Al-Qadisiyah Journal of Pure Science

Volume 29 | Number 1 Article 19

2024

The impact of anterior cruciate ligament injury on the serum level of some biochemical markers associated with cartilage turnover

Ahmed J. Al. Hamdaw
University of Al Qadisiyah College of Medicine, Al Diwaniyah, Qadisiyyah, IRAQ, hadeel.jabbar@qu.edu.iq

Ajil A. Alzamily
University of Al Qadisiyah College of Medicine, Al Diwaniyah, Qadisiyyah, IRAQ

Ishan Abdul Abbas Al Salman
University of Al Qadisiyah College of Medicine, Al Diwaniyah, Qadisiyyah, IRAQ

ARTICLE

The Impact of Anterior Cruciate Ligament Injury on the Serum Level of Some Biochemical Markers Associated With Cartilage Turnover

Ahmed J. Al Hamdawi*, Ajil A. Alzamily, Ishan A.A. Al Salman

University of Al Qadisiyah College of Medicine, Al Diwaniyah, Qadisiyyah, Iraq

Abstract

Background: **Anterior cruciate ligament injury patients' (ACL), meniscus, and collateral ligament tears are typical knee injuries. These injuries cause knee osteoarthritis and impede exercise. Knee stability comes from the ACL. Thrust or pace changes in sports may harm healthy young knees. Injuries primarily affect knee mobility and stability. The structural and functional changes in anterior cruciate ligament injury and rehabilitation are assessed using MRI and approved clinical imaging. Vital signs and an MRI may assess tissue inflammation and healing before and after surgery.**

Objective: **The study aimed to examine if IL-6 and IL-13 levels could be associated with the risk of ACL.**

Materials and methods: **120 participants—60 controls and 60 anterior cruciate ligament damage patients. They had 67 males and 53 females. Participants were 18—75 years old. This case-control study included patients at Al-Furat Al-Awsat and Royal Private Hospitals in Al-Diwaniyah Governorate, Iraq, from November 2022 to May 2023. Eliza and Elabscience kits were used to analyze IL-6 and IL-13. All patients were biochemically tested before and six weeks after ACLR. The Al-Qadisiyah University College of Medicine's Department of Medical Chemistry lab performed the tests.**

Results: **The study found a significant increase in serum biomarker Interleukin-6 (IL-6) levels in patients with anterior cruciate ligament injuries after surgery (6 weeks) compared to the control group ($P < 0.001$). Changes were significant between pre-surgery and control ($P < 0.01$). Data indicates substantial changes in IL-13 serum levels before and after surgery in knee patients with anterior cruciate ligament damage ($p < 0.05$). Considerable differences ($P < 0.001$) showed a considerable rise in the patient group compared to the control group.**

Conclusion: **We found that IL-6 and IL-13 may induce inflammation causing synovitis. High levels of these substances after an ACL injury may lead to continued inflammation and the development of post-traumatic osteoarthritis.**

Keywords: Anterior cruciate ligament injury, Interleukin-6 (IL-6), Interleukin 13 (IL-13)

1. Introduction

Knee joints are large, complex, hinged synovial joints. The knee has three joints that facilitate motion. In a wide range of situations, there are three articulations in the knee joint, i.e., two between the tibial and femoral condyles, and the third with the patella and femur. The knee's capacity to support flexion and rotation ensures full stability and control [1]. The thighbone (femur), shinbone (tibia), outer skin bone (fibula), and knee cap (patella) are the four primary bones that make up a knee [2,3]. Interactions between the tibia, patella, and femur are the primary components of the knee joint that allow for mobility. The patella is a little, flat, triangular-shaped bone that moves and rotates with the knee [4]. It is placed in the center of the body. The knee joint is composed of three functional compartments: the patellofemoral articulation, which connects the patella to the femur; the lateral femorotibial articulation; and the medial femorotibial articulation. As a whole, they form the knee [5]. Synovial gliding joints include the patellofemoral articulation, and synovial hinge joints include the

Received 22 February 2024; accepted 11 April 2024.
Available online 28 September 2024

* Corresponding author.
E-mail address: hadeel.jabbar@qu.edu.iq (A.J. Al Hamdawi).

