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ANALYSIS OF THE DEPENDENCE OF STATE OF LOCAL IMMUNITY AND MICROBIAL COENOSIS IN ORAL CAVITY ON THE ACTIVITY OF CARIES IN CHILDREN RESIDING PERMANENTLY IN CONDITIONS OF BIOGEOCHEMICAL FLUORINE AND IODINE DEFICIENCY

Abstract. *Aim of the study: to find out the dependence of local immunity state and microbial coenosis of oral cavity in children of different ethnic groups residing in conditions of biogeochemical fluorine and iodine deficiency on the level of activity of dental caries. Oral microbial coenosis and content of secretory immunoglobulin A in 146 children of different ethnic groups have been determined, correlations between the parameters with the level of caries activity have been revealed. When evaluating the state of local immunity in children with different levels of caries activity among the various ethnic groups it was found out that contents of secretory IgA in 100% of examined children are higher than normal; the direct correlation between the level of caries activity and sIgA concentration in the oral fluid has been revealed, moreover sIgA concentration increases with the complications of caries activity in direct proportion compared to normal healthy children (286.9 ± 6.7 ; 376.2 ± 7.8 ; 458.2 ± 9.3 ; 75.5 ± 2.7 ; $p < 0.05$, $p < 0.001$). In children of the Roma ethnic group it was found out a significant increase in sIgA content in the group of children with compensated caries activity compared to the group of healthy children (135.6 ± 4.5 ; 59.2 ± 1.2 ; $p < 0.05$), although the indicators are in the normal range (57-260 mg / ml).*

Key words: *children, caries, oral microbial coenosis, secretory immunoglobulin A, correlation dependencies, biochemical deficiency of fluorine and iodine.*

Introduction. The study is a part of scientific theme of the department of childhood stomatology of dental faculty of SHEI "Uzhhorod National University" "Improving the dental care to children residing in conditions of biogeochemical deficiency of fluorine and iodine" (state registration number 0114U004123), which was performed on the base of agreements on scientific and technical cooperation with the University of Presov (Slovakia, 2006), P.L. Shupyk Institute of dentistry NMAPE (2009), clinic of maxillofacial surgery of P.J. Safarik University (Slovakia, 2010), University of Debrecen (Hungary, 2010).

In the available sources of scientific literature much attention is paid to the study of oral microbial coenosis of children and adolescents in order to establish the prevalence of certain kinds of microorganisms as etiological factor of origin and progression of major dental diseases

[7, 11-15]. However, it was found out that the main role in causing the disease is played by the local immunity and physical health of the body, especially in childhood.

The structure and antigenic properties of sIgA fundamentally differ from the ones of serum sIgA, which is a complex of three molecules of different origin and structure. The structure of sIgA includes two (or 4) sIgA molecules with a molecular weight of 160 kDa each, J-chain (16 kDa), which connects them, and secretory component that joins during the transepithelial transport of polymer sIgA. sIgA plays an important role in local immunity of the body, preventing attachment and penetration of bacteria and viruses through the epithelium, binding the pathogens that get in the body fluids and neutralizing viruses that have penetrated into epithelial cells. Several parameters of body resistance to infections correlate with the

content of sIgA-antibodies in the extravascular fluids [9-12].

It was established that it is ethnic determinancy, which plays a significant role in causing the caries and its progression [1, 2, 8, 10]. Among the factors of local immunity determining by secretory immunoglobulin A [3 - 6], special attention should be paid to determining correlation dependencies of the concentration of microbial landscape and the level of activity of the dental caries in children of different ethnic groups.

Objective: to find out the dependence of local immunity and microbial coenosis of oral cavity in children of different ethnic groups residing in conditions of biogeochemical fluorine and iodine deficiency on the level of caries activity.

Materials and methods. In order to justify clinically and find out the reliability of the assumption the study of the state of oral biological coenosis and immunological state of 146 children aged 3-8 years of different ethnic groups with different levels of caries activity residing permanently in conditions of biochemical deficits was performed. The children examination was carried out after informed consent of parents or guardians in LLC "Dental clinic" of Dental Faculty of SHEI "Uzhhorod National University".

Determination of secretory IgA content was carried out by "sandwich" ELISA with the measurement of optical density value of content of plate wells with vertical scanning photometer at 450 nm.

Microbiological and immunological studies were performed in the microbiological laboratory of Transcarpathian branch of S.M. Vynogradskyi Society of Microbiologists of Ukraine (Chairman of the Society - doctor of medical sciences, professor Boiko N.V.).

Statistical analysis of the data was carried out using methods of parametric and nonparametric statistics. Consent of statistical distribution of empirical parameters with the theoretical Gaussian normal distribution was evaluated using the Kolmogorov-Smirnov (K.-S.) and Shapiro-Wilks (W) criteria, p is a reliability indicator.

