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Biochemistry Of Ligamentous Injury

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Abstract
Ligament injuries have multiple causes. The most usual injury mechanism in sports is abrupt tensile loading that exceeds the strength limit of the ligaments. Injuries should be accurately diagnosed and properly managed in the acute phase to facilitate favorable healing. Different ligaments have unique healing potentials, and for those that have poor natural healing potential, surgical repair or reconstruction may be indicated. Previous studies have revealed that the biomechanical performance of ligament grafts is inferior to normal tissue even with long-term follow-up. Prolonged immobilization of joints has a detrimental effect on the physical properties of ligaments and it takes a long time to recondition ligaments after immobilization. Appropriate exercise training has been shown to strengthen ligaments, and there is evidence to suggest that therapeutic ultrasound or laser can hasten ligament healing after injury, but the optimal parameters for these treatments are yet to be determined.

Keywords: Ligament, injury, healing, reconstruction, physiotherapy

1. Introduction
The natural course of the anterior cruciate ligament (ACL) deficient-knee has been well documented. Injury to the ACL often leads to the development of degenerative osteoarthritis (OA) [7, 8]. In addition, patients with ACL injury and posttraumatic OA are on average 15–20 years younger than patients with primary OA when they become symptomatic [5, 7]. Restoring knee-joint stability through ACL reconstruction has not been shown to decrease the incidence of posttraumatic OA in this patient population. However, some reports indicate that 50–60% of patients with ACL-reconstructed knees have radiographic evidence of OA after five years [1, 3]. These data suggest that something other than, or in addition to, a biomechanical disturbance is responsible for the osteoarthritic changes seen after ACL rupture. Various cytokines are involved in the aetiology of rheumatoid arthritis and osteoarthritis, and clinical trials using cytokines as arthritis markers for the staging of diseases have been performed [9]. Cartilage destruction progresses due to the activation of various proteases, and matrix metalloproteinases (MMPs) are key enzymes in cartilage destruction Studies concerning interactions between various cytokines and MMPs have also been carried out, and the mechanisms responsible for cartilage destruction and its prevention are being clarified. However, few reports [11, 12] exist concerning the concentrations of MMPs and tissue inhibitors of metalloproteinase (TIMP) in human knees with ACL injury, and there has been no study on their relationships with cytokines. The purpose of this study was to investigate the biochemical characteristics of human knees with ACL injury, including the concentrations of cytokines, MMPs, and TIMP in synovial fluid (SF), and to evaluate their relationships with each other. We also studied the relationships of their concentration profiles with the time after ACL injury to evaluate the appropriate timing for the treatment of ACL injury based on biochemical parameters.

Over the past five decades of experience in the management of extremity vascular trauma, a number of factors have been identified which directly influence patients' outcome. These include the time interval between injury and treatment, mechanism of injury, anatomic location, associated injuries, age, co-morbidities and clinical presentation [1]. Knowledge of these prognostic factors is essential for the appropriate evaluation and treatment of afflicted patients. The time interval from injury to treatment is perhaps the most critical determinant of salvage of both life and limb, following extremity vascular injury, as it is for all forms of trauma. This is explained by the time-dependent nature of the two major consequences of vascular injury, hemorrhage and ischemia [2]. Strong correlation between delay in treatment of arterial injuries and limb loss was shown in multiple studies such as, World War II, Afghanistan War, etc. Several civilian clinical series have confirmed close correlation of limb loss with delay in revascularization, especially when extremity arterial injury is complicated by associated injuries to vein, soft tissue, and bone [1]Even salvaged limbs are subject to functional disability, following treatment delay, due to nerve and muscle damage, as well as the development of potentially dangerous vascular complications such as pseudoaneurysms and arteriovenous fistulas (AVFs) [3]. Many studies have established the critical time interval, for restoration of limb perfusion and optimal limb salvage, to be at most 6-8 hours following extremity vascular trauma [4]. The degree of ischemia and extent of collateral circulation affect tissue tolerance of delay. Therefore, prompt diagnosis and treatment of vascular injuries must be a major goal of management of all extremity traumas [4]. Mechanisms of blunt injury involve a wider application of force, with greater damage to extremity vessels and surrounding structures than is imparted by penetrating trauma. Blunt vascular injuries are associated with a more difficult diagnosis and higher rates of amputation and severe dysfunction than simple penetrating vascular injuries which are typically clean, isolated, and more easily diagnosed and repaired [1]. Among Penetrating injuries, stabs impart the least destructive force and are associated with a small and discrete area of injury. High-velocity gunshot and shotgun wounds create a level of damage similar to blunt trauma, in terms of the

