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Original article

The role of matrix metalloproteinases in early and late gonarthrosis manifestations

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Abstract: The *aim* of this research was to study the specifics of the matrix metallopeptidase system in early and late gonarthrosis (GA) manifestations.
Material and Methods — 37 patients of the main group (0-I stages of GA), 30 patients of the comparison group (III-IV stages of GA) and 30 healthy individuals of the control group underwent sonography and T2-relaxometry of their knee joints. The 24-h excretion of urinary C-telopeptide fragments of type II collagen (CTX-II) as well as serum concentrations of matrix metalloproteinases-3 and -8 (MMP-3, MMP-8), and tissue inhibitor of metalloproteinases (TIMP-1) were also registered.
Results — We detected increases in urinary CTX-II to 3.9 [2.5; 4.5] mg/d, MMP-8 to 364.7 [215; 434] pg/ml and TIMP-1 to 806 [559; 1157] pg/ml in the main group; to 9.7 [6.8; 10.2] mg/d, 579 [463; 663] pg/ml and 1256 [1029; 1330] pg/ml in the comparison group. The presence ($p=0.04$) of strong positive correlation ($R=0.7$) between the 24-h urinary CTX-II excretion and the change of MRI-signal characteristics for the areas of hyaline cartilage under the heaviest load on knee joints T2 relaxometry evidence was observed as well as the presence ($p=0.001$) of strong positive correlation ($R=0.7$) between MMP-8 and 24-h urinary CTX-II extraction. In early GA stages a strong positive correlation ($R=0.6$) was observed between MMP-8 and TIMP-1 ($p=0.04$) as well as 24-h urinary CTX-II excretion and TIMP-1 ($R=0.5$) at $p<0.001$).
Conclusion — Type II collagen degradation with MMP-8/TIMP-1 imbalance is one of the relevant mechanisms of the hyaline articular cartilage extracellular matrix disorganization in early and late GA evidences. The rearrangement of correlations between MMP-8 and TIMP-1 indicates the change in interaction mechanisms for GA proteolytic enzyme system components.

Keywords: gonarthrosis, type II collagen, matrix metalloproteinases, tissue inhibitor of metalloproteinases.

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Introduction

Gonarthrosis (GA) is osteoarthrosis (OA) that primarily involves knee joints; it is a widespread and socially significant musculoskeletal disease of polyetiologic genesis characterized with progressive degenerative changes of the articular components: hyaline cartilage, subchondral bone and capsular ligamentous apparatus [1]. The changes of GA pathology mainly occur in articular hyaline cartilage along with the interaction of few unfavorable biological and mechanical factors. The cartilage metabolism is characterized by anabolic/catabolic imbalance in its extracellular matrix resulting in the inhibition of chondrocyte metabolic activity that progresses along with the acute inflammation [2]. The irreparable loss of structural proteoglycans (PGs) in cartilage tissue results from the decrease in output as well as the intensification of polypeptide aggregates destruction, and also the emergence of 'perverse' reparative response leading to the synthesis of structurally incomplete macromolecules and accumulation of sulfated glycosaminoglycan degradation impurities, hyaluronic acid, and osteopontin in biological media [3]. The key pathogenic factor of destruction progression in articular connective tissue components is the system of zinc metalloproteinases with substrate specificity for the main interstitial I and II collagens as well as minor collagens. Several experimental studies had proven correlations between OA progression and accumulation of interstitial matrix metalloproteinases-1 (MMP-1) synthesized with chondrocytes as well as mesenchymal fibroblast gelatinizes (MMP-2) [4]. The crucial role in OA progression is played by the degradation of these collagen types, fibronectin, and aggrecan resulting from hyper MMP-7 [5]. In experimental AO simulations induced with papain injections in rabbits we determined the role of MMP-13 in the progression of collagen II and PGs loss involving β-catenin pathway [6]. The unquestionable fact proves that with the late signs of degenerative-dystrophic damage of large joints constantly affected by proinflammatory cytokines with the dominance of interleukin-1β (IL-1β) [7] and growth factors along with MMP tissue inhibitors deficit, the effect of collagenases MMP-1, -8, -13, gelatinizes (MMP-2, -9) and stromelysins-1 (MMP-3) is activated.

