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THE VALUE OF MATRIX METALLOPROTEASES AND CONNECTIVE TISSUE MARKERS IN THE PATHOLOGY OF TEMP-JAW JOINT IN CHILDREN

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Abstract – The study of the characteristics of some markers of matrix metalloproteases and connective tissue in the development of the pathology of the temporomandibular joint in children is an important problem in pediatric dentistry. In children with a disease of the temporomandibular joint, there is a change in the activity of type 1, 3, 9 matrix metalloproteinases in the blood serum, indicating remodeling of connective tissue, indicating metabolic disturbances.

Key words: metalloproteases, pathology, metabolic disturbances, blood serum, pediatric dentistry, proprioceptors.

I. Introduction

The temporomandibular joint (TMJ) is a three-directional receptor organ connected with the proprioceptors of periodontal, chewing muscles and transmitting information to the central nervous system about the position of the lower jaw to control and regulate chewing movements [3,8,14]. This joint is formed by the head of the condylar process of the lower jaw, the mandibular fossa of the temporal bone and the posterior slope of the articular tubercle lying anteriorly. The bone structure is covered by the joint capsule, which is attached at the top to the base of the temporal bone, at the bottom to the neck of the condylar process. The articular disc divides the TMJ into two spaces (upper, lower), both spaces are filled with synovial fluid, the capsular-ligamentous apparatus of the temporomandibular joint consists of extra- and intracapsular ligaments.

The main pathogenetic factor in the development of internal TMJ disorders is the discoordination of the masticatory muscles and their spasmodic contraction. Of great importance in this mechanism is the spasm of the external pterygoid muscle, especially its upper head, which can lead to the development of pain, and with the development of the pathological process, to displacement of the articular disc, stretching of the capsule, the appearance of articular noise, that is, to internal disturbances of the TMJ [16,20,24].

II. Literature review

According to most authors, the temporomandibular joint dysfunction is a polyetiological disease with a complex and diverse development mechanism, including the state of muscles, occlusion, the elements that form the joint itself, psycho-emotional status, which are inextricably linked [8,14,22,23].

E.A. Bulycheva (2010) established a previously undetected pattern of development of the psychosomatic nature of the temporomandibular joint dysfunction, which consists in the emergence of a pathogenetic chain under the influence of chronic emotional stress (repeated mental trauma): chronic stress - parafunction of the masticatory muscles - dysfunction of the chewing muscles - dysfunction of the temporomandibular joints combined with hypoplasia of the heads of the lower jaw.

A number of authors suggest that connective tissue dysplasia, which is often manifested by pathology of the musculoskeletal and ligamentous apparatus, is one of the factors in the development of internal disorders in the temporomandibular joint [3,10,12,13]. As a result of the research, Kupriyanov I.A. et al. (2003), Ivasenko P.I. et al. (2007) found that in patients with connective tissue dysplasia and internal disorders of the temporomandibular joint, catabolic processes prevail in the tissues, chronic inflammation with a pronounced productive component, manifested by connective tissue edema, perivascular infiltrates, collagen fiber homogenization, hyalinosis, expressed sclerosis, is diagnosed. which causes a more severe course of the disease due to obliteration of blood vessels, deterioration of trophism of the capsular-ligamentous apparatus. The consequence is a more dynamic development of the pathological process, the occurrence of complications such as osteoarthritis, synovitis, the rapid appearance of irreversible deformations in the temporomandibular joint.

III. Analysis

In case of TMJ pathology, cartilage metabolism is disturbed, the activity of enzymes involved in the breakdown of aggrecan changes. A number of authors determined the molecular forms of the matrix of metalloproteinases (MMRs), including collagenases (MMR-1, MMP-8 and MMR-13) and (MMP-2 and MMP-9), in synovial fluid in patients with internal disorders of the temporomandibular joint of the lung and moderate severity. As a result of the studies, it was found that elevated levels of MMP-2, MMP-9 and MMR-8 in the synovial fluid of patients with TMJ pathology reflect the destruction of type I collagen in the active phase of the destruction of the temporomandibular joint elements, which can be used for diagnostic purposes [17,18,19].

