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MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS IN THE PATHOGENESIS OF PERIODONTAL DISEASES IN TYPE 1 DIABETES MELLITUS

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ABSTRACT — The item presents the results of a comparative immune enzymometric assay of the matrix metalloproteinases (MMP-1, MMP-2, MMP-8, MMP-9) and tissue inhibitors (TIMP-1, TIMP-2) levels in blood serum, oral fluid of children belonging to Health Groups I and II and children with type 1 diabetes mellitus (disease course within the range of 1 to 10 years) in projection onto the level of hygiene and the periodontal status. The role of MMP-2, MMP-8, MMP-9 in the pathogenetic mechanisms of inflammatory periodontal diseases development has been identified. Children with a short experience of endocrinopathy were found to have a balanced synthesis of matrix metalloproteinases and their tissue inhibitors in case of reversible inflammatory alterations in periodontal tissues. Children with a longer disease course were found to feature an imbalance between matrix metalloproteinases and tissue inhibitors, which provokes disturbances in remodeling and structural organization of the extracellular matrix in periodontal tissues, along with the development of inflammatory destructive issues.

KEYWORDS — child population, type 1 diabetes mellitus, periodontal issues, matrix metalloproteinases, tissue inhibitors, oral fluid, blood serum

INTRODUCTION

Type 1 diabetes mellitus (DM) in children is one of the most complex and urgent issues faced by medical science, health care and social protection, while its importance has been enforced through international regulatory documents (WHO St. Vincent Declaration, 1989; Weimar Initiative, 1997; UN Resolution, 2007). As stated by the International Diabetes Federation (IDF, 2016), the number of reported Type 1 diabetes cases in the world's child population has reached 549,000, whereas annual diabetes costs have exceeded 12% of total global health care spending. According to the data provided by the Federal National Register, by the year 2017, there had been 22,969 children and 8,758 adolescents registered as outpatients suffering from Type 1 DM, while the annual increase rate was 2.82% in children, and 0.97% in adolescents, i.e. 86.73 and 203.29 per 100,000, respectively. The dramatic part about Type 1 DM in children is due to clinical polymorphism, widespread prevalence, the progressing increase in the incidence, significant lability of the disease course, a tendency to the development of autoimmune reactions combined with increased responsiveness of the immune system, the trouble in achieving clinical and metabolic compensation, which results in complications and early disability within a life period that is active from the social stance, reduced life expectancy and quality, impaired sexual and physical development, and increased mortality rate [3,5,10,14,18,21,35,37].

The WHO experts claim (2015) that over 94% of children with Type 1 DM have periodontal issues of inflammatory, dystrophic, and tumor origin. The etiopathogenesis of periodontal diseases in childhood is due to the fact that the disorders develop in constantly rearranging, rapidly growing, morphologically and functionally immature tissues, which are not capable to offer a proper response even to minor damaging factors. Besides, periodontopathies can develop against oral microbiocenosis issues, endocrine and neurohumoral regulation, hemodynamics changes, connective tissue metabolism, mineral metabolism, vitamin deficiency, poor nutrition, occlusion anomalies, imbalanced growth and maturation of the elements that make up the periodontal structure [1,4,6,15,17,20,33,36].

All the above leaves no doubt regarding the relevance of well-planned diagnostics and treatment of dental diseases in the child population, viewing the body as a whole [11,19,22-32,38-40,46-51].

The search for diagnostically significant periodontal disease markers is due to investigating the role that collagen metabolism disorders play in the periodontopathy pathogenesis. The biological functions of collagen, the basic structural protein of the intercellular matrix, include the support & mechanical role, involvement in the intercellular interaction, effect on cell proliferation and differentiation, as well as organs

and tissues morphogenesis through the body growth and development. The rate of the metabolism of collagen, an actively renewing protein, depends on both biosynthesis and biodegradation, while the content of collagen fractions allows making judgment not only concerning the periodontal bone tissue inflammatory destruction, yet also regarding the osteoblasts functional activity. Type I collagen proteolytic degradation has been proven to be one of the key factors in the uncontrolled destruction of the extracellular periodontal matrix [9, 16, 34, 41, 42].