complexity and extent of the damage, the difficulty of diagnosis and treatment, and the higher rates of limb loss. If the interval between trauma and vascular reconstruction is prolonged beyond 4-6h, the possibility of surgical success is gradually reduced with increasing risk of kidney damage or even death. In these situations physical examination by surgeon is mainstay of decision making [8]. Vascular reconstruction has to be performed when ischemic limb seems viable by examination and post-traumatic interval of less than 4-6 hours. On the other hand, when the limb is necrotic the decision making becomes very difficult [5, 6]. But between these two states, taking decision to restore blood to the injured limb is controversial especially when the surgeon lacks extensive experience. Therefore, we should weigh the risk of reperfusion syndrome against the possibility of saving the borderline viable limb. In limb injuries involving amputation, laceration injuries, or compartment syndrome a circulatory insufficiency with a total or subtotal ischemia may occur and jeopardize the result of reconstructive surgery.

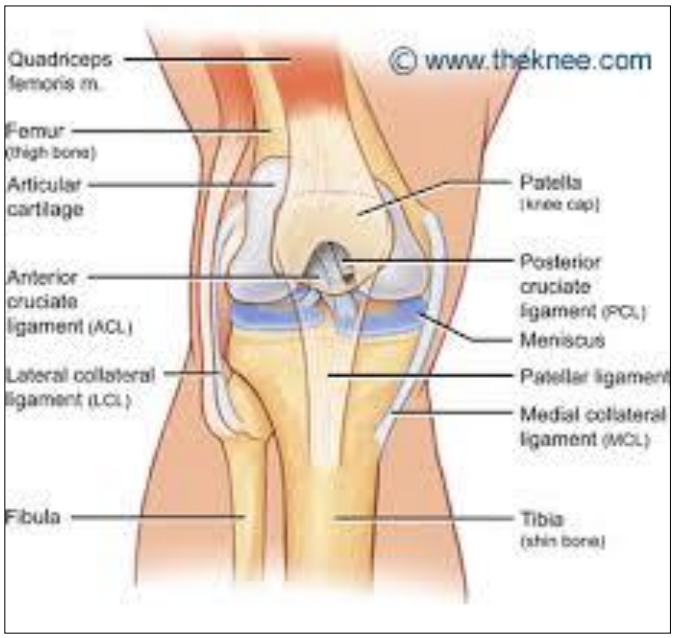


Fig 1

2. Materials and methods

A) Patient profiles

We included 32 knees with complete ACL (Anterior cruciate Ligaments) rupture confirmed arthroscopically in our study. The patients were 20 males and 12 females with a mean age of 26.5 (17–42) years. The patients did not have any previous history of knee injury. The mean period from injury to the sampling of SF was 32 (2–134) weeks. Although injury to the meniscus was noted arthroscopically in eight knees (lateral in seven, medial in one), no ligament other than the ACL was damaged. Radiography of the knee was normal in 29 knees and grade I in three knees according to the International Knee Documented Committee (IKDC) scale [9]. None of the patients had received intra-articular injection of steroid or hyaluronic acid. Control samples of six healthy volunteers comprising five males and one female with no history of knee injury with a mean age 24.3 (15–37) years were also evaluated.

