SERUM MMP-7 LEVELS IN PATIENTS WITH KNEE OSTEOARTHRITIS ACCORDING TO THE RADIOGRAPHIC DISEASE SEVERITY

Rheumatology

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ABSTRACT

The objective of this study was to investigate serum MMP-7 levels in patients with knee osteoarthritis (OA) according to the radiographic disease severity. A cross-sectional study was done on 60 consecutive knee OA patients and 60 age and sex-matched healthy control participants. Disease severity in the knee OA population was assessed using the Kellgren–Lawrence (K–L) grading scale. Circulating MMP-7 levels were examined in serum using the Quantikine Human MMP-7-ELISA. Serum MMP-7 levels were significantly higher in knee OA patients than control groups. Significantly higher MMP-7 levels in severe degree were compared with mild degree of knee OA patients. Our results indi¬cated that MMP-7 may play impor¬tant roles in the pathogenesis of OA and also MMP-7 can be a potential biomarker to predict the severity of knee OA.

KEYWORDS

Osteoarthritis, MMP-7, Kellgren-Lawrence Grading Scale

Introduction

Osteoarthritis (OA) is a degenerative disease of the joints characterized by changes in chondrocytes and degradation of cartilage extracellular matrix (ECM) causing damage to articular cartilage.[1] The prevalence of OA increased with increasing age of life expectancy in both developed and developing countries. OA was one of the causes of disability that can impair quality of life.[2]

Molecular mechanisms in OA were not fully understood, need validated markers to predict diagnosis and disease progression.[3] The etiology of cartilage damage in OA was unclear, presumably due to ECM destruction by inflammatory cytokines secreted by cartilage cells that cause joint inflammation and cartilage cell destruction, resulting in OA. If ECM production such as type 2 collagen, proteoglycan, and aggrecan decreases, and ECM decomposition is activated by matrix metalloproteinase (MMP) due to cartilage cell destruction, then the cartilage structure will be damaged, thus the patient may experience OA.[4]

The MMP consists of 28 members, and all amino acid sequences of MMP have 2 domains, catalytic and prodomain domains, which are important as substrates, including gelatinases, collagenases, matrilysins, stromelysins, membrane-type MMP, and metalloelastases.[5] MMP-7, also known as matrilysin, is a member of the MMP. MMP-7 is the smallest member that only consists of the com-mon catalytic domain and zinc-binding region.[6] MMP-7 has a specific ability to degrade various ECM components such as cartilage proteoglycan.[7]

MMP is considered as one of the potential biomarkers involved in the pathogenesis of OA. The role of MMP including MMP-7 in the occurrence of OA has been widely studied.[8-10] However, studies that evaluated MMP-7 levels in severity of knee OA were limited. This study aims to investigate serum MMP-7 levels in patients with knee OA according to the radiographic disease severity.

Methods

Patient Selection

A cross-sectional study was done on sixty consecutive knee OA patients that were admitted to Rheumatology division at Adam Malik General Hospital Medan, Indonesia from October 2017 and March 2018. This study also included 60 age and sex-matched healthy control participants with no symptoms of joint disease (pain, swelling, tenderness, or restriction of movement) in whom standard X-rays of knee joints confirmed the absence of radiographical knee OA. Subjects in both groups with evidence of malignancy, metabolic disorders, and systemic diseases were exluded. Subjects with other etiologies of knee joint diseases such as rheumatoid arthritis, gouty arthritis, septic arthritis, post traumatic or dysplasias also were excluded. All patients gave informed consent and the study was approved by the local ethical committee.

Diagnosis of knee OA

The diagnosis of knee patient with OA was confirmed by clinical examination and radiographic, which also fulfilled the criteria of the American College of Rheumatology (1987). Radiographic severity was assessed according to the Kellgren-Lawrence (K-L) grading system: grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. An experienced observer, who was blinded to the source of participants, scored the grading of radiographs. OA were classified into mild (K-L grade 2), moderate (K-L grade 3), severe (K-L grade 4). Only patients with radiographic OA, defined as KL score of ≥2, were included in the study.

Serum levels of MMP-7

Venous blood was drawn using a serum separator tube and allowed to clot for 30-45 minutes at room temperature before centrifugation for 15 minutes at approximately 1,000g. Serum was immediately stored frozen in aliquots at $-20^{\circ}C$ until assays for MMP-7 were performed. Circulating MMP-7 levels were examined in serum using the Quantikine Human MMP-7-ELISA (Quantikine, R&D System, Inc., Minneapolis). Serum levels were expressed as pg/ml.