Collagenolytic activity is typical of Matrix metalloproteinases (MMP), extracellular Zn^{2+}- and Ca^{2+}-dependent endopeptidases containing 25 isoforms and involved in connective tissue remodeling through destroying its organic components at physiological pH values. Together with other extracellular proteinases, MMPs under physiological conditions play a key role in the following processes: morphogenesis (embryogenesis); tissue resorption and remodeling; proliferation; membrane receptors cleavage; connective tissue protein metabolism; apoptotic ligands release; cell migration and differentiation; protein matrix proper development and functioning; oncogenic transformation; angiogenesis; immune response implementation; coagulation; cytokines and chemokines deactivation and activation. In case of inflammation, MMPs are involved in the destruction of all types of extracellular matrix proteins. The regulation of MMP activity is carried out at various levels, including protein activation, transcription, and interaction with specific endogenous tissue inhibitors of metalloproteinases (TIMP) — structurally related proteins, three of which (TIMP-1, 2, and 4) are secreted in a soluble form, with another one (TIMP-3) being associated with the extracellular matrix [8, 12, 43, 45].

Neutrophilic collagenase (MMP-8), which is closely associated with pathological changes in periodontium, has the highest proteolytic activity in relation to Type I collagen. A significant contribution to the destruction of collagen fibrils and the degradation of the collagen matrix through periodontitis is made by interstitial collagenase (MMP-1), which is capable of hydrolyzing Types I, II, III collagen, as well as gelatinase (MMP-2 and MMP-9), for which Type IV collagen is a substrate. The wide substrate specificity of MMPs determines their involvement not only in the processes of periodontal destruction, yet also in inflammatory response modulation [2, 7, 13, 44].

Experts claim that examination of the oral fluid is an informative and non-invasive way to assess the proteolytic status and activity in periodontium. Molecular & biochemical changes in the oral cavity have been proven to be affected not by the actual pathological processes in the periodont only, but also by somatic diseases, Type 1 DM in particular. Monitoring the collagen content by the level of MMP and their tissue inhibitors in children with Type 1 DM will allow proper assessment of the imbalance between the MMP and TIPM activity as projected onto the periodontal status. This will facilitate the identification of children with Type 1 DM with a high risk of developing periodontal diseases, also allowing prediction of the course and evaluating the effectiveness of treatment and preventive measures for periodontopathy.

Aim of study:
to identify the dependence between the content of matrix metalloproteinases, their tissue inhibitors and the periodontological status in children with Type 1 DM through different endocrinopathy stages.

MATERIALS AND METHODS

Subject to informed consent obtained from the parents, 129 children (57 boys, 72 girls) aged 8–12 were examined. The objectification of periodontal status in children in a mixed occlusion was performed taking into account the range and classification of periodontal pathology approved by the Presiding Panel of the Periodontics Section, Dental Association of Russia (2001) based on ICD-10 (WHO, 1997) using radiological criteria for evaluating the disease severity (Rabukhina N.A., Arzhantsev A.P., 2003). The oral hygiene status was evaluated based on indicators of the Oral Hygiene Index-Simlified (OHI-S, Green-Vermillion, 1964) and the Hygiene Index (HI, Yu.A. Fedorov, V.V. Volodkina, 1976). The clinical assessment of the periodontal tissues status was calculated by adding up the following values: Periodontal Index Russell (PI Russel, 1956); Papillary Marginal Alveolar Periodontal Index (PMA, Schour, Massler, 1948) Parma modification (C. Parma, 1960); Silness & Loe Plaque Index (PI J. Silness, H. Loe; 1964); Sulcus Bleeding Index (SBI Muhlemann and Son, 1971) modified by Cowell (1975); Schiller-Pisarev test and Svrakov Iodine Value (D. Svrakov, Yu. Pisarev, 1963); Community Periodontal Index of Treatment Needs (CPITN, WHO, 1989). 2% solution of methylene blue and 5% solution of erythrosine pink were used as plaque indicators.

Apart from the dental patient medical record, each child had an electronic periodontal chart filled out for them, containing information about the periodontal status at examination with the Florida probe automated system (FloridaProbe Corporation, USA). To identify the intensity of the inflammatory pathology in the periodont, orthopantomography was performed with a panoramic X-ray device Orthophos XG Plus DS / Ceph (Sirona).