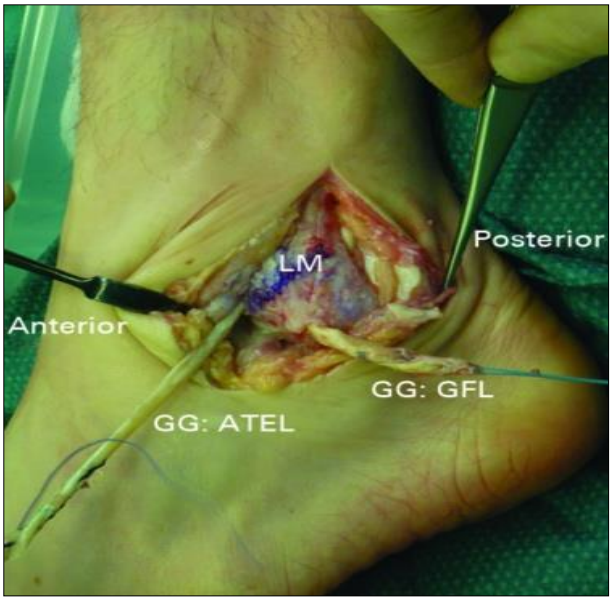


Fig 2

Details of the patients and healthy controls

The IL-6, MMP-3, and TIMP-1 concentrations were higher in the knees with ruptured ACL than in the normal knees. TNF-α was not detected in the normal knees but was noted at a mean level of 10.4 pg/ml in the knees with ruptured ACL. The IL-1β concentration was below the detectable range in all normal knees and in 25 knees with ruptured ACL. In the knees with ruptured ACL, the synovial fluid MMP-3 concentration was significantly correlated with the IL-6 concentration among various cytokines (P<0.01), but not with IL-1β and TNF-α [3]. The MMP-3 concentration showed a strong positive correlation (P<0.001) with the TIMP-1 concentration (Fig. 1), and a significant positive correlation (P<0.01) was also noted between the IL-6 and TIMP-1 concentration [2]. Moreover, in the ruptured ACL knees the synovial fluid MMP-3 concentration was elevated regardless of the time after injury. The TIMP-1 concentration tended to decrease with time, but it remained high regardless of the time after injury without a statistically significant decrease. The IL-6 concentration was high in many knees within 50 weeks after ACL injury but decreased thereafter (P<0.01).

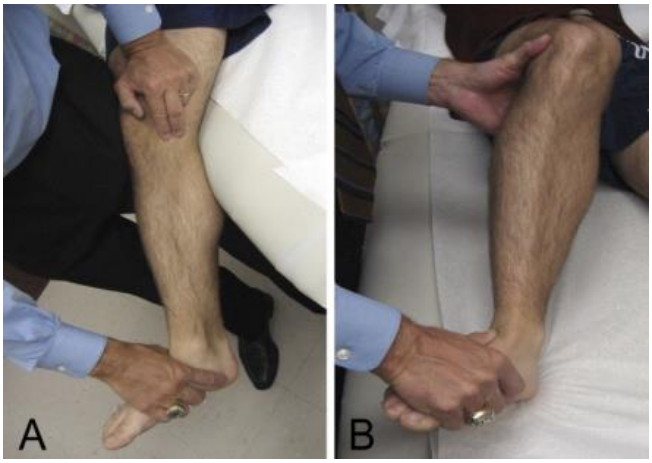
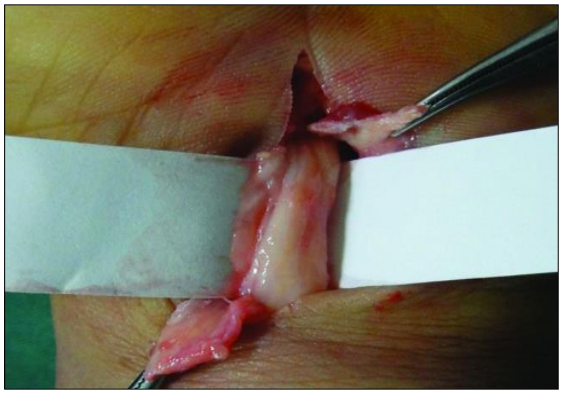


Fig 3



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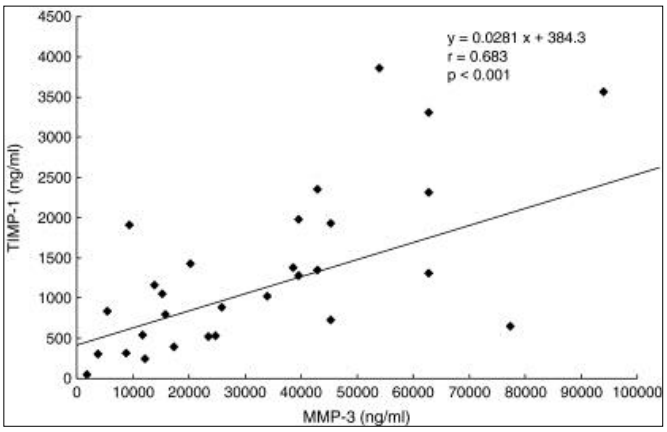

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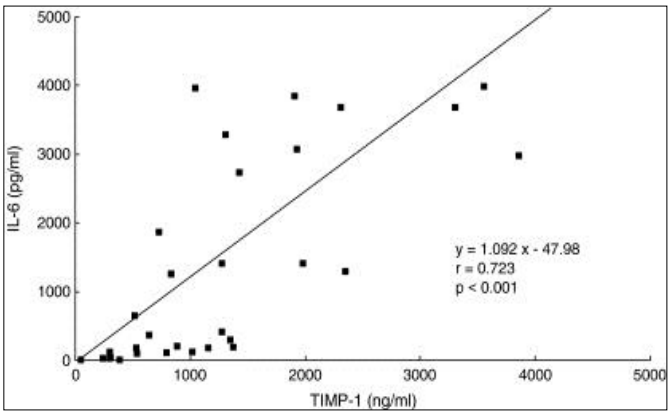

