ORIGINAL ARTICLE

THE LEVEL OF INTERLEUKIN-18 IN THE ORAL FLUID IN PRIMARY SCHOOL CHILDREN WITH CHRONIC CATARRHAL GINGIVITIS AND TYPE I DIABETES MELLITUS

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ABSTRACT

The aim: Of our research work was to study the level of proinflammatory interleukin-18 (IL-18) in the oral fluid of children with type I diabetes mellitus (DM), and to determine their periodontal status and the level of oral hygiene.

Materials and methods: 82 children were examined, they were divided into groups by presence of gingivitis and diabetes mellitus. The level of interleukin-18 in oral fluid was determined by immunoassay.

Results: In patients with chronic catarrhal gingivitis and type I diabetes mellitus the level of interleukin-18 in oral fluid is the highest (70.91±7.48 pg / ml); the level of interleukin-18 in children with diabetes mellitus and healthy gums is high enough too, it is 14.87±1.11 pg / ml. Interleukin-18 is 3.41±0.25 pg / ml in healthy children with healthy gums. It is 5.74±0.27 pg / ml in somatically healthy children with chronic catarrhal gingivitis.

Conclusions: We indicated that an increase in the value of interleukin-18 in oral fluid is associated with the presence of diabetes mellitus in children. Moreover, this cytokine can be considered as a potential biomarker of gum inflammation in children with diabetes mellitus.

KEY WORDS: type I diabetes mellitus, cytokines, gingivitis, gums

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INTRODUCTION

Type I diabetes mellitus is an autoimmune disease in genetically susceptible individuals, which leads to the destruction of pancreatic β-cells. It leads to absolute insulin deficiency [1, 2].

The data of Ukrainian researchers indicate that 8-10 % of all patients with type I diabetes mellitus are children, that is, one child out of every 500 and, accordingly, one out of 200 adolescents in Ukraine suffer from diabetes mellitus.

Dental manifestations of diabetes are noted in the overwhelming majority of patients, and some dentists indicate organs and tissues of the oral cavity are damaged in 100% of cases [1 - 4].

The presence of type I diabetes mellitus in children is the main risk factor for the occurrence of inflammatory periodontal diseases. Metabolism in the periodontal tissues is disturbed due to hypoglycemia, which leads to the progression of inflammatory and degenerative processes in the oral cavity [1, 3 - 5]. The common features for endocrinological diseases and periodontal pathologies are: angiopathy (namely at the level of vascular microcirculatory trackt), metabolic disorders, changes in claim of lipid peroxidation, autoaggression and occurrence of secondary immunodeficiency [6 - 9].

It is also known, that type I diabetes affects oral homeostasis. Several studies have shown the prevalence of gum disease in diabetic patients. Hygienic indices, periodontal indices, bleeding indices – all these indicators are increased in groups of adolescents with type I diabetes [2].

Oral fluid consists of mixed saliva, that is, a mixture of secretions from three pairs of large glands and many small ones in the oral cavity. It is a biological fluid that has great potential for scientists to study [10 - 12]. In addition to the fact that the composition of the oral fluid can change in the presence of systemic diseases (especially those that affect the function of the salivary glands) [13]. The possibility of simple, safe and non-invasive collection of the test material is valuable. This fluid is suitable for studying many biochemical parameters, for example, cytokines [8, 14, 15].

Periodontal monocytes, macrophages, fibroblasts, endothelial cells respond to microorganisms, lipopolysaccharides and other antigens of dental plaque, they secrete numerous chemokines and inflammatory cytokines [8, 14, 16, 17]. The final products of glycolysis accumulate in monocytes due to hyperglycemia [3 - 5]. They increase oxidative stress in cells and activate transcriptional nuclear factor kappa B [18], which influences the phenotype of macrophages and leads to increasing the production of inflammatory cytokines (such as IL-18) [2, 17, 19].

THE AIM

The aims of this scientific work were to determine the dental status and to study the content of proinflammatory interleukin-18 (IL-18) in the oral fluid in primary school children with insulin-dependent diabetes mellitus.

MATERIALS AND METHODS

82 children aged from six to twelve years old were examined (56 children with type I diabetes mellitus and 26 children without concomitant somatic diseases) during our research work.

Participants of the study and / or their caregivers (parents) responded to the questions about their childrens' dental and health status. We determined the index of hygiene according to Fedorov-Volodkina (1968) and simplified hygiene index OHI-S (Green, Vermillion, 1964). Periodontal index was assessed using the PMA index (papillary-marginal-alveolar index) modified by Parma (1960), which helps to assess the severity of gingivitis.

Bleeding index was determined according to Loe, Silness (1967), (it is also called gingival index (GI)), and according to Muhleman H.R. (1971). Both indices give an indication of severity of gingivitis.