The examined children were divided into two groups. The comparison group included 38 healthy children (Group I health) and basically healthy (Group II health) (Y.E. Veltishchev, 1994). In the comparison group, the diagnosis to the patients was given based on the pediatrician's conclusion. The main group (91 persons) included children with a diagnosis of Type 1 DM, who were undergoing treatment in the endocrinology department of Filippsky Child Clinical Hospital (City of Stavropol, Russia) within the period of 2010–2019. The diagnosis of Type 1 DM was given by endocrinologists following the clinical and laboratory criteria of WHO (1999). Depending on the duration of Type 1 DM, the patients in the main group were divided into two subgroups. The first subgroup included 43 children (47.3%) who had had the disease for up to two years; the other subgroup — 48 children (52.7%) with the disease duration of 3–10 years.

The material for the study was blood serum and unstimulated oral fluid (UOF). Blood sampling was done from the ulnar vein using a BD Vacutainer® vacuum system (BectonDickinson) with a clotting accelerator (Sarstedt) following the algorithm for morning vein blood sampling (on at empty stomach). UOF, too was taken on an empty stomach in the morning into the Saliva RNA Collection and Preservation Devices (Norgen Biotek) system. The collected UOF was poured into 200–250 μl aliquots in plastic tubes and stored frozen at –80° C. Further, the UOF aliquots were thawed at room temperature and centrifuged at 10,000 rpm for two minutes. The levels of MMPs and their tissue inhibitors in blood serum and in the supernatant were assessed by enzyme-linked immunosorbent assay (ELISA) following the manufacturers' requirements. The following standard kits were used: for MMP-1 — RayBio® Human Fas ELISA; for MMP-2 — Human/Mouse/Rat MMP-2 (total) (Quantikine, Research & Diagnostics Systems, Inc.); for MMP-8 — Human MMP-8 (total) (Quantikine, Research & Diagnostics Systems, Inc.); for MMP-9 — Human MMP-9 (Quantikine, Research & Diagnostics Systems, Inc.); for TIMP-1 — Human TIMP-1 (Quantikine, Research & Diagnostics Systems, Inc.); for TIMP-2 — Human TIMP-2 (Quantikine, Research & Diagnostics Systems, Inc.). The optical density was measured on a Sunrise (Tecan) automatic universal microplate reader at λ = 450 nm with correction at λ = 540 nm, followed by data processing with specialized Magellan™ software. The measurements calculation was performed subject to the formula below:

$$Y = a + bX + cX2$$

Y, the optical density; X, the level of MMP and tissue inhibitors.

The statistical analysis was performed using the SPSS Statistics 21.0 software package. The nature of the sample distribution was identified through the Shapiro-Wilk test. The comparison of groups with a normal distribution of quantitative traits was evaluated based on the Student t-test. The results are presented as the arithmetic mean and its standard error. An investigation of the relationship between the MMPs activity indicators and their tissue inhibitors, as well as index assessment of the periodontal status were carried out employing Pearson's parametric correlation analysis for normally distributed quantitative characteristics. To identify the differences between the two groups, the Mann-Whitney U-test was used; in case of over two groups, the Kruskal-Wallis test was used. Group differences were considered statistically significant at a 95% probability of an error-free forecast ($p < 0.05$).

RESULTS AND DISCUSSION

Table 1 below offers a view on the index evaluation of the oral cavity hygienic status.

An analysis of the oral hygiene status indicates that in Health Groups I and II, "very poor" and "poor" hygiene level were observed in 3 (7.9%) and 7 (18.4%) children, respectively. In the 1st subgroup of the main group, "very poor" and "bad" hygiene levels were detected in 6 (14.0%) and 9 (20.9%) children, in the 2nd subgroup – in 4 (8.3%) and 8 (16.7%) children, respectively. It is to be noted that the "good" level of hygiene was observed most often in Groups I and II – 15 (39.5%) children, while in the 1st subgroup this indicator was 14 (32.6%) children; in the 2nd subgroup — 9 (18.8%) children. Therefore, an increase in the disease severity in children with Type 1 DM is associated with a slight deterioration in the hygiene status, which is due to lacking full-range individual hygiene resulting from pain in the oral cavity; a decrease in the protective, secretory function of the salivary glands, and weakened motivated control.