Fig 6

The relationship between cytokine levels and other joint disorders

In the ACL-injured knees, the mean concentrations of each cytokine in the eight cases with meniscal tears were 42,250.0±21,942.3 ng/ml for MMP-3, 1,354.0±1,066.6 ng/ml for TIMP-1, and 2,201.3±1,252.9 ng/ml for IL-6. In contrast, the mean concentrations in 24 knees without meniscal tear were 27,617.5±23,336.6 ng/ml for MMP-3, 1,232.2±955.2 ng/ml for TIMP-1, and 1,057.5±1,435.0 ng/ml in IL-6. The IL-6 concentration in the cases of a meniscal tear was significantly higher than in the cases without tear (P<0.05). The mean concentration of each cytokine in the three cases with radiographic degenerative changes was 44,933.3±28,517.8 ng/ml for MMP-3, 730.0±258.7 ng/ml for TIMP-1, and 438.7±370.6 ng/ml for IL-6. On the other hand, in the 29 cases without radiographic changes they were 29,862.8±23,096.6 ng/ml for MMP-3, 1,317.8±999.4 ng/ml for TIMP-1, and 1,437.1±1,502.5 ng/ml for IL-6, respectively. There was no significant difference between radiographic changes and cytokine concentrations.

B) Patient profiles

We included 32 knees with complete ACL rupture confirmed arthroscopically in our study. The patients were 20 males and 12 females with a mean age of 26.5 (17–42) years. The patients did not have any previous history of knee injury. The mean period from injury to the sampling of SF was 32 (2–134) weeks. Although injury to the meniscus was noted arthroscopically in eight knees (lateral in seven, medial in one), no ligament other than the ACL was damaged. Radiography of the knee was normal in 29 knees and grade I in three knees according to the International Knee Documented Committee (IKDC) scale [9]. None of the patients had received intra-articular injection of steroid or hyaluronic acid. Control samples of six healthy volunteers comprising five males and one female with no history of knee injury with a mean age 24.3 (15–37) years were also evaluated.

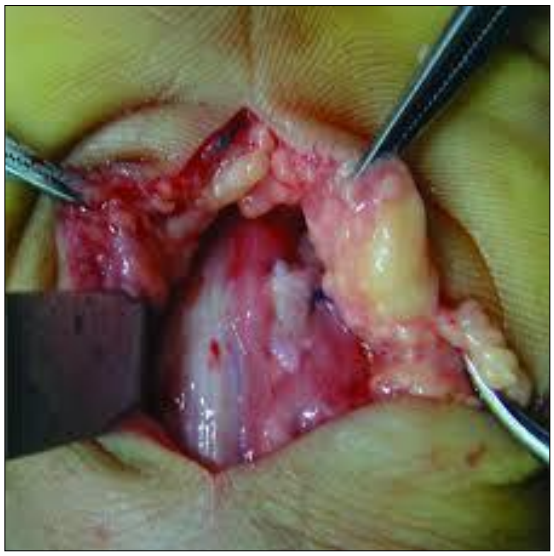

Fig 7

Statistics

A nonparametric method, the Mann-Whitney U test, was used to calculate statistical differences between unpaired groups. The two-tailed Student's *t*-test was used for comparisons within and between the groups, and Pearson's correlation coefficients were calculated[7]. All statistical procedures were performed using Stat-View software. A *P* value of less than 0.05 was considered to indicate statistical significance.

3. Results

The IL-6, MMP-3, and TIMP-1 concentrations were higher in the knees with ruptured ACL than in the normal knees. TNF-α was not detected in the normal knees but was noted at a mean level of 10.4 pg/ml in the knees with ruptured ACL. The IL-1β concentration was below the detectable range in all normal knees and in 25 knees with ruptured ACL. Mean synovial fluid concentrations of cytokines, MMP-3, TIMP-1in ACL-deficient

knees and normal knees. In the knees with ruptured ACL, the synovial fluid MMP-3 concentration was significantly correlated with the IL-6 concentration among various cytokines ($P<0.01$), but not with IL-1β and TNF-α [3]. The MMP-3 concentration showed a strong positive correlation ($P<0.001$) with the TIMP-1 concentration, and a significant positive correlation ($P<0.01$) was also noted between the IL-6 and TIMP-1 concentration. Moreover, in the ruptured ACL knees the synovial fluid MMP-3 concentration was elevated regardless of the time after injury. The TIMP-1 concentration tended to decrease with time, but it remained high regardless of the time after injury without a statistically significant decrease. The IL-6 concentration was high in many knees within 50 weeks after ACL injury but decreased thereafter ($P<0.01$).

