ACTA
ORTHOPAEDICA
et
TRAUMATOLOGICA
TURCICA

Author's translation
Acta Orthop Traumatol Turc 2009;43(3):214-218
doi:10.3944/AOTT.2009.214

Evaluation of intra-articular collagenase, TIMP-1, and TNF-α levels before and after anterior cruciate ligament reconstruction

Ön çapraz bağ cerrahisinde eklemiçi sıvıda ameliyat öncesi ve sonrası kollajenaz, TIMP-1 ve TNF-α düzeyleri

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Amaç: Bu çalışmada, ön çapraz bağ (ÖÇB) cerrahisinden sonra kemik-tendon iyileşmesinde etkili olduğunu düşündüğümüz kollajenazların, bu kollajenazların salınımını düzenleyen sitokinlerden olan tümör nekroz faktör-alfa (TNF–α) ve kollajenaz enzimlerinin doğal inhibitörü olan TIMP-1 enzimi düzeyleri sinovyal sıvıda araştırıldı.

Çalışma planı: Çalışmaya, izole ÖÇB cerrahisi uygulanan hastalar arasından, ameliyat sonrası takiplerde diz ekleminde efüzyon gelişen ve bu nedenle ameliyat sonrası artrosentez yapılan 16 hasta (15 erkek, 1 kadın; ort. yaş 27; dağılım 17-40) alındı. Bu hastalardan ameliyat sırasında ve sonrasında alınan sinovyal sıvı örneklerinde kollajenaz, TNF–α ve TIMP-1 düzeyleri uygun kitler kullanılarak ölçüldü. Ön çapraz bağın kopması ile ameliyat arasında geçen süre ortalama 21±10 ay (dağılım 1-72 ay), ameliyattan sonra artrosentez sırasında eklemiçi sıvı alma için geçen süre ortalama 18 saat (dağılım 12-36 saat) idi.

Sonuçlar: Ameliyat sırasında ve sonrasında alınan eklemiçi sinovyal sıvı örneklerinde ölçülen değerler kollajenaz için sırasıyla 1.49±0.06 ng/ml ve 1.45±0.05 ng/ml, TIMP-1 için 12±5 ng/ml ve 22±9.5 ng/ml, TNF-α için 10.4±7.1 pr/ml ve 14.11±6.1 pr/ml bulundu. Kollajenaz ve TNF-α açısından ameliyat sonrasındaki değişimlerde anlamlı farklılık görülmedi (sırasıyla, p=0.098 ve p=0.069); TIMP-1 düzeyindeki artış ise anlamlıydı (p=0.026).

Çıkarımlar: Çalışmamızdaki dizlerde ÖÇB kopuk olduğundan, TNF-α, kollajenaz, TIMP-1 düzeyleri yüksek bulunmuştur. Verilerimiz bu değerlerin ameliyat sonrası yakın dönemde nasıl değişim geçirdiğini göstermiştir.

Anahtar sözcükler: Ön çapraz bağ/yaralanma; matriks metalloproteinazları; diz eklemi; sinovyal sıvı; metalloproteinaz doku inhibitörü; tümör nekroz faktörü-alfa.

Objectives: We investigated intra-articular levels of collagenase, which presumably promotes bone-tendon healing, and collagenase mediators involved in its production (tumor necrosis factor-alpha, TNF-α) and inhibition (TIMP-1 enzyme) in patients following anterior cruciate ligament (ACL) reconstruction.

Methods: The study included 16 patients (15 males, 1 female; mean age 27 years; range 17 to 40 years) who underwent arthrocentesis due to effusion that developed following reconstruction of isolated ACL injuries. Intra-articular levels of collagenase, TNF-α, and TIMP-1 were measured using appropriate activity assay and immunoassay kits in synovial fluid samples obtained intraoperatively and during arthrocentesis. The mean time from ACL injury to surgical repair was 21±10 months (range 1 to 72 months). Arthrocentesis was performed in a mean of 18 hours (range 12 to 36 hours) following ACL repair.

Results: Measurements in synovial fluid samples obtained intra- and postoperatively yielded 1.49±0.06 ng/ml and 1.45±0.05 ng/ml for collagenase, 12±5 ng/ml and 22±9.5 ng/ml for TIMP-1, and 10.4±7.1 pr/ml and 14.11±6.1 pr/ml for TNF-α, respectively. Postoperative changes in the levels of collagenase (p=0.098) and TNF-α (p=0.069) were not significant, whereas increase in the TIMP-1 level was significant (p=0.026).