Table 2 shows index evaluation of the periodontal tissues status.

Analysis of the periodontal pathology structure revealed that in the comparison group the ratio of children with periodontopathy (11 children – 29.0%) and children with intact periodontium (27 children — 71.0%) was 0.4 units. In Groups I and II, chronic catarrhal gingivitis was diagnosed in 7 (18.4%) children, exceeding the prevalence of hypertrophic gingivitis (2 children — 5.3%) by 3.5 times. In the 1st subgroup, periodontopathy was observed in 13 (65.1%) children, while periodontal disease was not detected in 25 (34.9%) children. The ratio of children with periodontopathy against the number of children with intact periodont in the 1st subgroup (1.8 units)

went beyond the same index in the comparison group by 4.5 times. In the 1st subgroup only reversible inflammatory changes (chronic catarrhal and hypertrophic gingivitis) were detected, while the chronic catarrhal gingivitis incidence (19 children – 44.2%) exceeded the occurrence of hypertrophic gingivitis (4 children — 9.3%) by 4.7 times. In 7 (35.4%) children of the 2nd subgroup, inflammatory and irreversible destructive periodontal lesions were diagnosed — chronic localized mild periodontitis was detected in 4 (8.3%) children; chronic localized periodontitis of moderate severity — in 7 (14.6%) children; chronic generalized periodontitis – in 6 (12.5%) children. The incidence of chronic catarrhal gingivitis (18 children – 37.5%) in children of the 2nd subgroup exceeded the occurrence of hypertrophic gingivitis (5 children — 10.4%) by 3.6 times, while in comparison with similar parameters in the 1st subgroup there was a decrease in these values by 1.2 and 1.1 times, respectively. The recession as a nosological type of periodontopathy was recorded in 8 (16.7%) children of the 2nd subgroup, compared with 5 (11.6%) children of the 1st subgroup and 2 (5.3%) children in the comparison group.

Analysis of periodontal status indicates that children with Type 1 DM had a worsening periodontal status along with an increase in the disease course. In the 1st and 2nd subgroups, gum recession was diagnosed, as well as chronic catarrhal and hypertrophic gingivitis. The 3rd subgroup children featured a tendency towards transition of reversible inflammatory forms into irreversible inflammatory and destructive periodont lesions. The following factors determine periodontal status deterioration in children with a long-term course of Type 1 DM:

— Diabetic microangiopathies. The plasmorrhages that underlie microcirculatory disorders are manifested by primary plasma lesions of the vascular bed basal membrane, as well as vascular wall hyalinosis and sclerosis. These morphological changes against the unchanged vascular lumen reduce the transcapillary exchange intensity, contribute to thickening of the blood vessels walls, reducing their permeability. This slows down the nutrients transport, reduces tissue resistance to endotoxins produced by conditionally pathogenic (pathogenic) microflora of the gingival sulcus, aggravating the severity of inflammatory and destructive changes in periodontal tissues.

— Dysbiotic disorders. Hyposalivation, increased level of glucose in the gingival fluid and saliva, occurring against carbohydrate metabolism decompensation, change the microbiota composition. These changes, combined with an increase in the non-enzymatic proteins glycation, reduce the local protection of the oral cavity. This is accompanied with a decrease in the level of normal symbionts (dysbiosis), followed by colonization with conditionally pathogenic microflora. It is important to note that patients with decompensated diabetes have an increased level of spirochetes and motile bacteria localized in the apical part of the periodontal pockets. Given increased phagocytosis, the Porphyromonas gingivalis bacteroid and the Treponema denticola spirochete, belonging to the resident microflora, activate the MMPs neutrophilic matrix, aggravating the inflammation.

— Impaired immune response. Given carbohydrate metabolism decompensation, there is a decrease in the function of monocytes/macrophages, neutrophils, polymorphonuclear neutrophilic leukocytes; an increase in the content of IgA, IgG; a decrease in the level of IgM, T and B lymphocytes, while the synthesis of glycosaminoglycans and collagen is getting reduced. Besides, due to non-enzymatic glycation, there is also weakening in the function of cells involved in the immune defense system.