https://doi.org/10.29350/2411-3514.1254

femorotibial articulations [6]. The knee joint is an example of a triple-articulation joint. Two connecting the quadriceps and the thighbone, and a third linking the patella to the femur [7]. Lower femur ends, upper tibial surfaces, and patellar posterior surfaces all contribute to the knee joint's articulating surfaces [8]. The condyles and the menisci, which are passive supporting structures, provide joint stability in the knee. The four ligaments mostly serve as passive supporting entities. The medial/tibial collateral ligament (MCL), the lateral/fibular collateral ligament (LCL), the anterior cruciate ligament (ACL), and the posterior cruciate ligament (PCL) are the three. A ligament that links the tibia to the femur is called the medial/lateral ligament [9]. Aids in maintaining knee stability on the inside face for knee stability on the outside, go no farther than the Lateral/Fibular Collateral Ligament [9]. The knee is made up of ligaments that are meant to give passive stability and musculotendinous components that are meant to give dynamic stability [10]. The parts of the joint that are under the cartilage, the medial and lateral menisci, and the articular surfaces of the distal femur and proximal tibia are the ones that get the most stress during this interaction [11]. In the sagittal plane, knee movement includes flexion and extension; in the frontal plane, abduction, and adduction; and the horizontal plane, medial and lateral rotation are included. The joint's mobility and stability are regulated by the articular surfaces, menisci, ligaments, tendons, and musculotendinous structures' profiles [12,13]. There is an articulation between the patella and the femoral bone just in front of the knee, and the patella increases the moment arm of the muscles that extend the knee and protect the joint from harm [14]. Injury typically affects more than one structure in the knee joint, which is in line with its architecture and the relationships between its many components. Injuries to the intercondylar notch—where the anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL) cross—are among the most common and serious [15]. Both main and secondary stabilizers work together to keep the knee joint stable. Aside from the menisci and ligaments that support the knee joint, the surrounding muscles, including those of the hips and the gastrocnemius, have an important supporting role as well. Despite these supports, the knee is not well protected and, as a result of its position between the tibia and femur, the two longest bones in the body, is particularly susceptible to trauma and sports injuries [9]. Cartilage covers the uneven surfaces of the knee joint. The meniscus is a wedge-shaped fibrotic cartilaginous structure that joins the two main forms of knee cartilage, the first being the articular cartilage that covers the ends of bones [17]. When we walk, we bend at the knees, which also bear almost all of our body weight [18]. Among its many roles, the multifunctional cytokine interleukin-6 (IL-6) regulates hematopoiesis, acute phase reactions, and immunological responses. Hepatocytes, B cells, and T cells are all targets of interleukin-6 (IL-6) production [19,20]. This gene code for human Genomic analysis has pinpointed IL-6 to chromosome 7p21. The single-chain protein IL-6 that the IL-6 gene encodes can have a molecular mass of between 21 and 28 KDa due to variations in glycosylation and phosphorylation. The total IL-6 length in humans is 212 aa, with 184 aa forming the mature segment and 28 aa serving as the signal peptide [21]. In addition to influencing immune system function, tissue healing, and metabolism, IL-6 is a pleiotropic cytokine with anti-inflammatory and pro-inflammatory roles. It controls bone homeostasis, accelerates B cell development into plasma cells, and activates cytotoxic T cells. Il-6, like other proinflammatory cytokines, has been linked to inflammatory disorders such as rheumatoid arthritis and Crohn's disease [13,22]. A protein that is very similar to gp130, the receptor subunit of the interleukin-6 signal transducer (IL-6ST), is used by interleukin-6 (IL-6) and other cytokines to talk to cells. Eleven members make up the family: IL-11, IL-27, IL-31, CNTF, CT-1, CLC, LIF, NPN, OSM, and viral vIL-6 of Kaposi sarcoma-associated herpesvirus [23]. Out of a panel of seventeen inflammatory molecules, four specific cytokines (interleukin-6 (IL-6), interferon-ɾ (IFN-Ɣ), monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1 (MIP-1β)) are increased in knees following acute ACL injury (within six weeks of the acute event) [24]. While most cases of acute inflammation resolve within a day or two, some might last for weeks [25]. The fact that levels of intra-articular IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) were considerably elevated over three months following injury implies a highly protracted inflammatory state, which may cause structural cartilage degradation [26]. The 15q31 locus encodes the cytokine IL-13, which has a molecular weight of 14–40 kDa [27]. Besides activated Th2 cells, eosinophil granulocytes can also release IL-13 when GM-CSF and IL-5 are added to them [28]. It inhibits the production of both monocyte cell lines and inflammatory cytokines [29]. Crucial in allergic reactions, it causes B-lymphocytes to transition to the IgE class [30]. Allergic reactions and asthma might potentially be alleviated with the use of IL-13 antagonists. Transformed T cells release interleukin-13, which has a strong effect

on human monocytes and B-cell activity in laboratory settings [31]. The molecular weight of this 132-amino-acid nonglycosylated protein is about 10 KDa [32]. One reason why these two anti-inflammatory cytokines are so similar is because they both have a cellular receptor, the IL-4 type 1 receptor [33]. Interleukin-4 (IL-4) and interleukin-13 (IL-13), two components of Th-2-mediated immunity, have a significant impact on allergic inflammation [30]. These molecules are released by different kinds of cells and exert their effects via the STAT6 signaling route. They influence many different types of cells, including B cells, monocytes, basophils, eosinophils, and fibroblasts. If you suffer from a Th-2-induced condition like asthma or atopic dermatitis, blocking IL4 and IL-13 may help. The pathophysiology of autoimmune-mediated illnesses, such as inflammatory arthritis, may nevertheless include them, according to mounting evidence [34].

2. Methods

2.1. Study design

This study was a case-control study conducted on patients who were admitted to Al-Furat Al-Awsat Private Hospital and Royal Private Hospital. The patients were diagnosed with cartilage turnover in patients with anterior cruciate ligament damage by specialist physicians.

2.2. The setting of the study

Cartilage turnover in the anterior cruciate ligament of the knee was the subject of this study, which collected comprehensive patient data including gender, age, weight, height, and injury history. The labs of the Department of Medicinal Chemistry at the University of Al-Qadisiyah's College of Medicine were the sites of all these experiments.

2.3. Sample collections

The research lasted from November 2022 to May 2023. Sixty patients, ranging in age from eighteen to fifty-two, were included, all of whom had suffered anterior cruciate ligament damage to the knee. Experts in orthopedic and joint surgery make all of the patient's diagnoses based on their clinical history and x-ray results. Two dependable radiological methods for examining the ACL's anatomy are preoperative magnetic resonance imaging (MRI) and postoperative computed tomography (CT) scans [35]. Patients were categorized into two groups based on when they had surgery: those who had it done before and those who had it done after. It was also necessary to collect data from a healthy control group of sixty people. The average age in the study was between twenty and seventy-five years old. Healthy individuals were selected at random to serve as control subjects. No disorders affecting the bones or anterior cruciate ligaments are present in their bodies. Private clinic doctors in the Al-Diwaniyah Governorate made the diagnosis.

2.4. Blood collection

Each patient had this procedure twice. The first was taken just before surgery, and the second was taken again six weeks later. Each participant in the present investigation had 5 mL (ml) of blood collected by intravenous puncture and left to cool to room temperature for 15 min. To extract serum, the blood was spun in a centrifuge at 4000 rpm for 5 min. The serum that was collected was transferred to an Eppendorf tube and labeled before being kept at −80 °C until it was needed.

2.5. Interleukin 6 and interleukin 13

Sandwich-ELISA is the principle utilized by this ELISA reagent. Antibody-specific for human IL-6 and human IL-13 have been pre-coated onto the ELISA plate included in this kit.

