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ORIGINAL ARTICLE

Serum TNF-alpha levels: potential use to indicate osteoarthritis progression in a mechanically induced model

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Abstract To investigate plasma tumor necrosis factor-alpha (TNF-α) levels as an indicator to reflect the magnitude of the destructive inflammatory phase and articular cartilage damage after a knee trauma. Eighteen mature Wistar Albino male rats were divided into two groups equal in number. Nine animals underwent anterior cruciate ligament transection (ACLT) of the right knees, while nine animals had a sham procedure. All animals were killed at the end of 8 weeks; serum TNF-α levels were analyzed with enzyme linked-immunosorbent assay, and the osteoarthritic changes of articular cartilage were evaluated by a histopathological method using OARSI (Osteoarthritis Research Society International) osteoarthritis cartilage histopathology assessment system score. Serum TNF-α levels and OARSI scores showed significant difference between two groups. Despite 8 weeks after the initial trauma, ACLT group still demonstrated elevated levels of plasma TNF-α indicating the ongoing inflammatory phase. Serum TNF-α levels were also found to be correlated with the OARSI osteoarthritis cartilage histopathology assessment system scores. Post-traumatic local TNF-α overproduction as a proinflammatory cytokine is known to have a major role in cartilage matrix degradation. In this study, elevated plasma TNF-α levels were considered as the consequence of the early local inflammatory response to altered knee biomechanics. Degree of articular cartilage damage found to be consistent with plasma TNF-α levels suggest that monitoring plasma TNF-α levels may be a simple and reliable method to reflect the magnitude of destruction during the ongoing inflammatory phase of OA.

Keywords Tumor necrosis factor-alpha · Knee osteoarthritis · ACL injury

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Introduction

Characterized by progressive loss of articular cartilage that leads to functional restrictions, chronic pain and joint contractures, OA still remains to be a burden for patients and orthopedic surgeons. Although malalignment, muscle strength is known to influence the onset and progression of OA and knee traumas that are not treated adequately are prone to come up with OA [1–3]. In this study, abnormal knee kinematics and alteration in joint loading initiated the proinflammatory cytokine-mediated catabolic pathways and came up with various degrees of osteoarthritic changes in knee cartilage consistent with the current literature. Especially, shear and tangential traction forces on the surface are known to start the catabolic biochemical cascade and upregulate proinflammatory cytokines (TNF-α, IL-1) and proteolytic enzymes such as matrix metalloproteinases and aggrecanases [4, 5]. Among these cytokines, TNF-α has a major role and is able to induce apoptotic cell death, inflammation, and matrix degradation by stimulating proteolytic enzyme secretion from chondrocytes and sinovial fibroblasts. As a consequence of this biochemical disturbance and molecular interaction, the early inflammatory phase of osteoarthritis takes place, and as

an important fact, the magnitude of this inflammatory response is believed to influence further osteoarthritic changes. Detection of the degree of this early inflammatory phase is vital because damage is still reversible and structural changes of the cartilage are minor. Therefore, a reliable and simple indicator reflecting the inflammatory phase and degree of cartilage damage is essential.

Though post-traumatic local biochemical parameters have been measured in various studies, systemic response of proinflammatory cytokine TNF-α and its relation with articular damage are first to be described in this study.

Plasma TNF levels as a consequence of abnormal knee kinematics and the ongoing local inflammation can be of potential use indicating the degree of cartilage damage.

We hypothesize that serum TNF-α levels can reflect the magnitude of the ongoing destructive inflammatory phase of OA after a local trauma, help predict the potential outcomes, and guide us to select the appropriate treatment modality.

Materials and methods

All procedures were in compliance with Turkish Law 6343/2 Veterinary Medicine Deontology Regulation 6.7.26. Study was in compliance with Gazi University Ethical Council regulations and principles of guide for the care and use of the laboratory animals. Approval was obtained from Gazi University Experimental Animals Ethical Council and prior to performing the study. Study was carried on in Gazi University Experimental Animal Research Laboratory.

Surgical procedure

Eighteen knees of 18 Wistar Albino rats were included in the study. In group 1, 9 animals underwent anterior cruciate ligament transection (ACLT) of the right knees via median parapatellar approach to mimic a mechanically induced osteoarthritis (OA). The animals were allowed daily unrestricted cage activity.

In group 2, 9 animals were taken as sham-operated group. The sham operation consists of an arthrotomy only, using the same approach. All animals were killed 8 weeks after surgery. Distal femur and proximal tibia were dissected free from muscle, and articular cartilage was harvested from both femoral condyles and tibial plateaus.