The content of IL-18 in the oral fluid was determined to characterize inflammation in the organs of the oral cavity. Determination of the concentration of IL-18 in the oral fluid was performed by ELISA. An unstimulated oral fluid was taken on an empty stomach in the morning, at the same time. Patients were asked to rinse the mouth beforehand. The sampling was made by spitting 4 ml of oral fluid into plastic sterile tubes. They were hermetically sealed, carried out after 30 minutes. The collected oral fluid was delivered to the laboratory.

The study of IL-18 in the oral fluid was carried out by adding the test material in parallel with the control samples into special containers with immobilized antibodies. The binding of IL-18 with conjugate No. 1, wich contained antibodies to human IL-18 with biotin was done at the next stage. The new conjugate was formed as a result, then it interacted with the conjugate No. 2, which contained streptovidine with horseradish peroxidase. The concentration of IL-18 in the oral fluid was determined by colorimetric reaction. This reaction was done, by using the substrate horseradish peroxidase-hydrogen peroxide and chromogen-tetrametilbenzidine by enzyme immunoassay analyzer STATFax 303 Plus (USA) at a wavelength of 450 mm.

The exclusion criteria were: children who had prior orthodontic treatment or were undergoing orthodontic treatment at the time of examination; smoking; treatment of periodontium or antibiotic therapy in the last 6 months; any other systemic diseases, except diabetes mellitus; eruptive gingivitis. We also excluded children with diabetes mellitus with any other complications than periodontal inflammation.

The results of the study were statistically processed using the methods of the standard package for statistical calculations Statistica 5.0 Microsoft Office Exel 2003. We calculated such statistical variables as mean values (M) and standard error of the average value (m). We used the t-criterion of Student to compare mean absolute values. The difference in results was considered statistically significant, if the value of index was $p \leq 0.05$

Our research work was performed in compliance with the principles of bioethics and legal norms and requirements of clinical / biomedical research, namely: the Declaration of Helsinki (1964-2013), the Constitution of Ukraine and the Civil Code of Ukraine (2006), Fundamentals of Legislation of Ukraine on Health Care (1992), Guidelines for Clinical Trials of the Ministry of Health of Ukraine № 42-7.0: 2005 "Drugs. Clinical Practice" (2005), Model Regulations on Ethics commissions at medical institutions (Order of the Ministry of Health of Ukraine № 690 of September 23, 2009).

RESULTS AND DISCUSSION

All the patients were divided into the following subgroups:

Group 1 – 13 children with clinically healthy periodontium and no concomitant diseases;

Group 2 – 13 children without concomitant diseases, but with chronic catarrhal gingivitis;

Group 3 – 26 children with type I diabetes mellitus and healthy gums;

Group 4 – 30 children with type I diabetes mellitus and chronic catarrhal gingivitis.

We have determined the hygienic status of the oral cavity, the state of the periodontium and the content of IL-18 in the oral fluid of children. The data are shown in Table I.

The index of hygiene according to Fedorov-Volodkina in the group of somatically healthy children without chronic catarrhal gingivitis is 1.48±0.05 points, which corresponds to good hygiene of the oral cavity (Table 1). The Fedorov-Volodkina hygiene index is slightly worse and is, respectively, 1.84±0.06 points and 1.65±0.07 points, which can be interpreted as a satisfactory level in somatically healthy with chronic catarrhal gingivitis and children with type I diabetes mellitus without chronic catarrhal gingivitis. The above index is 2.20±0.08 points, which corresponds to unsatisfactory oral hygiene in the group of patients with type I diabetes mellitus and chronic catarrhal gingivitis. Such a value can be explained by the fact that bleeding of the gums, their edema and pain in children with type I diabetes mellitus does not allow to make oral hygiene qualitatively.

The results of the simplified index of oral hygiene OHI-S (Green-Vermillion) confirm the identified trend. This index is 0,69±0,06 points in somatically healthy children with healthy gums, that is good level of hygiene. The OHI-S (Green-Vermillion) is 0,88±0,07 points I children with type I diabetes mellitus and healthy gums and 1,60±0,06 points in somatically healthy children with chronic catarrhal gingivitis, that is the satisfactory level of hygiene. OHI-S in the group of children with type I diabetes mellitus and chronic catarrhal gingivitis is 1.87±0.05 points, and it can be interpreted as bad level of hygiene.

Table I. Condition of periodontium, oral hygiene and content of IL-18 in the oral fluid of somatically healthy children and children with type I diabetes

Indicators	Group			
	Healthy children with healthy gums n = 13	Healthy children with chronic catarrhal gingivitis n= 13	Children with type I diabetes mellitus and healthy gums n = 26	Children with type I diabetes mellitus and chronic catarrhal gingivitis n = 30
	1	2	3	4
Hygiene index (F-V), points	1.48 ± 0.05 * *****	1.84 ± 0.06 ****	1.65 ± 0.07 ***	2.20 ± 0.08
OHI-S (G, V), points	0.69 ± 0.06 * *****	1.60 ± 0.06 ** **	0.88 ± 0.07 ***	1.87 ± 0.05
PMA, %	0 * *****	20.52 ± 0.83 ****	0 ***	40.47 ± 0.96
GI (S, L), points	0 * *****	0.84 ± 0.05 ****	0 ***	1.83 ± 0.04
Bleeding index (Muhleman H.R.), points	0 * *****	0.71 ± 0.05 ****	0 ***	1.07 ± 0.05
IL-18, pg / ml	3.41 ± 0.25 * ** *****	5.74 ± 0.27 *** *	14.87 ± 1.11 ***	70.91 ± 7.48