2.6. Assay procedure

1. Establish which wells will contain the sample, blank, and diluted standard. Make sure to test all samples and standards in triplicate by adding 100 μL of each dilution of standard, blank, and sample into the corresponding wells. It is advised to find out the sample dilution ratio by conducting preliminary experiments or following the suggestions of technical assistance. Use the sealant that is included with the package to cover the plate. Keep the mixture in an incubator set at 37 degrees Celsius for 90 min. Please be advised to add solutions to the well at the base of the micro ELISA plate, being sure not to contact the inner wall as this might lead to excessive foaming.
2. Pour out the contents of each well without washing. Straight away, fill up every well with 100 μL of the Biotinylated Detection Ab working solution. Apply a fresh coat of sealant to the dish. Allow to sit at 37 °C for 30 min.
3. Transfer 350 μL of wash buffer to every well after removing the solution from each well. After 1 min of soaking, drain the solution from each well by aspirating or decanting it, and then pat

the items dry using clean absorbent paper. Wash the item three times more. Please be aware that this and subsequent washing processes may be performed using a microplate washer. As soon as the wash process is complete, put the tested strips to use. Make sure that wells are never dry.
4. Including 100 μL of HRP Add a functional conjugate to every well. Apply a fresh coat of sealant to the dish. Stir occasionally, and let stand for 30 min at 37 degrees Celsius.
5. After removing the solution from each well, carry out the wash operation five times, just as in step 3.
6. Add 90 μL of substrate reagent to each well. Freshly seal the dish. Wait 15 min at 37 °C. Avoid the light on the plate. The reaction time depends on the color change, which may be shortened or extended up to 30 min. Let the microplate reader warm for 15 min before the OD measurement.
7. Add 50 μL of Stop Solution to each well. Please add the stop solution in the same order as the substrate.
8. Find the OD value of each well at the same time using a micro-plate reader set to work at 450 nm

3. Results

3.1. Serum level of interleukin-6 (IL-6) in anterior cruciate ligament injury patients

The result of the present study showed an increase in the serum of interleukin-6 between before-surgery and after-surgery patient groups compared to control, with a high increase indicated in the after-surgery group ($P < 0.001$) (Fig. 1). Significant changes were indicated between the before-surgery and control ($P < 0.01$).

3.2. Comparison of serum level of interleukin-6 according to age

A comparison of serum IL-6 between the control group and the patients' group (before and after surgery) according to age intervals is shown in Table 1 and Fig. 2. In the age interval, 20–39 years, the mean serum level was significantly higher in the patient's group before surgery in comparison with the control group ($p = 0.002$), and after surgery it became further significantly higher in the patient group in comparison with the control group ($p = 0.001$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.211$).

In the age interval 40–59 years, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p = 0.832$), and after surgery, it remains not significantly different in the patient's group in comparison with the control group ($p = 0.139$); however, there was a significant difference in the serum level before surgery and after surgery ($p = 0.047$).

3.3. Comparison of serum level of interleukin-6 according to sex

Comparison of serum IL-6 between the control group and the patients' group (before and after

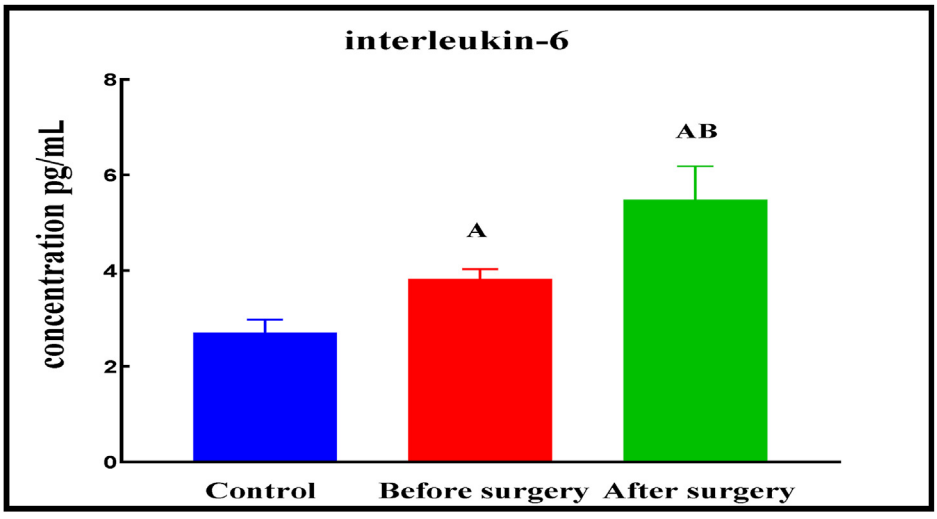

*Fig. 1. **Estimation serum concentrations of interleukin-6 [IL-6 (pg/mL)].** Levels in anterior cruciate ligament injury of the knee patients with before-surgery and after-surgery groups and control. Data are expressed as means ± SD, indicating a significant change between patient groups and control ($P < 0.001$). Significant changes were indicated between the before-surgery and control ($P < 0.01$). Letter (A) indicates a significant difference when comparing the control group to the patient's group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

Table 1. Comparison of serum IL-6 between the control group and patients' group (before and after surgery) according to age intervals.

Age	Control group	Patient group before	Patient group after	*p1*	*p2*	*p3*
20–39	2.63 ± 1.43	4.10 ± 1.53	5.81 ± 2.30	0.002 **	0.001 ***	0.211
n	20	25	25			NS
40–59	2.96 ± 2.08	3.17 ± 0.32	4.55 ± 0.80	0.832	0.139	0.047 *
n	7	5	5	NS	NS	

n: number of cases; data were presented as mean ± standard deviation; *p*1: comparison between control group and patients before surgery; *p*2: comparison between control group and patients after surgery; *p*3: comparison between patients group before surgery and after surgery; NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

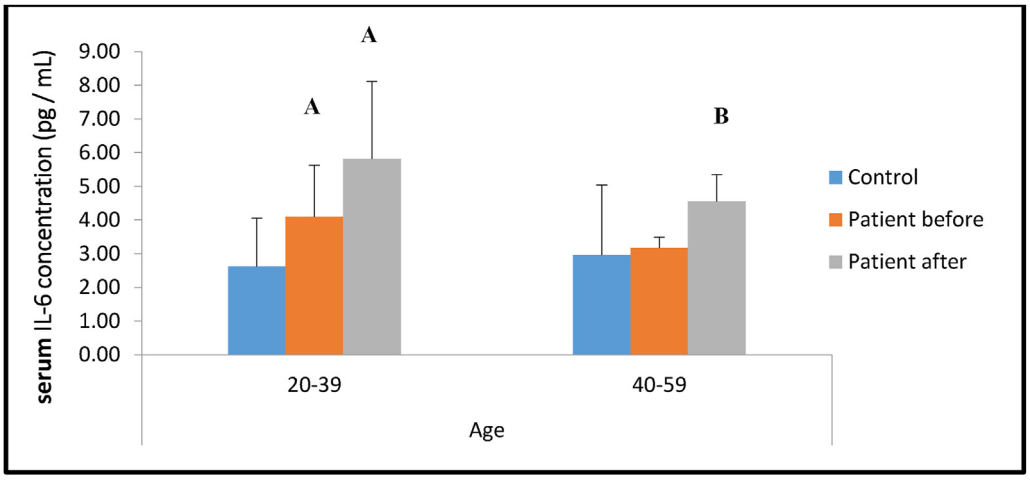

*Fig. 2. **Comparison of serum IL-6 between the control group and patients' group (before and after surgery) according to age intervals.** Letter (A) indicates a significant difference when comparing the control group to the patient's group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

surgery) according to sex is shown in Table 2 and Fig. 3.

