Matrix Metalloproteinase Levels as a Marker for Rotator Cuff Tears

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Abstract

Although many studies report the role of matrix metalloproteinases (MMPs) in rotator cuff tears, a paucity of data exists correlating the clinical severity of the disease with the implicated MMP levels. The purpose of this study was to investigate and compare the levels of expression of MMP-1, -3, -9 and -13 in patients with rotator cuff tears. We hypothesized that patients with clinically worse symptoms as measured by a standardized pain and function scale would have a higher expression of MMPs.

Rotator cuff specimens were obtained from 16 consecutive patients undergoing rotator cuff repair. Total protein was extracted from these specimens and quantified. Equalized total protein extracts were used for performing enzyme-linked immunosorbent assay for quantitative determination of MMP-1, -3, -9 and -13. Preoperatively, the University of California, Los Angeles (UCLA) Shoulder Rating Scale was administered to each patient. Statistical comparisons were performed using analysis of variance. The expression of MMP-13 was notably increased in the rotator cuff extracts of all patients (P < 0.02). In addition, MMP-13 levels showed a significant proportional correlation with the patient pain score as per their UCLA ratings (r = 0.5). Although higher MMP-9 levels were assayed, this was not statistically significant. Expression of MMP-1 and-3 was insignificant.

Our data suggest a critical role for MMP-13 in rotator cuff tears; elevated levels are a possible indicator for an impending tear. Further studies with increased sample size are warranted to prove the possible use of MMP-13 as therapeutic targets that may be inhibited by anti-inflammatory agents to limit disease progression.

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Rotator cuff tears are a common cause of disability in adults. Decreased arm range of motion (ROM) combined with pain results in functional impairment. Untreated tears can progress to cuff tear arthropathy, causing further functional impairment.

Rotator cuff tears can be caused by trauma or overuse, with an increasing incidence of injury occurring in patients older than 60 years. Underlying processes, such as diminished blood supply with aging, may be involved in making these patients more susceptible to rotator cuff tears. Studies have also shown microvascular changes in the rotator cuff as patients age. Additional studies have been performed on the role of an imbalance in degradation and regeneration of normal rotator cuff tissue, in which peptides known as matrix metalloproteinases (MMPs) play an important role.

The families of zinc-dependent proteases known as MMPs are important for the maintenance and repair of tendons. They collectively degrade components of the tissue extracellular matrix and play an important role in the morphogenesis and remodeling of organs in normal and pathological states. In many pathological conditions, an imbalance exists between the synthesis and degradation of the matrix, leading to net tissue degradation. The important role of MMPs in connective tissue turnover has been the focus of several studies reporting a correlation between increased MMP activity and matrix degradation, representing part of the remodeling process in wound healing.

Studies have identified the potential role of these enzymes in tumor growth, vascular aneurysmal disease, embryonic development, and tissue remodeling, and many studies have reported elevated MMP levels in patients with rotator cuff tears. Yoshihara et al and Osawa et al reported elevated levels of MMP-1, MMP-3, and glycosaminoglycans in the synovial fluid of patients with massive rotator cuff tears. Gotoh et al found MMP-1 mainly in the granulation tissue of the torn supraspinatus tendon, and Blaine et al reported an increase in expression of MMP-1 and MMP-9 in bursa specimens of patients with rotator cuff tears. Tillander et al downplayed the role of MMP-1 in the pathogenesis of rotator cuff tears and implicated fibronectin, whereas other studies associate MMP-13 with signs of degeneration. Thus, because MMPs play a critical role in maintaining the dynamic homeostasis and integrity of the extracellular matrix, loss of this balance resulting in elevated MMP activity has been associated with numerous pathologic conditions of connective tissue, including degenerative tendinopathy and rotator cuff tears.

In patients with increased activity and age—and, as a result, more rotator wear—the presence of certain MMPs subsequently increased, indicating a relationship between MMPs and rotator cuff wear. Although the responsible MMPs have not been identified, studies have reported the possible association of MMPs in rotator cuff tears. Therefore, the role of MMPs in rotator cuff injury and disease cannot be downplayed.

We hypothesized that patients with full-thickness rotator cuff tears and presenting with clinically worse symptoms as measured by a standardized pain and function scale would have a higher MMP expression. We measured the levels of MMP-1, -3, -9 and -13 in torn rotator cuff samples and performed a quantitative comparison of the MMPs, correlating them with the patients’ functional impairment and pain level as measured by their University of California, Los Angeles (UCLA) shoulder score.

**Total Protein Extraction**

Total protein was extracted from the samples by grinding and homogenizing the rotator cuff tissue in a mammalian protein extraction reagent. It was quantified by Bradford’s technique using a protein assay kit as per the manufacturer’s instructions (Thermo Fisher Scientific Inc, Rockford, Illinois). Briefly, the quantitative level of protein in each sample was determined by a microassay mixing 100 µl of tissue extract, buffers, and Coomassie plus reagent in microplate wells and measuring the absorbance at 595 nm on a spectrophotometer (Packard Inc, Meriden, Connecticut). The concentration of protein in the samples was determined from a standard curve plotted using an array of standard protein solutions made with bovine serum albumin.