The literature data support the immediate participation of stromelysins-1 (MMP-3) in deterioration of articular hyaline cartilage strength properties due to its capacity to participate in the degradation of some sophisticated molecules in the connective tissue structures (type I, II, IV, IX, X collagens, PG, fibronectin, and others [8]) if the effect of tissue inhibitor of metalloproteinases

(TIMP) is insufficient. In its turn MMP-3 is capable of activating other proteolytic enzymes (MMP-1, matrilysin and gelatinase B) contributing to further progression of inflammatory and degradation changes in joint tissues in OA [9]. The dynamic MMP-3/TIMP balance underlies normal remodeling of joint tissues while their imbalance due to the increase in expression influenced by inflammation mediators (cytokines and growth factors) results in cell apoptosis and collagen structural integrity disorders. It is also known that the biological effects of MMP-3 are enforced by the activation of key enzyme precursors (MMP-1, 7, 8, 13) that are the main mediators of collagenous in articular hyaline cartilage tissue [10].

It is assumed that OA early stages are mainly associated to disorganized collagen matrix represented with its types I, IV, and V as a result of angiogenesis-stimulating gelatinase A (MMP-2) influence due to the release and transition of endothelial cells as well. The crucial role in OA progression is therefore played by the degradation of molecules of these collagen types, fibronectin, and aggrecan as a result of hyper MMP-7.

Meanwhile the literature sources [11] only provide individual references to the specifics of MMP-8, -3, and TIMP-1 roles in the pathogenesis of early OA signs compared to late stages of inflammatory destructive changes in joints, and therefore this research has been undertaken.

Material and Methods

Patients

37 patients of both sexes with early GA presentations (0-I stages, the main group) and 30 patients with III-IV GA stages (the comparison group) voluntarily participated in the research. 30 individuals who had neither articular pathology presentations nor other diseases able to affect the examined values made up the control group. GA diagnosis was proven with the patients' complaints, their medical histories and laboratory tests under the Federal Clinical Guidelines on osteoarthrosis diagnostics and treatment confirmed and approved on October 05, 2013 [12] at the session of the Association of Rheumatologists of Russia/APP plenum in cooperation with the Ministry of Healthcare of the Russian Federation Special Rheumatology Board. The mean age of the surveyed cohort was 46.0±3.4 years old. The exclusion criteria were oncology diseases, chronic inflammatory diseases during an acute exacerbation, s/p surgeries within 12 months before the research as well as clinically relevant liver, kidney, cardiovascular or respiratory diseases.

Assessment of the research participants' orthopedic status

The articular symptom load was assessed using the global score WOMAC (Western Ontario and McMaster Universities Arthrose index) as well as KOSS (Knee Injury and Osteoarthritis Outcome Score) questionnaires.

Radiological methods for the articular structures examination

The objectification of articular structures condition was performed using both the instrument and diagnostic methods including standard 2-projection radiography of knee joints with Apelem DX-90 (France) apparatus equipped with a digital processing option. The basal areas of articular hyaline cartilages were scanned with the 9-12 MHz linear array probe of Siemens-2000 (Germany) as well as Hitachi Echelon 1.5T (Japan) tomography scanner followed by T2-relaxometry color mapping (Relax MAP) of the weight-bearing areas around femoral condyles and patellofemoral junctions. The morphometry in the regions of interest was also performed.

Analysis of the body fluids chemical composition

The intensity of cartilage tissue type II collagen disorganization at GA was defined with its C-terminal telopeptides (Urine CartiLaps CTX II EIA) 24-h extraction, their concentration being detected with ELISA on multifunctional Anthos 2020 (UK) spectrophotometer. We also employed this method to find fasting serum concentrations of matrix metalloproteinase MMP-3 (Human MMP-3 Platinum ELISA/eBioscience), MMP-8 (RayBio Human MMP-8 ELISA Kit/RayBiotech) and tissue inhibitors of metalloproteinases TIMP-1 (Human TIMP-1 ELISA KIT/Aviscera Bioscience) in the morning. The wavelengths of 620 and 450 nm were employed to assess the optical density of the test reactants as recommended by the manufacturers of commercial reagent kits.

Statistical analysis

We ran the Shapiro-Wilk tests for normality of results distribution. The nonparametric Mann-Whitney U-test (two-tailed test) was used to find the differences between the groups; the results were presented in the form of the median with low and upper quartiles – Me (LQ, UQ). To estimate the strength and direction of interrelations between the studied parameters we employed the Spearman's rank correlation coefficient (R). The differences were considered significant at $p<0.05$.

