATALIAY KOVALENKO[1,A,C], TETYANA BOCHAROVA[1,2,C,D], ANDRIY BONDARENKO[1,B,E], VIKTOR SHEVCHUK[1,B,F], NATALIIA ODUSHKINA[1,B,F], ROZANA NAZARYAN[1,A,E], VITALIY GARGIN[1,2,E,F]

[1]Kharkiv National Medical University, Kharkiv, Ukraine; [2]Kharkiv International Medical University, Kharkiv, Ukraine

A – research concept and design, B – data collection, C – data analysis and interpretation, D – article writing, E – critical review of the article, F – final approval of the article

Importance of antimicrobial factors for microbiocenosis and local immunity of the oral cavity in children with mucoviscidosis

Khmiz T[1], Tkachenko M[1], Kovalenko N[1], Bocharova T[1,2], Bondarenko A[1], Shevchuk V[1], Odushkina N[1], Nazaryan R[1], Gargin V[1,2].

[1]Kharkiv National Medical University, Kharkiv, Ukraine; [2]Kharkiv International Medical University, Kharkiv, Ukraine

The involvement of commensals and opportunistic pathogens and the role of protective mechanisms in the development of dental diseases in children with cystic fibrosis require more detailed study.
***The aim** of the study was to determine the ecological characteristics of the oral microbiota and some antimicrobial factors of saliva in children with mucoviscidosis.*
***Materials and methods.** The study involved an assessment of oral microbiota as complex ecological system that protects the human body from colonization by pathogenic flora in children with cystic fibrosis. Bacteriological studies have been performed on clinical material from 30 children with mucoviscidosis diagnosed with dental and periodontal diseases.*
***Results.** In the microbiological study of plaque microbiota, 70 strains of opportunistic pathogens were isolated in patients with mucoviscidosis. The most significant were alpha-hemolytic Streptococci (40%). The proportion of bacteria of Neisseria genus in patients with cystic fibrosis was lower and amounted to 24.3%. C. albicans fungi were isolated in comparable values (18.5%), S. aureus (8.5%), as well as gram-negative strains of E. aerogenes (4.3%) and E. coli (4.3%) significantly dominated. The results indicate that opportunistic pathogens S. aureus, E. aerogenes and E. coli partially replaced the representatives of the normal oral microbiota alpha-hemolytic streptococci and non-pathogenic species of Neisseria genus in patients with mucoviscidosis.*
***Conclusions.** Microbiota of plaque in children with mucoviscidosis is characterized by an expansion of the spectrum of opportunistic pathogens due to Staphylococcus aureus, enterobacteria and C. albicans fungi, which indicates a violation of the microbiocenosis due to reduced mucosal immunity. Mucosal immunity of the oral cavity in children with mucoviscidosis is characterized by a 1.5-fold decrease in lysozyme activity and the level of secretory IgA in the saliva of children.*

Key words: *children, microbiocenosis, local immunity, oral cavity, mucoviscidosis*

Pol Med J, 2022; L (297); 190–194

Znaczenie czynników przeciwdrobnoustrojowych dla mikrobiocenozy i odporności miejscowej jamy ustnej u dzieci z mukowiscydozą

Khmiz T[1], Tkachenko M[1], Kovalenko N[1], Bocharova T[1,2], Bondarenko A[1], Shevchuk V[1], Odushkina N[1], Nazaryan R[1], Gargin V[1,2].

[1]Charkowski Narodowy Uniwersytet Medyczny, Charków, Ukraina; [2]Charkowski Międzynarodowy Uniwersytet Medyczny, Charków, Ukraina