**Quantitative Determination of MMPs**

The MMP levels in the patient tissue sample extracts were determined by enzyme-linked immunosorbent assay. The protein concentrations were equalized in all samples before determining the MMP levels. This was accomplished by tabulating the protein concentrations in all samples, then equalizing the concentrations with enzyme-linked immunosorbent assay diluents.

Human MMP-1, -3, -9 and -13 enzyme-linked immunosorbent assay kits (RayBiotech, Norcross, Georgia) were used to detect the levels of MMP-1, -3, -9, and -13. The families of MMPs were predicted to be present in rotator cuff disease; therefore, additional studies have been performed on the role of an imbalance in degradation and regeneration of normal rotator cuff tissue, in which peptides known as matrix metalloproteinases (MMPs) play an important role. They collectively degrade components of the tissue extracellular matrix and play an important role in the morphogenesis and remodeling of organs in normal and pathological states. In many pathological conditions, an imbalance exists between the synthesis and degradation of the matrix, leading to net tissue degradation.
-9 and -13. Standards and samples were pipetted into the wells, followed by incubation for 2.5 hours to enable the MMP in the sample to be bound to the immobilized specific MMP antibodies in the wells. The wells were washed, and biotinylated antihuman specific MMP antibody was added and incubated for an hour. The unbound biotinylated antibody was washed, and horseradish peroxidase–conjugated streptavidin was added to the wells and incubated for 45 minutes. The wells were washed again, and a tetramethylbenzidine substrate solution was added to the wells and observed for the development of color in proportion to the amount of MMP bound. Following incubation for 30 minutes, the stop solution was added to obtain a color change from blue to yellow. Subsequently, the intensity of the color was measured by a spectrophotometer at 450 nm.

This procedure was followed for the individual detection of MMP-1, -3, -9, and -13. Following the analysis of the MMP concentrations via a microplate reader, the MMP concentrations were tabulated and plotted against the corresponding patient UCLA shoulder pain score for further correlation analysis.

Statistical Analysis
Statistical comparisons were performed with analysis of variance (ANOVA) using SPSS version 16.0.2 software (SPSS, Inc, Chicago, Illinois). Significance was set at \( P < .05 \).

RESULTS
Levels of MMP-1, -3, -9, and -13 by Enzyme-linked Immunosorbent Assay
Among the MMPs tested, elevated levels of MMP-13 were noted for all samples (Figure 1). The expression of MMP-13 was statistically significant \( (P = .02) \). A noticeable increase in the levels of MMP-9 was also observed, but the levels were lower when compared with MMP-13 and were not statistically significant. MMP-1 and -3 showed insignificant expression levels.

Correlation of MMP-1, -3, -9, and -13 With UCLA Shoulder Rating Scale
The expression of MMP-13 in the rotator cuff samples showed a significant proportional correlation with the pain score reported by patients according to the UCLA Shoulder Rating Scale \( (r = −0.53) \) (Figure 2). The relationship of MMP-9 was almost proportional but was not statistically significant (Figure 3). No direct relationship was observed between the levels of pain exhibited in the patients and the levels of MMP-1 and -3 in their rotator cuff specimens.

DISCUSSION
Various studies have investigated the role of MMPs in the pathogenesis of rotator cuff tears and provided evidence for its association in the degradation of tendon tissue. However, a quantitative determination and comparison of the concentrations of the associated MMPs in torn rotator cuff samples has not been reported. In the current study, we analyzed tissue rotator cuff specimens taken from around an observed tear in 16 patients prior to repair. This was performed to compare the levels of MMP-1, -3, -9, and -13 and to test the possibility of an elevated MMP level as a marker for the severity of clinical symptoms.

The correlation of the severity of pain and functional impairment was rated by the UCLA Shoulder Rating Scale, which
has demonstrated good internal consistency when compared with the gold-standard Shoulder Pain and Disability Index. A score >27 is considered excellent, and a score <27 is fair or poor. The score is derived from the patient’s self-reported level of pain, upper-extremity function, and physician assessment of ROM. To determine how MMPs affect rotator cuff injury and patient pain, the UCLA shoulder values were plotted against the MMP values extrapolated off the standard graph for each of the samples. The enzyme-linked immunosorbent assay demonstrated that among the MMPs tested, the concentration of MMP-13 was significantly high when compared with the others. A significant relationship was also found between increased levels of MMP-13 and UCLA shoulder score. This relationship could be indicative of the role of MMP-13 in a rotator cuff injury and their elevated levels as a possible indicator for an impending tear. Matrix metalloproteinase-1, -3, and -9 showed no direct correlation with UCLA score.