The relationship between cytokine levels and other joint disorders

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4. Observation

MMP-3, also called proteoglycanase, is a key enzyme involved in cartilage destruction [7]because it has the ability to decompose important molecular structures of the cartilage matrix, such as aglycan, type IX collagen, and fibronectin, and is involved in the activation of other MMPs [13]. Many cells including synovial fibroblasts produce MMP-3. Chondrocytes also have the ability to produce MMP-3, and its production is induced and promoted by the action of cytokines such as IL-1 and TNF [4, 8]. Furthermore, immunohistochemical studies have shown that a positive correlation exists between the intensity of MMP-3 staining and matrix degradation in cartilage tissues [6]. Since MMP-3 plays a central role in the destruction of cartilage matrix, clinical attempts to use it as an arthritis marker for the staging of arthritic diseases have been reported The MMP-3 concentration in synovial fluid is higher in patients with rheumatoid arthritis than in those with osteoarthritis [2], suggesting an association with the degree of progression of synovitis and joint destruction. Lohmander *et al.* also found that the MMP-3 concentration in the synovial fluid of patients with osteoarthritis of the knee increases as the disease progresses, suggesting that MMP-3 is an index of cartilage destruction [11]. TIMP, on the other hand, acts as a regulator of MMP activity and as a protective factor against metastasis and invasion of malignant neoplasms [2, 10] in cartilage; TIMP-1 not only prevents matrix destruction as an MMP-inhibiting protein but also contributes to matrix maintenance by promoting cell proliferation [4]. Transcutaneous oxygen monitoring has been shown to reflect tissue perfusion and has been recommended for predicting the final outcome of major vascular trauma of the limb. The effect of transient iatrogenic ischemia, caused by tourniquet pressure, on local and systemic blood gas analysis has been demonstrated in animals [7]. But similar studies have not yet been reported in humans. The present study was thus performed to determine whether biochemical assay can predict the outcome of traumatic acute ischemic limb after vascular reconstruction.

5. Discussion

In this study, the synovial fluid MMP-3 concentration was markedly higher in the knees with ruptured ACL than in the normal knees, indicating that an increase in MMP-3 may have a destructive effect on the cartilage matrix. Synovial fluid MMP-3 concentration is increased in the knees with ruptured ACL similarly to knees with osteoarthritis and is markedly higher than in normal knees [11] and our results support this. Similarly, the TIMP-1 concentration was markedly increased in the knees with ruptured ACL compared with the normal knees. In addition, a strong positive correlation was observed between the MMP-3 and TIMP-1 concentration. Lohmander et al. found a correlation between the MMP-3 and TIMP-1 concentrations with a molar ratio of 1 or less in normal knees [11] suggesting a protective reaction of TIMP-1 against MMP-3. In this study, the MMP-3/TIMP-1 ratio (molar ratio) was markedly increased to 17 in the knees with a ruptured ACL. This imbalance between MMP-3 and TIMP-1 may make the environment of the knee with a ruptured ACL more susceptible to cartilage destruction of the cytokines examined in this study, only IL-6 was increased in the knees with a ruptured ACL compared with the normal knees. IL-6 was discovered as a cytokine that promotes B-cell differentiation and proliferation and induces antibody production, but was subsequently shown to be produced by a variety of cells including Chondrocytes and synovial cells [6]. Since IL-6 has biological activities similar to those of IL-1, such as inducing the production of pyrogenic factors and acute phase proteins, and is elevated in morbid synovial fluid, such as that of patients with rheumatoid arthritis, it initially attracted attention due to its possible relationship with cartilage destruction. In addition, IL-6 is a pre-inflammatory cytokine, and an IL-6 receptor antagonist is being used as an anti-inflammation drug for RA [4, 5]. In this study, the IL-6 concentration was high in many knees within 50 weeks after ACL injury but decreased thereafter, while the MMP-3 concentration was elevated regardless of the time after injury.

6. Conclusion

We suggest that, to prevent the development of OA after ACL injury, it is important to start treatment of the ACL-injured knee as soon as possible because cytokines such as IL-6 and MMP-3 induce the cartilage destruction. However, this study was designed in a cross-sectional manner and was performed at random times after ACL injury. Changes in cytokines levels with time are still unknown. In the future, a biochemical study based on cohort analysis after ACL injury would clarify the relationship between the ACL-injured knee and the development of OA.

7. References

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