Al-Qadisiyah Journal of Pure Science

Manuscript 1242

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ARTICLE

Assessment of Tissue Inhibitor of Metallopeptidase-1 in Patients With ACL Injury, Meniscal Tear, and Cartilage Damage

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Abstract

Background: **The knee is one of the largest and most intricate joints in the body. The knee is the joint that joins the femur (thigh bone) to the tibia (shin bone). The fibula (smaller bone that runs alongside the tibia) and patella (kneecap) are two more bones that make up the knee joint. Biomarkers like Tissues inhibitor of metallopeptidase-1 (TIMP-1) might be provided diagnostic, prognostic, or burden information for knee disease prior to radiographic changes becoming apparent. Few studies have clarified which biomarkers may be most informative following injury.**

Materials and methods: **During the period from November 2022 into May 2023, 76 Iraqi participants were invited from the AL-Furat AL-Awsat Hospital and Royal Hospital in Al-Qadisiyah governorate. The patients were divided into three groups. Group I: Less than 6 months, group II: 6−12 months and group III: More than a year. Permissions were obtained prior the beginning of the tests. Inflammatory parameters such as Tissue inhibitor of metallopeptidase were detected by ELISA. The Health and Medical Human Research Ethics Committee of the Faculty of Medicine, University of Qadisiyah, authorized the project.**

Results: **The levels of TIMP-1 were very low in patients whose injury duration was Group I; 5.336 ± 1.69 pg/mL as compared with another group II: (8.485 ± 1.6 pg/mL), and group III: (9.57 ± 0.90 pg/mL). Significant differences were observed ($P < 0.0001$).**

Conclusions: **The current study identified one synovial fluid biomarker (TIMP-1), whose concentrations after anterior cruciate ligament injury differ depending on the duration of the injury.**

Keywords: Tissue inhibitor of metallopeptidase-1, ACL injury, Cartilage damage

1. Introduction

Anatomy of the Knee: The knee is one of the largest and most intricate joints in the body. The knee is the joint that joins the femur (thigh bone) to the tibia (shin bone). The fibula (smaller bone that runs alongside the tibia) and patella (kneecap) are two more bones that make up the knee joint [1]. The knee is important in activity that involves carrying the body weight in both horizontal (running and walking) and vertical (jumping) orientations. In the flexed position, the knee allows for flexion and extension around a virtual transverse axis, as well as a modest medial and lateral rotation about the axis of the lower leg [2]. Tendons are fibrous bands that link the knee bones to the leg muscles that move the joint. Ligaments connect and support the knee bones [3]. Two muscle groups are involved in the knee joint. These are the quadriceps (located on the front of the thighs) and hamstring (located on the back of the thighs) muscles, which assist straighten the legs and flex the leg at the knee [4,5]. By linking the bones and reinforcing the joint against aberrant forms of movement, the four main

Received 7 December 2023; accepted 11 April 2024.
Available online 20 August 2025

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https://doi.org/10.29350/2411-3514.1242

ligaments serve a vital function in regulating movement [6].

1. The ACL keeps the femur from slipping backwards on the tibia and the tibia from sliding forwards on the femur [7].
2. The posterior cruciate ligament keeps the femur from moving forward on the tibia and the tibia from sliding backward on the femur [8].
3. Medial collateral ligaments prevent the femur from sliding to one side [9].
4. Lateral collateral ligaments: These connect the femur and tibia on the outside. It isn't connected to the joint capsule [10].

Tissues inhibitor of metallopeptidase-1 (TIMP-1), commonly known as TIMP1, is a glycoprotein with a molecular weight of 28 kDa that acts as a tissue inhibitor of metalloproteins [11]. TIMP1 is found in a variety of tissues throughout the body. This protein belongs to the TIMP family. Glycoprotein is a natural inhibitor of matrix metalloproteinases (MMPs), a class of peptides involved in the breakdown of extracellular matrix [12]. In addition to inhibiting most known MMPs, the encoded protein can increase cell proliferation in a variety of cell types and may have anti-apoptotic properties [13].