In males, the mean serum level was significantly higher in the patient's group before surgery in comparison with the control group ($p = 0.034$); and, after surgery, it became further significantly higher in the patient's group in comparison with the control group ($p = 0.030$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.151$).

In females, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p = 0.073$); but, after surgery, it became significantly higher in the patient's group in comparison with the control group ($p = 0.035$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.863$).

3.4. Serum level of interleukin-13 in anterior cruciate ligament injury patients

The result of the present study showed an increase in the serum of interleukin-13 after surgery as compared to before surgery in both before and after anterior cruciate ligament injury of the knee patients, at a significant difference ($p < 0.05$). As shown in (Fig. 4), Significant changes were indicated in the after-surgery group and the before-surgery compared to the control ($P < 0.001$).

Table 2. Comparison of serum IL-6 between the control group and patients' group (before and after surgery) according to sex.

Sex	Control group	Patient group before	Patient group after	*p1*	*p2*	*p3*
Male	2.74 ± 1.65	3.89 ± 1.44	5.70 ± 2.07	0.034 *	0.030 *	0.151
n	12	27	27			NS
Female	2.68 ± 1.47	4.44 ± 1.67	4.68 ± 0.85	0.073	0.035 *	0.863
n	18	3	3	NS		NS

n: number of cases; data were presented as mean ± standard deviation; *p*1: comparison between control group and patients before surgery; *p*2: comparison between control group and patients after surgery; *p*3: comparison between patients group before surgery and after surgery; NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

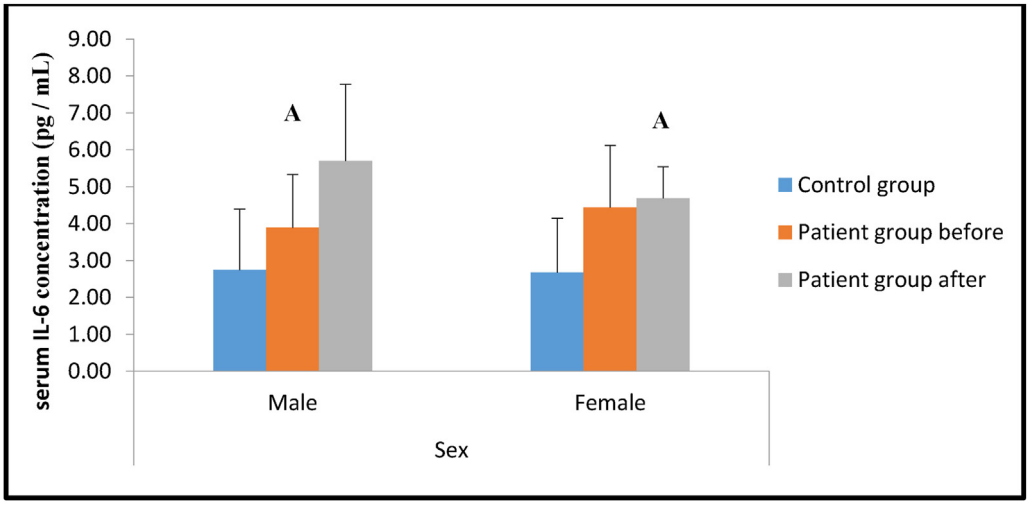

Fig. 3. ***Comparison of serum IL-6 between the control group and patients' group (before and after surgery) according to sex.*** *Letter (A) indicates a significant difference when comparing the control group to the patient group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

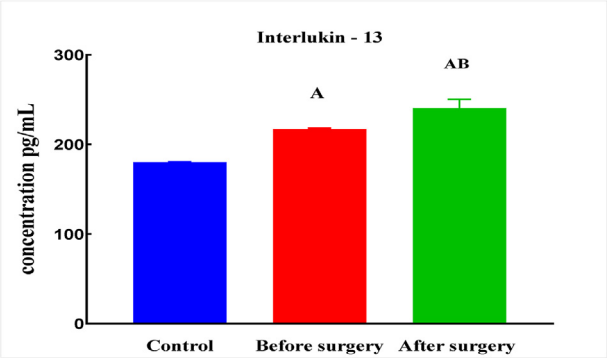

Fig. 4. ***Estimation serum concentrations of interleukin-13 [IL-13 (pg/mL)].*** *Levels in anterior cruciate ligament injury of the knee patients with before-surgery and after-surgery groups and control. Data are expressed as means ± SD, indicating a significant change between patient groups and control (P < 0.001), and Significant changes were indicated between patient groups (P < 0.05). Letter (A) indicates a significant difference when comparing the control group to the patient's group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

3.5. Comparison of serum level of IL-13 according to age

Comparison of serum IL-13 between the control group and the patients' group (before and after surgery) according to age intervals is shown in Table 3 and Fig. 5. In the age interval 20–39 years, the mean serum level was significantly higher in the patient's group before surgery in comparison with the control group ($p < 0.001$), and after surgery, it became further significantly higher in the patient's group in comparison with the control group ($p < 0.001$); in addition, there was significant difference in the serum level before surgery and after surgery ($p = 0.038$).

In the age interval 40–59 years, the mean serum level was significantly higher in the patient's group before surgery in comparison with the control group ($p < 0.001$), and after surgery, it became further significantly higher in the patient group in comparison with the control group ($p < 0.001$); in addition, there was a significant difference in the serum level before surgery and after surgery ($p = 0.021$).

3.6. Comparison of serum level of IL-13 according to sex

Comparison of serum IL-13 between the control group and the patients' group (before and after surgery) according to sex is shown in Table 4 and Fig. 6.

Table 3. Comparison of serum IL-13 between the control group and patients' group (before and after surgery) according to age intervals.