Evaluation of serum TNF-α levels, tissue preparation, histopathology of cartilage

Serum TNF-α levels (pg/ml) were obtained from group 1 and 2 at the end of 8 weeks before killing the animals. Serum TNF-α levels were evaluated using enzyme linked-immunosorbent assay (ELISA) using commercial kits (California, Biosource).

Excisional biopsy specimens of knee were fixed in 10% formalin during 1 week and then were processed in 10% formic acid solution for decalcification. Formic acid solution was renewed in every 24 h, and the consistency of the bone was checked everyday. The bone specimens were cut at 72 h into 2 pieces in coronal plane and embedded in paraffin blocks to be sectioned on their cut surfaces. Three-micron-thick sections were cut on 2 slides and stained with hematoxylin/eosin. The slides were screened by one pathologist, and histopathological assessment was made using the OARSI (Osteoarthritis Research Society International) cartilage OA histopathology assessment system. System is based on 6 grades and 4 stages reflecting the severity and extent of OA over the joint surface. Score is defined as the assessment of combined OA grade and OA stage (grade × stage) [6, 7].

Statistical analysis

Statistical comparisons were generated using Statistical package for Social Sciences-11 for Windows program (SPSS,Chicago,IL,USA). All data are expressed as median (range). TNF-alpha and OARSI histopathological assessment score data were analyzed using a non-parametric Mann–Whitney *U* test. *P* values less than 0.05 were considered statistically significant. Spearman correlation test and linear regression analysis were used to evaluate the relationship between TNF-α and OARSI histopathology scores in groups 1–2.

Results

Although the baseline serum TNF-α levels were undetectable, 8 weeks after the initial local trauma, the systemic inflammatory response was found to be active in both groups and serum TNF-α levels became detectable. Serum TNF-α levels (pg/ml) and OARSI histopathology scores in groups 1 and 2 are presented in Table 1.

Levels of serum TNF-α were found to be significantly higher in the ACLT group compared with those of sham-operated group, supporting the fact of the ongoing inflammatory process in molecular level. Median serum TNF-α levels were 25.1 pg/ml (20.0–55.2) in the ACLT group while 13.4 pg/ml (8.3–24.9) in the sham-operated group (*P*: 0.001).

Compared with the sham-operated group, ACLT group demonstrated higher OARSI scores (*P*: 0.005). Median OARSI histopathology scores were 6.0 (4.0–16.0) in the

Table 1 Serum TNF levels (pg/ml) and OARSI histopathology scores in groups 1 and 2

	Group 1	Group 2	*P* value (Mann–Whitney *U*)
TNF-alpha level (pg/ml)	25.1 pg/ml (20–55.2)	13.4 pg/ml (8.3–24.8)	0.001
OARSI Histopathology scores	6 (4–16)	3 (1–9)	0.005

ACLT group while 3.0 (1–9) in the sham-operated group. Various degrees of matrix discontinuity at superficial zone, vertical, and branched fissures into mid zone (moderate osteoarthritic changes) were observed in all sections in ACLT group compared with the normal cartilage structure of the sham-operated group (Fig. 1a, b).

Serum TNF-α levels in the ACLT group showed statistically significant correlation with OARSI cartilage OA histopathology assessment system scores in the ACLT group, indicating the degree of articular cartilage damage (*P*: 0.011, *R*: 0.792, *R*sq: 0.525). On the other hand, positive correlations were found between same parameters in the sham-operated group but these were not statistically significant (*P*: 0.069, *R*: 0.630, *R*sq: 0.754). Furthermore, statistically significant correlation was found when correlation analysis was performed also on cumulative data (ACLT and sham-operated knees together) (*P*: 0.001, *R*: 0.895, *R*sq: 0.713).

Discussion

Articular cartilage damage has been reported in 23% of knees with an acute ACL injury and 54% of those with chronic ACL laxity. Knee instability and alteration in physiologic loading lead to irreversible structural and functional changes in chondrocytes and extracellular matrix of cartilage in a short period of time as demonstrated in several studies.