Udział patogenów komensalnych i oportunistycznych oraz rola mechanizmów ochronnych w rozwoju chorób zębów u dzieci z mukowiscydozą wymagają badań szczegółowych.
***Celem** pracy było określenie cech ekologicznych mikroflory jamy ustnej i niektórych czynników przeciwdrobnoustrojowych śliny u dzieci z mukowiscydozą.*
***Materiał i metody.** W pracy dokonano oceny mikroflory jamy ustnej jako złożonego układu ekologicznego, który chroni organizm człowieka przed kolonizacją florą patogenną u dzieci z mukowiscydozą. Przeprowadzono badania bakteriologiczne na materiale klinicznym u 30 dzieci z mukowiscydozą z rozpoznaniem chorób zębów i przyzębia.*
***Wyniki.** W badaniu mikrobiologicznym mikrobioty płytki nazębnej u chorych na mukowiscydozę wyizolowano 70 szczepów patogenów oportunistycznych. Najbardziej znaczące były paciorkowce alfa-hemolizujące (40%). Odsetek bakterii z rodzaju Neisseria u chorych na mukowiscydozę był niższy i wyniósł 24,3%. Grzyby C. albicans wykryto w znacznych ilościach (18,5%), natomiast St. aureus (8,5%) oraz szczepy gram-ujemne E. aerogenes (4,3%) i E. coli (4,3%) istotnie dominowały. Wyniki wskazują, że patogeny oportunistyczne S. aureus, E. aerogenes i E. coli częściowo zastąpiły mikroflorą prawidłową jamy ustnej, takie jak paciorkowce alfa-hemolizujące i niepatogenne gatunki z rodzaju Neisseria u chorych na mukowiscydozę.*
***Wnioski.** Mikrobiota płytki nazębnej u dzieci z mukowiscydozą charakteryzuje się poszerzonym spektrum patogenów oportunistycznych wywołanych przez St. aureus, enterobakterie i grzyby C. albicans, co wskazuje na naruszenie mikrobiocenozy z powodu obniżonej odporności błony śluzowej jamy ustnej. Odporność błony śluzowej jamy ustnej u dzieci z mukowiscydozą charakteryzuje się 1,5-krotnym spadkiem aktywności lizozymu i poziomu wydzielniczego IgA.*

Słowa kluczowe: *dzieci, mikrobiocenoza, odporność miejscowa, jama ustna, mukowiscydoza*

Pol Merkur Lekarski, 2022; L (297); 190–194

The oral microbiota is a complex ecological system that protects the human body from colonization by pathogenic flora. On the other hand, its representatives are the cause of caries and periodontitis, the most common diseases of the oral cavity [6,23]. In addition, systemic disorders such as cardiovascular disease, diabetes, tumors may be associated with disruption of the oral ecosystem [1,17]. One of such diseases is mucoviscidosis, a genetic disease that mainly affects the respiratory and digestive systems, but all patients have lesions of the oral cavity [20,37]. Systemic diseases characterized by inflammatory processes disrupt the balance of microbiota [7,18].

The most common diseases of the oral cavity, namely dental caries and periodontal diseases associated with microorganisms that form plaque on the tooth surface, which meets all the criteria

of the biofilm [4,31]. The ability of microorganisms to form biofilms is especially evident in mucoviscidosis. Chronic colonization of aggressive pathological microflora in children with cystic fibrosis results in impairment of oral microbiocenosis, which contributes to the development of inflammatory diseases of periodontal tissues, in particular, chronic gingivitis [22,33]. Microbial biofilms are characterized by significant resistance to various factors of local immunity and to antimicrobial agents.

Immunoglobulins sIgA and IgG are one of the main factors of specific protection of the oral cavity, which regulate the adhesion of microorganisms to teeth and mucous membranes [27,39]. Children with hypersensitivity to caries were shown to have a deficiency of secretory immunoglobulins and their increased expression in caries. However, there are opposite data on the correlation between sIgA levels and caries [34,35]. Other antimicrobial factors in saliva are defensins, histatins, cathelicidins, as well as the proteins lactoferrin, lysozyme and lactoperoxidase. Any disturbances in the homeostasis of the immune system or nonspecific resistance can lead to colonization by opportunistic pathogens and infectious diseases of the oral cavity [30,38]. The involvement of commensals and opportunistic pathogens and the role of protective mechanisms in the development of dental diseases in children with cystic fibrosis require more detailed study.

The aim of the study was to determine the ecological characteristics of the oral microbiota and some antimicrobial factors of saliva in children with mucoviscidosis.

MATERIALS AND METHODS

The research involved assessment of bacteriological studies of clinical material from 30 children with mucoviscidosis (main group) diagnosed with dental and periodontal diseases, and 23 children with inflammatory diseases of the oral cavity without mucoviscidosis (control group). The children under study aged 2 to 17 years. Dental plaque smears were used as clinical material. The etiological significance of the disease was taken into account at the level of microbial count 10^5 CFU for bacteria and 10^3 CFU for fungi. Isolation of microorganisms and their identification was performed on morphological, cultural and biochemical properties according to regulations [36]. Ecological analysis of plaque microbiota was performed by studying its composition and functional characteristics using the constancy index [31] and the Berger-Parker dominance index [29].