Statistical analysis

SPSS version 22 (SPSS Inc., Chicago) was used for analysis. The data were analysed using univariate and bivariate analysis with 95% confidence intervals. The results were expressed as the mean $\pm$ standard deviation. Bivariate analysis was carried out using the independent t-test and ANOVA test with a p-value < 0.05 was considered statistically significant.

Results

Cohort and control cases were homogeneous by age and sex. BMI was significantly higher in the OA knee group than controls. Most OA patients (48.3%) with mild OA (K-L grade 2).

Table 1. Baseline characteristics in knee OA patients and controls

Variable	Cases	Controls	p
Age (years)	57 $\pm$ 4.01	56.2 $\pm$ 3.98	0.769
Gender Female Male	 30 23	 37 30	0.968
Body mass index	26.8 $\pm$ 4.2	24.3 $\pm$ 3.8	<0.001*
Kellgren-Lawrence grading			
2	29 (48.3%)	–	
3	19 (31.7%)	–	
4	12 (20%)	–	

*p<0.05

There were significant differences in serum MMP-7 levels between knee OA patients and control groups (p<0.001). Serum MMP-7 levels were significantly higher in knee OA patients than control groups (Table 2 and Figure 1A).

Table 2. Serum levels of MMP-7 in knee OA and control groups

|	Knee OA	Controls	p
MMP-7 (pg/mL)	15.7 ± 4.63	7.2 ± 2.24	<0.001*

*p<0.05

There were significant differences in serum MMP-7 levels in various severities of knee OA patients (p=0.032). Significantly higher MMP-7 levels in severe degree were compared with mild degree of knee OA patients. There were no significant difference in MMP-7 levels between patients with severe and moderate degree of knee OA and also between moderate and mild degree of knee OA (Table 3 and Figure 1B).

Table 3. Serum MMP-7 levels in patients with knee OA according to the radiographic disease severity

	Grade 2	Grade 3	Grade 4	p
MMP-7 (pg/mL)	10.68 ± 8.5	14.86 ± 7.94	20.87 ± 6.37#	0.032*

*p<0.05, #the result of this category was significantly different with grade 2

1(A)   1(B)
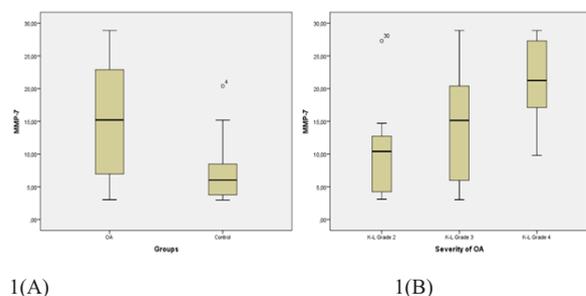
Figure 1(A) Serum levels of MMP-7 in knee OA and control groups (B) Serum levels of MMP-7 in various severities of knee OA

Discussion

MMP are functionally and structurally associated with zinc endopepticases that cleave proteins of the extracellular matrix, including collagens, elastin, matrix glycoproteins and proteoglycans and are thought to be responsible for the degeneration of articular cartilage.[11] Most MMP are consist of three distinct domains: an amino-terminal propeptide involved in the maintenance of enzyme latency; a catalytic domain that binds zinc and calcium ions and a hemopexin-like domain that is located at the carboxy terminal zone of the protease and that plays a role in substrate binding.[12]

Previous studies have reported an increase in levels of MMP protein in OA patients. Those MMP could degrade the components of extracellular matrix, including proteoglycans, vitronectin, fibronectin, laminin, and collagens.[13,14] MMP were involved in the progression of OA.[15] Zheng et al showed that high protein levels of MMP in OA might accelerate the pathogenesis of the disease.[10]

This study showed that Serum MMP-7 levels were higher in knee OA patients than control groups. These results are consistent with previous studies. Ohta et al reported that MMP-7 levels were overproduced in OA cartilage and MMP-7 might play a significant role in the ECM degradation in OA.[8] Tao et al found that expression protein levels and mRNA levels of MMP-7 were significantly increased in OA patients compared with control group.[16] Ling et al showed that high expression levels of MMP-7 enhanced plasmin activation to render extracellular matrix vulnerable to degeneration and injury.[17]

This study found that serum MMP-7 levels were significantly higher in severe degree than mild degree of knee OA patients. Our results indicated that MMP-7 may play important roles in the pathogenesis of OA

and also MMP-7 can be a potential biomarker to predict the severity of knee OA. Further research are needed to determine the sensitivity and specificity of MMP-7 levels for predicting severe OA, as well as to determine the role of MMP-7 as an predictor for early OA.

Conclusion

Serum MMP-7 levels were significantly higher in knee OA patients than control groups. Significantly higher MMP-7 levels in severe degree were compared with mild degree of knee OA patients.

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