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Serum and knee synovial fluid matrix metalloproteinase-13 and tumor necrosis factor-alpha levels in patients with late-stage osteoarthritis

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Objective: The purpose of this study was to compare levels of matrix metalloproteinase-13 (MMP-13) and tumor necrosis factor-alpha (TNF-α) in 2 stages of osteoarthritis, define their predominant pathways, and investigate their correlation with Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores.

Methods: Forty-two patients (mean age: 64±8.8 years) with grade 3 and grade 4 knee osteoarthritis according to Kellgren-Lawrence criteria were enrolled in the study and underwent total knee arthroplasty. TNF-α and MMP-13 levels were measured preoperatively from venous blood samples and intraoperatively from knee synovial fluid via enzyme-linked immunosorbent assay. Preoperative and 1-month postoperative knee functions were assessed by WOMAC.

Results: Grade 4 synovial fluid MMP-13 (4.76±5.82 pg/ml) was elevated compared to grade 3 (3.95±4.45), whereas grade 3 serum MMP-13 (1.128±0.308 pg/ml) was found elevated compared to grade 4 (1.038±0.204) (p=0.438, p=0.430, respectively). Grade 4 serum TNF-α (0.253±0.277) was elevated compared to grade 3 (0.206±0.219), whereas grade 3 synovial fluid TNF-α (0.129±0.052) was elevated compared to grade 4 (0.118±0.014). Positive correlation was observed between synovial fluid MMP-13 levels and postoperative WOMAC scores. Mean serum TNF-α (0.226±0.246 pg/ml) was elevated compared to synovial levels (0.124±1.59), and synovial MMP-13 (4.31±1.24) was elevated compared to serum (1.089±1.519).

Conclusion: Despite the systemic increase in TNF-α levels concordant with osteoarthritis grade, MMP-13 levels are elevated via local manner, with a significant correlation with WOMAC scores.

Keywords: Matrix metalloproteinase; TNF-alpha; knee osteoarthritis.

Level of Evidence: ????

Osteoarthritis (OA) is widely known as a common disease among the elderly which mainly involves the cartilage. However, recent data suggest an important role for subchondral bone and synovial membrane in the pathogenesis and initiation of the disease.[1–3] Furthermore, periarticular and bifocal localized dense bony

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islands are also proven to initiate cartilage erosion and contribute to OA progression.[4] The influence of instability and abnormal tibiofemoral kinematics on joint cartilage loading can initiate complex pathophysiologic mechanisms that lead to cartilage degradation.[5,6] Extracellular matrix (ECM) turnover is regulated by matrix metalloproteinases (MMPs) that degrade essential ECM proteins. MMPs are secreted from macrophages, fibroblasts, and chondrocytes via the stimulatory effect of interleukin-1 beta (IL-1ß) and tumor necrosis factor-alpha (TNF-α). Studies reveal significantly elevated MMP 1, 3, 9, and 13 levels in the subchondral bone, cartilage, and synovial membrane specimens in osteoarthritic patients compared to control groups, and degradation of ECM has been proven to be initiated mainly by MMPs secreted from chondrocytes.[7,8] In addition to MMPs, proinflammatory cytokines such as TNF-α, IL-1ß, and interferon-gamma (IFN-γ) become active, as increase in mechanical stress on joint cartilage can initiate the inflammatory phase.[9–12] TNF-α is known to be the most potent and active cytokine secreted from M1 macrophages in response to IL-1 and bacterial toxins (e.g, lipopolysaccharide).[13–15] In a study by Alaaeddine et al., when compared to nonarthritic tissues, elevated TNF-α receptors were found in chondrocytes and synoviocytes derived from osteoarthritic joints. Chondrocytes derived from osteoarthritic joints also upregulated TNF-α receptors in normal human chondrocytes when incubated together.[16,17] To bind TNF-α receptors, 100 μg ^{99m}technetium human anti-TNF antibody was used, and elevated levels of TNF-α were detected in arthritic joints using scintigraphy in OA and rheumatoid arthritis patients.[18]

Although various proinflammatory cytokines described above have been documented to play a major role in OA pathogenesis, comparison between local/systemic MMP-13 and TNF-α levels in grades 3 and 4 osteoarthritic knees and their relation with Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores have not been investigated to date. In addition to environmental and patient-specific factors, we believe that MMP-13 and TNF-α are the 2 primary mediators in OA progression. The aim of the present study was to compare serum and synovial fluid levels of MMP-13 and TNF-α in 2 grades of the disease, identify their correlation with WOMAC scores, and define their possible predominant pathways (local–systemic).