According to modern concepts, the pathogenesis of TMJ dysfunction consists of sequential events starting with the development of occlusal disorders, stress leading to spasm of the masticatory muscles, manifested by muscle pain, displacement and restriction of the movements of the lower jaw, which, in turn, disrupts dynamic occlusion, in the future internal disturbances occur, manifested by clicking, which is replaced by intermittent blocking in the joint of a displaced disk and ends with the development of destructive changes in bone structures, i.e. osteoarthritis [15,20]. The scientific literature confirms that the reactivity of a macroorganism is largely determined by the state of the dentofacial system (DFS) and its interactions with ecosystems [7, 10]. Epidemiological studies conducted in European countries also indicate a high prevalence of TMJ pathology among children [3,8,12]. Therefore, in recent years, many works on the problem of TMJ and factors affecting their development, especially among children, have been published [12,15,22].

TMJ pathologies make up a rather large part of the maxillofacial region pathologies, while a carious index is higher in this group of people than in children without TMJ pathology [13,17,22].

In addition to carious disease, in children with TMJ pathology, periodontal diseases are more common [18,20]. The effectiveness of preventive measures among children with TMJ pathology is much lower, which is explained by the use of standard methods of prevention, which do not take into account the peculiarities of the patient's dental status.

When taking children with TMJ pathology, as a rule, doctors are faced with the final results of processes that began as early as embryonic development or at an early age. In the formation of the TMJ pathology, stable pathogenetic mechanisms act that maintain a consistently high level of prevalence and are associated with genetic factors and the deterioration of the health of women during pregnancy and children [14,19]. An important role in the development of TMJ pathology is played by endocrinopathies, impaired formation of bone and connective tissue, as well as nerve fibers in the prenatal period [8,12,19]. Acquired TMJ diseases more often develop against the background of external influences, inflammatory diseases, and injuries that act at different age periods [8,17,20].

Studies on the prevalence of TMJ diseases among children, as well as on the establishment of their etiopathogenesis, are ongoing, since there is still no consensus on the causes, the increasing number of this pathology. Based on this, the problem posed to us is relevant.

The aim of this study was to study the characteristics of some markers of matrix metalloproteases and connective tissue in the development of pathology of the temporomandibular joint in children.

The work is based on the results of a survey of 78 children aged 6 to 18 years with TMJ pathology. The observation group included 45 (57.3%) girls and 33 (42.7%) boys. Examination of sick children included a survey, examination, assessment of the state of hard tissues of teeth, noted the presence of abnormalities and deformations of the dentition, the condition of the temporomandibular joint. When collecting an anamnesis, complaints were clarified when pain or noise in the temporomandibular joint first appeared, how often they occur, whether treatment was carried out, how effective it was.

An examination of the oral cavity was carried out in a dental office, with artificial lighting using a standard set of dental instruments - a mirror and a dental probe. Data on the displacement of the lower jaw in the vertical, sagittal and transversal planes was obtained during external examination of the face with closed dentition in the position of relative physiological rest and with maximum opening of the mouth. Patients were examined according to a single protocol, which included: determining the type of bite, KPU index, the ratio of the first permanent molars according to Engle's classification, KPU index, IROPZ index of 1 and 2 molars according to V.Y. Milikevich, 1984. Palpation of the temporomandibular the joints were passed through the skin, in front of the tragus of the ear or through the front wall of the external auditory canal when the lower jaw was closed and with its movements. Palpating the masticatory muscles, painful and compacted areas, the presence of trigger points were determined. The degree of dysfunction of the temporomandibular joint was determined using the Helkimo clinical index. Questioning of the subjects was carried out using a specially designed questionnaire to identify TMJ pathology, the assessment was carried out in points.

Morphometric methods - a biometric study of the control and diagnostic models of the jaws, analysis of photographs of the face and occlusion of the dentition, radiological methods, dental computed tomography. Clinical observations of children with TMJ and difficulty in nasal breathing due to adenoids have shown that the most common pathology in these children is distal occlusion of the dentition, characterized by maxillary anthesis,

retroposition of the lower jaw, a tendency to vertical type of growth of the jaw, biprotrusion with incisors, jaws, narrowing of the upper jaw in the area of the Pont points on premolars and molars.

X-ray examination of the temporomandibular joint in 45 sick children aged 6 to 15 years with internal disorders of the temporomandibular joint. The results of a dental examination of patients were entered on the medical record of the outpatient patient F-043 / u-2/88, for school students - on the rehabilitation card (registration form 267) and a specially designed card for assessing dental status. In the passport part of the card, an identification number, last name, first name, middle name, year of birth, date of filling, address, transferred and associated diseases were recorded.