Results

Instrumental assessment of the joint structures

We revealed a considerable ($p<0.05$) reduction of the mean articular hyaline cartilages thickness at knee joint scanning in GA patients of the main group as compared to the individuals of both the comparison and the control groups (*Table* 1). The changes in patients of the main group were less prominent than these of the comparison group individuals compared to the healthy ones. The hyaline cartilages in individuals of the main and the comparison groups had been generally thinned in the medial femoral condyles which were proven when the areas of the changed MR-signal were found as well as the sites of chondromalacia on patellofemoral junctions. The changes were more evident in patients of the comparison group.

Body fluids biochemical analysis

The biochemical analysis of the patients' body fluids revealed a more prominent increase in concentrations of 24-h urinary CartiLaps (CTX II) excretion in patients of the comparison group than these in patients of the main group as compared to the results of healthy individuals (*Table* 2).

The research of matrix metallopeptidases admission to the bloodstream of the examined individuals showed the increase ($p<0.05$) in MMP-8 concentrations for patients of both the main and the comparison groups as compared to the control group patients, this being more evident at the late stages of the disease. No significant differences in concentrations of MMP-3 participating in proteolysis of non-collagenous proteins in the articular hyaline matrix were observed; this could be associated

with the low local inflammatory activity in the absence of prominant radiographic evidences of degenerative-dystrophic changes in early GA. Interestingly, the increase ($p<0.05$) in TIMP-1 admission to serum at GA as compared to that of the healthy control group individuals was detected.

Correlation analysis of the results

The correlation analysis revealed ($p=0.04$) strong positive correlation ($R=0.7$) between 24-h urinary CartiLaps (CTX II) extraction and the characteristics of MRI signal change for the most overloaded areas of hyaline cartilage on knee joints T2 relaxometry data in patients of the main group. There was ($p=0.021$) a negative mean ($R=-0.6$) correlation between the thickness of the articular hyaline cartilage upon knee joints ultrasonic data and 24-h urinary CartiLaps (CTX II) extraction with the dependency ($p<0.001$) between articular hyaline cartilage ultrasonic measurement data and T2 relaxometry results in this group to be characterized as negative mean ($R=-0.5$). The research of the proteolytic system components revealed ($p=0.001$) a strong positive ($R=0.7$) correlation between MMP-8 and 24-h urinary CartiLaps (CTX II) extraction as well as TIMP-1 ($R=0.5$) at $p<0.001$ in early stages of the disease.

However the decrease of correlation force ($R=-0.5$) between T2 relaxometry characteristics and fragments of collagen type II admission into biological media was observed in later stages of GA which was probably associated with systemic evidence of the pathological process including inflammatory and degradation changes in other large joints. We revealed ($p<0.001$) the rearrangement of interaction mechanisms between MMP-8 and TIMP-1 at the assessment of proteolytic enzyme system components in the comparison group. The process was reflected in changes of correlation nature and its direction ($R=-0.4$) in the signs of late GA. As the articular pathology progressed, we also observed ($p=0.03$) the decrease in correlation force ($R=-0.17$) between MMP-8 circulation in serum and the level of TIMP-1. Therefore we assumed that the MMP-8 collagenase/stromelysins-1 (MMP-3) system imbalance progresses due to their hyperproduction along with the decrease of TIMP-1 circulation. At the same time we detected the decrease ($R=-0.2$) of TIMP-1 ($p=0.04$) relevance for disorganization of collagen matrix; it was calculated using 24-h urinary CartiLaps (CTX II) extraction. No significant correlations between serum MMP-3 and MMP-8 ($R=0.12$) concentrations as well as MMP-3 and TIMP-1 ($R=-0.3$) concentrations was observed in GA patients.

Discussion

The differences in changes of telopeptides CartiLaps (CTX II) 24-h extraction in GA attested the intensification of disorganization of the connective tissue components containing type II collagen as the articular pathology progressed. Considering the comparison of the laboratory and instrumental test results of the patients, the possible involvement of other musculoskeletal components into the inflammatory and degradation processes at late evidence of degenerative-dystrophic joint lesions may be assumed.

The references mention [13] the correspondence of the cartilage and bone tissue metabolic products accumulation in biological media to GA radiological stages. This sets significant limits to the researching of articular structure damaging mechanism specifics in the pathogenesis of its early evidence. The assessment of the revealed correlations between the studied indicators enables claiming that the disorganization of type II collagen serves as one of the patterns for destructive and inflammatory changes of articular hyaline cartilage ECM in GA. This process is triggered by the imbalance of the proteolytic enzyme (MMP) system and the enzyme inhibitors (TIMP). The hyperproduction of neutrophilous collagenase MMP-8 found in both groups of GA patients and the nature of its correlations with the rates of type II collagen 24-h excretion allows considering this proteolytic enzyme to be one of the significant factors of cartilage tissue ECM destabilization.