The constancy index was calculated by the formula: C% = p·100 / P, where C% is the constancy index, p is the number of samples in which a certain species was detected, P is the total number of analyzed samples. Depending on the obtained values, all species were divided into dominant or constant (C> 50%), rare or additional (25% <C <50%) and random (C <25%).

The colonization level or population density of microorganisms was determined by counting colony-forming units of bacteria in 1 ml of clinical material and expressed in decimal logarithm (lg CFU / ml). The relative importance of the most common species was determined by the Berger-Parker dominance index, which was calculated by the formula: $d = N_{max}/N$, where d is the dominance index, N_{max} is the number of individuals of the most common species, N is the number of individuals under study.

Determination of lysozyme activity was performed by bacteriolytic method by hydrolysis of *Micrococcus lysodeikticus* [25] by the formula: $L_{rel} = L_o / L_k$, where L_o is an indicator of lysozyme activity in children with mucoviscidosis, and L_k is an indicator of lysozyme activity in children of the main group. Immunoglobulin levels of IgM, IgG, IgA and sIgA in saliva were determined by enzyme-linked immunosorbent assay.

Statistical processing of the findings was performed using the system software package "STATISTICA 10 Enterprise 10.0.1011.6" The results are presented as arithmetic mean±standard deviation (M±m). Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In the microbiological study of plaque microbiota, 70 strains of opportunistic pathogens were isolated in patients with mucoviscidosis and 58 strains of opportunistic pathogens in patients with inflammatory diseases of the oral cavity. In children of the main group the most significant were alpha-hemolytic streptococci (40%) (tab. 1), while in patients of the control group the leading place belonged to *Neisseria spp.* (39.7%) and alpha-hemolytic streptococci (36.2%) (tab. 2). The proportion of bacteria of *Neisseria* genus in patients with cystic fibrosis was lower and amounted to 24.3%. In patients of both groups, *C. albicans* fungi were isolated in comparable values (18.5% in the main group, 18.9% in the control group). *S. aureus* (8.5% and 1.7%, respectively), as well as gram-negative strains of *E. aerogenes* (4.3%) and *E. coli* (4.3%) significantly dominated in patients of the main group compared with the control group (tab. 1 and 2). The results indicate that opportunistic pathogens *S. aureus*, *E. aerogenes* and *E. coli* partially replaced the representatives of the normal oral microbiota alpha-hemolytic streptococci and non-pathogenic species of *Neisseria* genus in patients with mucoviscidosis. The growth of the role of *C. albicans* in infectious processes of the oral cavity in patients with mucoviscidosis is also indicated by other researchers [11,28].

Since most oral α-hemolytic streptococci and non-pathogenic species of *Neisseria* genus play a significant role in maintaining oral homeostasis, reducing their microbial load can lead to caries and mucosal diseases [18,21]. The most common microorganisms were isolated in two- and three-component associations in both groups of children. In most cases, they were joined by staphylococci, *C. albicans* fungi and gram-negative rods in the main group (tab. 1 and 2).

Microorganisms isolated from plaque in patients of the control group were identified only in associations, while in patients with mucoviscidosis 90% of microorganisms were found in associations, and 10% in monoculture (tab. 3). In patients of the control group, most associations were represented by gram-positive and gram-negative bacteria (56.5%), in patients of the

Table 1. Species composition of the microbiota isolated from plaque in patients with mucoviscidosis
Tabela 1. Skład gatunkowy mikroflory izolowanej z płytki nazębnej u chorych na mukowiscydozę

Microorganism	Frequency of microorganisms isolation (absolute value /%)			
	General	In monoculture	In two-component associations	In three-component associations
Alpha-hemolytic streptococci	28/40	3/10.7	13/46.4	12/42.9
Neisseria spp.	17/24.3	-	7/41.1	10/58.9
S. aureus	6/8.5	-	2/33.3	4/66.7
E. aerogenes	3/4.3	-	1/33.3	2/66.7
E. coli	3/4.3	-	3/100	-
C. albicans	13/18.6	-	2/15.4	11/84.6
Total	70	3	28	39