Conclusion: This study showed elevated levels of TNF-α, collagenase, and TIMP-1 due to the presence of ruptured ACL. Our findings showed how these levels changed in the acute postoperative period.

Key words: Anterior cruciate ligament/injuries; matrix metalloproteinases; knee joint; synovial fluid; tissue inhibitor of metalloproteinases; tumor necrosis factor-alpha.

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Submitted / *Başvuru tarihi:* 16.08.2008 **Accepted / *Kabul tarihi:*** 06.03.2009

Recently, important researchs have been published in order to evaluate tunnel-tendon healing in anterior cruciate ligament (ACL) reconstructions. These studies include evaluating biologic process and its agents affecting the healing after placing and fixation of the graft.[1]

It has been well established that sinovial fluid between the tendon-bone interface can affect the healing and incorporation by biologic agents and enzymes.[2,3] Most important agents affecting this healing porcess are members of the matrix collagenase (MMP1,MMP8,MMP13). The natural inhibitor of these enzymes is tissue metalloproteinase inhibitor (TIMP-1). There is an equilibrium between these matrix collagenases and their inhibitor.[4]

In the present study, we quantitatively evaluated the collagenase levels, which we believe that they may have an effect on tendon-bone healing, at intrarticular synovial fluid at the early post-operative period after ACL reconstruction. For this purpose we also measured levels of tumor necrosing factor alfa (TNF-α) and TIMP-1in intraarticular synovial fluid pre and post-operatively.

Materials and method

In this study, 16 patients, who underwent isolated ACL reconstruction, were included. Post-operatively arthrocentesis was performed in all patients as they had post-operative knee effusion. Study was conducted using intrarticular fluid of these knees. One of the patient was femal and the 15 were male. Avarage age of the patients was 27 years (range; 17-40 years). The avarage time between ACL rupture and the time of the surgery was 21±10 months (range; 1-72 months).

Pre-operative synovial fluid was obtained at the of artroscopic procedures taking all measure for sterilization. Synovial fluid samples were centrifuged at 10.000 rpm and stored at -80C°. Same procedures were performed for synovial fluids obtained post-operatively.

Collagenase enzyme levels were measured by Cheemican International Type I Collojenase Activity Assay Kit and Cheemican International Type I Collojenase (MMP-8) Activity Assay Kit. TIMP-1 levels were measured by Cheemican International Human TIMP-1 Immunassay Kit. TNF-α levels were measured by Biosource International Human TNF-α Immunassay Kit.

After measurements were completed pre-operative and post-operative results were compared statistically. Wilcoxon, NPar, and Ki-square tests were used for statistical analysis.

Results

Pre-operative and post-operative average level of collagenase enzymes in synovial fluids was 1,49 ± 0,06 ng/ml (range; 1,34 -1,50), and 1,45 ng/ml + 0,05 (range; 1,41 - 1,50) respectively (Figure-1). The difference was not statistically significant (P=0.098). In 11 knees collagenase enzyme level increased and decreased in 5 knees. This difference was not statistically significant (P>0.05).

Pre-operative and post-operative average level of TIMP-1 in synovial fluids was 12 ± 5 ng/ml (range; 2,4- 55,9) and 22 ± 9.5 ng/ml (range; 3,6-34,5) respectively (Figure-2). The difference was statistically significant (P=0.026). In 12 knees inhibitor enzyme level increased and decreased in 4 knees. This difference was not statistically significant (P>0.05).

Pre-operative and post-operative average level of TNF-_ in synovial fluids was 10,4 ± 7,1 pr/ml (3,1-28,1) and 14,11± 6.1 (3,1-58,1) respectively (Figure-3). The difference was not statistically significant (P=0.069). In 7 knees TNF-α levels increased and decreased in 7 knees whereas there was no change in 2 knees.

Discussion

Collagenase enzymes (MMP8, MMP1, MMP13) and their natural inhibitors TIMP-1 have been discussed at large in literature published for osteoarthritis and cartilage damage.[5-8] There is a potential risk of osteoarthritis after ACL rupture. Biomechanical instability and high concentration of inflamatuary agents and MMP-TIMP in intraarticular synovial fluid are belived to be responsible for the process. We compared the pre-operative and 18 hours post-operative

collagenase enzym levels in synovial fluid and did not detected any significant change. Also in the present study we measured TNF-α which is responsible for the release of collagenase enzymes and TIMP-1 levels demonstrated that TIMP-1 increased after ACL reconstruction. In our best knowledge we Are not aware of any study measuring TIMP-1 level post-operatively in synovial fluid.