Age	Control group	Patient group before	Patient group after	*p1*	*p2*	*p3*
20–39	179.06 ± 1.82	216.98 ± 5.98	243.53 ± 58.90	<0.001 ***	<0.001 ***	0.038 *
n	20	25	25			
40–59	182.43 ± 6.30	216.89 ± 5.48	224.60 ± 4.47	<0.001 ***	<0.001 ***	0.021 *
n	7	5	5			

n: number of cases; data were presented as mean ± standard deviation; *p*1: comparison between control group and patients before surgery; *p*2: comparison between control group and patients after surgery; *p*3: comparison between patients group before surgery and after surgery; NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

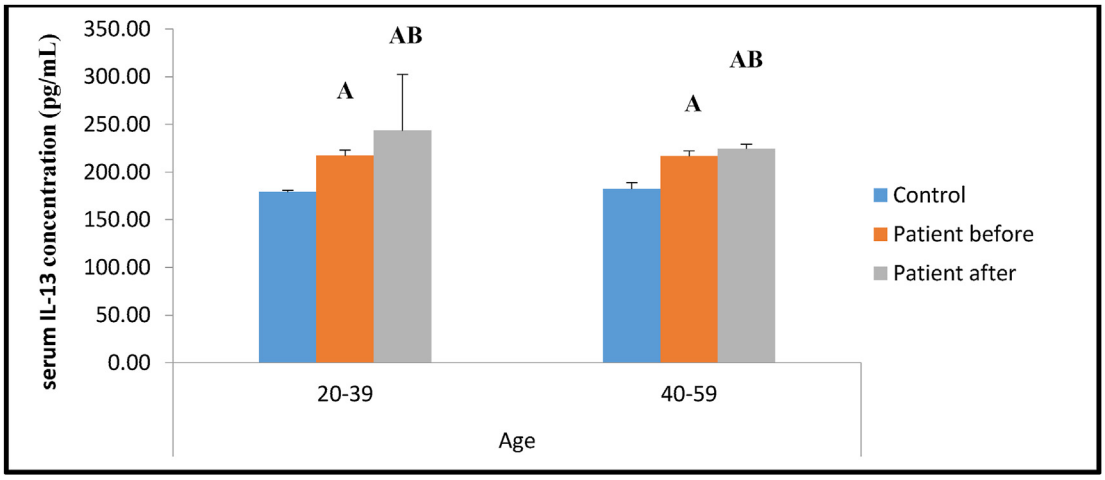

Fig. 5. ***Comparison of serum IL-13 between the control group and the patients' group (before and after surgery) according to age intervals.*** *Letter (A) indicates a significant difference when comparing the control group to the patient group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

Table 4. Comparison of serum IL-13 between the control group and patients' group (before and after surgery) according to sex.

Sex	Control group	Patient group before	Patient group after	*p1*	*p2*	*p3*
Male	178.83 ± 1.33	217.02 ± 5.72	233.83 ± 41.35	<0.001 ***	<0.001 ***	0.049 *
n	12	27	27			
Female	180.76 ± 4.59	216.43 ± 7.94	299.29 ± 120.01	<0.001 ***	<0.001 ***	0.366
n	18	3	3			NS

n: number of cases; data were presented as mean ± standard deviation; *p*1: comparison between control group and patients before surgery; *p*2: comparison between control group and patients after surgery; *p*3: comparison between patients group before surgery and after surgery; NS: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

In males, the mean serum level was significantly higher in the patient's group before surgery in comparison with the control group ($p < 0.001$); and, after surgery, it became further significantly higher in the patient's group in comparison with the control group ($p < 0.001$); in addition, there was a significant difference in the serum level before surgery and after surgery ($p = 0.049$).

In females, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p < 0.001$); but, after surgery, it became significantly higher in the patient's group in comparison with the control group ($p < 0.001$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.366$).

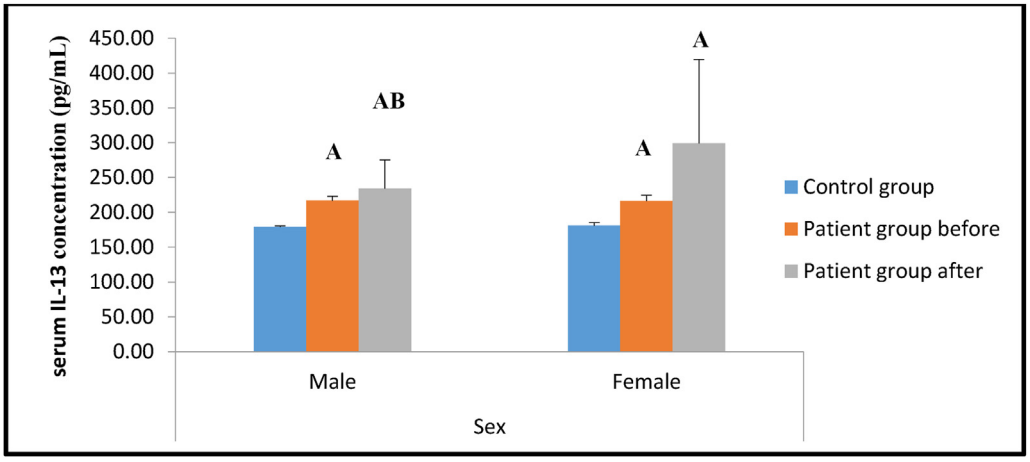

Fig. 6. ***Comparison of serum IL-13 between the control group and patients' group (before and after surgery) according to sex.*** *Letter (A) indicates a significant difference when comparing the control group to the patient's group before or after surgery; letter (B) indicates a significant difference when comparing patients before surgery to patients after surgery.*

4. Discussion

Playing activities like soccer, football, and skiing may put a strain on the anterior cruciate ligament (ACL) and other ligaments in the body [36]. An increase in inflammatory cytokines and collagen breakdown signals inside the joint after injury initiates a detrimental cascade of events that starts with an ACL rupture. It is very uncommon for individuals with ACL tears to also have meniscus tears in addition to cartilage damage [37]. It is evident that ACL repair surgery causes inflammation and may reduce cartilage degradation that follows [38]. While anterior cruciate ligament repair cannot stop osteoarthritis from developing, it may enhance knee kinematics and lessen the likelihood of cartilage and meniscus injuries. More precise magnetic resonance imaging (MRI) has made it possible to see knee degeneration sooner after an ACL injury, which has prompted studies into whether or not novel treatments may slow or stop this process [39].

