

CORRECTION OF IMMUNOLOGICAL REACTIVITY DISORDERS IN CHILDREN AFFECTED BY DENTAL CARIES

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Absrtact: Dental caries is one of the most prevalent diseases affecting children and adolescents. The dramatic improvement in dental health, especially in children, in many countries during last 20 years is proof that prevention works. Dental caries is not inevitable; the causes are well known, discouraging caries development and discouraging caries healing are realities to be grasped. We would like to introduce the results on our investigation of the probiotic "Lactobacterine" in correction of the disorders of general and local immunity of the blood serum and oral liquid in children from bad environmental regions. We have evidence of the high efficiency in recommended caries prophylaxis that has been proved by positive indicators of immunological data of the blood serum and oral liquid in children from areas with various environmental pollutions. The results were obtained after 30 month since the beginning of the research. We have observed the decrease of the circulating immune complexes, increased amount of the complementum as well as the immunoglobulin G and M in the blood serum and have noticed that the activity of the lysozyme and immunoglobuline A in oral liquid were on the growth in oral liquid of children from the prophylactic group comparing with those from the control groups.

Key words: dental caries, oral liquid, prophylaxis, probiotic.

Introduction

There is a great industrial and agricultural development over the period of last decade as well as a big economical progress that have a massive impact on the ecology and society [1,7]. The influence of anthropogenic pollution can not be underestimated. Such progress highly affects the oral organs and tissues as well as prevalence of stomatological disorders on the whole. [3,6].

The role of eco-pathogenic risks of vital importance in pediatric stomatology as the maxilla-facial area is in the constant state of growing and development therefore any hazardous

substances may be extremely harmful in any amount an doses to the child's well being. Moreover, the younger is the child the more sensitive and disadvantageous it can be. The high sensitivity of the child to xenobiotics is defined by critical periods of development, specific of the metabolism in the growing body, the immaturity of a number of enzyme detoxication systems, limited function of liver and kidney, immaturity of the immune system and ability to accumulation of xenobiotics and radionuclides by hard bone and dental tissues. [4,5].

Ukraine has a history of Chornobyl

catastrophe that is intensified by its terrible consequences [12]. There is an evidence of its negative effect such as decline of the birth rate and increase in mortality, increased number of genetic disorders, inherited abnormalities, the growth of oncological, endocrine and other diseases as well as dental caries, enamel hypoplasia and maxilla-facial anomalies. The prevalence of caries in children from the polluted areas is recorded at 33,8-96,7%, and reaches even 100%. The intensity of dental caries in 12 years old children is about $4,57 \pm 0,38 - 7,24 \pm 0,40$ teeth. The statistic of enamel hypoplasia is fluctuated within 6,47-62,2%, periodontal disorders are observed in 14,3-77,2% of examined children, malocclusion is diagnosed around by 47,53-59,98% [7].

Considering the suppression of immunity practically in all children from the industrial regions were commend measures on improvement of general and local immunity as prophylaxis of dental caries.

The article demonstrates the correction by probiotic of the general and local immunity indexes in the blood serum and oral liquid of 12 years old children diagnosed with dental caries from Ivano-Frankivsk region concerned by radiation and chemical pollution. The results demonstrate that rational use of "Lactobacterine" leads to the increase of the complementum, as well as to the growth of Ig G and Ig M and s-IgA in the blood serum, decrease of circulating immune complexin oral liquid of children from the prophylactic groups comparing with those from control groups.

Objective.

Improvement of the efficiency in caries prophylaxis in children by correction of immunological parameters of saliva and blood serum by administration of probiotic "Lactobacterine".

Materials and methods.

We have examined 120 12 years old children affected by dental caries. There were 59 individuals from Snyatyn region affected by Chernobyl catastrophe and 61 children from

Kalush chemically polluted region. The number of children in the main groups in every region was 30. Control groups contained 29 children from both Snyatyn and Kalush regions. For more reasonable findings we calculated the average statistic norm in 30 healthy children with DMF-0.