Based on the obtained research results, for a differentiated approach to the planning of comprehensive rehabilitation of children with TMJ, we found it appropriate to conduct additional laboratory tests. The content of MMR-1, MMR-9, TIMR-1 and the concentration of C-RB in the blood serum were determined by enzyme-linked immunosorbent assay using standard test kits (HUMAN, Germany) in accordance with the attached instructions.

The solid-state immunoassay method used is based on the "sandwich" principle. The degree of color was proportional to the concentration of C-RB in the analyzed sample. ELISA results are recorded using a spectrophotometer, measuring the optical density in the two-wave mode: the main filter is 450 nm, the reference filter is in the range of 620 - 655 nm. After measuring the optical density of the solution in the wells, the C-RB concentration in the analyzed samples was determined on the basis of a calibration graph. Glycosoaminglycan (GAG) was determined by a carbazole reaction according to the method of E.A. Kosyagina [Sharaev et al., 1990]. The following reagents were used: 94% ethanol, 5% trichloroacetic acid (TCA), concentrated sulfuric acid, 0.2% solution of carbazole in 94% ethanol, standard solutions with 5, 20, and 50 µg of glucuronic acid in 1 ml of water. 0.5 ml of the test liquid and 2 ml of chilled (in ice-water) ethanol were collected in a centrifuge tube. Stirred for 5-6 minutes, centrifuged at 3000 rpm for 10 minutes. The precipitate was emulsified in 3 ml of 5% TCA, the resulting mixture was hydrolyzed in a boiling water bath for 30 minutes, with a droplet separator. Then it was cooled at room temperature and re-centrifuged (3000 rpm, 10 minutes). The content of HA was determined in the supernatant. In 2 tubes (1 - control), 1 ml of the obtained supernatant and 5 ml of reagent 3 were added while cooling in ice water and stirring. They were heated for 10 minutes in a boiling water bath and cooled to room temperature. 0.1 ml of reagent 4 was added to the test tube, 0.1 ml of ethanol was added to the control. The contents were mixed, heated for 10 minutes at 65-70 ° C. A violet-pink color appeared. After 10 minutes, the samples were photometric at 530 nm (light green filter) in cuvettes with a layer thickness of 1 cm. Differences in the values of the experimental and control samples were found, and the concentrations were determined using a calibration graph. The concentration of GAG in serum is on average up to 41.3 ± 2.3 µmol / L.

Statistical processing of the obtained data was performed using the SPSSStatistics 21.0 program. Differences were considered statistically significant at $p < 0.05$.

IV. Discussion

In our study, the choice of MMR-1, which is interstitial collagenase and MMR-9, acting on the collagen of the basement membranes, was carried out taking into account the fact that the extracellular matrix and basement membrane have a different structure and composition, and TIMR-1 is able to inhibit both of these proteinases. In 45 examined children with TMJ, serum matrix metalloproteinases of types 1 and 9 (MMP-1 and MMP-9) were studied, which play a central role in the metabolism of connective tissue proteins and are specific markers of collagen breakdown (Gasanov A.G., 2010). The biochemical parameters of blood serum in children with TMJ are presented in table 1. A significant increase in the content of MMP-1 in children with TMJ was noted as the main enzyme that denatures fibrillar collagen of the extracellular matrix. Similar changes were revealed in the study of the content of MMP-9, the concentration of which in children of the main group was 1.6 times higher than in children of the comparison group, which, according to N.I. Solovieva and O.S. Ryzhakova (2010), may indicate the activation of type IV collagen hydrolysis. The concentration of TIMR-1 in cases of TMJ decreased as compared with the control group of children. The increased coefficients MMR-1 / TIMR-1 and MMR-9 / TIMR-1 confirm the possibility of exceeding the rate of collagen degradation by matrix proteinases at the rate of its synthesis.

Table 1
Comparative characteristics of the content of matrix metalloproteinases in blood serum in children with TMJ, M ± m

Index	Survey groups	
	Children with TMJ, n = 45	Healthy children (control) n = 15
MMP-9, ng / ml	118,17±8,63*	73,97±5,19
MMP-1, ng / ml	11,11±1,08*	4,37±0,53
MMP-3, ng / ml	38,04±3,14*	7,72±0,61
TIMR-1, ng / ml	598,62±18,91	728,32±19,13
MMR-1 / TIMR-1, conv. Food	0,003	0,002
MMP-9 / TIMP-1, conv. units	1,33*	0,54