Patients and methods

Forty-two patients (38 female, 4 male; mean age: 64±8.8 years) with grade 3 and grade 4 knee OA according to Kellgren-Lawrence criteria were enrolled in a prospective interventional clinical study with secondary data analysis. Patients with diabetes mellitus, Addison's disease, or currently taking any medication that could affect the cytokine profile were excluded from the study. Ten patients were previously diagnosed with hypertension and received medical treatment to regulate blood pressure. All patients were hospitalized 2 days prior to surgery for preoperative planning, received subcutaneous low-molecular-weight heparin 12 hours prior to surgery, and were provided the same diet. All patients had a history of sedentary lifestyle due to pain, and none had experienced any kind of sports-related trauma. During their presurgical hospitalization, patients were not allowed to engage in strenuous activity that could affect inflammatory cytokine levels.

All patients underwent single-stage bilateral posterior cruciate ligament-retaining total knee replacement surgery. Preoperative 3-cc venous blood sample was taken from the antecubital vein from each patient immediately before the first tourniquet was inflated. Blood sample collection was synchronized, and all patients were scheduled to undergo surgery at 8:00 AM, with a maximum delay of 5 min. Synovial fluid samples were collected with a needle intraoperatively from each knee after the capsule was exposed. TNF-α and MMP-13 levels were analyzed using enzyme-linked immunosorbent assay (ELISA). Immediately after collection, samples were centrifuged for 10 minutes at 1500 rpm and stored at −80 °C. Collection and storage of the samples were performed using the same protocol for each patient. Preoperative and 1-month postoperative knee functions were assessed by WOMAC score. WOMAC index is compromised of 24 parameters that include pain (score range: 0–20), stiffness (score range: 0–8), and functional impairment (score range: 0–68).[19]

Preoperative and postoperative WOMAC knee function scores from 42 patients were correlated with both serum TNF-α and MMP-13 levels and synovial fluid TNF-α and MMP-13 levels. The significance between serum and synovial fluid MMP-13 levels and serum and synovial fluid TNF-α levels was evaluated separately in grade 3 and 4 patients. The significance between serum and synovial fluid TNF-α and serum and synovial fluid MMP-13 levels was analyzed between grade 3 and grade 4 patients. Statistical comparisons were generated using SPSS software (SPSS Inc., Chicago, IL, USA). All data are expressed as means±SD. Paired sample t-test was used to evaluate preoperative and postoperative follow-up data, whereas Student's t-test was used to analyze the significance of MMP-13 and TNF-α levels at the 2

different grades of OA. Pearson correlation coefficient was used to analyze the correlation between WOMAC scores and variables from TNF-α and MMP-13 levels. A p value less than 0.05 was considered statistically significant.

The current study was approved by the local ethics committee, and informed consent form was obtained from each patient.

Results

In both grade 3 and 4, mean TNF-α levels (0.226±0.246 pg/ml) were found to be significantly higher compared to mean synovial fluid TNF-α levels (0.124±1.59 pg/ml) (p=0.011). Contrary to the above finding, mean synovial fluid MMP-13 levels (4.31±1.24 pg/ml) were found to significantly higher compared to serum MMP-13 levels (1.089±1.519 pg/ml) (p=0.001) (Table 1). Compared to serum, synovial MMP-13 levels were found to be higher in both grade 3 and grade 4, whereas TNF-α was found to be higher in synovial fluid compared to serum in grade 3 but higher in serum compared to synovial fluid in grade 4 (Table 2). Compared to grade 3 (3.95±4.45), grade 4 synovial fluid MMP-13 (4.76±5.82) was elevated, whereas grade 3 serum MMP-13 (1.128±0.308) was elevated compared to grade 4 (1.038±0.204) (p=0.438, p=0.430, respectively). Compared to grade 3 (0.206±0.219), grade 4 serum TNF-α (0.253±0.277) was elevated, whereas grade 3 synovial fluid TNF-α (0.129±0.052) was elevated compared to grade 4 (0.118±0.014) (p=0.548, p=0.363, respectively). There was no significant correlation between preoperative WOMAC score (69.9±7.3), postoperative WOMAC score (40.2±14.5), serum, synovial fluid TNF-α, and serum MMP-13 levels; nor was there a significant correlation between preoperative WOMAC score and synovial fluid MMP-13 levels. Contrary to blood MMP-13 levels, a positive correlation was observed between synovial fluid MMP-13 levels and postoperative WOMAC score (p=0.038, r=0.321) (Table 3).