4.1. Serum level of interleukin-6 (human IL-6) in anterior cruciate ligament injury patients

IL-6 is a multi-functional cytokine that has emerged as a promising therapeutic target for a range of illnesses, including RA [40]. Along with its roles in cell proliferation and differentiation, it has an anti-inflammatory effect and a pro-inflammatory one in autoimmune diseases and infections [41]. A wide range of cells that are involved in the start and management of inflammation and the immune response produce IL-6. These cells include keratinocytes, endothelial cells, monocytes/macrophages, fibroblasts, and smooth muscle cells [21]. Infectious agents, bacterial byproducts, and proinflammatory cytokines all have a role in inducing IL-6 gene expression. In response to infections, the body often produces IL-6, an early-inducible cytokine that quickly accumulates in the bloodstream [42]. In models of experimental chronic inflammation, IL-6 promotes the development of disease, indicating a pro-inflammatory role. However, IL-6 has immunoregulatory or even anti-inflammatory actions in some models of acute inflammation. This is likely due to its capacity to bridge the gap between the acute and chronic phases of inflammation, facilitating the creation of mononuclear granulomas and other forms of chronic inflammation. Therefore, IL-6 may play a role in transitioning defense responses from innate immune responses and early-stage inflammation to adaptive immunological responses [19,43]. During the inflammatory phase of wound healing, the inflammatory cytokines (TNF-a, IL-6, IL-1b, and IL-1a) are particularly significant, according to 44. By analyzing the concentrations of inflammatory cytokines within the joint immediately following an anterior cruciate ligament (ACL) injury, we aimed to comprehend the posttraumatic healing process of a knee that had suffered an ACL injury [45,46]. Preventing the catabolic response after an ACL injury has recently been shown to delay the early and permanent loss of cartilage proteins seen following an ACL injury [47].

4.2. Serum level of interleukin-13 in anterior cruciate ligament injury patients

People who have eosinophilic disorders, like eosinophilic esophagitis, asthma, and atopic dermatitis, are more likely to have inflammation because of IL-13. On the other hand, IL-13's anti-inflammatory effect on macrophages comes from blocking pro-inflammatory cytokines like TNFα and IL-1β [48,49]. It was found that IL-13 had anti-inflammatory effects on arthritis [34,50]. This study looked at how a single proinflammatory cytokine (TNF-α), an anti-inflammatory cytokine (IL-13), and several proinflammatory cytokines (IL-1α, IL-6, and TNF-α) affect the function of chondrocytes. Chondrocytes are the exclusive cellular constituents found in articular cartilage that are tasked with the synthesis and upkeep of the cartilage matrix. Pro-inflammatory cytokines, such as IL-6, TNF-α, and IFN-γ, cause collateral ligament degeneration by stopping the production of proteoglycans and increasing the activity of enzymes that break down matrixes. It is important to note that the purported anti-inflammatory effects of IL-4 and IL-13 on cartilage may be indirect due to the in vivo nature of some of these experiments, which permitted intricate interactions with synovial inflammation. On the other hand, cytokines with anti-inflammatory properties, including IL-13, IL-4, and IL-10, exert the opposite influence on cartilage metabolism as IL-1, TNF-α, and IFN-γ. Conversely, prior research has elucidated the pivotal role that pro-inflammatory and anti-inflammatory cytokines play in the process of cartilage synthesis [53,54].

4.3. Comparison of all marker concentrations in patient's anterior cruciate ligament injury of the knee between the control group and patients' group (before and after surgery) according to age intervals

4.3.1. Serum interleukin-6 (IL-6) concentration

Our study found that the concentration of serum IL-6 increased with age in the 20–39 age group. Compared to the control group, patients had significantly higher serum levels before and after

surgery. However, within the 20–39 age group, there was no significant difference in serum levels before and after surgery. Biomarkers of healing were shown to correlate with participants' ages in this research. Adolescents undergo major physiological changes, such as growth plate activity and hormonal alterations, throughout puberty, making their age a crucial factor in the research. During endochondral bone formation, the growth plates of the long bones in the leg release type II collagen and chondrocytes that are very active [55]. In contrast, between the ages of 40 and 59, there is a statistically significant difference between the pre-and post-operative serum IL-6 concentration levels, but no such difference between the control and patient groups. Patients' joints deteriorate with age, which is the main cause. This study's findings are in line with those of Barakat AM et al. [56]. There is a strong correlation between the presence of IL-6 in the synovial fluid and serum of RA patients and the progression of disease activity as patients age.

4.3.2. Serum interleukin-13 (IL-13) concentration

Our study looked at serum IL-13 levels in people of different ages and found that patients' levels were significantly higher than the control group's levels both before and after ACL reconstruction surgery. However, these levels were very different between the two groups. These findings are in line with those of previous research by Barker T, Martins TB et al. [57,58].

4.4. Comparison of all marker concentrations in patient's anterior cruciate ligament injury of the knee between the control group and patients' group (before and after surgery) according to sex

4.4.1. Serum interleukin-6 (IL-6) concentration

There was no significant difference in the serum level of IL-6 concentration between male and female patients before and after ACL repair surgery. However, the serum level of IL-6 concentration was significantly higher in both male and female patients before and after surgery compared to the control group. Our data demonstrates sex disparities in the timing and cellular makeup of the inflammatory response in the affected joint following anterior cruciate ligament reconstruction (ACLR) surgery. These findings align with recent research indicating that females experience a faster resolution of acute inflammation compared to males. Notwithstanding these innate disparities in gender [59,60].

4.4.2. Serum interleukin-13 (IL-13) concentration

Males' blood IL-13 concentrations varied significantly between the pre-and post-ACL repair surgery groups, as compared to the control group. There was a notable disparity in serum IL-13 levels before and during anterior cruciate ligament repair (post-ACLR) surgery [61,62]. On the other hand, serum IL-13 levels did not change significantly in females before or after ACL regeneration surgery. The current research's findings are in agreement with those of a previous study by Hagemans FJA, Larsson S et al. [63].

4.5. Conclusion

The cytokines interleukin-6 (IL-6) and interleukin-13 (IL-13) may be involved in the inflammatory process that leads to synovitis, according to our data. Furthermore, persistently high levels of these substances after an anterior cruciate ligament (ACL) injury imply that they may contribute to the development of post-traumatic osteoarthritis by maintaining the inflammatory environment.

Funding

The study was funded by the researchers themselves.

Acknowledgments

Al-Qadisiyah University/College of Medicine provided facilities, which helped in enhancing the quality of this work, are highly appreciated by the authors.

