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MICROBIAL CARIOGENIC FACTORS AND THE MECHANISMS OF THEIR COUNTERACTION IN THE ORAL FLUID OF CHILDREN AT EARLY AGE

O. I. Godovanets, L. G. Hrynkevych, O. T. Khomyshyn

Bukovinian State Medical University, Chernivtsi, Ukraine

Objective *– to determine the balance between pro- and antibacterial agents influencing upon the development of a carious process in children at an early age.*
Materials and methods. *117 children 2-3 years of age were examined. Two observation groups were formed depending on the condition of the hard dental tissues. I group included 52 children without caries, group II– 65 children with caries. The oral hygiene of the children from the observation groups was assessed by means of Kuzmina EM index. Lysozyme activity in the oral fluid was studied by means of bacteriological method. sIgA content was determined by means of radial immunodiffusion method. Streptococcus mutans concentration in the oral fluid of children was determined by means of «Saliva-Check Mutans» set. All the manipulations were carried out after an informed consent of parents was obtained.*
Results. *Oral hygiene in children during the period of the temporal occlusion formation is found to be unsatisfactory – (0,48±0,20) points according to Kuzmina EM index. The level of colonization of the oral cavity with Streptococcus mutans is high in 34,48 % of stomatologically healthy children and in 73,33 % of children with caries. Under conditions of caries process development, the main protective components in the oral fluid change. In particular, sIgA level 17,14 % increases concerning the index of healthy children ($p<0,05$), and lysozyme activity 1,25 times decreases respectively ($p<0,05$).*
Conclusions. *All the children examined present soft dental plaque, which is confirmed by the values of hygienic indices and levels of Streptococcus mutans concentration, which is reliably higher in children from group II. Under conditions of caries process development, changes of the major protective components of the oral cavity appear. They promote effective action of cariogenic factors.*

Key words:
children, early caries, oral hygiene, oral fluid, immunoglobulins, lysozyme.

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E-mail: godovanec.oksana@bsmu.edu.ua

МІКРОБНІ КАРІЄСОГЕННІ ЧИННИКИ ТА МЕХАНІЗМИ ЇХ ПРОТИДІЇ У РОТОВІЙ РІДИНІ ДІТЕЙ РАННЬОГО ВІКУ

О. І. Годованець, Л. Г. Гринкевич, О. Т. Хомишин

Буковинський державний медичний університет, м. Чернівці, Україна

Мета роботи – *визначити баланс між про- та антибактеріальними агентами, що впливають на розвиток каріозного процесу в дітей раннього віку.*
Матеріал та методи. *Обстежено 117 дітей віком 2-3 роки. Сформовано дві групи спостереження залежно від стану твердих тканин зубів: до І групи віднесено 52 дитини без карієсу, до ІІ групи – 65 дітей із карієсом. У дітей груп спостереження здійснено оцінку стану гігієни ротової порожнини за допомогою індексу ЕМ Кузьміної, досліджено ротову рідину щодо рівня активності лізоциму бактеріологічним методом, умісту sIgA методом радіальної імунодифузії. За допомогою тестового набору «Saliva-Check Mutans» визначали рівень концентрації Str. mutans у ротовій рідині дітей. Усі маніпуляції здійснювали після одержання проінформованої згоди батьків.*
Результати. *Установлено, що в дітей у період формування тимчасового прикусу гігієна ротової порожнини є незадовільною – (0,48±0,20) бала за індексом МЕ Кузьміної. Рівень колонізації ротової порожнини Str. mutans є високим у 34,48 % стоматологічно здорових дітей та у 73,33 % дітей, уражених карієсом. За умов розвитку каріозного процесу в дітей спостерігаються зміни щодо основних захисних компонентів ротової рідини, зокрема підвищення рівня sIgA на 17,14 % відносно показника у здорових дітей ($p<0,05$) та зниження активності лізоциму в 1,25 раза ($p<0,05$).*
Висновки. *У всіх обстежених дітей виявлено м'які зубні відкладення, що підтверджується значеннями індексів гігієни та рівнем концентрації Str. mutans, які є вірогідно більшими в осіб ІІ групи. За умов розвитку каріозного процесу спостерігаються зміни основних захисних компонентів ротової порожнини, що, безумовно, є сприятливим фоном для результативної дії карієсогенних чинників.*

Ключові слова:
діти, ранній дитячий карієс, гігієна ротової порожнини, ротова рідина, імуноглобуліни, лізоцим.

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Introduction

Microorganisms of the oral cavity are known to play a crucial role in the development of carious process [1-2]. *Streptococcus mutans* is a key factor launching demineralization process. *Lactobacilli* and *Candida fungi* significantly affect the development of caries in children as well [3-6].

At the same time, numerous innate protective mechanisms function in the oral cavity. They produce both a direct antimicrobial effect and indirect influence on the microbial colonization. These include such proteins as defensins, histatins, glycoproteins of the saliva, proline rich proteins, immunoglobulins, cystatins and lysozyme. Lysozyme, sIgA and lactoferrin are the first-line protectors in the oral cavity of a child [7-9].