Discussion

There is now strong evidence that the structural changes

Table 1. Mean serum and knee synovial fluid TNF-α and MMP-13 levels of osteoarthritic patients.

	Serum	Synovial fluid	p
	Mean±SD	Mean±SD	
Tumor necrosis factor-alpha (n=42)	0.226±0.246	0.124±1.59	0.011
Metalloproteinase-13 (n=42)	1.089±1.519	4.31±1.24	0.000

SD: Standard deviation; TNF-α: Tumor necrosis factor-alpha; MMP-13: Metalloproteinase-13.

Table 2. Mean serum and knee synovial fluid TNF-α and MMP-13 levels in grade 3 and grade 4 osteoarthritis classified according to Kellgren-Lawrence system.

Grade	Parameter	Serum	Synovial fluid	p
		Mean±SD	Mean±SD	
Grade 4 (n=24) (57.2%)	TNF-α	0.253±0.277	0.118±0.014	0.046
	MMP-13	1.038±0.204	4.76±5.82	0.01
Grade 3 (n=18) (42.8%)	TNF-α	0.206±0.219	0.129±0.052	0.118
	MMP-13	1.128±0.308	3.95±4.45	0.003

SD: Standard deviation; TNF-α: Tumor necrosis factor-alpha; MMP-13: Metalloproteinase-13.

Table 3. Pre- and postoperative WOMAC index knee function scores and their correlation with knee synovial fluid MMP-13 levels.

	n=42	Synovial fluid MMP-13 (4.31±1.24 pg/ml)
Preoperative WOMAC	r	0.211
(69.9±7.3)	p	0.179
Postoperative WOMAC	r	0.321
(40.2±14.5)	p	0.038

WOMAC: Western Ontario and McMaster Universities Arthritis Index; MMP-13: Metalloproteinase-13.

observed in OA are due to a combination of mechanical factors and biochemical pathways. Despite advancements in molecular studies, the pathophysiology of OA and factors that contribute to the disease progression are still unknown. Experimental studies reveal that there is an ongoing inflammatory process that triggers both anabolic and catabolic events.[20] Recently, several specific mediators such as MMP-13, MMP-3, IL-1ß, IFN-γ, and TNF-α have been distinguished from synovial membrane and joint cartilage to explain the pathophysiological mechanisms leading to OA and to elucidate their potential use in detecting the extent of the disease.[21] A model of human culture of synovial cells from digested osteoarthritis synovium demonstrated that both inflammatory and destructive responses are cytokine-driven through a combination of IL-1 and TNF-α.[22]

Several variables including obesity, diurnal rhythm, diet, medications, collection- storage of samples, time point of collection, activity level, trauma, and gender can affect the levels of these cytokines in patients.[23–25] Patients in the present study were selected in accordance with these criteria, and the collection protocol was standardized to achieve a homogeneous group (with the exception of gender of patients, which could not be standardized). Although IL-1 and TNF-α can be detected in synovial fluid and serum from OA patients, levels of these synovium-chondrocyte-derived mediators vary in different stages of the disease, as demonstrated in the present study. A study conducted with 10 early-stage and 15 advanced-stage OA patients revealed that early and advanced stages of the disease demonstrate different levels of inflammation. Especially in the advanced stages, proinflammatory cytokines such as TNF-α and IL-1ß are highly expressed as a consequence of CD4+ T cell infiltration.[26] Similarly, Aktas et al. concluded that in the advanced stages, MMPs play a key role in the inflammatory process, joint destruction, and blockade of this enzyme by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor may be chondroprotective and decelerate the progression of the disease, as demonstrated in an animal knee OA model.[27]