— Oxidative stress. Decreased production of reactive oxygen species, a decrease in the oxygen-dependent phagocyte metabolism activation, incomplete phagocytosis mechanisms, correlating with an increase in the area of insulin-producing β-cells destruction in patients with long-term experience of Type 1 DM, indicates the onset of Stage 3 (depletion stage) in oxidative stress.

Table 3 shows the level of matrix metalloproteinases and their tissue inhibitors in blood serum.

Table 4 offers a view on the level of matrix metalloproteinases and their tissue inhibitors in the oral fluid.

Analysis of the MMP-1 (intestinal collagenase) level in children with Type 1 DM in blood serum and UOF, if compared with children of Health Groups 1 and 2, shows overproduction of MMP-1, while the increase in the indicators in the 2nd subgroup (38.6 ± 3.7% and 176.2 ± 10.4%, respectively) reaches the highest level with respect to the growth in patients of the 1st subgroup (6.8 ± 0.9% and 14.3 ± 1.3%). The increase in the level of MMP-1 along with an increase in the disease duration, which correlates with deteriorating periodontal status, is due to an increase in collagen fibrils degradation through remodeling of extracellular matrix and the development of a chronic inflammatory process in periodontal tissues.

Compared to the children in Health Groups I and II, children with Type 1 DM had a statistically significant increase in the MMP-2 (Gelatinase-A) level only in the oral fluid (children of the 1st subgroup – 120.6±8.3%; children of the 2nd subgroup — 696.9±37.2%), while there was no statistically significant increase in the serum MMP-2 levels in children of the 1st and 2nd subgroups. An increase in the level of MMP-2 involved in

Table 1. *Index evaluation of the oral cavity hygienic status (points), (M ± m)*

Index	Children I, II Health groups	Children with experience of type 1 diabetes up to 2 years	Children with experience of type 1 diabetes 3–10 years
OHI-S, Green-Vermillion	1,27±0,03	1,61±0,04*	2,29±0,07*
HI, Yu.A. Fedorov, V.V. Volodkina	1,74±0,16	2,08±0,13*	2,43±0,12*

Note: ** — p≤0.05 is statistically significant in comparison with the patients of the comparison group (Newman-Cales test, Dunn test).*

Table 2. *Index evaluation of the periodontal tissues status, (M ± m)*

Index	Children I, II Health groups	Children with experience of type 1 diabetes up to 2 years	Children with experience of type 1 diabetes 3–10 years
PI Russel, points	1,44±0,13	2,91±0,28**	3,86±0,17**
PMA Parma C., %	18,21±1,07	27,56±1,73*	43,97±3,09*
PI J. Silness, H. Loe, points	0,43±0,02	0,94±0,04**	2,17±0,07**
SBI Muhlemann-Cowell, points	0,76±0,04	1,23±0,06**	2,08±0,13**
CPITN, WHO, points	0,24±0,03	0,73±0,07**	1,36±0,16*
Schiller-Pisarev test and Svrakov Iodine Value, points	1,29±0,08	1,76±0,13*	3,62±0,21*

Note: ** — p≤0.05 is statistically significant in comparison with the performance of patients in the comparison group; ** — p≤0.01 is statistically significant in comparison with the patients of the comparison group (Newman-Keyles test, Dunn test).*

Table 3. *The level of matrix metalloproteinases and their tissue inhibitors in blood serum (ng/ml), (M ± m)*

Index	Children I, II Health groups	Children with experience of type 1 diabetes up to 2 years	Children with experience of type 1 diabetes 3–10 years
MMP-1	8,68±0,11	9,27±0,52*	12,03±0,94*
MMP-2	231,96±14,27	234,41±17,13*	249,82±13,67*
MMP-8	9,27±1,08	11,06±2,74*	27,48±6,41*
MMP-9	326,13±18,53	339,48±32,28*	386,35±24,31*
TIMP-1	161,35±12,84	174,06±8,97*	213,16±17,36*
TIMP-2	116,74±8,73	129,64±10,69*	187,43±16,28*

Note. ** - p≤0.05 is statistically significant in comparison with the patients of the comparison group (Newman-Cales test, Dunn test).*

Table 4. *The level of matrix metalloproteinases and their tissue inhibitors in the oral fluid (ng/ml), (M ± m)*

