ORIGINAL ARTICLE

Statistical model of caries formation and progression in children of preschool and early school age domiciled in biogeochemical deficiency of fluorine and iodine*

Statystyczny model powstawania i rozwoju próchnicy u dzieci w wieku przedszkolnym i wczesnoszkolnym zamieszkałych w obszarach ubogich we fluor i jod*

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Abstract

Aim of the study. *To create a statistical and prognostic model of caries formation and progression in children of preschool and early school age domiciled in biogeochemical deficiency of fluorine and iodine.* ***Materials and methods.*** *Microbial landscape was identified with biochemical parameters and immunological status of saliva in 146 children aged 3-8 years of different ethnic groups. Statistical evaluation of the results was conducted.* ***Conclusions.*** *For the formation and progression of caries the presence of Str. sobrinus is necessary plus dysbiotic changes in the oral cavity (decrease of concentrations of Bifidobacterium and Lactobacillus salivarius, emergence of conditionally pathogenic and pathogenic microorganisms), increasing concentration of ionized calcium, alkaline phosphatase, and lower levels of phosphorus and pH.*

Streszczenie

Cel pracy. *Stworzenie statystycznego i progostycznego modelu powstawania i rozwoju próchnicy u dzieci w wieku przedszkolnym i wczesnoszkolnym zamieszkujących obszary ubogie we fluor i jod.* ***Materiał i metody.*** *Środowisko mikrobiologiczne zostało określone za pomocą parametrów biochemicznych i immunologicznego statusu śliny u 146 dzieci w wieku 3-8 lat reprezentujących różne grupy etniczne. Przeprowadzono analizę statystyczną otrzymanych wyników.* ***Wnioski.*** *Dla powstania i rozwoju próchnicy niezbędna jest obecność Str. sobrinus wraz z dysbiotycznymi zmianami w obrębie jamy ustnej (zmniejszenie stężenia Bifidobacterium i Lactobacillus salivarius, pojawienie się warunkowo patogenów i patogennych mikroorganizmów), wzrost stężenia jonów wapnia, fosfatazy zasadowej, niższe poziomy fosforu i pH.*

KEYWORDS:
children, caries, biogeochemical deficiency of fluorine and iodine, formation of caries, progression of caries

HASŁA INDEKSOWE:
dzieci, próchnica, biogeochemiczny niedobór fluoru i jodu, tworzenie próchnicy, rozwój próchnicy

Introduction

Leading dental researchers pay much attention to microbiological status of children and adolescents to establish the prevalence of certain types of microorganisms as etiological factors of emergence and progression of chief dental diseases.[1-4,11-17] It was established that biochemical parameters of saliva and oral cavity's local immunity play an important role.[6-8,11,17,18]

Epidemiological research among children of Transcarpathian region revealed that there are significant ethnic factors in causing the disease. In particular, in children of Romany ethnic group the incidence of caries was significantly lower comparing to children of Ukrainian, Hungarian, Slovak, and Polish ethnic groups. Separating a pure ethnic group is difficult, but with the prevalence of Romany group the incidence and caries activity is lower.[9,10]

Since there is not enough scientific medical dental research based on statistical parameters of the formation and progression of caries in children who permanently live in conditions of biogeochemical fluorine and iodine deficiency it justifies the validity of such studies.

The study aimed at creating a statistical model of caries formation and progression in children of preschool and early school age domiciled in biogeochemical deficiency of fluorine and iodine.

Methods

To achieve the aim biochemical parameters were identified, plus biocenosis and immunological status of saliva in 146 children aged 3-8 years of different ethnic groups with different level of caries's activity who live in conditions of biogeochemical fluorine and iodine deficiency.

Examination of children was conducted after obtaining consent of parents or guardians in "Dental Clinic" of Dental Faculty of Higher Educational Establishment "Uzhhorod National University". Microbiological research was conducted in microbiological laboratory of the S.M. Vinogradsky Transcarpathian Department of microbiologists's community of Ukraine (Head of community Ph.D., professor N. V. Boyko).

Among 146 children surveyed, 37 belonged to the Romany ethnic group, accounting for 25.3%, 109 – other ethnicities – 74.7%. Children were divided into groups depending on the activity of caries 35 – with compensated caries (24.0%), 26 – with subcompensated (17.8%), 35 – with decompensated caries (24.0%) and 50 healthy children, 25 of Romany ethnic group and 25 of other ethnic groups – control group.