The stomatological examination was conducted according to methods recommended by WHO. Caries activity was assessed by T.Vinogradova: compensated (DMF1-3); sub-compensated (DMF 4-6); de-compensated (DMF 7-9). The children from the main groups were treated and provided by professional oral hygiene. They were also prescribed with probiotic "Lactobacterine" ("Biopharma", Ukraine) as endogenic prevention. The medication itself is a yellowish-beige powder with sour-milk smell and taste and contains lyophilically dried microbial mass of living lactobacilli, that when added water forms a homogeneous curvature of yellowish-beige color. It also includes dry strains of *Lactobacillus plantarum* (strains 8P – A3 and 38) or *Lactobacillus fermentum* (strains 9OT – C4 and 39) that have proved to have anti-caries effect by inhibiting of *Streptococcus mutans*. One dose consists not less than $2 \cdot 10^9$ colony forming unit of Lactobacteria. The contents of the bottle should be dissolved for 5 minutes in boiled water at room temperature (20-25°C) at the rate of 1 teaspoon of water per 1 dose of the drug. After obtaining a homogeneous mixture with lactic smell, the content of the vial is poured into a glass and mixed. The solution has to be used immediately after preparation. It is recommended to administer "Lactobacterine" by 3 doses per day 60 minutes before meal for children with compensated form of caries; for children with sub-compensated caries is recommended to have 5 doses a day and for decompensated caries – 5 doses twice a day 60 minutes before meal. It is advised to have a medication with milk. The duration lasted for 14 days twice a year in the fall and spring.

We have to admit that such therapeutic effect of this probiotics provided by Lactobacteria that have antagonistic activity towards

pathogenic and conditionally pathogenic microorganisms and as a result they create appropriate environment to good microflora of intestine. The Lactobacteria produce lactic acid that is considered as a strong antiseptic that is efficient against pathogenic and conditionally pathogenic microbes founded and activated by prolonged disfunction of intestine.

The lactic acid also participates in exchange of Calcium by transformation of Calcium from food into Calcium Lactate that is better absorbed by organism (prevention of Rickets). This medication is important in production of vitamins and splitting of proteins with the formation of easily digestible amino acids including the irreplaceable, synthesized by the body.

Children from the control groups were introduced with individual oral hygiene and were provided by the dental treatment.

The efficiency of the conducted method was assessed by the amount of circulating immune complexes (CIC) as selective precipitation of complexes in 3,75% solution of polyetyleneglicole (PEG) of molecular mass 6000 followed with measurement of light absorption of samples on a spectrophotometer. Determination of the titer of the complement was carried out by colorimetric investigation of hemolysis produced by the interaction of the curvature of the ram's erythrocytes, hemolytic serum of rabbit and blood serum of the patient. The activity of lysozyme in saliva was determined by biological method of serial dilutions with microbe "Micrococcus luteus" test. Quantitative results of the content of immunoglobulins in blood serum was carried out by radial immunodiffusion. The measure of the secretory immunoglobulin A (SIgA) was conducted by radial immunodiffusion according to Mancini with the help of a monospecific serum. Oral liquid in children was collected in the morning before breakfast with a sterile pipette in sterile containers. We also had written permission from the parents for the examination of the gained biological material.

The duration has lasted for a period of 2,5 years.

Results

It has been proved that after the administered preventive measures the amount of lysozyme in oral liquid has increased from $(12,82 \pm 1,30)$ up to $(18,60 \pm 1,16)$ points in children from Snyatyn region ($p < 0,05$). On the other hand, there was a raise of lysozyme from $(12,70 \pm 1,30)$ upto $(14,20 \pm 1,80)$ points in children from the control group which was significantly lower than the data of the main group after the treatment ($p \leq 0,001$). All results are assembled and demonstrated in table 1.

The lysozyme has also extended in children from Kalush region from $(13,80 \pm 1,22)$ up to $(17,90 \pm 1,21)$ points ($p < 0,05$) and the control group demonstrated the statistic $(13,80 \pm 1,61)$ that did not differ from the data before the treatment ($p \leq 0,001$).

Furthermore, our study proved that secretory IgA has enhanced in children from Snyatyn region from $(0,92 \pm 0,07)$ up to $(1,36 \pm 0,05)$ g/l ($p < 0,001$) and in children from Kalush region it has changed from $(1,05 \pm 0,07)$ up to $(1,29 \pm 0,05)$ g/l ($p \leq 0,01$). In control group this index is within a norm ($p < 0,001$). The IgA in the blood serum of the examined children has proved it significant raise from $(1,21 \pm 0,19)$ up to $(1,83 \pm 0,18)$ g/l in children from Snyanty region ($p < 0,05$) comparing to $(1,28 \pm 0,18)$ up to $(1,79 \pm 0,17)$ in children from Kalush region ($p < 0,05$). After the appointed treatment there was a positive ($p < 0,001$) dynamics within this index.