The nutritional hormone ACTH induces the production of TIMP-1 in adrenocortical cells, and an increase in TIMP expression is also related to a decrease in collagenase activity [14]. TIMP1 overexpression is associated with a poor prognosis in a variety of malignancies, including laryngeal carcinoma and melanoma [15]. In ACL-injured knee fluid, mean concentrations of TIMP-1, IL-6 and MMP-3 were significantly higher than in normal standing. IL-6 and MMP-3 concentrations were strongly linked. The levels of IL-6 and TIMP-1 have been linked [16]. Data reveal that aggrecan, COMP, and MMP-3 concentrations are elevated in the intact contralateral knee of ACL rupture patients, possibly as a result of altered loading [17]. Have postulated that the tissue mineral protein (MMP) family plays an important role in cartilage matrix disintegration [18]. The concentrations of TIMP-1 and MMP-3 in synovial fluid increase immediately after acute ACL damage and remain elevated for many years, most likely due to chronic low-grade synovitis produced by increasing biomechanical stresses in the joint [19]. ACL injuries can result in altered proprioception, intra-articular fibrosis, sagittal and rotational plane knee instability, thigh muscle atrophy, and, in rare cases, altered joint congruency, particularly in the postero-lateral tibia region due to injury-induced cartilage and subchondral bone compression [20,21].

2. Materials and methods

2.1. Methods (Patients)

During the period from November 2022 to May 2023, the Croos Sectional Study was performed. The Health and Medical Human Research Ethics Committee of the College of Medicine, University of Al-Qadisiyah, Iraq is authorized of the study. A 76 participtant were invited in Al-Qadisiyah Governorate's at AL-Furat AL-Awsat pravite Hospital and Royle prvivate Hospital. Before the experiments began, permissions were sought.

2.2. Exclusion criteria were included

1. Any other ligament injury requires surgical treatment.
2. Cartilaginous and osteochondral lesions.
3. Bones fractures.
4. Degenerative meniscus tears, previous knee surgery, previous meniscus injury, or ACL injury to the same knee.
5. Chronic inflammatory diseases within the joint or outside the joint immune diseases or tumors.
6. Use of immunomodulatory drugs or aspirin, intra-articular injections of corticosteroids and other drugs.
7. Radiological and endoscopic signs of osteoarthritis.

Medical seniors diagnosed patients based on clinical features by a senior orthopaedic surgeon based on present history, physical examination, magnetic resonance imaging (MRI), and confirmation by arthroscopic examination. The history of patients included the following: Age and Body mass index. The ages were included in the study. A total of 76 Iraqi participants suffered from ACL injuries, meniscal tears, and cartilage damage. They were invited from the Al-Furat Al-Awsat Privat Hospital in the Al-Qadisiyah governorate, Iraq. Three groups of patients were created according to the duration of the disease: less than six months for Group I (25 patients), six-twelve months for Group II (26 patients), and more than one year for Group III (25 patients). During the ACL restoration process, synovial fluid was taken from each patient's injured knee.

2.3. Determination of human tissues inhibitor of metallopeptidase-1

Human (TIMP-1) Tissues inhibitor of metallopeptidase-1 by ELISA (USA/Elabscience).

2.4. Ethical approval

The college of medicine, university of Qadisiyah granted ethical approval. Before taking the sample, the patients and his relative were asked for their permission. Sampling, health and safety precautions were implemented. The date for this study was 28-11-2022 and the approval number was 4409/30.

2.5. Statistical analysis

The statistical analysis was carried out using Microsoft Office Excel 2013 and Graph Pad Prism 9.2.0 to compile the data. Data were presented numerically as mean standard deviation. One-way ANOVA and post hoc analysis using the Tukeys test were used to identify significant differences between groups. When the P value was 0.05, all data were deemed as significant.

3. Results

3.1. Tissues inhibitor of metallopeptidase-1 level

The results of this study show an increase in levels of TIMP-1 (9.57 ± 0.9021) ng/mL in patients with more than 1 year compared with other groups from 6 to 12 months (8.485 ± 1.657) ng/ml and less than 6 months (5.336 ± 1.69) ng/ml. TIMP-1 concentrations in our study differed significantly (p value 0.0001) from all other groups investigated.

