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Synovial Fluid Macrophage Migration Inhibitory Factor Levels Correlate with Severity of Self-Reported Pain in Knee Osteoarthritis Patients

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Funds Collection G

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Background: Inflammation is considered as one of the main pathogeneses in OA-induced pain. Macrophage migration inhibitory factor (MIF) is a well known pro-inflammatory cytokine. We aimed to determine whether MIF levels in serum and synovial fluid (SF) are associated with severity of OA-induced pain.

Material/Methods: We recruited 226 patients with knee OA and 106 controls. Self-reported pain severity of OA patients was evaluated using the Western Ontario McMaster University Osteoarthritis (WOMAC) pain scores. MIF levels were detected using enzyme-linked immunosorbent assay (ELISA).

Results: OA patients had similar serum MIF levels compared to controls (11.93 [5.68–18.10] *vs.* 10.06 [6.60–14.61] ng/ml, P>0.05). In OA patients, MIF levels in SF were dramatically lower compared to paired serum samples (3.39 [1.87–5.89] *vs.* 11.93 [5.68–18.10] ng/ml, P<0.01). MIF levels in SF were significantly correlated with WOMAC pain scores (r=0.237, P<0.001), but MIF levels in serum had no significant correlation with WOMAC pain scores (r=0.009, P=0.898).

Conclusions: MIF levels in SF, but not in serum, were independently associated with the severity of self-reported pain in OA patients. The inhibition of MIF signaling pathways may be a novel therapeutic approach for ameliorating OA-induced pain.

MeSH Keywords: Biological Markers • Cytokines • Knee • Osteoarthritis, Knee

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Background

Osteoarthritis (OA) is a chronic degenerative joint disorder characterized by articular cartilage degeneration, subchondral bone abnormalities, and nonspecific synovial inflammation [1]. As a part of the ageing process, OA is the leading cause of physical disability and impaired quality of life in the elderly [2]. Of all the severe symptoms caused by OA, the pain sensation is the primary complaint that leads to a significantly reduced quality of life.

As OA is a continuing pathological process, there has been a long-standing interest in identifying new specific biological markers for symptomatic severity to facilitate the early diagnosis of joint destruction and to evaluate disease. The establishment of reliable biomarkers could facilitate investigating the etiology and pathogenesis underlying OA and thus determine possible new therapeutic targets and improve clinical outcomes [3].

Although many underlying mechanisms, such as bone alterations, contribute to OA-induced pain [4], inflammation is now believed to play an important role in the pathogenesis of OA-induced pain [5]. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine produced by macrophages within innate and adaptive immune responses [6]. MIF can induce the production of a large number of pro-inflammatory molecules and thus may be involved in the pathophysiology of arthritis by promoting inflammation and angiogenesis [7–9]. Moreover, Liu et al. recently revealed that serum and synovial fluid (SF) MIF levels were significantly associated with the radiographic severity of OA [10]. Therefore, we hypothesized that MIF might be implicated in the pathophysiology of OA-induced pain. The present study thus aimed to investigate the possible correlation between MIF levels in serum and SF and self-reported pain intensity in OA patients.

Material and Methods

Subjects

A total of 226 primary knee OA patients undergoing diagnostic or therapeutic arthroscopy or total knee replacement in our hospital from April 2010 to November 2012 were recruited. Knee OA was diagnosed based on the American College of Rheumatology published criteria of knee OA [11]. As controls, we enrolled 106 age- and sex-matched volunteers without clinical and radiological evidence of OA. Participants were excluded if they had rheumatoid arthritis (RA), post-traumatic arthritis, previous joint infection, crystal deposition arthritis, enteropathic arthritis, hemophilic arthropathy, acute inflammatory disease, malignant tumor, end-stage renal or hepatic disease, or used corticosteroid medication for pain control. The Ethics Committee of Weifang People's Hospital approved the protocol. All participants gave their informed consent.

Pain evaluation

The pain severity of each patient was evaluated according to the Western Ontario McMaster University Osteoarthritis Index (WOMAC) pain score [12]. The items were scored with the use of a 0–4 rating scale, where 0 represents no pain and higher scores represent greater pain. The psychometric properties of the WOMAC score, including reliability, validity, and responsiveness, are all well established in an OA population [12].

Laboratory examinations

After overnight fasting, venous blood samples were obtained from all subjects. SF samples were obtained from OA patients before the treatment with hyaluronic acid injection or surgery. SF samples were immediately centrifuged at 3000 rpm for 15 min to remove cell debris, and the supernatant was aliquoted into 1.5-mL micro-centrifuge tubes and frozen at –80°C until use. We did not collected SF samples from the controls due to ethical concerns. Serum was obtained by centrifugation at 3000 rpm and then stored at –80°C before measuring. Serum and SF MIF levels were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. All ELISA determinations were performed in triplicate and the results were averaged.