As a result of the introduced prevention and treatment there is an expressive growth of IgM- and IgG in children of the main groups: in Snyatyn region from $(0,58 \pm 0,05)$ up to $(0,86 \pm 0,05)$ g/l ($p < 0,001$) and from $(8,60 \pm 0,40)$ up to $(11,60 \pm 0,41)$ g/l ($p < 0,05$) accordingly; In Kalush region the amount of IgM has changed from $(0,61 \pm 0,05)$ up to $(0,79 \pm 0,04)$ g/l ($p < 0,01$) and IgG – from $(9,20 \pm 0,35)$ up to $(10,80 \pm 0,36)$ g/l ($p < 0,01$). In the control groups of both regions, the rates were equal before the treatment and did not respond to the statistical norm.

After the "Lactobacterine" was administered the amount of the circulating immune complexes in oral liquid of children from Snyatyn

region has decreased from (16,8±0,65) points up to (7,4±0,63) (p<0,05). It has also declined in the control group after the investigation from (16,2±0,65)p. up to (14,9±0,63), although the difference is statistically unreliable.(p>0,05).

Table 1. Dynamic of indicators of general and local humoral immunity in children from Snyatyn and Kalush regions influenced by the treatment and prophylactic measures

Region	Snyatyn				Kalush				Average stati stic norm (n=30)
	Main group (n=30)		Control group (n=29)		Main group (n=30)		Control group (n=31)		
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	
Content of lysozyme in saliva, points	12,82 ±1,30***	18,60 ±1,16	12,70 ±1,30	14,20 ±1,80+++	13,80 ±1,22*	17,90 ±1,21	13,90 ±1,21	13,80 ±1,6+++	19,45 ±1,22
Content of IgA in saliva, g/l	1,21 ±0,19*	1,82 ±0,18	1,23 ±0,19	1,27 ±0,18+++	1,28 ±0,18*	1,79 ±0,17	1,29 ±0,16	1,28 ±0,14+++	2,26 ±0,24
Content of SIgA in saliva, g/l	0,92 ±0,07***	1,36 ±0,05	0,95 ±0,06	1,12 ±0,07+++	1,05 ±0,07**	1,29 ±0,05 ⁺	1,27 ±0,06	1,14 ±0,04+++	1,49 ±0,08
Content of IgG in blood serum, g/l	8,16 ±0,04***	11,60 ±0,41	8,50 ±0,35	8,90 ±0,34+++	9,20 ±0,35**	10,80 ±0,36 ⁺	8,90 ±0,34	8,80 ±0,32+++	12,70 ±0,90
Content of IgM in blood serum, g/l	0,58 ±0,05***	0,86 ±0,05	0,60 ±0,36	0,63 ±0,35 ⁺	0,61 ±0,09	0,79 ±0,04 ⁺	1,62 ±0,05***	0,64 ±0,05+++	0,97 ±0,07
M-CIC in blood serum, points	122,80 ±4,9***	87,30 ±4,1 ^{ooo}	122,30 ±4,7	119,80 ±4,6+++	112,00 ±4,6***	90,30 ±4,2+++	112,60 ±4,2	112,90 ±4,2+++	58,0 ±0,45
Content of M-CIC in saliva, points	16,80 ±0,65***	7,40 ±0,63 ^o	16,20 ±0,65	14,90 ±0,63+++	19,10 ±0,57***	8,90 ±0,62+++	19,00 ±0,58***	13,90 ±0,52+++	5,80 ±0,45

Content of complement in blood serum, p.opt density.	0,67 ±0,06	0,84 ±0,07	0,66 ±0,04	0,58 ±0,04 ⁺⁺⁺	0,68 ±0,05 [*]	0,81 ±0,04	0,64 ±0,04	0,64 ±0,03 ⁺⁺⁺	0,90 ±0,08
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* - between the indicators before and after treatment within the group * - ($p < 0,05$); ** - ($p < 0,01$); *** - ($p < 0,001$);

+ - between the indicators after the treatment and according to statistical norm; + - ($p < 0,05$); ++ - ($p < 0,01$); +++ - ($p < 0,001$).