Findings suggest that most primary OA diseases have underlying predisposing traumas that are not treated properly such as ACL rupture, ligamentous injury, and meniscal tears that strongly influence disease progression. OA must be considered as a multifactorial disease that also has an important inflammatory component which is usually underestimated by most orthopedic surgeons. OA not only a degenerative disease but also having an ongoing inflammatory phase has been proven in several studies [8–12]. Current studies involve a number of proinflammatory cytokines, receptors, and enzymes that contribute in this complicated degradation process [5]. Known important catabolic mediators of this process include TNF-α, metalloproteinases, interleukin (IL)-1beta, IL-17, and IL-18. Among these, TNF-α controls the homeostasis of matrix synthesis and matrix degeneration in articular cartilage in concert with other cytokines, such as interleukin 1 (IL-1), transforming growth factor β, or insulin-like growth factor1, and has to be focused on [13–15]. Changes of these biochemical parameters after ACL injury in humans have been studied and found to be elevated in synovial fluid [16]. Dysregulation of TNF-α production after trauma has been implicated with OA, and elevated levels of TNF-α have been shown in samples of synovial fluid in ACL deficient human knees. ACL transection has shown to upregulate mRNA levels for TNF-α in MCL scar [17]. Though in studies, TNF-α elevation was local and evident in the first 24 h, and this study reveals that the inflammatory phase is still active in a systemic fashion after 2 months. In most of these studies, instability of the knee is proven to come up with local inflammatory responses and is focused on the local cytokine levels. Local overproduction of TNF-α and IL-1 has shown to direct the balance toward ECM degradation. As a part of the post-traumatic local inflammatory response, we believe that levels of circulating catabolic proinflammatory mediators and proteinases also have vital importance in disease progression. In the current study, we focused on plasma levels of TNF-α as to reflect the degree of post-traumatic inflammatory response and cartilage damage after ACL rupture. The

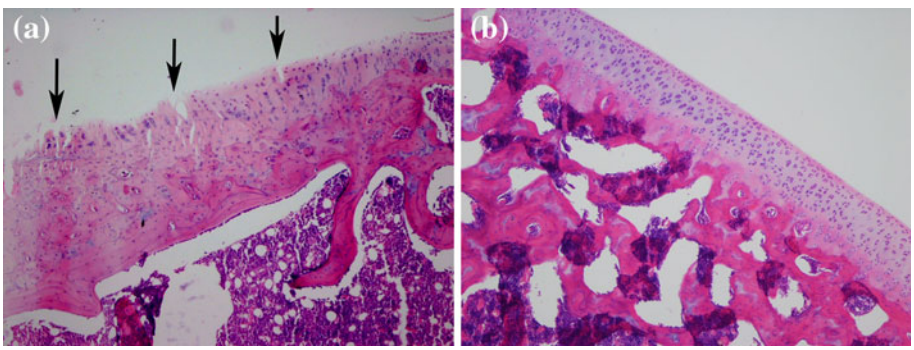

Fig. 1 Micrographs from group 1 (ACLT) showing matrix discontinuity at superficial zone, matrix vertical, and branched fissures into mid zone (*arrows*) (**a**), normal matrix architecture in group 2 (**b**) (Hematoxylin/eosin ×10)

circulating TNF-α is the production of the activated synoviocytes, mononuclear cells, and by articular cartilage itself as a response to instability and alteration of loading but as demonstrated in the current study, detectable elevation in serum TNF-alpha levels require adequate stimulus either mechanical or chemical.

We aimed to promote the systemic post-traumatic inflammatory response mechanically and created an unphysiologic loading and kinematic of the knee joint by transecting the anterior cruciate ligament. We believe that post-traumatic serum TNF-α levels, as part of the systemic inflammatory response, can reflect the ongoing inflammation and are correlated with the degree of matrix degradation and severity of articular cartilage damage.

The real issue is to furtherance the balance between synthesis and degradation of articular cartilage in molecular level to achieve favorable results after trauma. Based on this idea, novel treatment strategies are focused on anticytokine therapy [18, 19]. Though these treatment options have proven to have beneficial effects on disease progression, the main idea is to be aware of the magnitude of the degradative process that takes place in the molecular level during the inflammatory phase. Monitoring systemic proinflammatory cytokine levels such as TNF-α can guide us for the course, and the magnitude and duration of this inflammatory phase help us predict the possible outcomes of the disease in an early manner and help choose the appropriate treatment option. Since the goal is to be aware of the magnitude of the early inflammatory phase and its proven negative effects on articular cartilage, measuring serum TNF-α levels can be of value as a simple and reliable method. To predict the degree of the inflammatory phase and related cartilage damage in molecular level with a simple blood test after knee trauma for each individual can offer the advantage to focus on preventive measures for OA progression. Furthermore, the selected therapeutic efficacy of treatment modalities during the inflammatory phase can also be evaluated by screening plasma TNF-α levels in each patient.

Conflict of interest No funds were received in support of this study. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

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