Table 2. Species composition of the microbiota isolated from plaque of patients in the control group
Tabela 2. Skład gatunkowy mikroflory izolowanej z płytki nazębnej chorych z grupy kontrolnej

Microorganism	Frequency of microorganisms isolation (absolute value /%)			
	General	In monoculture	In two-component associations	In three-component associations
Neisseria spp.	23/39.7	-	12/52.2	11/47.8
Alpha-*hemolytic streptococci*	21/36.2	-	10/47.6	11/52.4
S. epidermidis	2/3.5	-	2/100	-
S. aureus	1/1.7	-	-	1/100
C. albicans	11/18.9	-	-	11/100
Total	58	-	24	34

Table 3. Composition of the microflora (association) isolated from dental plaque of patients
Tabela 3. Skład mikroflory (związku) izolowanego z płytki nazębnej chorych

Microorgnisms	Frequency of microorganisms isolation			
	Main group		Control group	
	Absolute quantity	%	Absolute quantity	%
Monoculture	3	10	0	0
Associations:	27	90	23	100
Bacteria + *C. albicans*	13	48.1	10	43.5
Gram-positive cocci	1	3.8	0	0
Gram-positive cocci + Gram-negative bacteria	13	48.1	13	56.5
Two-component	14	46.7	12	52.2
Three-component	13	43.3	11	47.8

main group such associations were found in 48.1% of cases. In 48.1% of patients with mucoviscidosis and 43.5% of patients with inflammatory diseases of the oral cavity the bacterial microflora was accompanied by *C. albicans* fungi.

The literature provides data on the diversity of microbial consortia that inhabit the oral cavity of patients with mucoviscidosis and which play an active role in the development of inflammatory processes in the oral cavity and respiratory infections [12]. Thus, oral commensal streptococci and *Pseudomonas aeruginosa* mutually contribute to the formation of biofilms and colonization [5] with increasing pathogenicity of the latter [9]. Since interactions between different types of microorganisms can potentially affect disease progression, the identification of key agents can help improve the outcome of therapeutic intervention.

Evaluation of the ecological characteristics of plaque microbiota showed that dental and periodontal disease in both groups caused a violation of the microbiocenosis towards a reduction in the role of dominant species. Thus, according to the constancy index, alpha-hemolytic streptococci and non-pathogenic species of *Neisseria* genus in the control group belonged to additional species, and in the main group alpha-hemolytic streptococci also belonged to additional, and *Neisseria spp*. together with other species were rare (tab. 4). *C. albicans* dominated among the rare species in both groups of patients. The level of microbial colonization was significant for streptococci, *Neisseria* and *C. albicans* in both groups (tab. 4).

There are different mechanisms of interspecific interaction in biotopes that affect the population of microorganisms, so the study of these mechanisms and the time course of microbial populations may affect the effectiveness of treatment and disease outcome [24]. The study of immune defense in mucoviscidosis has always received a great deal of attention due to the difficulties in treating bacterial infections [26].

Immune protection of the oral cavity is carried out by antibodies of different classes [19] Secretory IgA is a central component of the immunity of mucous membranes and the first link of protection against cariogenic bacteria [13]. They prevent the penetration of opportunistic pathogens and their toxins into the mucous membranes, and also control the symbiotic relationship between commensals and the host [15,27]. Increased activity and sIgA levels have been found in patients with caries [10]. Secretory immunoglobulins act synergistically with lactoferrin and lysozyme [2].

Table 4. Ecological characteristics of the microbiota isolated from dental plaque of patients
Tabela 4. Charakterystyka ekologiczna mikroflory izolowanej z płytki nazębnej chorych

Microorgnisms	Groups of patients					
	Main			Control		
	constancy index	dominance index	microbial colonization density lg CFU/g (M + m)	constancy index	dominance index	microbial colonization density lg CFU/g (M + m)
Alpha-hemolytic streptococci	40	1.0	6.18±0.6	36.2	1.09	6.14±0.5
Neisseria spp.	24.3	1.65	5.24±0.8	39.7	1.0	5.48±0.7
S. aureus	8.5	4.67	4.33±0.8	1.7	23.0	4.0±0.0
S. epidermidis	-	-	-	3.5	11.5	4±0.0
E. aerogenes	4.3	9.33	4.67±0.6	-	-	-
E. coli	4.3	9.33	4±0.0	-	-	-
C. albicans	18.6	2.15	4.38±0.7	18.9	2.09	4.45±0.9