In the current study, as the grade of the disease advanced, levels of TNF-α in the serum elevated, whereas levels of MMP-13 in the synovial fluid elevated. These findings support the findings that both local and systemic inflammation take place simultaneously during OA progression. Despite limited human studies, animal studies reveal elevated levels of TNF-α and MMP-13 levels in the osteoarthritic group compared to the control.[28,29] Our study shows the failure to compare serum and synovial fluid TNF-α and MMP-13 levels between grades 3–4 and grades 1–2 knees. Vast majority of grade 1–2 osteoarthritic knees are considered to be treated conservatively, thus collection of serum and synovial TNF and MMP13 is not applicable. While JuHee et al. demonstrated that MMP-13 was highly expressed in rats with OA, Masahiko proved that inhibition of IL-1ß and TNF-α in a degenerative OA chondrocyte cell culture decreased MMP-13 and found that the degeneration decreased as a consequence of MMP-13 down-regulation.[30,31] Although these studies highlight the importance of the inhibition of several mediators (IL-1, TNF-α, MMP-13, MMP-3), to date, no specific enzyme has been described to correlate with the grade of OA and WOMAC scores. Data from the current study describe TNF-α and MMP-13 to have the potential to correlate with the grade of the disease and WOMAC scores. Compared with synovial fluid, elevated serum levels of TNF-α in both grades 3 and 4 demonstrate ongoing systemic inflammation during both stages. Elevated synovial MMP-13 levels in grade 4 and elevated serum MMP-13 levels in grade 3 reveal that their pathways are both systemic and local and work simultaneously. Elevated grade 4 serum TNF-α levels compared to grade 3 and elevated synovial fluid TNF-α levels in grade 3 compared to grade 4 show that these proinflammatory cytokines are locally elevated during the early stages, whereas systemically elevated in the advanced stages. In light of these findings, anti-inflammatory cytokine treatment modalities targeting the joint may be effective in the early stages, whereas additional systemic drug usage may be appropriate in the advanced stages. Rutgers et al. concluded that only by inhibiting TNF-α using a serum injection composed of intra-articular anti-inflammatory cytokine can be beneficial over cartilage metabolism.[32] Contrary to this finding, in the current study, non-significant correlation between serum and synovial fluid TNF-α levels and preoperative and postoperative WOMAC indices prove that TNF-α is not the sole factor to impact knee functions. The positive correlation between elevated synovial MMP-13, advanced disease grade, and increased postoperative WOMAC scores show that MMP-13 may be one of the major endopeptidases that contribute to OA in a local manner. Although MMP-13 is known to be secreted from chondrocytes via the stimulation of TNF-α produced from macrophages and T cells, there was no significant correlation between TNF-α and MMP-13 levels in our study. This finding demonstrates that TNF-α alone is insufficient for MMP-13 secretion from chondrocytes, and it is highly possible that various cytokines such as IL-1ß and IFN-γ may have a stimulatory effect.

Compared to synovia, elevated serum TNF-α in concordance with the grade of the disease shows that TNF-α is responsible for systemic inflammation but does not have a major effect on OA and knee functions. As the grade of the disease advances, increase in synovial MMP-13 and serum TNF-α levels become pronounced. Despite systemic increase in TNF-α levels concordant with OA grade, MMP-13 levels are elevated via local manner. Different from TNF-α, although synovial MMP-13 levels were found to be correlated with WOMAC scores independent from the grade of the disease, it must be recognized that various factors such as surgical technique, alignment of the lower extremity, contractures, muscle strength, and accompanying comorbidities strongly influence knee functions. To homogenize the treatment group in terms of these variables will enable further studies to focus directly on the impact of these molecules on knee functions and investigate the use of local anti-MMP-13 and systemic TNF-α as treatment modalities during the early stages of the disease before the vicious cycle begins.

Conflics of Interest: No conflicts declared.

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