References

[1] Juneja P, Munjal A, Hubbard JB. Anatomy, joints. In: StatPearls [Internet]. StatPearls Publishing; 2023.
[2] Bammidi R, Jakka M, Balaga VR, Barla N, Vittanala VS, Pampana SJS, et al. Design and analysis of knee cap. Int J Med Rev Case Reports 2020;4(6):41.
[3] Albdeery A, Alzamily A. Evaluation of the effect of injections of both platelet-rich plasma and hyaluronic acid in patients with early knee osteoarthritis via the concentration of interleukin-1β in serum. J Biomed Biochem 2022;1(3):39–49.
[4] Vaienti E, Scita G, Ceccarelli F, Pogliacomi F. Understanding the human knee and its relationship to total knee replacement. Acta Bio Medica Atenei Parm 2017;88(Suppl 2):6.
[5] Sciarretta FV, Lane JG. Anatomy of the knee. In: The art of the musculoskeletal physical exam. Springer; 2023. p. 393–402.
[6] Webster J. The influence of soft tissue balancing on post-operative in vivo tibiofemoral contact kinematics. In: Total knee arthroplasty. Canada: The University of Western Ontario; 2018.
[7] Abulhasan JF, Grey MJ. Anatomy and physiology of knee stability. J Funct Morphol Kinesiol 2017;2(4):34.
[8] Ragab Y, Emad Y, Alhusseiny K, Elnaggar A, Saad AA, Saad OA, et al. Bilateral extensive steroid-associated osteonecrosis (SAON) of the femur, tibia, and patella: successful early management with combined antiresorptive and anabolic bone agents. Egypt Rheumatol 2023;45(4):324–8.

[9] Prathap Kumar J, Arun Kumar M, Venkatesh D. Healthy gait: review of anatomy and physiology of knee joint. Int J Curr Res Rev 2020;12(6):1–8.
[10] Kositsky A. Semitendinosus structure and function in healthy and anterior cruciate ligament reconstructed individuals. Itä-Suomen yliopisto 2023. Online published dissertation https://erepo.uef.fi/handle/123456789/29252.
[11] Mohamadi A, Momenzadeh K, Masoudi A, Walley KC, Ierardi K, Ramappa A, et al. Evolution of knowledge on meniscal biomechanics: a 40-year perspective. BMC Musculoskelet Disord 2021;22:1–13.
[12] Guyot J. Atlas of human limb joints. Springer Science & Business Media; 2013.
[13] Yousif HA, Alzamily AA, Alsalman IA. Interleukin-4 enhances the production of anti-inflammatory macrophages that inhibit the growth of osteoclasts to improve knee functionality. J Biomed Biochem 2022;1(3):50–6.
[14] Wheatley MGA, Thelen DG, Deluzio KJ, Rainbow MJ. Knee extension moment arm variations relate to mechanical function in walking and running. J R Soc Interface 2021; 18(181):20210326.
[15] Chng YL. Biomarkers of anterior cruciate ligament injury and recovery. Queensland University of Technology; 2019.
[17] Fox AJS, Bedi A, Rodeo SA. The basic science of human knee menisci: structure, composition, and function. Sports Health 2012;4(4):340–51.
[18] Wu X, Cao W, Yu H, Zhang Z, Leng Y, Zhang M. Generating electricity during locomotion modes dominated by negative work via a knee energy-harvesting exoskeleton. IEEE/ASME Trans Mechatronics 2022;27(6):4451–61.
[19] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspect Biol 2014;6(10):a016295.
[20] Alzamily AA, Al-Delfi MN, Al-Barqaw AR. A role for inflammatory IL-6 in the development of coronary artery disease: a case-control study at Al-Qadisiyah governorate, Iraq. Mil Med Sci Lett 2022;91(4):293–304.
[21] Chauhan P, Nair A, Patidar A, Dandapat J, Sarkar A, Saha B. A primer on cytokines. Cytokine 2021;145:155458.
[22] Wiegertjes R, van de Loo FAJ, Blaney Davidson EN. A roadmap to target interleukin-6 in osteoarthritis. Rheumatology 2020;59(10):2681–94.
[23] Caveney NA, Glassman CR, Jude KM, Tsutsumi N, Garcia KC. Structure of the IL-27 quaternary receptor signaling complex. Elife 2022;11:e78463.
[24] Alonso B, Bravo B, Mediavilla L, Gortazar AR, Forriol F, Vaquero J, et al. Osteoarthritis-related biomarkers profile in chronic anterior cruciate ligament injured knee. Knee 2020; 27(1):51–60.
[25] Bigoni M, Sacerdote P, Turati M, Franchi S, Gandolla M, Gaddi D, et al. Acute and late changes in intraarticular cytokine levels following anterior cruciate ligament injury. J Orthop Res 2013;31(2):315–21.
[26] Wei G, Lu K, Umar M, Zhu Z, Lu WW, Speakman JR, et al. Risk of metabolic abnormalities in osteoarthritis: a new perspective to understand its pathological mechanisms. Bone Res 2023;11(1):63.
[27] Mohammed Ali SH. Assessment of interleukin-13 (rs20541) genomic polymorphism in patients with acute respiratory distress syndrome about COVID-19 infection. Arch Razi Inst 2022;77(6):2291.
[28] Ayakannu R, Abdullah NA, Radhakrishnan AK, Raj VL, Liam CK. Relationship between various cytokines implicated in asthma. Hum Immunol 2019;80(9):755–63.
[29] Fu C, Tao Y, Chen H, Li M, Xiao Y, Yao Y, et al. Monocyte differentiation-surveilling nano-shuttles for activatable targeted inhibition of atherosclerotic plaque progression. Adv Funct Mater 2023:2303150.
[30] Cernescu LD, Haidar L, Panaitescu C. Dendritic cell-CD4+ T cell interaction: the differential role of IL-4/IL-13 in serum IgE levels in house dust mite allergic patients. Exp Ther Med 2021;21(1):1.
[31] Shankar A, McAlees JW, Lewkowich IP. Modulation of IL-4/IL-13 cytokine signaling in the context of allergic disease. J Allergy Clin Immunol 2022;150(2):266–76.
[32] Nganvongpanit K, Phothawan A, Pradit W, Eitssayeam S, Settakorn J, Chomdej S, et al. Using alumina and zirconia ceramic composite in dogs: a biocompatibility study. Chiang Mai J Sci 2013;40(4):763–74.
[33] Shaikh PZ. Cytokines & their physiologic and pharmacologic functions in inflammation: a review. Int J Pharm Life Sci 2011;2(11).
[34] Iwaszko M, Biały S, Bogunia-Kubik K. Significance of interleukin (IL)-4 and IL-13 in inflammatory arthritis. Cells 2021; 10(11):3000.
[35] Ahmed A, Razzaque MA, Kaleem M, Zaman AU, Akram R, Javed S. Diagnostic accuracy of magnetic resonance imaging in detecting anterior cruciate ligament injuries. Med J Indones 2017;26(3):218–23.
[36] Gill VS, Tummala SV, Boddu SP, Brinkman JC, McQuivey KS, Chhabra A. Biomechanics and situational patterns associated with anterior cruciate ligament injuries in the National Basketball Association (NBA). Br J Sports Med 2023;57(21):1395–9.
[37] Hunt ER, Jacobs CA, Conley CE, Ireland ML, Johnson DL, Lattermann C. Anterior cruciate ligament reconstruction reinitiates an inflammatory and chondrodegenerative process in the knee joint. J Orthop Res 2021;39(6):1281–8.
[38] Hunt ER. Anterior cruciate ligament injury-induced alterations in inflammation and muscle physiology. 2020.
[39] Cheung EC, DiLallo M, Feeley BT, Lansdown DA. Osteoarthritis and ACL reconstruction—myths and risks. Curr Rev Musculoskelet Med 2020;13:115–22.
[40] Danese S, Argollo M, Le Berre C, Peyrin-Biroulet L. JAK selectivity for inflammatory bowel disease treatment: does it clinically matter? Gut 2019 Oct;68(10):1893–9. https://doi.org/10.1136/gutjnl-2019-318448. Epub 2019 Jun 21. PMID: 31227590.
[41] de Oliveira dos Santos AR, de Oliveira Zanuso B, Miola VFB, Barbalho SM, Santos Bueno PC, UAP Flato, et al. Adipokines, myokines, and hepatokines: crosstalk and metabolic repercussions. Int J Mol Sci 2021;22(5):2639.
[42] Simbirtsev AS, Kozlov IG. Cytokine system. Mech Stretch Cytokines 2012:1–33.
[43] Forcina L, Franceschi C, Musarò A. The hormetic and hermetic role of IL-6. Ageing Res Rev 2022:101697.
[45] Lieberthal J, Sambamurthy N, Scanzello CR. Inflammation in joint injury and post-traumatic osteoarthritis. Osteoarthritis Cartilage 2015;23(11):1825–34.
[46] Jian J, Konopka J, Liu C. Insights into the role of progranulin in immunity, infection, and inflammation. J Leukoc Biol 2013; 93(2):199–208.
[47] Haslauer CM, Proffen BL, Johnson VM, Hill A, Murray MM. Gene expression of catabolic inflammatory cytokines peak before anabolic inflammatory cytokines after ACL injury in a preclinical model. J Inflamm 2014;11(1):1–10.
[48] Omata Y, Frech M, Saito T, Schett G, Zaiss MM, Tanaka S. Inflammatory arthritis and bone metabolism regulated by type 2 innate and adaptive immunity. Int J Mol Sci 2022;23(3):1104.
[49] Gill PA, Inniss S, Kumagai T, Rahman FZ, Smith AM. The role of diet and gut microbiota in regulating gastrointestinal and inflammatory disease. Front Immunol 2022;13:866059.
[50] Chen Z, Bozec A, Ramming A, Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol 2019;15(1):9–17.
[53] Schuerwegh AJ, Dombrecht EJ, Stevens WJ, Van Offel JF, Bridts CH, De Clerck LS. Influence of pro-inflammatory (IL-1α, IL-6, TNF-α, IFN-γ) and anti-inflammatory (IL-4) cytokines on chondrocyte function. Osteoarthritis Cartilage 2003; 11(9):681–7.
[54] Wu Y, Li J, Zeng Y, Pu W, Mu X, Sun K, et al. Exosomes rewire the cartilage microenvironment in osteoarthritis: from intercellular communication to therapeutic strategies. Int J Oral Sci 2022;14(1):40.

