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Correlation of synovial fluid HMGB-1 levels with radiographic severity of knee osteoarthritis

Abstract

Purpose: This study measured high-mobility group box 1 (HMGB-1) levels in serum and synovial fluid (SF) in patients with primary knee osteoarthritis (OA) and correlated these levels with radiographic disease severity.

Methods: Seventy-eight OA patients and 30 controls were enrolled in this study. All OA patients were scored according to the Kellgren-Lawrence (KL) grading system. HMGB-1 levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: SF HMGB-1 levels were significantly higher in knee OA patients, compared with controls ($P < 0.01$). Moreover, SF HMGB-1 levels were positively associated with KL scores ($P < 0.01$). Multinomial logistic regression demonstrated that the SF HMGB-1 level was an independent factor for radiographic severity of OA ($P = 0.002$); however, serum HMGB-1 levels did not differ significantly between OA patients and controls and did not correlate with KL scores ($P > 0.05$).

Conclusion: These results demonstrate that HMGB-1 levels in SF of knee OA patients are independently associated with radiographic disease severity.

Manuscript submitted 16th May, 2011

Manuscript accepted 11th August, 2011

Clin Invest Med 2011; 34 (5): E298-E303.

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Osteoarthritis (OA) is a common degenerative joint disease characterized by articular cartilage destruction, osteophyte formation and subchondral bone remodeling. The principal clinical features of OA include pain, joint stiffness, limited motion, swelling and crepitus [1]. OA is the leading cause of disability among the elderly and the knee is the most clinically significant site of primary OA involvement [2].

Increasingly, evidence in both experimental and clinical studies suggests that inflammatory molecules such as pro-inflammatory cytokines are among the critical mediators of the disturbed processes implicated in OA pathophysiology, suggesting that OA is a chronic inflammatory process [3]. High-mobility group box 1 (HMGB-1), a highly conserved protein that was previously known as a DNA-binding protein and that is involved in DNA transcription, replication, and repair [4], was recently identified as a potent pro-inflammatory cytokine during the inflammatory process [5]. The receptors of HMGB-1 include receptors for advanced glycation end products (RAGE), Toll-like receptor (TLR)2, TLR4 and syndecan [6]. The interaction between HMGB-1 and its receptors and the subsequent downstream signaling can lead to an augmented inflammatory reaction [7].

HMGB-1 can be translocated from the nucleus to the cytosol and then released extracellularly [8]. It has been reported that extracellular HMGB-1 is present in serum and synovial fluid (SF) of patients with OA [8]; however, there have been no such studies on serum and SF levels of HMGB-1 in the various clinical stages of primary knee OA and the relationship between serum and SF levels of HMGB-1 and disease severity of primary knee OA remains entirely obscure. This study aimed to assess whether serum and SF levels of HMGB-1 are correlated with disease severity in knee OA patients.

Materials and Methods

Study subjects

This study was approved by the ethics committee of Renji Hospital. All patients gave their informed consent. Seventy-eight patients with primary knee OA undergoing total knee arthroplasty in Renji Hospital from April 2008 to April 2011 were enrolled in the study. All primary OA patients met the American College of Rheumatology clinical symptomatic and radiographic criteria for OA [9]. Participants were excluded if they had secondary post-traumatic OA, systemic inflammatory or autoimmune disorders, previous knee injury or joint infection, or history of corticosteroid medication.

SF samples were taken from the most affected knee during the surgery. As controls, SF samples were also obtained from 30

patients who underwent arthroscopy to treat traumatic intra-articular knee joint injury such as meniscal or cruciate ligament tears during the same period. All the controls underwent conventional x-rays examination before the surgery, and these patients showed no abnormalities of articular cartilage during arthroscopic examination and were evaluated as radiologically normal [Kellgren-Lawrence (KL) grade 0] [10]. Fasting venous blood samples were also drawn from all participants 1-2 h before surgery.

Radiographic assessment

All participants underwent weight-bearing anteroposterior radiographs to assess the structural changes of the affected knee. Radiographic severity was assessed according to the KL grading system [10]: grade 1, questionable 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.

Biochemical investigation

SF and blood samples were centrifuged and stored at -80°C until analysis. HMGB-1 levels in serum and SF were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (HMGB-1 ELISA kit II; Shino-Test Corporation, Tokyo, Japan) with an intra-assay and inter-assay CV of both <10% [11]. All samples were routinely analyzed by ELISA in triplicate, and the results were averaged. ELISAs were conducted in Shanghai Jiaotong University School of Medicine using a Multiskan MK3 automatic microplate reader (Thermo Scientific, Waltham, USA).