Index	Children I, II Health groups	Children with experience of type 1 diabetes up to 2 years	Children with experience of type 1 diabetes 3–10 years
MMP-1	0,21±0,02	0,24±0,03*	0,58±0,11*
MMP-2	0,97±0,33	2,14±0,19*	7,73±2,86*
MMP-8	187,62±16,71	241,91±19,44*	352,47±24,06*
MMP-9	294,83±21,23	370,63±41,71*	533,29±47,88*
TIMP-1	190,54±14,68	217,36±20,32*	261,83±31,27*
TIMP-2	19,36±3,04	26,58±4,29*	41,66±7,01*

Note: ** — p≤0.05 is statistically significant in comparison with the patients of the comparison group (Newman-Cales test, Dunn test).*

the degradation of collagen in UOF — which offers a proper reflection of the inflammatory and destructive process intensity in periodontal tissues — is associated with activation of local compensation & regulatory mechanisms, which is aimed at restoring periodontal tissues. The change dynamics pertaining to the MMP-2 content in the oral fluid offers a reliable reflection of clinical manifestations (the depth of periodontal pockets, the degree of bleeding of the gums), and can be used in clinical practice as a marker to evaluate the effectiveness of dental treatment.

Evaluation of the MMP-8 (neutrophilic collagenase) content taken as a marker for chronic periodontitis, and a marker for impaired neutrophil secretion, in the children with Type 1 DM (in blood serum and UOF), if compared to the children in Groups I and II, revealed an overproduction of MMP-8, while an increase in the values in the 2nd subgroup (196.4 ± 10.4% and 87.8 ± 6.1%, respectively) went up to the most significant level, if matched against a similar increase in the 1st subgroup (19.3 ± 1, 7% and 28.9 ± 1.9%). An increase in the salivary and serum MMP-8 levels along with the progression of endocrinopathy, as a key factor in the extracellular matrix destruction, is due to inflammatory activity in the pancreas islet β-cells, which is accompanied with resorption and destruction of the alveoli osteoid tissue along with activated osteoclast function, as well as dental plaque development, increased dentine demineralization and inflammatory response in the gum tissue. A significant increase in the MMP-8 level and the transition to the active form is a marker of chronic generalized periodontitis in its acute stage and rapidly progressive periodontitis, which can be used as an informative indicator for the pathological process intensity (resorption of interalveolar septa; expansion of periodontal cracks; damage to the bone tissue of the jaw alveolar processes, cortical plates destruction, and presence of osteoporosis foci).

Compared with the children of Health Groups I and II, the children with Type 1 DM had their serum and UOF overproducing MMP-9 (Gelatinase-B), while the increase in the indicators in the 2nd subgroup (18.5 ± 1.4% and 80.9 ± 6.3%, respectively), matched against the children of the 1st subgroup (4.1 ± 0.3% and 25.7 ± 1.6%), appeared the most significant. MMP-9 been proven to be involved in processes like inflammation, recovery, remodeling and osteoclastic bone resorption, while the presence MMP-9 in gingival fluid with periodontitis (up to 99%) and squamous cell carcinoma (up to 93%), allow it to be classified as a marker risk for progression of inflammatory periodontal diseases and marker of squamous cell carcinoma. An increase in the MMP-9 concentration at the local and systemic levels with an increase in Type 1 DM experience in children serves a proper reflection of a decrease in the number of degradation products of native collagens (Type IV, V, VII, X, XI), which means an increase in the intensity of inflammatory and destructive processes in periodontal tissues along with physiological protective and adaptive mechanisms failure. An increase in the levels of TIMP-1 and TIMP-2 involved in the MMP enzymatic activity regulation, in the blood serum and UOF of children with Type 1 DM who belong to Health Groups I and II, means accumulation of extracellular collagen along with extracellular matrix destruction, an increase in the antigenic load, an increased inflammatory response, periodontal tissues degradation, as well as the development of vascular intimal hyperplasia. The most significant increase in TIMP-1 (blood serum, 32.1 ± 1.8%; UOF, 37.4 ± 2.1%) and TIMP-2 (blood serum, 60.6 ± 3.4%; UOF, 115.2 ± 6.3%) was recorded in the 2nd subgroup, which reflects the degree of Type 1 DM advance and the intensity of the morphological structure destruction in the periodont, along with disturbed protective, barrier, trophic, plastic and shock-absorbing functions. Along with an increase in Type 1 DM history, the most significant increase in the serum and UOF TIMP-2 — compared with TIMP-1 — was due to the following factors: the production of TIMP-1, which is involved in the native collagen breakdown, prevails in children with a short disease history, where reversible inflammatory changes in periodontal tissues are dominant; the expression of TIMP-2, which is involved in the denatured collagen breakdown, dominates in children with a long history of endocrinopathy, where (along with the initial signs of inflammation) inflammatory and destructive irreversible periodontal lesions are diagnosed, too. The prominent response of TIMP-2, which is an inhibitor of MMP-2 and MMP-9, can be accounted for by the accumulation of high molecular weight collagen and its degradation products, as well as by the duration and the chronic nature of the inflammation.