Note: * - significance of differences P <0.05 with respect to control data

Representatives of the MMR groups are also interstitial collagenase MMR-3, which break down the fibrillar collagen of the corresponding types, also naproteoglycans, laminin, fibronectin and amorphous collagen. In our studies, presented in table 1 indicated a significant increase in MMP-3 in children with TMJ pathology, when compared with healthy children. The revealed imbalance of type I and type III collagens due to the high activity of metalloproteases with PG indicates a predominance of synthesis of type III collagen, which relates to embryonic proteins with low strength, which correlates with a systemic decrease in the level of collagen, which determines the integrity of connective tissue in the dentofacial system. Decreased synthesis of total collagen and the predominance of its immature fraction with a deficiency of components of the intracellular matrix, which determine the weakening and overstretching of connective tissue. The differences in the number, nature of distribution and localization of collagen and elastic fibers along with impaired expression of protein-coding genes, in particular, the MMR and TIMR families, determine the multilevel changes in the microarchitectonics of the dentofacial system in children with TMJ.

As you know, the systemic metabolism of connective tissue in patients with DST is characterized by the release of glycoproteins, a decrease in sulfated GAG. In addition, they determine the rheological properties of blood, which serves as an explanation for the occurrence of typical hemostasis disorders in the dentition, which affects thrombophilia due to a systemic inflammatory response, which explains the predominance of GAG destruction over their synthesis. Table 2 shows the dynamics of inflammatory markers in children with TMJ disease.

Table 2
The dynamics of GAG, C-RB and ESR in sick children with TMJ disease

Groups	The level of serum GAG, μmol / L	erythrocyte sedimentation rate mm / hr	C-RB highly sensitive, ng / ml
Children with TMJ, n = 48	53,65±21,5*	16,34 ±3,51*	35,39±4,42*
Healthy children, n = 15	24,15±1,79	7,08±0,53	9,73±0,18

Note: * - significance of differences P <0.05 when compared with control

As can be seen from the presented research results, in children with TMJ, there was a significant increase in the level of glycosoaminglycan (GAG) in the blood serum by an average of 2.2 times when compared with a group of healthy children. The obtained research results indicate that degenerative processes in the substance of the connective tissue are associated with a violation of the structure or function of the GAG and their involvement in the

pathological process. In addition, the dynamics of GAPG in the blood serum of the examined children indicates its high specificity, which indicates cartilage damage and indicates the development of connective tissue dysplasia.

All children examined were examined for the main indicators of inflammation by determining C-RB and ESR using a standard technique. The results are presented in table 2. When assessing the level of inflammation, the ESR index in groups of children with TMJ pathology was increased relative to the control group. When determining serum concentrations of C-RB using a highly sensitive ELISA, a consistent increase was observed in the results from the control group to the group with TMJ pathology. High concentrations of C-RB in the examined children with TMJ corresponds to the role of inflammation in damage to the connective tissue of the dentition.

As a rule, GAG are degradation products of collagen and intercellular substance, which represent an extensive heterogeneous group of substances that form the intercellular matrix of connective tissue. The role of serum GAG concentrations as a biochemical marker in patients with dentoalveolar anomalies was studied mainly by domestic scientists. In sick children with TMJ pathology, compared with healthy children, there is an increased level of GAG in the blood. Therefore, in our opinion, a study of the concentration of GAG and its fractional spectrum can be used as additional tests in the diagnosis of the activity of the pathological process, its severity, nature of the course, and the effectiveness of treatment for TMJ.

V. Conclusion

In recent years, there has been an increase in the importance of inflammation in the pathogenesis of dentofacial anomaly. Sensitive markers can detect inflammatory changes. The most sensitive markers of inflammation are levels of C-RB and interleukin-6 (IL-6). In the study of children with TMJ, we found an increase in levels of C-RB.

The latter indicates the presence of inflammation and immune disorders, which are associated with an increase in the rate of progression of DST lesion. Scientists from Iran tested clinical nutrition with a high content of sesame seeds to reduce inflammation in patients with DST, however, they received a decrease in the levels of IL-6 only, while the levels of C-RB and the total antioxidant activity of serum did not change after 2 months of treatment with 40 grams of sesame seeds seed [Haghighianetal., 2013], which indicates a high sensitivity of C-RB with not only high inflammation, but also a degenerative process of connective tissue.

Thus, in children with TMJ, there is a change in the activity of type 1, 3, 9 matrix metalloproteinases in the blood serum, indicating remodeling of connective tissue, indicating metabolic disturbances.

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