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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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ABSTRACT

Objective: To compare the levels of MMP-13 and TNF- α in late stage osteoarthritis, define their predominant pathways and investigate their correlation with McMaster Universities Arthritis Index scores. **Patients and methods:** A total of 42 patients (mean age 64 ± 8.8) with grade 3 and grade 4 knee osteoarthritis according to Kellgren- Lawrence criteria and who were scheduled for total knee arthroplasty were enrolled in the study. TNF-alpha and MMP-13 levels were measured preoperatively from venous blood samples and intraoperatively from knee synovial fluid via ELISA. Preoperative and 1 month postoperative knee functions were assessed by McMaster Universities Arthritis Index.

Results: Grade 4 synovial fluid MMP-13 (4.76 ± 5.82) was elevated compared to grade 3 (3.95 ± 4.45) ($p = 0.438$), whereas grade 3 serum MMP-13 (1.128 ± 0.308) was found elevated compared to grade 4 (1.038 ± 0.204) ($p = 0.430$). 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 level (0.226 ± 0.246 pg/ml) was found higher compared to synovial level (0.124 ± 1.59), synovial MMP-13 level (4.31 ± 1.24) was found higher compared to serum level (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.

Level of evidence: Level IV, Diagnostic study.

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Introduction

Although Osteoarthritis (OA) is generally known to be a common disease among the elderly, mainly involving the cartilage, recent data suggest an important role for subchondral bone and synovial membrane in its pathogenesis and initiation of the disease.^{1–3} Furthermore, periarticular and bifocal localized dens bony 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 start complex pathophysiologic mechanisms that lead to cartilage degradation.^{5,6} Extracellular matrix (ECM) turnover is regulated by matrix metalloproteinases (MMP) that degrade essential ECM proteins. MMP's are secreted from macrophage, fibroblast and chondrocytes via the stimulatory effect of Interleukin-1beta (IL-1 β) and Tumor Necrosis Factor-alpha (TNF- α). Studies reveal significant elevated MMP 1,3,9,13 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 MMP's, secreted from chondrocytes.^{7,8} Besides MMP's, pro-inflammatory cytokines such as TNF- α , IL-1 β , IFN-gama become active as, increase in mechanical stress on joint cartilage can initiate the inflammatory phase.^{9–12} TNF- α is known to be the

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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 conducted by Alaaeddine et al, compared to non-arthritis 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} 100 microgram (99 m) Tc human anti-TNF antibody was used to bind TNF- α receptors and elevated levels of TNF- α was detected in arthritic joints using scintigraphy in OA and Romatoid Arthritis patients.¹⁸

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

Material and methods

42 patients (38 female, 4 male), mean age 64 ± 8.8 years with grade 3 and grade 4 knee OA according to Kellegren–Lawrence criteria were enrolled in a prospective interventional clinical study with secondary data analysis. Patients with DM, Addison disease and under any medication that could effect the cytokine profile were excluded from the study. 10 patients were previously diagnosed with HT with blood pressures regulated with medical treatment. All patients were hospitalized 2 day prior surgery for preoperative planning, received s.c. low molecular weight heparin 12 h before surgery and the same diet. All patients had a history of sedentary life style due to pain, none experienced trauma and were related with any kind of sports activity and during their hospitalization were not allowed strenuous activity that could effect inflammatory cytokine levels prior surgery.

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 just before the first tourniquet was inflated. The time of day the blood samples collected were synchronized and all patients underwent surgery as the first patient 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 10 min at 1500 rpm at the operating room and stored at -80° . Collection, storage of the samples were carried out using the same protocol for each patient. Preoperative and 1 month postoperative knee functions were assessed by WOMAC score. WOMAC index is comprised 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 serum TNF- α , MMP-13 and synovial fluid TNF- α , MMP-13 levels. The significance between serum and synovial fluid MMP-13 levels, serum and synovial fluid TNF- α levels were evaluated separately both in grade 3 and 4 patients. Significance between serum TNF- α , synovial fluid TNF- α and serum MMP-13, synovial fluid MMP-13 levels was analyzed between grade 3 and grade 4 patients. Statistical comparisons were generated using Statistical package for Social Sciences-11 for

Windows programme (SPSS, Chicago,IL,USA). All data are expressed as means \pm 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. P values less than 0.05 were considered statistically significant.

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

Results

In both grade 3 and 4, mean TNF- α levels ($0,226 \pm 0,246$ pg/ml) were found to be significantly higher compared to mean synovial fluid TNF- α levels ($0,124 \pm 1,59$) ($p = 0,011$). Contrary to the above finding, mean synovial fluid MMP-13 levels ($4,31 \pm 1,24$ pg/ml) were found to significantly higher compared to serum MMP-13 levels (1089 ± 1519) ($p = 0,001$) (Table 1). Compared to serum, synovial MMP-13 levels were found to be high both in 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 ($3,95 \pm 4,45$), grade 4 synovial fluid MMP-13 ($4,76 \pm 5,82$) was elevated, whereas grade 3 serum MMP-13 ($1128 \pm 0,308$) was found elevated compared to grade 4 ($1038 \pm 0,204$) ($p = 0,438$, $p = 0,430$). Compared to grade 3 ($0,206 \pm 0,219$) grade 4 serum TNF- α ($0,253 \pm 0,277$) was elevated whereas, grade 3 synovial fluid TNF- α ($0,129 \pm 0,052$) was elevated compared to grade 4 ($0,118 \pm 0,014$). ($p = 0,548$, $p = 0,363$). There was no significant correlation between preoperative WOMAC (69.9 ± 7.3), postoperative WOMAC index (40.2 ± 14.5) and serum, synovial fluid TNF- α and serum MMP-13 levels and also between preoperative WOMAC Index and synovial fluid MMP-13. Contrary to blood MMP-13 levels, a positive correlation was observed between synovial fluid MMP-13 levels and postoperative WOMAC scores ($p = 0,038$, $r = 0,321$) (Table 3).