Statistical analysis

Data are presented as mean values ± standard deviation, median and the inter-quartile range, or frequency, as appropriate. The statistical significance of differences between groups was analyzed by parametric tests (unpaired t-test), nonparametric tests (Mann-Whitney U test), or chi square test and Wilcoxon signed rank test for paired samples, as appropriate. Differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis, Kruskal-Wallis analysis, or chi square test, as indicated. Associations between MIF levels and WOMAC pain scores were examined by Spearman's rank correlation and expressed as correlation coefficients. To assess the independent predictors of WOMAC pain scores, multivariate stepwise linear regression analysis was used. SPSS 16.0 for windows (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. A P value <0.05 (2-tailed) was considered as statistical significance.

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Table 1. Clinical characteristics and MIF levels.

	Controls (n=106)	OA patients Total (n=226)	KL grade 2 (n=99)	KL grade 3 (n=74)	KL grade 4 (n=53)
Age (years)	63.61±11.15	64.01±9.49	64.64±10.31	63.86±8.53	63.06±9.27
Female (n,%)	61, 57.55%	139, 61.50%	64, 64.65%	45, 60.81%	30, 56.60%
BMI (Kg/mm^2)	22.43±2.28	23.10±2.04*	23.31±2.24	22.94±1.78	22.96±1.99
MIF in serum (ng/mL)	10.06 (6.60–14.61)	11.93 (5.68–18.10)	12.41 (6.68–16.98)	10.41 (5.30–18.08)	15.09 (6.99–21.40)
MIF in SF (ng/mL)		3.39 (1.87–5.89)	2.73 (1.76–4.31)	3.79 (1.37–8.69)a	4.16 (2.70–6.19)**

All values are expressed as mean value ±SD, median (interquartile range) or n(%). OA – osteoarthritis; KL – Kellgren-Lawrence; BMI – body mass index; MIF – macrophage migration inhibitory factor; SF – synovial fluid. * P<0.01 compared with controls; ** P<0.01 compared with KL grade 2.

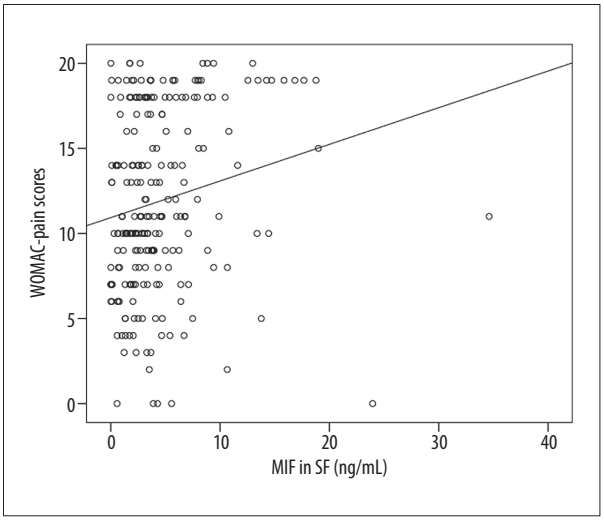

Figure 1. Correlations of MIF levels in SF (ng/ml) with WOMAC pain scores (SF – synovial fluid).

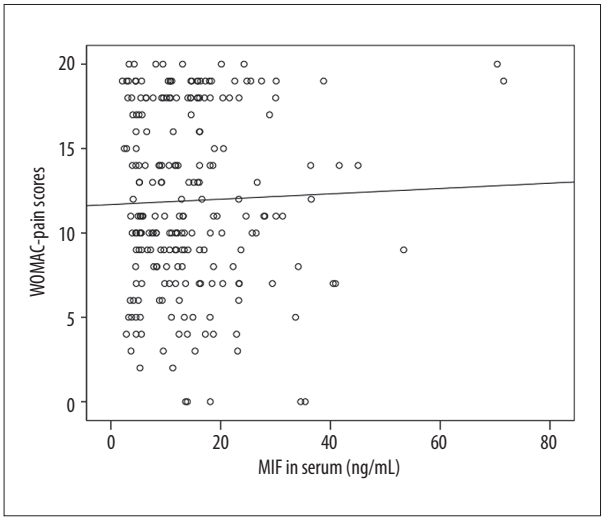

Figure 2. Correlations of MIF levels in serum (ng/ml) with WOMAC pain scores.

Results

Baseline clinical and demographic characteristics

The baseline clinical and demographic parameters of controls and OA patients are displayed in Table 1. OA patients had significantly higher body mass index (BMI) compared to controls. No statistically significant differences were found between the 2 groups in terms of age or sex distribution. In OA patients, clinical and demographic characteristics were further analyzed based on Kellgren and Lawrence (KL) classification [13]. We demonstrated that there were no significant differences in baseline clinical characteristics among OA patients with various radiographic KL grades (P>0.05).