Potential limitations of this study include the amount of tissue sampled and the fact that variable concentrations of protein could be present in different parts of the cuff. Although the samples were taken from the leading edge of the tear in all patients, the degree of injury was not defined for the study. Variability may have existed in the tears and in the amount of degenerative changes they underwent. To account for this, we quantified the total protein content present in each rotator cuff extract by Bradford’s technique using a protein microassay kit. The results showed varied amounts of protein concentration between the samples. To control for this variable, the dilution factor necessary to equalize the protein content in each sample was calculated. This was followed by the addition of the calculated amounts of the enzyme-linked immunosorbent assay diluents to each sample to obtain the equalized samples for the quantitative determination of MMPs.

Normal healthy tendons are mostly composed of parallel arrays of collagen fibers, and the collagen portion is made up of 65% to 80% type I collagen, with small amounts of other types of collagen and elastin. These include type II collagen in the cartilaginous zones, type III collagen in the reticulin fibers of the vascular walls, type IX collagen, type IV collagen in the basement membranes of the capillaries, type V collagen in the vascular walls, and type X collagen in the mineralized fibrocartilage near the interface with the bone.

A likely explanation for our results lies in substrate specificity of the various MMPs. In the MMP family, subdivisions exist based on the substrate preference of each enzyme. The collagenases contain 3 members: fibroblast collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13). The collagenases are the primary proteinases responsible for cleavage of the triple helix fibrillar collagens into 3 quarters. Similarly, the stromelysins contain 3 members: MMP-3, MMP-10, and MMP-11. These enzymes are known to have a broad range of substrate specificity.

Matrix metalloproteinase-13 plays a role in tumor invasion and extracellular degradation. It is classified as a collagenase that breaks down collagen I, II, and III in the extracellular matrix. This renders them agents of extracellular matrix degradation, causing injury and damage to the tissue. Although MMP-1 is also specific for collagen types I, II, and III, MMP-13 cleaves gelatin 40 times more efficiently than MMP-1 and may be further involved in the degradation of collagen. This helps explain our results demonstrating the dominance of MMP-13 acting on its specified substrate collagen I, which is present abundantly in the extracellular matrix of the rotator cuff tissue. The low levels of expression of other MMPs are likely the result of the unavailability of the specified substrate. Matrix metalloproteinase-3 degrades fibronectin; laminin; collagen types III, IV, IX, and X; and cartilage proteoglycans. Matrix metalloproteinase-9 degrades collagen types IV and V. Although MMP-9 shows some relationship with UCLA shoulder score, the levels are lower when compared with MMP-13. Therefore, for indicative roles in rotator cuff injury, MMP-13 seems to be a more viable option.

Our study validates the findings of Tillander et al, who reported that MMP-1 was found infrequently in patients with rotator cuff damage and pain. This was demonstrated by immunolocalization of MMP-1 on the rotator cuff tissue. Lo et al obtained tissue samples from 10 patients with variable rotator cuff tissue damage. To survey for certain proteins, they used reverse transcription polymerase chain reaction and found that the presence of MMP-13 was increased in patients with more rotator cuff tissue damage. The results of these studies are concordant with our data for MMP-1 and MMP-13.

Tissue inhibitors of metalloproteinase (TIMPs) are also present in the extracellular matrix. These are the natural endogenous inhibitors of the MMPs, and studies show that they can inhibit all MMPs in vitro. They provide a checks-and-balances system to modulate the reparative and degradative processes and thus are responsible for maintaining the dynamic homeostasis of the extracellular matrix. Four TIMPs have been identified in humans. The relative balance between the MMPs and TIMPs is thought to play an essential role in development, morphogenesis, tissue remodeling, and disease processes such as rheumatoid arthritis and osteoarthritis. Hence, the identification of TIMPs and their action to curb the MMPs and restore balance may be an area of study to which our findings could be directed.

Bedi et al reported distinct and better histologic differences on local delivery of a universal MMP inhibitor on tendon-to-bone surface healing after acute rotator cuff repair in a rat model. They reported that the biologic modulation of endog-
enous MMP activity to basal levels could reduce pathologic tissue degradation and favorably influence healing after rotator cuff repair. Recent investigations with tetracyclines and tetracycline derivatives, such as doxycycline, show that in addition to their antibiotic properties, these can also inhibit MMP activity. In vitro, these appear to have the ability to disrupt bacterial biofilms, which are often present in chronic wounds. Although studies and clinical trials on drugs that inhibit MMPs are being performed, these data suggest that the important targets in preventing the destruction of the extracellular matrix are the MMPs, which can be inhibited by endogenous and synthesized inhibitors.

**CONCLUSION**

Our study demonstrates the critical role of MMP-13 in rotator cuff tears; elevated levels are a possible indicator for an impending tear. Although our study lacked a control group to correlate the elevated MMP-13 levels in patients with severe rotator cuff tears, the data demonstrated the potential of high MMP-13 levels as an indication of the clinical severity of the rotator cuff tear. Further studies with an increased sample size, cadaveric controls, and the use of endogenous or synthetic inhibitors could prove the possible use of MMP-13 as a therapeutic target to inhibit or decrease tear progression and possibly influence healing response after rotator cuff repair.

**REFERENCES**