At small volumes of samples the distribution was assumed to be close to normal, the arithmetic mean and the median are supposed to be nearly the same, maxima relatively to the mean values are symmetric, skewness and kurtosis do not significantly exceed 2 by their absolute value. The following methods of descriptive statistics were used: estimation of the arithmetic mean (m), confidence interval (p), median (M), minimum (\min) and maximum (\max), standard deviation (σ^2), skewness and kurtosis. Pearson's paired and partial correlations (r) with the confidence interval (p) were also analyzed based on the absolute data. The method of nonparametric statistics, namely the Spearman rank correlation (r_s), was used too. Correlation coefficients were marked as Sk . An analysis of factor loadings, cluster analysis - hierarchical classification were performed. Allowable confidence level (p) of null statistical hypothesis (absence of significant heterogeneity or factor influence) was set equal to 0.05.

All the calculations were performed on a personal computer using licensed software "MS Excel 7" for «Windows XP» operating system.

Results and discussion. Among the 146 examined children 37 children are of the Roma ethnic group, which is 25.3%, 109 children are of other nationalities - 74.7%. The children were divided into groups due to the activity of caries: 35 ones with compensated caries (24.0%), 26 children with sub-compensated caries (17.8%), 35 children with decompensated caries (24.0%) and 50 healthy children: 25 ones of Roma ethnic group and 25 ones of other ethnic groups, which are the control group.

There are significant differences in the concentration between the children of the Roma ethnic group, the ones of mixed ethnic groups and a control group of healthy children without caries.

Immunological studies of the content of secretory IgA in the oral fluid of examined children have found out that in children with caries the sIgA content is significantly higher compared with that of healthy children ($p < 0.05$) and higher than normal levels. In 100% of children of mixed ethnic groups contents of secretory immunoglobulin A are higher than

normal.

Concentration of sIgA in the children of mixed ethnic groups increased in direct proportion to the complexity of caries activity (286.9 ± 6.7 ; 376.2 ± 7.8 ; 458.2 ± 9.3 ; 75.5 ± 2.7 ; $p < 0.05$, $p < 0.001$). That is, there is a direct correlation between the level of caries activity in children of mixed ethnic groups and concentration of secretory immunoglobulin A in saliva, which is a marker of violations of oral microbial coenosis and indicates the further progression of lesions of dental hard tissues.

In children of the Roma ethnic group there is a significant increase of sIgA content in the group of children with compensated caries compared to the group of healthy children (135.6 ± 4.5 ; 59.2 ± 1.2 ; $p < 0.05$), although the indicators are within normal limits (57 - 260 mg / ml).

When evaluating the state of local immunity in children with different levels of caries activity among the various ethnic groups it was found out that contents of secretory IgA in 100% of examined children are higher than normal; the direct correlation between the level of caries

activity and sIgA concentration in the oral fluid has been revealed, moreover sIgA concentration increases with the complications of caries activity in direct proportion compared to normal healthy children (286.9 ± 6.7 ; 376.2 ± 7.8 ; 458.2 ± 9.3 ; 75.5 ± 2.7 ; $p < 0.05$, $p < 0.001$).

In children of the Roma ethnic group it was found out a significant increase in sIgA content in the group of children with compensated caries activity compared to the group of healthy children (135.6 ± 4.5 ; 59.2 ± 1.2 ; $p < 0.05$), although the indicators are in the normal range (57-260 mg / ml).

Statistically significant correlations have been revealed between the following parameters: *Streptococcus sobrinus* and sIgA with $r = 0.57$, $p = 0.01$; *Streptococcus sobrinus* and *Enterococcus faecalis* and *Proteus vulgaris* with sIgA with $r = -0.48$, $p = 0.07$; with correlation coefficients $r = (-0.57)$, $p = 0.01$.

Table 1 shows the microorganisms having statistically significant correlative relationships one between another and with content of immunoglobulin A and the level of caries activity.

Table 1

Pearson correlation coefficients "r" and the level of reliability "p" for different pairs of microorganisms

Microorganisms	TMC	<i>Str. sobrinus</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Proteus vulgaris</i>	<i>S. aureus</i>
TMC	1.0000	0.1004	0.4509	-0.2190	0.4482	0.7332
	p= -	p=0.598	p=0.012	p=0.245	p=0.013	p=0.000
<i>Str. sobrinus</i>	0.1004	1.0000	-0.0313	0.3673	-0.1683	-0.1974
	p=0.598	p= -	p=0.870	p=0.046	p=0.374	p=0.296
<i>Pseudomonas aeruginosa</i>	0.4509	-0.0313	1.0000	-0.0929	-0.1782	0.4562
	p=0.012	p=0.870	p= -	p=0.625	p=0.346	p=0.011
<i>Proteus vulgaris</i>	0.4482	-0.1683	-0.1782	-0.3729	1.0000	0.3852
	p=0.013	p=0.374	p=0.346	p=0.042	p= -	p=0.036
<i>S. aureus</i>	0.7332	-0.1974	0.4562	-0.2361	0.3852	1.0000
	p=0.000	p=0.296	p=0.011	p=0.209	p=0.036	p= -

According to Table 1 concentration of *Streptococcus sobrinus* significantly correlates with the concentration of *Enterococcus faecalis* in the oral fluid with the Pearson coefficient $r = 0.37$, $p = 0.046$. In its turn the concentration of *Enterococcus faecalis* significantly correlates

with the content of *Proteus vulgaris* in oral fluid with the Pearson coefficient $r = -0.37$, $p = 0.42$, though in the opposite direction.