Biochemical parameters of saliva, such as total and ionized calcium, phosphorus, alkaline phosphatase and measured hydrogenous pH were assessed with Dimension RxL Max Integrated Chemistry System (Siemens) device by immunofluorescence method.

Microbial environment was determined by selective culture of saliva on the following nutrient media: meat peptone agar, selective agar, semi-selective medium, semi Blaurock environment, Endo environment, bismuth-sulfite agar, enterococcal agar, laktobak-agar and bifidum agar.

Determination of secretory IgA was carried out with «sandwich» variant of enzyme-linked immunosorbent assay by measuring of value of optical density of holes tablet content by vertical scanning on the photometer with wavelength of 450 nm.

Statistical analysis of obtained data was conducted using methods of parametric and nonparametric statistics. Compliance of statistical distribution empirical indicators according to theoretical normal Gaussian distribution was assessed using Kolmogorov-Smirnov criterion (K.-S.) and Shapiro-Wilks (W), with p – an indicator of reliability.

For small volumes of sample distribution it was estimated as close to normal at about equality of the arithmetic mean and median, symmetry of the maximum values relative to the average value and at condition that coefficient of asymmetry and kurtosis at absolute value does not significantly exceed 2. Methods of descriptive statistics include the arithmetic mean estimation (C), reliability interval (p), median (M), minimum (min) and maximum (max), standard deviation ($\sigma2$), asymmetry and kurtosis. Paired and Pearson's partial correlations (r) were also analysed with reliability interval (p), based on absolute data.

Methods of nonparametric statistics – Spearman's rank correlation (rs) were also used. Correlation coefficients were marked as $C_к$. Also performed was factor loadings analysis, cluster analysis - hierarchical classification. Admissible level of reliability (p) statistical null hypothesis (absence of significant heterogeneity factor or influence) equaled 0.05.

Interpretation calculations were performed on a personal computer using licensed software "MS Excel 7" operating system "Windows XP".

Results and discussion

The results of biochemical, microbiological and immunological analysis of saliva in Romany children group and mixed ethnic groups residing in conditions of biochemical deficiency fluorine and iodine, revealed significant differences reflected in the following statistical model.

In children of the Romany ethnic group there is presence of *Str. mutans* y 100% in quantity $2×10^3±2×10^2$ CFU (colony forming unit), *Str. salivarius* $4×10^3±4×10^2$ CFU, *Bifidobacterium* $15×10^6±1×10^5$ CFU, *Lactobacillus salivarius* $1×10^4±1×10^3$ CFU, *Str.mitis* $1×10^3±1×10^2$ CFU, *Enterococcus faecalis* $4×10^4±4×10^3$ CFU. The average value of total microbial number is $2.5×10^5±1×10^3$ CFU. In children of Romany ethnic control group in the oral cavity dysbiotic changes were observed.

In children of Romany ethnicity with compensated degree of caries's activity we observed the presence of *Str. mutans* in 100% in quantity of $2×10^6±2×10^3$ CFU, *Str. salivarius* $4×10^4±4×10^3$ CFU, *Bifidobacterium* $5×10^4±1×10^3$ CFU, *Lactobacillus salivarius* absent, *Str.mitis* $1×10^3±1×10^2$ CFU, *Enterococcus faecalis* $4×10^6±4×10^3$ CFU. The average value of total microbial number is $4×10^6±1×10^3$ CFU. These indicators indicate oral dysbiosis of I and II degree, because of the absence of *Lactobacillus salivarius* and presence of large numbers of *Enterococcus faecalis*.

In children of control mixed ethnic groups 100% of cultures are *Str. sobrinus* in quantity $1×10^4±1×10^3$ CFU, *Str. mutans* is not present, *Str. salivarius* $3×10^3±1×10^2$ CFU, *Bifidobacterium* $2×10^4±1×10^3$ CFU, *Lactobacillus salivarius* $1×10^3±1×10^2$ CFU, *Str.mitis* $1×10^3±1×10^2$ CFU. The average value of total microbial number is $3×10^5±1×10^3$ CFU, that indicates oral dysbiosis of I and II degree. The amount of alkaline phosphatase 21.6±1.02 unit of activity, ionized Ca 0.96±0.05 mmol/l, phosphorus 6.15±0.37 mmol/l.