MIF concentrations

OA patients had similar serum MIF levels compared to controls (11.93 [5.68–18.10] *vs.* 10.06 [6.60–14.61] ng/ml, P>0.05; Table 1). In OA patients, MIF levels in SF were dramatically lower than those in paired serum samples (3.39 [1.87–5.89] *vs.* 11.93 [5.68–18.10] ng/ml, P<0.01; Table 1). The correlation between serum and SF MIF levels was not statistically significant (r=0.127, P=0.057). In OA patients with various KL grades, there were no significant differences in serum MIF levels among the subgroups (P>0.05; Table 1). However, OA patients with KL grade 3 and KL grade 4 had significantly higher SF MIF levels compared to those with KL grade 2 (P<0.01; Table 1).

Correlation of MIF levels with WOMAC-pain score

As demonstrated in Figure 1, SF MIF levels were significantly correlated with WOMAC pain scores (r=0.237, P<0.001).

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Table 2. Multivariate linear regression analysis for the determinants of WOMAC-pain scores.

Variables	β	t	P value
Age	0.047	0.670	0.503
Gender	0.047	0.670	0.503
BMI	–0.087	–0.841	0.401
MIF in serum	0.019	0.284	0.777
MIF in SF	0.175	2.626	0.009

Abbreviations are shown in Table 1.

However, there was no significant association between serum MIF levels and WOMAC pain scores in OA patients (r=0.009, P=0.898; Figure 2). Multivariate stepwise linear regression was then used to adjust for potential confounders. The analysis revealed that MIF levels in SF were still the significant determinants of WOMAC pain scores (t=2.262, P=0.009; Table 2).

Discussion

We investigated the correlation between MIF levels in serum and SF and severity of OA-induced pain. We demonstrated that MIF levels in SF but not in serum were correlated with the severity of self-reported pain in knee OA patients. These findings indicate that the increased expression of MIF in arthritic joints may be responsible for the higher pain intensity of OA.

It is now well-known that OA is also a chronic inflammatory process [14]; therefore, pro-inflammatory cytokines have potential as OA-related biomarkers. MIF is a pro-inflammatory cytokine and glucocorticoid-induced immunomodulator, mainly produced by macrophages. Serum MIF levels have been reported to be elevated in several inflammatory disorders, such as coronary artery disease, metabolic syndrome, and rheumatoid arthritis [8,15,16]. In the present study, we demonstrated that OA patients had similar serum MIF levels compared with controls, suggesting that systemic circulating levels of MIF have no relationship with OA. We also revealed that in OA patients, MIF levels in serum were significantly higher than those in paired SF samples, and the correlation between serum and SF MIF levels was not statistically significant. These results suggest that MIF has limited ability to transfer across the synovial membrane due to its molecular weight or complex structure; MIF levels in SF might reflect the intra-articular expression of MIF.

These results are different from those of a study by Liu et al., which reported that OA patients had significantly higher serum MIF levels compared with controls [10]. As we mentioned above, serum MIF level is a sign of inflammatory response in systemic circulation rather than in local arthritic joints. We excluded patients with acute inflammatory disease from our study, but Liu et al. did not [10]. The difference in systemic inflammatory state of the subjects might partially explain the conflicting data. These conflicting data may also be attributable to differences in disease advancement, populations, or assays used. In addition, we demonstrated that OA patients with KL grade 3 and KL grade 4 had significantly higher SF MIF levels compared to those with KL grade 2. This result is in accord with the results of Liu et al., which revealed that SF MIF levels rose as KL grade increased [10].

The most intriguing finding of our study was that SF MIF levels were positively correlated with WOMAC pain scores in OA patients. This correlation was still significant after adjusting for age, sex, and BMI as potential confounders in multivariate linear regression analysis. These results indicate that OA patients with higher SF MIF levels may have more severe self-reported pain. Increasing evidence shows that the over-expressed inflammatory cytokines in the inflamed joints have an important pathophysiological role in the generation and maintenance of OA-induced pain by acting on nociceptive nerve cells [18,19]. MIF is a potent pro-inflammatory cytokine that can induce the release of several inflammatory cytokines, such as interferon (IFN)-γ, interleukin (IL)-1β, 6, 8, and tumor necrosis factor (TNF)-α, through activating an inflammatory cascade [20,21]. Therefore, the pro-inflammatory role of MIF might explain the relationship between MIF levels in SF and pain intensity of OA observed in our study.

Some inherent limitations of this study should be considered. First, the cross-sectional design and relatively small sample size are the major limitations of our study; therefore, it is necessary to repeat this research in prospective longitudinal studies with larger populations. Second, we did not examine the correlation of MIF levels with other inflammatory cytokines, such as IFN-γ, IL-1β, and TNF-α. Further studies examining these correlations could provide more useful information on the potentially pathogenic role of MIF in OA-induced pain.

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Conclusions

MIF levels in SF, but not in serum, were independently associated with the severity of self-reported pain in OA patients. If these results are confirmed by further studies, the inhibition of MIF signaling pathways may be a novel therapeutic approach for ameliorating OA-induced pain.

Conflict of interest

The authors declare that they have no conflicts of interest.

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