Lysozyme is a factor of nonspecific resistance with antimicrobial activity aimed at maintaining microbiocenosis of the oral cavity [11,32]. Low levels of local nonspecific immunity are characterized by decreased enzyme activity. Assessment of lysozyme activity in the saliva of children showed that in patients with mucoviscidosis, this figure was probably 1.5 times lower than in children with oral diseases, but who were not diagnosed with mucoviscidosis (tab. 5). Decreased enzyme levels in adults with mucoviscidosis who had high caries rates have been reported by other researchers [14].

Table 5. The content of lysozyme and immunoglobulins in the saliva of children of the main and control groups, mg/l
Tabela 5. Zawartość lizozymu i immunoglobulinu w ślinie dzieci z grup głównych i kontrolnych, mg/l

Groups	*Lysozyme, s.u./l*	*sIg A*	*Ig A*	*Ig M*	*Ig G*
Control	15.75 ± 1.441	123.2± 9.41	1.95± 0.46	3.32± 0.60	2.02± 0.32
Main	10.47 ± 1.758*	93.03± 15.98*	4.05± 0.77*	5.05± 0.53*	2.92± 0.39*

Note: * probability of change (p <0.05) compared with the control group

When determining the content of immunoglobulins sIgA, IgA, IgM and IgG in the saliva of children, there were significant differences in the levels of all antibodies in children with mucoviscidosis, compared with the control group. Thus, the level of sIg A in children of the main group was reduced by 1.5 times, and the concentration of antibodies of other classes was increased, namely IgA by 2 times, IgM by 1.5 times and IgG by 1.4 times compared to indicators of the control group (tab. 5). Decreased activity of secretory immunoglobulins in the saliva of children with cystic fibrosis indicates a weakening of local immunity of the oral cavity, which may contribute to the development of infectious processes. The increase in the level of other classes of antibodies compared to the control group is due to their penetration through the epithelial barrier from the bloodstream to replace the deficiency of sIg A [16].

The data of the study show that children with mucoviscidosis develop plaque microbiome from two- and three-component associations of opportunistic pathogens, namely *Staphylococcus aureus, Enterobacter, Escherichia coli* and *C. albicans*. Decrease in colonization resistance of the oral cavity is caused by weakening of separate links of local immunity which provide resistance of oral tissues to infectious diseases.

CONCLUSIONS

1. Microbiota of plaque in children with mucoviscidosis is characterized by an expansion of the spectrum of opportunistic pathogens due to *Staphylococcus aureus, enterobacteria* and *C. albicans* fungi, which indicates a violation of the microbiocenosis due to reduced mucosal immunity.
2. Mucosal immunity of the oral cavity in children with mucoviscidosis is characterized by a 1.5-fold decrease in lysozyme activity and the level of secretory IgA in the saliva of children.