In children of mixed ethnic groups with compensated degree of caries activity in 100% of *Str.sobrinus* culture in quantity $1×10^5±1×10^3$ CFU, *Str. mutans* was absent, *Str. salivarius* $4×10^4±4×10^2$ CFU, *Bifidobacterium* $2×10^3±1×10^2$ CFU, *Lactobacillus salivarius* was absent, *Str.mitis* $1×10^3±1×10^2$ CFU, *Escherichia coli* $1×10^3±1×10^2$ CFU. The average value of total microbial number is $4×10^5±1×10^3$ CFU, that indicates oral dysbiosis of I and II degree. Alkaline phosphatase 27.6±1.02 unit of activity, Ca 1.06±0.05 ionized mmol/l, phosphorus 5.85±0.37 mmol/l.

In children of mixed ethnic groups with subcompensated degree of caries activity in 100% of *Str. sobrinus* culture in quantity $1×10^9±1×10^3$ CFU, *Str. mutans* was absent, *Str. salivarius* $4×10^2±4×10^1$ CFU, *Bifidobacterium*, *Lactobacillus salivarius* was absent, *Str.mitis* $1.5×10^3±1.5×10^2$ CFU, *Escherichia coli* $1×10^4±1×10^2$ CFU. *Pseudomonadas aeruginosa* $2×10^3±1×10^2$ CFU, *S.aureus* $1×10^3±1×10^2$ CFU, *Citrobacter Freundi* $1.5×10^3±1,5×10^2$ CFU *Proteus vulgaris* $2×10^3±2×10^2$ CFU, *Enterococcus faecium* $1×10^3±1×10^2$ CFU. Number of alkaline phosphatase 45.0±0.81 unit of activity, ionized Ca 1.35±0.09 mmol/l, phosphorus 5.03±0.1 mmol/l.

In children of mixed ethnic groups with decompensated degree of caries activity in 100% of *Str. sobrinus* culture in quantity $5×10^5±5×10^3$ CFU, *Str. mutans* was absent, *Str. salivarius* $1.5×10^3±1.5×10^1$ CFU, *Bifidobacterium*, *Lactobacillus salivarius* was absent, *Str.mitis* $1×10^2±1×10^1$ CFU, *Escherichia coli* $1×10^5±1×10^2$ CFU. *Pseudomonadas aeruginosa* $2×10^{10}±1×10^2$ CFU, *S.aureus* $1×10^5±1×10^2$ CFU, *Citrobacter Freundi* $1×10^4±1×10^2$ CFU *Proteus vulgaris* $1×10^9±1×10^2$ CFU. Number of alkaline phosphatase 52.0±1.98 unit of activity, ionized Ca 1.95±0.09 mmol/l, phosphorus 5.33±0.1 mmol/l.

The average value of total microbial number is $3\times10^{10}\pm1\times10^{3}$, that indicates oral dysbiosis of III and IV degree.

Conclusions

For the formation of caries the following conditions are necessary: the presence *Str. sobrinus* in quantity of 1×10^{4} CFU, dysbiotic changes in the oral cavity, accompanied by decreased concentration of *Bifidobacterium* less than $2\times10^{3}\pm1\times10^{2}$ CFU, reduction or total absence of *Lactobacillus salivarius*, appearance of conditionally pathogenic and pathogenic microflora – *Escherichia coli* and increasing concentration of ionized calcium in saliva, compared with healthy children (p<0.05) increase of alkaline phosphatase, and reduction of phosphorus and pH_{pp}. (r=[0.52-0.84]).

Progression of caries that leads to the development of sub- and decompensated form of caries is characterized by the following parameters: increasing number of *Str. sobrinus* from 1×10^{4} CFU, increasing the total microbial number in the oral cavity to 1×10^{9} and a large number of pathogenic microorganisms, in particular *Pseudomonadas aeruginosa*, *Escherichia coli*, *S .aureus*, *Proteus vulgaris*, accompanied by a significant decrease of pH, and significantly higher calcium-phosphate coefficient (0.54; 0.21; p>0.05).

In children with sub- and decompensated caries TMN does not significantly differ (p>0.05), though the species composition of microorganisms in subcompensated level of caries activity is significantly higher, with prevalence of pathogenic organisms.

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