Thus, there is an inverse dependence of the concentration of microorganisms in the oral fluid, i.e., the more the first micro-organism

content in the oral cavity is, the less that of the other microorganism is, in the opposite case they have antagonistic nature of the interaction in the environment.

Reliable correlations have been revealed between: concentration of *Str. sobrinus* and the amount of sIgA (coefficient $r = 0.57$, $p = 0.01$); concentration of *Str. sobrinus* and the amount of alkaline phosphatase ($r = 0.40$, $p = 0.29$); *Enterococcus faecalis* content and the amount of alkaline phosphatase ($r = -0.49$, $p = 0.06$), and the concentration of *Enterococcus faecalis* and amount of sIgA - $r = -0.48$, $p = 0.07$. The

concentration of *Proteus vulgaris* demonstrates counteraction to pH factors, alkaline phosphatase, Ca, P with correlation coefficient $r = -0.57$, $p = 0.01$.

As the calculation of Pearson correlation dependencies indicates no significant correlation between the factors and caries activity, the correlation dependence has been analyzed by Spearman (S_k , confidence interval was set to be $p < 0.05$).

Table 2 shows the Spearman correlation parameters for factors that demonstrate reliable correlation with the incidence of caries.

Table 2

Spearman rank correlation

Microorganisms	<i>Streptococcus sobrinus</i>	<i>Enterococcus faecalis</i>	sIgA
<i>Streptococcus sobrinus</i>	1.000000	0.336344	0.537879
<i>Pseudomonas aeruginosa</i>	-0.133978	-0.101252	-0.072316
<i>Enterococcus faecalis</i>	0.336344	1.000000	0.446572
<i>Lactobacillus salivarius</i>	0.121825	0.046287	0.461528
<i>Lactobacillus acidophilus</i>	0.340715	-0.077074	0.096279
sIgA	0.537879	0.446572	1.000000
Caries intensity	0.575502	0.556694	0.620255

Note. * MD removed in pairs. Correlations at $p < 0.05$ have been detected.

Thus, the Spearman correlation have been revealed between: caries intensity and concentration of *Streptococcus sobrinus* ($S_k = 0.575502$); content of *Enterococcus faecalis* in oral fluid ($S_k = 0.556694$); content of sIgA ($S_k = 0.575502$); sIgA amount in its turn correlates with the content of *Streptococcus sobrinus* ($S_k = 0.537879$) and content of *Enterococcus faecalis* in oral fluid ($S_k = 0.446572$).

Amount of *Streptococcus sobrinus* has the relationship with the contents of *Enterococcus faecalis* and *Lactobacillus acidophilus* in oral fluid ($S_k = 0.336344 - 0.340715$ $S_k =$ respectively).

Conclusions. When evaluating the state of local immunity in children with different levels of caries activity among the various ethnic groups it was found out that contents of secretory IgA in 100% of examined children are higher than normal; the direct correlation between the level of caries activity and sIgA concentration in the oral fluid has been revealed, moreover sIgA concentration increases with the complications of caries

activity in direct proportion compared to normal healthy children (286.9 ± 6.7 ; 376.2 ± 7.8 ; 458.2 ± 9.3 ; 75.5 ± 2.7 ; $p < 0.05$, $p < 0.001$).

In children of the Roma ethnic group it was found out a significant increase in sIgA content in the group of children with compensated caries activity compared to the group of healthy children (135.6 ± 4.5 ; 59.2 ± 1.2 ; $p < 0.05$), although the indicators are in the normal range (57-260 mg / ml).

An increase in the concentration of secretory immunoglobulin A is a marker of violations of oral microbial coenosis and indicates the further progression of lesions of dental hard tissues.

The Spearman correlation coefficients (S_k) has been found out between the following parameters: the caries intensity and content of *Streptococcus sobrinus* ($S_k = 0.575502$); content of *Enterococcus faecalis* ($S_k = 0.556694$); amount of sIgA ($S_k = 0.575502$). Concentration of sIgA in its turn correlates with amount of *Streptococcus sobrinus* ($S_k = 0.537879$) and *Enterococcus faecalis* ($S_k = 0.446572$); contents of

Enterococcus faecalis and *Lactobacillus acidophilus* have relationship with amount of *Streptococcus sobrinus* ($S_k = 0.336344$ and $S_k = 0.340715$, respectively; confidence interval is set to be $p < 0.05$).

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