REFERENCES

1. Bakirov V, Chumachenko D, Chumachenko T, et al.: Toward reference architecture of control system socio-epidemic processes of emergent infections. CEUR Workshop Proceedings 2021(3038):109-115.
2. Bove M, Mhillaj E, Tucci P, et al.: Effects of n-3 PUFA enriched and n-3 PUFA deficient diets in naďve and Aβ-treated female rats. Biochem Pharmacol. 2018 Sep;155:326-335. doi: 10.1016/j.bcp.2018.07.017.
3. Boyko VV, Mykytyn VZ, Pak VY, et al.: Differentiated approach to the treatment of the adhesive obstruction. Azerbaijan Med J. 2020; 2020(2):11-18.
4. Chevalier M, Ranque S, Pręcheur I.: Oral fungal-bacterial biofilm models in vitro: a review. Med Mycol. 2018 Aug 1;56(6):653-667. doi: 10.1093/mmy/myx111.
5. Chumachenko D., Chumachenko T.: Intelligent Agent-Based Simulation of HIV Epidemic Process. Adv Intell Sys Comput 2020;1020:175-188. doi: 10.1007/978-3-030-26474-1_13
6. Denga O, Pyndus T, Gargin V, et al.: Influence of metabolic syndrome on condition of microcirculatory bed of oral cavity. Georgian Med News. 2017;(273):99-104.
7. Fesenko D, Glazunov O, Nakonechna O, et al.: Consequences of microsequences of microcirculatory disturbances of oral mucosa in modeling of rheumatoid arthritis. Georgian Med News. 2019;(295):137-140.
8. Gargin V., Muryzina I., Shcherbina N., et al.: Relationship between bone density of paranasal sinuses and adrenal steroids pattern in women during menopausal transition. Anthropological Review. 2020;83(4):407-418. doi: 10.2478/anre-2020-0031
9. Gargin V., Radutny R., Titova G., et al.: Application of the computer vision system for evaluation of pathomorphological images. 2020 IEEE 40th International Conference on Electronics and Nanotechnology, ELNANO 2020 – Proceedings; 2020. 469-473, doi:10.1109/ELNANO50318.2020.9088898.
10. Gutarova N, Kryvenko L, Kovach I, et al.: Features of the morphological state of bone tissue of the lower wall of the maxillary sinus with the use of fixed orthodontic appliances. Pol Merkur Lekarski. 2020;49(286):232-235.
11. Kolupayev SM, Yaroslavska JJ, Mikhailenko NM, et al.: Etiopathogenesis of stone formation of combined localization. Azerbaijan Med J 2021(4):50-56.
12. Kon K, Rai M.: Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches. Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches; 2016. p. 1-413.
13. Krivenko S, Lukin V, Krylova O, et al.: A fast method of visually lossless compression of dental images. Appl Sci. 2021;11(1):1-14.
14. Kuzenko Y, Mykhno O, Sikora V, et al.: Dental terminology „discoloration" or „pigment dystrophy" – a review and practical recommendations. Pol Merkur Lekarski. 2022;50(295):65-67.
15. Lenters V, Thomsen C, Smit LA, et al.: Serum concentrations of polybrominated diphenyl ethers (PBDEs) and a polybrominated biphenyl (PBB) in men from Greenland, Poland and Ukraine. Environ Int. 2013;61:8-16. doi:10.1016/j.envint.2013.09.001
16. Ludwicki JK, Góralczyk K, Strucíński P, Hazard quotient profiles used as a risk assessment tool for PFOS and PFOA serum levels in three distinctive European populations. Environ Int. 2015;74:112-118. doi:10.1016/j.envint.2014.10.001
17. Merkulova TV, Peresypkina TV, Cherniakova GĚ, et al.: Socio-psychological determinants of adolescent health at the initial stage of professional education. Wiad Lek. 2021;74(5):1147-1151.
18. Morgese MG, Schiavone S, Bove M, et al.: Sub-chronic celecoxib prevents soluble beta amyloid-induced depressive-like behaviour in rats. J Affect Disord. 2018 Oct 1;238:118-121. doi: 10.1016/j.jad.2018.05.030.
19. Nazaryan R, Kryvenko L, Zakut Y, et al.: Application of estimated oral health indices in adolescents with tobacco addiction. Pol Merkur Lekarski. 2020;48(287):327-330.
20. Nazaryan R, Tkachenko M, Kovalenko N, et al.: Analysis of local immunity indicators of the oral cavity and degree of gingivitis depending on mutation of cftr gene in children with cystic fibrosis. Georgian Med News. 2019;(296):27-31.
21. Nechyporenko AS, Alekseeva VV, Sychova LV, et al.: Anatomical prerequisites for the development of rhinosinusitis. Lek Obz. 2020;6(10):334-338.
22. Nechyporenko AS, Nazaryan RS, Semko GO, et al.: Application of spiral computed tomography for determination of the minimal bone density variability of the maxillary sinus walls in chronic odontogenic and rhinogenic sinusitis. Ukrainian journal of radiology and oncology. 2021;29(4):65–75. DOI: https://doi.org/10.46879/ukroj.4.2021.65-75
23. Nechyporenko AS, Reshetnik VM, Alekseeva VV, et al.: Implementation and analysis of uncertainty of measurement results for lower walls of maxillary and frontal sinuses. 2020 IEEE 40th International Conference on Electronics and Nanotechnology, ELNANO 2020 – Proceedings; 2020. DOI: 10.1109/ELNANO50318.2020.9088916
24. Pelchen-Matthews A, Ryom L, Borges ÁH, et al.: Aging and the evolution of comorbidities among HIV-positive individuals in a European cohort. AIDS. 2018;32(16):2405-2416. doi:10.1097/QAD.0000000000001967
25. Pokhil SI, Bondarenko AV, Bocharova TV, et al.: Implementation and analysis of Babesia immunoassay testing. Pol Merkur Lekarski. 2020;48(285):170-173.
26. Polyvianna Y., Chumachenko D., Chumachenko T. Computer aided system of time series analysis methods for forecasting the epidemics outbreaks. 2019 15th International Conference on the Experience of Designing and Application of CAD Systems, CADSM 2019:1-4. doi: 10.1109/CADSM.2019.8779344
27. Popova TM, Kryvenko LS, Tishchenko OV, et al.: Effect of Electronic Cigarettes on Oral Microbial Flora. J Pharm Nutr Sci. 2021; 11(1): 54-64. doi:10.29169/1927-5951.2021.11.08
28. Reiner RC, Jr., Wiens KE, Deshpande A, et al.: Mapping geographical inequalities in childhood diarrhoeal morbidity and mortality in low-income and middle-income countries, 2000-17: Analysis for the Global Burden of Disease Study 2017. Lancet 2020;395(10239):1779-1801.