Discussion

There is now strong evidence that the structural changes, globally observed in OA are due to a combination of mechanical factors and biochemical pathways. Despite the ongoing advanced molecular studies, the pathophysiology of OA and factors that contribute to disease progression is 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-1 β , IFN-gama ve 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 response 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,

Table 1

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

	Serum	Synovial fluid	p
TNF- α (n = 42)	$0,226 \pm 0,246$	$0124 \pm 1,59$	0,011
MMP-13 (n = 42)	1089 ± 1519	$4,31 \pm 1,24$	0,000

Table 2

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

Grade	Parameter	Serum	Synovial fluid	p
Grade-4 n = 24 (%57,2)	TNF-alfa	0,253 \pm 0,277	0118 \pm 0,014	0046
	MMP-13	1038 \pm 0,204	4,76 \pm 5,82	0,01
Grade-3 n = 18 (%42,8)	TNF-alfa	0,206 \pm 0,219	0129 \pm 0,052	0118
	MMP-13	1128 \pm 0,308	3,95 \pm 4,45	0,003

Table 3

Preoperative 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 \pm 1.24 pg/ml)
Preoperative	r	0.211
WOMAC (69.9 \pm 7.3)	p	0.179
Postoperative	r	0.321
WOMAC (40.2 \pm 14.5)	p	0.038

activity level, trauma, gender can effect all the levels of these cytokines in these patients.^{23–25} The patients were selected in concordance with these criteria and the collection protocol was standardized to achieve a homogeneous group except from the gender of patients that could not be standardized. Although IL-1 and TNF- α can be detected in synovial fluid and serum from OA patients, these synovium-chondrocyte derived mediators vary in different stages of the disease as demonstrated in our study. A study conducted with 10 early stage and 15 advanced stage OA patients revealed that early and advanced stages of the disease demonstrates different levels of inflammation. Especially in the advanced stages, proinflammatory cytokines such as TNF- α and IL-1 β are highly expressed as a consequence of CD4+ T-cell infiltration.²⁶ Similarly Aktas et al concluded that during the advanced stages, MMPs play a key role in inflammatory process, joint destruction and blockade of this enzyme by a HMG-CoA reductase inhibitor may be chondroprotective and slow down 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, TNF- α was found to be elevated in the serum whereas MMP-13 in the synovial fluid. These findings supported the information that during OA progression, both a local and systemic inflammation takes place simultaneously. Despite limited human studies, animal studies reveal elevated levels of TNF- α and MMP-13 levels in the osteoarthritic group compared to control.^{28,29} Our study shows the lack to compare serum and synovial fluid TNF- α and MMP-13 levels between grade 3–4 and grade 1–2 knees due to ethical issues. While JuHee et al demonstrated that MMP-13 was highly expressed in rats with OA, Masahiko proved that inhibition of IL-1 β ve 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 point out the importance of the inhibition of several mediators (IL-1, TNF- α , MMP-13, MMP-3), up to date no specific enzyme has been described to be correlated with the grade of OA and WOMAC scores. Data from the current study define 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 grade 3 and 4 conclude that there is an 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 shows that these proinflammatory cytokines are locally

elevated during the early stages, whereas systemically elevated in the advanced stages. In the light of these findings, anti-inflammatory cytokine treatment modalities, targeting the joint may be effective in the early stages whereas additional systemic drug usage seems more logical in the advanced stages. Rutgers et al concluded that, only by inhibiting TNF- α , using a serum injection composed of intraarticular antiinflammatory cytokine can be beneficial over cartilage metabolism.³² Contrary to this finding, in the current study, nonsignificant correlation between serum and synovial fluid TNF- α levels and preoperative, postoperative WOMAC indices prove that TNF- α is not the only factor that has an impact on knee functions. The positive correlation between elevated synovial MMP-13, advanced disease grade and increased postoperative WOMAC scores conclude 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 macrophage and T-cells, there was no significant correlation between TNF- α and MMP-13 levels in our study. This finding certifies that solely TNF- α is not sufficient for MMP-13 secretion from chondrocytes and its highly possible that various cytokines such as IL-1 beta, IFN-gama may have a stimulatory effect.

Compared to synovia, elevated serum TNF- α in concordance with the grade of the disease conclude that TNF- α is responsible for the 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 has to be kept in mind that various factors such as; surgical technique, alignment of the lower extremity, contractures, muscle strength and accompanying comorbidities have strong influence on knee functions. To homogenize the treatment group in terms of these variables can lead to further studies that can focus directly on the impact of these molecules on knee functions and consider using local anti-MMP-13 and systemic TNF- α as treatment modalities during the early stages of the disease before the vicious cycle begins.

Conflict of interest

The authors declare there is no conflict of interest regarding the submission and publication of the manuscript and its potential implications.

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