TIMP-1 (NG/ML) measurements revealed a significant difference between less than 6 months and 6–12 months (p-value 0.0001) and a significant difference between less than 6 months and more than 1 year (p-value = 0.0309). As seen in Fig. 1.

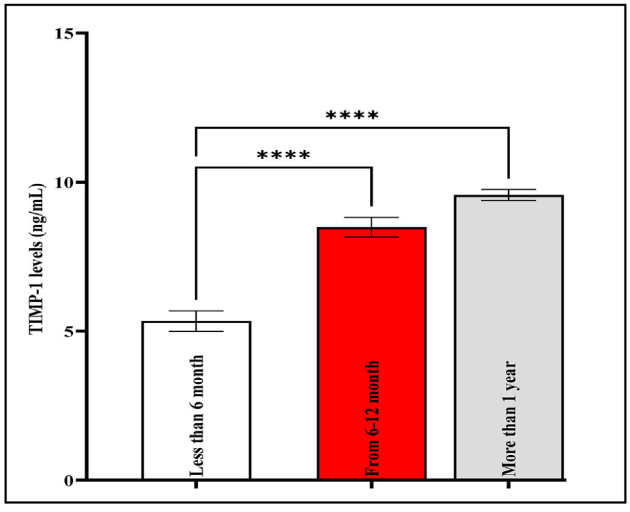

Fig. 1. ***Estimation of TIMP-1 concentrations (ng/mL) A group comparison.*** *A substantial difference (p-value 0.0001) was found when comparing the entire group. Data are given as means standard deviations (P0.05).*

3.2. Estimation of tissues inhibitor of metallopeptidase-1

Levels biomarker among all groups is shown in Table 1. TIMP-1 levels were very low in patients less than 6 months compared with other groups aged 6–12 months and age groups older than 1 year. A significant difference was observed (P < 0.0001).

4. Discussion

Synovitis has been linked to increased TIMP-1 concentrations in synovial fluid or a higher MMP-3 to TIMP-1 ratio (Haraden et al., 2019). TIMP-1, an anti-inflammatory protease inhibitor, was reported to be significantly elevated in synovitis [22]. In our current study, a comparison was made between three groups divided from the first day of injury to 6 months, from 6 months to 12 months, and injury over a year. We found that as the duration of the ingury increased, so did the amount of TIMP_1. The consequences of present study were consistent with results of previous studies in those with joint injury, showing elevated SF levels of TIMP-1 [23,24]. The study are similarly compatible with those of Higuchi et al., who investigated the biochemical effects of synovial fluid in knees with ACL rupture. According to their findings, following ACL rupture, MMP3 and TIMP levels both rose, but the balance between imbalance is caused by an increase in IL-6 [25]. TIMP-1 increase may be attributed to TNF-alpha elevation, as indicated in a prior study. TNF-alpha was found to be responsible for the release of collagenase enzymes and TIMP-1 levels [26,27]. Our findings are also consistent with a recent study that

Table 1. TIMP-1, significant differences were seen across all groups invtigated (p 0.05).

Characteristic	Less than 6 month	From 6 to 12 month	More than 1 year	P value
	N = 24	N = 25	N = 24	
TIMP-1 level (pg/ml)				
Range	2.168–7.828	6.29–10.95	8.139–10.74	P < 0.0001
Mean ± SD	5.336 ± 1.69	8.485 ± 1.657	9.57 ± 0.9021	

found that people with ACL injuries had higher synovial fluid levels of proteoglycan fragments, MMP-3, and TIMP-1 in the first week [28]. Patients observed higher synovial fluid concentrations of aggrecanepitope-846, MMP-3, and TIMP-1.2020 between one week and two months after ACL injury. Patients reported higher MMP-3TIMP-1 synovial fluid concentrations between two months and one year after ACL damage [29].

Funding

Self-funding.

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