Note:
* - the difference is significant between groups 1 and 2, p≤0.05;
** - the difference is significant between groups 1 and 3, p≤0.05;
*** - the difference is significant between groups 3 and 4, p≤0.05;
**** - the difference is significant between groups 2 and 4, p≤0.05;
***** - the difference is significant between groups 1 and 4, p≤0.05.

We compared the values of hygiene indices according to Fedorov-Volodkina and according to the simplified hygiene index OHI-S (Green-Vermillion). A statistically significant difference (p≤0.05) was found when comparing groups 1 and 2, 3 and 4, 2 and 4, 1 and 4, but it was not found (p≥0.05) between groups 1 and 3 (in children with healthy gums with type I diabetes mellitus and without type I diabetes mellitus).

This fact confirms the opinion of some authors, who note that the hygiene index does not differ significantly in patients with type I diabetes mellitus and in the control group of persons [2]. However, it contradicts the opinion of others, who found that the value of the hygiene index in patients with insulin-dependent diabetes mellitus (especially with poor metabolic control) was significantly higher than in healthy patients [4].

Our studies showed a statistically significant difference between the control group and patients with type I diabetes mellitus regarding periodontal indices and bleeding indices. Our results correspond with existing data in the scientific literature [2]. Inflammatory processes in the periodontium of children and adults with diabetes mellitus is elevated, therefore it is very important to recognize them and diagnose them early [1, 4].

The periodontal index and bleeding indices in children without chronic catarrhal gingivitis (both somatically healthy and children with type I diabetes mellitus) are equal to zero, and confirm the absence of inflammation. PMA is 20.50±0.83 % in somatically healthy children with chronic catarrhal gingivitis; gingival index (GI) according to Loe, Silness is 0.84±0.05 points; and bleeding index according to Muhleman H.R. is 0.71±0.05 points. These findings correspond to mild gingivitis. The complex of above-mentioned indices evidences that the severity of gingivitis in children with type I diabetes mellitus is moderate. Namely, PMA is 40.47±0.96 %, gingival index (GI) according to Loe, Silness is 1.83±0.04 points, bleeding index according to Muhleman H.R. is 1.07±0.05 points.

The defense mechanisms in patients with diabetes are altered. Children with this endocrine pathology suffer from increased exudation (edema) of the gums and note an increase in bleeding of the gums along with the development of the underlying disease compared to healthy children of the same age.

The level of IL-18 is 3.41±0.25 pg / ml in the oral fluid of healthy children with healthy gums. It is 5.74±0.27 pg / ml in somatically healthy children with chronic catarrhal gingivitis. The level of IL-18 in the oral fluid is much higher

and it is 14.87±1.11 pg / ml in the group of children with type I diabetes mellitus and healthy gums. Its level is really high - 70.91±7.48 pg / ml in the group of patients with type I diabetes mellitus and chronic catarrhal gingivitis. Statistically significant difference ($p \leq 0,05$) is detected after comparing all the groups (1 and 2, 1 and 3, 3 and 4, 2 and 4, 1 and 4) with each other.

It should be noted that no statistically significant difference was found when we compared the periodontal and bleeding indices in groups 2 and 4 (children with healthy gums without type I diabetes mellitus and with type I diabetes mellitus) ($p \geq 0.05$). But when comparing the values of the level of proinflammatory IL-18 in the oral fluid of children from the above groups, a statistically significant difference was found ($p \leq 0.05$).

CONCLUSIONS

Our results coincide with the data of Ukrainian, Russian and foreign literature sources about the relationship between type I diabetes mellitus and gingivitis.

The level of IL-18 grows along with the periodontal index, gingival index and bleeding index, both in the groups with insulin-dependent diabetes mellitus and in the control groups. Our results concur with the data of other scientific works about the level of IL-18 in the oral fluid of children and adults [17, 19]. The results of our study indicate that an increase in the value of IL-18 in the oral fluid is closely associated with the presence of type I diabetes in children.

Moreover, from our point of view, IL-18 can be considered as a potential biomarker of inflammation in periodontium in children with type I diabetes mellitus, when the clinical manifestations are not visible. We determined that the level of IL-18 is elevated even when there is an absence of clincal manifestations of gum inflammation. So, the study of the topic of the cytokine profile in children with type I diabetes mellitus deserves further consideration. New methods for the prevention and treatment of gingivitis should be developed, taking into account above-mentioned link in pathogenesis.

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