[55] Ek Orloff L. Biomarkers of knee joint healing in adolescents with anterior cruciate ligament injuries. Université d'Ottawa/ University of Ottawa; 2022.
[56] Barakat AM, Hasony HJ, Al-Khafaji AT, Mezal TJ. Diagnostic value of antibodies to citrullin-containing peptides in patients with rheumatoid arthritis in southern Iraq. Basrah J Surg 2009;15:43−50.
[57] Barker T, Martins TB, Hill HR, Kjeldsberg CR, Trawick RH, Leonard SW, et al. Vitamins E and C modulate the association between reciprocally regulated cytokines after an anterior cruciate ligament injury and surgery. Am J Phys Med Rehabil 2011;90(8):638−47.
[58] Liu BM, Martins TB, Peterson LK, Hill HR. Clinical significance of measuring serum cytokine levels as inflammatory biomarkers in adult and pediatric COVID-19 cases: a review. Cytokine 2021;142:155478.
[59] Morris JL, McEwen PC, Letson HL, Dobson GP. Adenosine, Lidocaine, and Magnesium (ALM) therapy modulates early sex-specific inflammatory and immune responses following experimental anterior cruciate ligament rupture and reconstruction. Transl Med Commun 2023;8(1):14.
[60] Morris JL, Letson HL, Biros E, McEwen PC, Dobson GP. Female rats have a different healing phenotype than males after anterior cruciate ligament rupture with no intervention. Front Med 2022;9:976980.
[61] Evans A. The effect of mechanical load on biomarkers of knee joint inflammation for individuals who are predisposed to knee cartilage degeneration: an exploratory study. Brigham Young University; 2018.
[62] Barker T. Oxidative stress and muscle dysfunction following anterior cruciate ligament surgery. Oregon State University; 2009.
[63] Hagemans FJA, Larsson S, Reijman M, Frobell RB, Struglics A, Meuffels DE. An anterior cruciate ligament rupture increases levels of urine N-terminal cross-linked telopeptide of type I collagen, urine C-terminal cross-linked telopeptide of type II collagen, serum aggrecan ARGS neoepitope, and serum tumor necrosis factor−α. Am J Sports Med 2021;49(13):3534−43.