Higuchi et.al searched the biochemical effects of synovial fluid in knees with ACL rupture. In their study they reported that after ACL rupture MMP3 and TIMP levels both increased but equilibrium between TIMP and MMP3 was disturbed in favor of MMP3. They concluded that increase in IL-6 is responsible for this unequilibrium.[10]

MMPs, under strict regulation of TIMP-1, play a big role in soft tissue regeneration which maintains tissue matrix turnover. This control mechanism is accomplished by 1:1 enzyme-inhibitor complexMMPs play dominant role during destruction phase of connective tissue remodelling whereas TIMP-1 is active in the restoration pahse in order to control proteolytic activity.[11-12]

MMPs are released from chondrocytes, fibroblasts, leukocytes, and synovial cells. Postoperative effusion is mainly hemorrhagic. It takes time for the effusion to be inflamatuary and to maintain cells potential for MMP source. Regarding this, in our study time for obtaining synovial fluid samples post-operatively may not be long enough This may explain why MMP increase was not significant in this study. In animal study it was reported that synovial fluid reaction becomes evident 13 weeks after cutting ACL.[14]

The pathology in the synovial membrane after ACL rupture may lead joint instabiltiy and joint surface irregularity which aggravates after release of biochemical mediators.

After ACL reconstruction synovial fluid containing cytokines, MMP, and TIMP counteracts with greft healing. Depending on the surgical technique graft healing inside tunnel occurs as tendon-bone or bone-bone. In tendon-bone healing synovial fluid runs between the graft and tunnel and negatively affects the healing which also may lead to tunnel enlargement(synovial bath effect).

Cameron et.al reported an increase in inflamatuary cytokines (TNF-α) and interleukines in synovial fluid after ACL rupture. These biologic agenst are well kown to enlarge the greft tunnel and bone resorption by inducing the osteoclastic activity.[15] Zysk et.al aimed to demonstrate the effects of biologic agents on tunnel enlargement by studying the cytokine concentration in synovial fluid and they observed an association between tunnel enlargement and increase in cytokine concentration 7 days after surgery.[16]

In animal studies and in revision ACL reconstruction surgeries tendon-bone healing has been shown to develop with a fibrovascular tissue at the beginning and then proceed with sharpey like fibers.[17] These different healing tissues may counteract with MMPs and be affected negatively.[2] Reviewing the results of our study under the light of data from the literature; it can be considered that increase of TIMP-1related to the increase of MMPs, helps to supress the negative effects of proteolytic activity. Also, we measured only the active MMPs in the present study which may explain nonsiginificant increase of MMPs. The acting mechanism fo the collagenases occurs by their shortly active forms being tied to the related tissues In this regard, it is necessary to measure active form of collagenase enzymes or to observe biologic reaction when they are blocked in order to prove their existence in synovial fluid.

TNF-α is one of the important inflamatuary cytokines which ,also induces MMP release. El Said et.al reported that TNF-α levels increased in the synovial fluid after ACL rupture in the early posttraumatic period. They stated increased TNF-α level is responsible for a decrease in lubricin level.[18] Irie et.al stated that inflamatuary cytokines increases significantly in the first 24 hours after ACL rupture and aimed to follow decrease pattern afterwards.[19]

However in the study of El Said it is reported that these cytokines kept their high levels for 6

months. In this study average time between ACL rupture and the surgery is 1 month. In this period TNF-α is supposed to decrease and come to its normal level in the synovial fluid. However reviewing the level of TNF-α in this study it can clearly be seen that inflamatuary process continues which explains the high levels of TNF-α in synovial fluid. As ACLs were ruptured in our study, levels of TNF-α and MMP, TIMP were found to be high. However these levels may decrease or increase in the hemorrhagic and partially inflamatuary nature of synovial fluid. This makes our study meaningfull.

In conclusion, we detected an increase in TIMP-1 levels in the present study. However, we did not find significant increase in MMPs and TNF-α levels and were not able make any correlation between them. This can be explained that measuremenst were made in the early post-operative course. It can be proposed to measure these biologic agents's level in synovial fluid at late post-operative period with advanced methods.

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