Table 5 shows the ratio (matrix metalloproteinases / tissue inhibitors) in blood serum and UOF.

An analysis of the MMP/TIMP quantitative ratios revealed that under physiological conditions, no active MMPs were to be found in the tissues, while the level of the precursors (pro-MMP) was minimal. As long as the length of Type 1 DM history in children increases, the ratio dynamics proves multidirectional: for the blood serum there is an increase in MMP-1/TIMP-1; MMP-8/TIMP-1; MMP-8/TIMP-2; decrease in MMP-2/TIMP-1; MMP-9/TIMP-1; MMP-1/IMP-2; MMP-2/IMP-2; MMP-9/IMP-2; for the oral fluid there is an increase in MMP-1/TIMP-1; MMP-2/TIMP-1; MMP-8/TIMP-1; MMP-9/

Table 5. *The MMP/TIMP ratio in blood serum and UOF*

Index	Children I, II Health groups	Children with experience of type 1 diabetes up to 2 years	Children with experience of type 1 diabetes 3-10 years
Blood serum			
MMP-1/TIMP-1	0,053 (1:18,7)	0,053 (1:18,7)	0,056 (1:17,7)
MMP-2/TIMP-1	1,44 (1,4:1)	1,34 (1,3:1)	1,17 (1,1:1)
MMP-8/TIMP-1	0,057 (1:17,4)	0,063 (1:15,7)	0,129 (1:7,8)
MMP-9/TIMP-1	2,021 (2,0:1)	1,950 (1,9:1)	1,812 (1,8:1)
MMP-1/TIMP-2	0,074 (1:13,4)	0,071 (1:13,9)	0,064 (1:15,6)
MMP-2/TIMP-2	1,987 (1,9:1)	1,808 (1,8:1)	1,332 (1,3:1)
MMP-8/TIMP-2	0,079 (1:12,6)	0,085 (1:11,7)	0,146 (1:6,8)
MMP-9/TIMP-2	2,793 (2,8:1)	2,618 (2,6:1)	2,061 (2,0:1)
Unstimulated oral fluid			
MMP-1/TIMP-1	0,001 (1:904,8)	0,001 (1:904,1)	0,002 (1:450,0)
MMP-2/TIMP-1	0,005 (1:196,4)	0,009 (1:101,6)	0,029 (1:33,8)
MMP-8/TIMP-1	0,98 (1:1,1)	1,11 (1,1:1)	1,34 (1,3:1)
MMP-9/TIMP-1	1,55 (1,5:1)	1,71 (1,7:1)	2,03 (2,0:1)
MMP-1/TIMP-2	0,011 (1:92,2)	0,009 (1:110,7)	0,014 (1:71,8)
MMP-2/TIMP-2	0,05 (1:19,9)	0,08 (1:12,4)	0,18 (1:5,4)
MMP-8/TIMP-2	9,69 (9,7:1)	9,10 (9,1:1)	8,46 (8,5:1)
MMP-9/TIMP-2	15,23 (15,2:1)	13,94 (13,9:1)	12,80 (12,8:1)

TIMP-1; MMP-1/TIMP-2; MMP-2/TIMP-2; decrease in MMP-8/TIMP-8; MMP-9/TIMP-2. The MMP–TIMP imbalance results in activated proteolysis system, which comes accompanied with disturbed connective tissue protein metabolism, increased bone resorption and remodeling processes, proteolytic degradation of Type I collagen, as the major factor involved in the uncontrolled destruction of the intercellular matrix, followed with periodontal structure disturbed arrangement.