Statistical analysis

For continuous variables, normal distribution was evaluated with the Kolmogorov-Smirnov test. Results of normally distributed continuous variables were expressed as the mean value $\pm$ SD, and those for non-normally distributed continuous variables were expressed as the median (interquartile range). Categorical variables were summarized as frequencies or percentages. Differences between the two groups were analyzed using unpaired t-test or Chi-square test when appropriate. Differences among groups were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis analysis followed by Tukey *post hoc* analysis. As the HMGB-1 levels were not normally distributed, logarithmic (log) transformed values were

TABLE 1. Baseline clinical characteristics of OA patients and controls

	Controls	OA patients			
	(n=30)	Total (n=78)	KL grade 2 (n=20)	KL grade 3 (n=27)	KL grade 4 (n=31)
Age (years)	39.07±10.57	68.77±8.44*	67.50±7.64*	67.81±9.25*	70.42±8.19*
Female, n (%)	17, (56.67%)	53, (67.95%)	16, (80.00%)	19, (70.37%)	18, (58.06%)
BMI (kg/mm ²)	23.33±2.14	23.41±2.02	22.99±2.10	23.31±2.13	23.76±1.87
Smoker, n (%)	6, (20.00%)	18, (23.08%)	4, (20.00%)	4, (14.81%)	10, (32.26%)
DM, n (%)	6, (20.00%)	23, (29.49%)	6, (30.00%)	8, (29.63%)	9, (29.03%)

All values are expressed as the mean value ±SD or n (%). OA= KL grade= Kellgren-Lawrence grade, BMI= body mass index, DM=Diabetes mellitus * P<0.01 *vs.* Controls

performed for multiple comparisons among the groups. Spearman rank correlation coefficient was employed to determine the correlation between HMGB-1 levels and KL scores. Multinomial logistic regression analysis was performed to assess the independent predictors of KL scores. As the HMGB-1 levels were not normally distributed, log transformations values were also performed for multinomial logistic regression. All statistical analyses were performed using SPSS 13.0 for windows (SPSS Inc., Chicago, USA). A value of P<0.05 (two-tailed) was considered statistically significant.

Results

Baseline clinical characteristics

Of the 30 controls, 25 exhibited isolated meniscal tears and five with combined meniscal and cruciate ligament tears, and the time since injury was 61.72±47.10 days and 23.20±23.74 days, respectively. The disease duration of OA patients was 48.36±30.93 months. Table 1 summarizes the baseline clinical characteristics of the study population. The mean age was significantly older in OA patients than in controls (P<0.05). In the OA group, there were no significant differences in baseline clinical characteristics among subgroups.

HMGB-1 levels in SF and serum

HMGB-1 levels in SF and serum were analyzed according to the KL classification. As shown in Table 2, OA patients had higher SF HMGB-1 levels compared with controls. Multiple comparisons among patients with different KL grades showed that SF HMGB-1 levels were significantly higher in the KL 4 subgroup when compared with the KL2 (P<0.01) and KL3 (P<0.05) subgroups. Levels of HMGB-1 in the KL3 subgroup were not significantly different from those in the KL2 subgroup and . serum HMGB-1 levels showed no significant differences between OA patients and controls (P>0.05).

Moreover, in the OA group, serum HMGB-1 levels did not show significant differences among subgroups (P>0.05).

Correlation of HMGB-1 levels with KL scores

As demonstrated in Fig 1, a significant positive correlation was found between SF HMGB-1 levels and KL scores (r=0.394, P<0.001); however, no correlation was found between serum HMGB-1 levels and KL scores (r= 0.091, P=0.426). As age, BMI and gender are known to influence OA severity, multinomial logistic regression was utilized to investigate the association between SF/serum HMGB-1 levels and KL scores in OA patients. Multinomial logistic regression showed that there was still a positive association between log (SF HMGB-1 levels) and the severity of OA disease, even after controlling for age, BMI and gender (P=0.002).

FIGURE 1. Correlation between SF HMGB-1 levels and radiographic severity of OA patients.

KL grade: Kellgren and Lawrence grading system

TABLE 2. Serum and SF HMGB-1 levels of OA patients and controls

	Controls	OA patients			
	(n=30)	Total (n=78)	KL grade 2 (n=20)	KL grade 3 (n=27)	KL grade 4 (n=31)
Serum HMGB-1 (ng/ml)	1.83 (1.11-4.52)	2.56 (1.37-6.24)	1.97 (1.24-4.06)	2.66 (1.36-7.82)	3.09 (1.84-6.24)
SF HMGB-1 (ng/ml)	4.79 (1.52-9.37)	18.65 (10.80-29.11)*	12.38 (8.59-21.68)	16.68 (9.77-24.11)	26.99 (16.01-45.66)**#

All values are expressed as the median (interquartile range). OA= osteoarthritis, KL grade= Kellgren-Lawrence grade, SF= synovial fluid

* P<0.01 vs. Controls, **P<0.01 vs. KL grade 2, # P<0.05 vs. KL grade 3

Discussion

In the present study, serum and SF levels of HMGB-1 were measured both in knee OA patients, presenting with different radiographic severities, and in controls. SF HMGB-1 levels were significantly higher in knee OA patients compared with controls. Moreover, there was a positive association of SF HMGB-1 levels with radiographic disease severity in knee OA patients.