Lysozyme, as a hydrolytic enzyme, breaks down cellular membranes of gram-positive and gram-negative bacteria, fungi and viruses. It is one of the key antimicrobial factors of the oral fluid [10]. Therefore, determination of the activity of this enzyme is informative concerning its effect on the state of local non-specific immunity, which is the first-line protection against microbe-associated pathological processes.

The antibodies, present in the oral cavity and attached to various surfaces by means of glycoproteins, are the second protective factors found in the oral cavity. sIgA fraction is the most numerous. It constitutes 90 % of all the immunoglobulins of the oral fluid [11]. Proteins are structural units of glycoproteins, and it forms a substrate for fixation of immunoglobulins and their performance of protective functions. Moreover, protein-carbohydrate complexes provide protective functions on a mechanical level.

The aim of the study

To determine the balance between pro- and antibacterial agents influencing upon the development of a carious process in young children.

Research materials and methods

2-3-year old children were examined at the Department of Pediatric Dentistry, Bukovinian State Medical University, in order to assess cariogenic situation in their oral cavities. Two observation groups were formed depending on the condition of the hard dental tissues. I group included 52 children (27 boys and 25 girls) without caries, II group – 65 children (31 boys and 34 girls) with caries. The oral hygiene of the children from the observation groups was assessed by means of Kuzmina EM index. Lysozyme activity in the oral fluid was studied by means of bacteriological method. sIgA content was determined by means of radial immunodiffusion method, and protein level – by means of biuret method [12]. *Streptococcus mutans* concentration in the oral fluid of children was determined by means of «Saliva-Check Mutans» set.

The results of the study obtained were statistically processed by means of the standard package of programs «Microsoft Office Excel». The following indices were calculated: means arithmetic value (M), arithmetic mean deviation (σ), mean error (m). Probability of differences (P) was determined by Student (t) criterion.

The conducted research complied with bioethical norms, that is indicated in the conclusion of Bukovinian State Medical University Bioethics Board, № 8 of 15.04.2021. All the manipulations were carried out on receiving an informed consent of parents.

Results and discussion

The simplest clinical method to assess the state of microbial load of the oral cavity is determination of hygienic index, and we have done it. Table 1 presents the results of determination of Kuzmina EM index among the children from the observation groups.

Table 1

The values of Kuzmina EM index in healthy and caries-afflicted children considering gender factor, M±m

Observation groups	Subgroups considering gender factor	Kuzmina EM index	
		value	hygiene assessment
I group	total (n=52)	0,33±0,02	satisfactory
	boys (n=27)	0,40±0,02	satisfactory
	girls (n=25)	0,29±0,01	satisfactory
II group	total (n=65)	0,65±0,04 *	unsatisfactory
	boys (n=31)	0,71±0,05 *	unsatisfactory
	girls (n=34)	0,62±0,03 *	unsatisfactory

*Note. * – reliable difference between I and II groups, p<0,05.*

According to the information, contained in the Table, oral hygiene was satisfactory in children without afflictions of the hard dental tissue. The values of the indices were the following: good oral hygiene was found in 7 (13,46 %) children, plaque was completely absent; 36 (69,23 %) children had satisfactory oral hygiene, plaque was found on a half of the teeth examined; and 9 (17,31 %) children had unsatisfactory oral hygiene as the majority of their teeth were covered with plaque. There was no significant difference in the indices between boys and girls.

In children of II group hygienic index was twice as much and indicative of unsatisfactory oral hygiene. Thus, satisfactory oral hygiene was determined in 15 (23,08 %) examined children, and 50 (76,92 %) of them had unsatisfactory oral hygiene, that was reliably more than among the children from I group (p<0,05). None of this group had good oral hygiene. Similar to the previous

group, there was no significant difference found in the indices between boys and girls.

One more informative method of assessment of microbial load in the oral cavity of children is determination of the content of *Streptococcus mutans.* The level of its colonization is an objective cariogenic marker. The results of the examination of this index are presented in Table 2.

As evidenced by the data in the Table, the percentage of caries-free children examined with a high titer of *Streptococcus mutans* was twice as low as in children with caries of temporary teeth. A reliable difference was observed between the indices in the groups and subgroups. There was no difference in the indices between boys and girls found.

Table 2

The level of Streptococcus mutans content in the oral fluid of healthy and caries afflicted children considering gender factor, M±m

Observation groups	Subgroups considering gender factor	High	Low
		>5x10^5 CFU / ml of saliva	<5x10^5 CFU / ml of saliva
I group	total (n=29)	34,48 %	65, 52 %
	boys (n=14)	35,71 %	64,29 %
	girls (n=15)	33,33 %	66,67 %
II group	total (n=30)	73,33 % *	26,67 % *
	boys (n=16)	75,00 % *	25,00 % *
	girls (n=14)	71,43 % *	28,57 % *

*Note. * – reliable difference between indices of I and II groups, p<0,05.*

Lysozyme activity of the oral fluid of healthy children (I group) was (37,15±1,82) units per liter. It was 25,34 % higher than in children afflicted with caries (II group) (29,64±1,53) units per liter (p<0,05).