In the 1st subgroup, the activity of tissue inhibitors and MMPs, which play a key role in chronic inflammation development and maintenance, is mainly observed in the early stages – *expression; proenzymes activation; matrixin active forms accumulation*. Compared with the children in Health Groups I and II, the change in MMP / TIMP ratios in blood serum and UOF, which reflects the mature enzyme activity forms suppression by tissue (endogenous) inhibitors through direct interaction with their active centers, was insignificant. The compensatory production of TIMP, which is sufficient to inactivate the active forms of MMP, supports proteolytic activity inhibition and prevents intercellular matrix proteins degradation in children at the initial stages of Type I DM course.

In the 2nd subgroup, serum and UOF revealed hyperproduction of the active matrixins and their tissue inhibitors. A significant difference in the MMP/TIMP ratios, if compared with Health Groups I and II, indicates a significant imbalance between the metalloproteinases production and their tissue inhibitors. Excessive or low expression of tissue inhibitors in the 2nd subgroup, including due to blockade by salivary proteolytic enzymes, does not offer a proper blockage of the active MMP forms. The accumulation of extracellular matrix proteins, which indicates the *proteolysis* activation and overstrain in the protective and adaptive mechanisms, confirms the key role played by morphological disorders of the intercellular matrix in the pathogenesis of inflammatory and destructive changes taking place in periodontal tissues in case of a long Type 1 DM history.

CONCLUSIONS

1. Children with Type 1 DM, under hyperglycemia and with a long disease history, feature disturbed regulation of matrix metalloproteinases synthesis (MMP-1, MMP-2, MMP-8, MMP-9) as well as their tissue inhibitors (TIMP-1, TIMP-2), which is due to disorders in the multicomponent system of intercellular matrix catabolism, and malfunctioning of its regulatory mechanisms with failure to maintain proper phase changes.
2. The expression of matrix metalloproteinases and their tissue inhibitors in blood serum and oral fluid reveals significant individual variability not in healthy

children alone, yet also in children with Type 1 DM.
3. The balance in the synthesis of matrix metalloproteinases and their tissue inhibitors in blood serum and oral fluid in children with short experience of Type 1 DM can be seen from the lack of statistically significant differences when compared with similar indicators for children of Health Groups I and II.
4. The biological fluids of children with long-term experience of Type 1 DM against chronic hyperglycemia and insulin hypoproduction reveal a statistically significant increase in matrix metalloproteinases (1.1–7.9 times), their tissue inhibitors (1.3–2.2 times), if compared with similar indices in children belonging to Health Groups I and II, indicating the proteolysis system activation.
5. An increase in the MMP-1, MMP-2, MMP-8, MMP-9, TIMP-1, TIMP-2 levels in blood serum and oral fluid along with an increase in the Type 1 DM history in children is accompanied by progressing inflammatory and destructive changes in periodontal tissues, which is associated with an imbalance between matrix metalloproteinases and their tissue inhibitors, impaired remodeling processes and structural organization of the extracellular matrix.
6. Rising index ratios (blood serum, MMP-8/TIMP-1, MMP-8/TIMP-2; oral fluid, MMP-2/TIMP-1, MMP-2/TIMP-1) can be considered as an early diagnostic sign (predictor) of inflammation, as well as a criterion for developing groups of *patients with a high risk of developing Type 1 DM*.
7. The MMP-8, MMP-9 levels in biological fluids offer a proper reflection to the severity of pathologies within periodontal disease, while an increase in the MMP-9 activity is a diagnostically significant marker for the progression of inflammatory and destructive changes in periodontal disease, accompanied by a decrease in the carbohydrate metabolism compensation.
8. An increase in MMP/TIMP ratios with an increase in Type 1 DM history means, on the one hand, a decrease in the tissue inhibitors receptor control, and on the other – proteolytic degradation of the extracellular matrix, as a result of a complex chain of reactions where MMP-2 and MMP-9 act synergistically, through a fibronectin-like fragment, taking a feedback-like turn and regulating the mechanism in vivo.

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