Given the fact that subchondral bone is as good a substrate of collagenolytic enzymes influence as articular hyaline cartilage, the augmentation of MMP-8 secretion could also be associated to the increase of osteoclastic activity further facilitating the disturbance of trophic interactions in the system of bone and cartilage tissues [14]. The specifics of interaction between the studied indicators have proven the priority of MMP-8 involvement in degradation reactions of type II collagen within the composition of connective tissues at both early and late GA evidence.

Table 1. The results of the knee articular hyaline cartilage measurements with radiological methods

Indicators/Group	*The main group n=35*	*The comparison group n=28*	*The control group n=21*
Articular hyaline cartilage thickness, scan results, mm	2.0 (1.4, 2.6)	1.3 (0.9, 1.7) *p=0.027	3.7 (3.1, 4.2) **p<0.001 ***p=0.001
Articular hyaline cartilage T2-relaxometry results Ave. (Rel), mm^2	685 (623, 736)	775 (727, 825)	506 (479, 556) **p<0.001, ***p<0.001

The results are presented as median with low and upper quartiles – Me (LQ, UQ). p-level shows the difference relevance of the studied parameters between * the main group and the comparison group, ** the comparison group and the control group, *** the main group and the control group.

Table 2. MMP-3, MMP-8, TIMP-1 serum concentrations and 24-h urinary CartiLaps (CTX II) excretion in patients with early and late gonarthrosis evidences

Groups / Studied indicators	*MMP-3, pg/ml*	*MMP-8, pg/ml*	*TIMP-1, pg/ml*	*Urine CartiLaps (CTX II), mg per day*
The main group (n=37)	0.79 (0.64, 1.15)	364.73 (220.48, 434.53)	806.12 (559.25, 1157.34)	3.9 (2.5, 4.5)
The comparison group (n=30)	0.76 (0.58, 0.94)	579.34 (463,84, 663,44) *p=0.004	1256.00 (1029.33, 1330.67) *p=0.004	9.7 (6.8, 10.2) *p<0.001
The control group (n=30)	0.68 (0.61, 0.70)	203.15 (154.33, 215.71) **p<0.001	530.61 (459.18, 590.20) **p=0.001, ***p<0.001	1.7 (1.3, 2.1) **p=0.004, ***p<0.001

The results are presented as median with low and upper quartiles – Me (LQ, UQ). p-level shows the differences relevance for the studied parameters between * the main group and the comparison group, ** the comparison group and the control group, *** the main group and the control group.

Despite the obtained proof of the increase of both MMP-8 and TIMP-1 syntheses in patients of the comparison group, we also observed the weakening of the relationship between these metabolites suggesting the formation of independent disorganization mechanisms of skeletal connective tissues with the involvement of matrix metalloproteinases and their inhibitors at late GA stages.

Conclusion

Type II collagen degradation at MMP-8/TIMP-1 imbalance is one of the most significant mechanisms of the articular hyaline cartilage extracellular matrix disorganization in both early and late GA evidence. The rearrangement of correlations between MMP-8 and TIMP-1 indicates the change in interaction mechanisms of proteolytic enzyme systems components in GA.

Limitations

The application of the research results is limited in respect of the patients with comorbid conditions (type I and II diabetes, arterial hypertension, general connective tissue diseases) that may significantly affect the studied indicators.

Conflict of Interests

This research was conducted in the Research Institute of Traumatology, Orthopedics and Neurosurgery, Federal State Budgetary Educational Institution of Higher Education «V.I. Razumovsky Saratov State Medical University» of the Ministry of Healthcare of the Russian Federation as a part of the governmental assignment for Designing of Integrated Early Diagnosis Technique for Articular Cartilage Remodeling Disorders in Persons with Higher Risk of Osteoarthrosis in Large Joints (Registration No. AAAA-A18-118020290176-9). The author has no conflict of interest to disclose.

Ethical Approval

The research was performed under the standards of Good Clinical Research Practice and the principles of the Declaration of Helsinki, the research protocol No.6 of February 6, 2018 approved by the Federal State Budgetary Educational Institution of Higher Education «V.I. Razumovsky Saratov State Medical University» of the Ministry of Healthcare of the Russian Federation's Ethics Committee. The written ICs were received from all participants of the research before their enrollment.

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