Our research demonstrated that an average sIgA content in the oral fluid of children was variable. The fig. 1 below presents average values of sIgA content in the oral cavity of children from the observation groups.

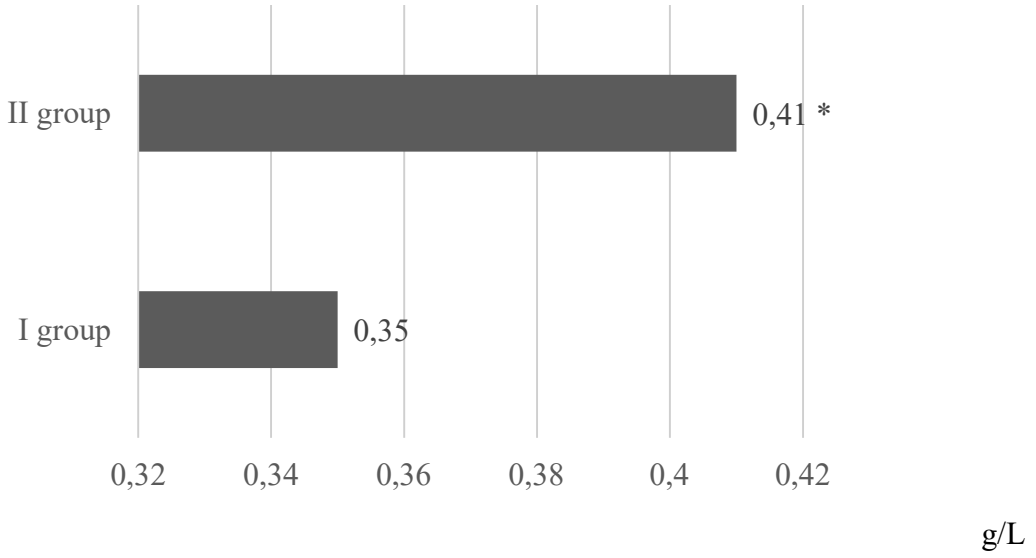

Fig. 1. sIgA content in the oral fluid of healthy (I group) and caries afflicted (II group) children, M±m.

*Note. * – reliable difference from the indices of I group, p<0,05.*

This index in children from II group was characterized by both decrease and increase. The changes correlated with the condition of the hard dental tissue. When the intensity of dental caries of a child was low, sIgA level increased, while with high intensity of lesions the level of this immunoglobulin mostly decreased. In general, in children from II group sIgA level was 17,14 % higher than in I group (p<0,05).

An average level of the whole protein in the oral fluid of healthy children was (2,67±0,15) g/L. With caries process development this index 41,95 % increased, it was (3,79±0,24) g/L in children of II group (p<0,05).

Thus, the oral fluid of children has a caries process trigger – dental plaque containing the main cariogenic microorganisms. Under favorable conditions, when local protective reactions are disturbed, demineralization of

the dental enamel is launched followed by its irreversible affliction.

Conclusions

Therefore, all the children under examination present soft dental plaque, that is confirmed by the values of hygienic indices and levels of *Streptococcus mutans* concentration, which is reliably higher in children from II group. Under conditions of caries process development, changes of the major protective components of the oral cavity appear. They promote effective action of cariogenic factors.

Prospects of further studies

It would be reasonable to continue the research and assess all the cariogenic factors concerning early caries in young children.

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Відомості про авторів:
Годованець О. І. – доктор медичних наук, професор, завідувач кафедри стоматології дитячого віку, Буковинський державний медичний університет, м. Чернівці, Україна.
E-mail: godovanec.oksana@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0002-1889-3893
Гринкевич Л. Г. – асистент кафедри стоматології дитячого віку, Буковинський державний медичний університет, м. Чернівці, Україна.
E-mail: grynkevych.l@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0003-2460-2105
Хомишин О. Т. – аспірант кафедри стоматології дитячого віку, Буковинський державний медичний університет, м. Чернівці, Україна.
E-mail: homyshyn_oleksandr@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0002-8144-4257

Information about authors:
Godovanets O. I. – Doctor of Medical Sciences, Professor, Head of the Department of Pediatric Dentistry, Bukovinian State Medical University, Chernivtsi, Ukraine.
E-mail: godovanec.oksana@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0002-1889-3893
Hrynkevych L. G. – Assistant, Department of Pediatric Dentistry, Bukovinian State Medical University, Chernivtsi, Ukraine.
E-mail: grynkevych.l@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0003-2460-2105
Khomyshyn O. T. – post-graduate, Department of Pediatric Dentistry, Bukovinian State Medical University, Chernivtsi, Ukraine.
E-mail: homyshyn_oleksandr@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0002-8144-4257

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