29. Romaniuk A, Lyndin M, Lyndina Y, et al.: Changes in the hematopoietic system and blood under the influence of heavy metal salts can be reduced with vitamin E. Turk Patoloji Derg 2018;34(1):73-81.
30. Schenström A, Rönnberg S, Bodlund O. Mindfulness-Based Cognitive Attitude Training for Primary Care Staff: A Pilot Study. Complement Health Pract Rev 2006;11(3):144-152.
31. Schiavone S, Morgese MG, Mhillaj E, et al.: Chronic Psychosocial Stress Impairs Bone Homeostasis: A Study in the Social Isolation Reared Rat. Front Pharmacol. 7:152. doi: 10.3389/fphar.2016.00152
32. Shepherd L, Borges Á, Ledergerber B, et al.: Infection-related and -unrelated malignancies, HIV and the aging population. HIV Med. 2016;17(8):590-600. doi:10.1111/hiv.12359
33. Shevchuk V, Odushkina N, Mikulinska-Rudich Y, et al.: A Method of increasing the effectiveness of antibacterial therapy with ceftriaxone in the complex treatment of inflammatory diseases of the maxillofacial area in children. Pharmacologyonline 2021;3:652-662.
34. Svitlana D, Ęateryna K, Dmitriy Z, et al.: Realization of the algorithm pharmakon (Medicine – poison) in modern antibiotic treatment. Pharmacologyonline 2021;2:1172-1178.
35. Tishchenko OV, Kryvenko LS, Gargin VV.: Influence of smoking heating up tobacco products and e-cigarettes on the microbiota of dental plaque. Pol Merkur Lekarski. 2022;50(295):16-20.
36. Tkachenko AS, Kot YG, Kapustnik VA, et al.: Semi-refined carrageenan promotes generation of reactive oxygen species in leukocytes of rats upon oral exposure but not in vitro. Wien Med Wochenschr. 2021;171(3-4):68-78. doi:10.1007/s10354-020-00786-7
37. Tkachenko M, Fomenko Y, Bondarenko A, et al.: The use of miramistin in the treatment of chronic gingivitis in children with cystic fibrosis. Pharmacologyonline 2021;3:398-404.
38. Yanko N, Artemyev A, Kaskova L.: Dental health indicators of the Chernyakhov population from Shyshaki (Ukraine). Anthropological Review. 2021;84(1): 17-28. doi:10.2478/anre-2021-0002
39. Yaroslavska Y, Mikhailenko N, Kuzina V, et al.: Antibiotic therapy in the complex pathogenic treatment of patients with sialolithiasis in the stage of exacerbation of chronic sialoadenitis. Pharmacologyonline 2021;3:624-631.

Acknowledgments: This study is a part of the scientific research work “Optimization of early diagnosis, prevention and treatment of oral tissue diseases with smoking addiction”, 0120U102057, Kharkiv National Medical University”.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29.03.2022
Revised: 04.04.2022
Accepted: 30.04.2022

Address for correspondence:
Gargin Vitaliy
Kharkiv National Medical Universit
Nauky av. 4, 61022, Kharkiv, Ukraine
phone: +(380)990498557
e-mail: vitgarg@ukr.net