In children of the main group from Kalush region has been observed a decrease of the circulating immune complexes in oral liquid from ($19,1 \pm 0,56$) p. Up to ($8,9 \pm 0,62$) p. ($p < 0,05$) after administration of the probiotic. In the control group the CIC was ($17,9 \pm 0,52$) p. and did not differ from the initial data ($19,0 \pm 0,58$) p. ($p < 0,001$).

The circulating immune complexes have changed also in blood serum of children from both regions comparing to the initial indicators ($p < 0,05$). On the other hand, this indexes remained high and did not differ from the original data that proved insufficient functional activity of phagocytic cells involved in the epithelization of pathogenic immune complexes.

The complement has increased in blood serum after the introduced scheme in children from Snyatyn region from ($0,67 \pm 0,06$) up to ($0,84 \pm 0,07$) p. ($p > 0,05$); in main of Kalush region from ($0,68 \pm 0,05$) up ($0,81 \pm 0,04$) ($p < 0,05$). This index remained the same in children affected by caries in the control groups.

The analysis of the results based on the indexes of general and local humoral immunity influenced by the recommended correction indicates its advantageous immune-correction effect.

Discussion.

The environmental pollution is of vital importance in Ukraine as amount of contamination on 1 sq. km. is about 6.5 times more than in the United States and 3,2 times more than in the European Economic Union. About 15% of the territory of Ukraine with a population of more than 10 million people is in a critical ecological

state, and 2% of these territories are recognized as an area of environmental disaster [3, 4].

Taking into consideration that the immune, endocrine and other systems remain immature in children, they are very sensitive to the bad environmental factors. The situation is worsened by summing of several factors at once: deterioration of socio-economic conditions of existence, quality of life and man-made environmental impact, which has a cumulative nature [2].

According to the last study the reis a confirmation that the use of probiotics is efficient as prevention of dental caries in children [9, 10]. There are numerous positive effect of probiotics such as production of various antimicrobial substances : organic acids, hydrogen peroxide and bacteriocin; competition with pathogenic microorganisms for the attachment to the mucous membrane; modification by changing pH that lead to the reduction of viability of pathogenic microorganisms; stimulation of non-specific immunity and change of humoral and cellular immune response [13]. The doctors-stomatologists are interested in probiotics based on their ability to adhere and colonize to various tissues of the oral cavity [12]. According to the literature, the *Lactobacillus paracasei* and *Lactobacillus rhamnosus* are capable of being embedded in a biofilm and to be to competitive with caries pathogenic or periodontal-pathogenic microflora including *Streptococcus mutans* и *Porphyromonas gingivalis* [11]. For example, Terekhova and co-authors have observed a rise in the pH of the plaque as well a decrease in the number of colonies of *Streptococcus mutans* up to 92% and *Lactobacteries* up

to 25% in oral liquid of pre-school children that used yoghurt with probiotic cultures. It has been established the high efficiency of *Lactobacillus reuteri* in caries prevention that can suppress cariogenic micro-flora such as *Streptococcus mutans* i *Streptococcus sobrinus* up to 80% selectively inhibiting periodontitis-associated microorganisms, decreasing inflammation of oral mucosa as well as formation of dental plaque and dental calculus and by improving appearance of gingiva by gingivitis [14]. The same results were gained by introduction of probiotics into the chewing gums that proves again its potential benefits in caries prophylaxis [15].

Thus, for the rational prevention of dental caries and achievement of a high level of dental health in children from bad environment, we recommend endogenous administration of probiotic.

Conclusions.

1. We have observed a significant decrease in the level of circulating immune complex within 36 month in the main group of children from the plains and foot hill areas diagnosed with compensated caries: in Snyatyn region in 2,3 times and in Kalush region - 2,1 times comparing to the initial level ($p < 0,05$).

2. Content of lysozyme in oral liquid of schoolchildren from Snyatyn has increased in 1,5 times after 2,5 years of preventive correction and in children from Kalush-in 1,3 times comparing to initial level ($p < 0,05$).

3. There was a tendency to an increase of s-IgA in oral liquid of children from the main groups after 36 month of correction, it has raised up to 33,5% in children from radiation-polluted area and in children from a chemically polluted region-up to 28,5% comparatively to the beginning of investigation ($p < 0,05$).

Changes of the immunological composition in saliva and blood serum after the proposed correction have proved the improvements of general and local immunity of the oral cavity followed by a raise of cariesres istancy of the hard dental tissues.

Prospects for further researches.

We would like to proceed our research on the efficiency of recommended correction in the future-3-4 years.

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