The development of preventive strategies and early-stage interventions for OA is largely dependent upon a better understanding of the molecular mechanisms and the identification of reliable biomarkers that reflect specific biological or pathological processes associated with OA. To date, few biomarkers have been clinically validated as reflecting the development and progression of OA and to distinguish different clinical stages of the disease.

Although the exact pathogenesis of OA is still not well understood, inflammation is believed to play a key mechanism in the development and progression of OA even in the early stages of the disease [12]. HMGB-1, a 30 kD member of the high mobility group non-histone chromosomal protein family [13], was identified as a potent pro-inflammatory cytokine that mediates delayed endotoxin lethality and systemic inflammatory response [6]. HMGB-1 can be activated in the pathophysiology process of chronic inflammatory diseases, including arthritis [14]. Kokkola *et al.* determined that HMGB-1 is abundantly expressed in synovial tissues from rheumatoid arthritis patients and from rats with experimental arthritis [15]. Taniguchi *et al.* confirmed this result and demonstrated that SF HMGB-1 levels are significantly elevated in patients with rheumatoid arthritis [8]. In the present study, HMGB-1 concentrations were measured in the SF and serum of study subjects using ELISA: SF HMGB-1 levels were found to be significantly higher in OA patients in comparison with controls. Serum HMGB-1 levels were demonstrated to be very low and not significantly different between OA patients and healthy controls. It could be speculated that HMGB-1 concen-

trations in SF reflect the extracellular levels of this putative pro-inflammatory mediator in arthritic joints rather than in HMGB-1 concentrations in systemic circulation. The present study has significant differences in ages between OA patients and the healthy controls; therefore, further studies are needed to clarify whether increased SF HMGB-1 levels in OA patients can be attributed to increased age.

The major finding of the present study was that SF HMGB-1 levels increased with the advancement of KL grade and were positively correlated with KL scores in OA patients. After adjusting for potential confounders, there was still a significant association between SF HMGB-1 levels and KL scores. These results indicate that the interaction between HMGB-1 and its receptors may also be a pathogenetic factor in the degenerative process of OA. Necrotic and/or activated chondrocytes and monocyte/macrophages release HMGB-1 into extracellular cartilage matrix [16]. After interacting with several different cell surface receptors, extracellular HMGB-1 can activate various signaling pathways such as protein kinase B (Akt), mitogen-activated protein kinases (MAPKs) and nuclear factor- κ B (NF- κ B) [17]. These pro-inflammatory signaling pathways play potent roles in chronic inflammation of synovial tissue [17] and particularly as stimuli for enchondral bone formation [16]. Moreover, ligand-receptor interaction has been found to stimulate synovial macrophages to produce and release HMGB-1, and other pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, thus acting as a positive feedback mechanism [14,18]. In addition, extracellular HMGB-1 can bind to components of the plasminogen activation system and enhance the activity of matrix metalloproteinases (MMPs) [14, 19], which are major mediators of cartilage destruction [20]. All these studies lend support to the hypothesis that HMGB-1 plays a significant role in both the inflammatory and destructive processes of OA and are consistent with the results observed in the present study.

The potential limitations of the present study merit consideration. Firstly, this cross-sectional study included only a small group of Chinese patients and further study with a larger

cohort would be needed to validate our data. Secondly, SF samples cannot be obtained from age-matched healthy controls for ethical reasons, resulting in a high percentage of elderly population in patients with knee OA in the research cohort, and the age difference between OA patients and controls might introduce some bias. Thirdly, only HMGB-1 levels in serum and SF were examined in the present study. Additional studies on other pro-inflammatory cytokines in the same samples may provide more valuable information on the disease-promoting role of HMGB-1 signaling pathways in OA and to truly appreciate HMGB-1 uniqueness. Fourthly, fasting venous blood samples were collected at different time points within the 1-2 h pre-surgery. Diurnal variations of biomarkers such as pro-inflammatory cytokines have been previously reported [21] and may skew the results; therefore, variability in the timing of specimen collection is an important limitation of the present study.

In summary, SF HMGB-1 levels in knee OA patients were significantly higher compared with controls. Moreover, SF HMGB-1 levels were positively associated with the disease severity of knee OA. These findings indicated that SF HMGB-1 levels may be useful for judging the severity of OA. Therapeutic interventions, via blockage of HMGB-1 signaling pathways to delay the degenerative process of OA, warrants further investigations.

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