

Assessment of MCP-1 and IL-20 Levels in Cartilage Turnover Among Patients with Anterior Cruciate Ligament Injuries

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ABSTRACT

Background: Anterior cruciate ligament (ACL) injuries, along with meniscus and collateral ligament tears, are among the most common knee injuries. These injuries significantly impact knee stability and mobility, often leading to osteoarthritis and limiting physical activity. The ACL provides critical stability to the knee, and its injury is frequently associated with structural and functional changes detectable via MRI and clinical imaging. Inflammation and healing in ACL injuries can be evaluated using biochemical markers. **Objective:** This study aimed to investigate the association of monocyte chemotactic protein-1 (MCP-1) and interleukin-20 (IL-20) levels with the risk and progression of ACL injuries. **Materials and Methods:** The study included 120 participants, comprising 60 patients with ACL injuries and 60 healthy controls (67 males and 53 females) aged 18–75 years. The participants were recruited from Al-Furat Al-Awsat Private Hospital and the Royal Private Hospital in Iraq's Al-Diwaniyah Governorate between November 2022 and November 2023. MCP-1 and IL-20 levels were measured using Elabscience ELISA kits in the Department of Medical Chemistry at Al-Qadisiyah University College of Medicine. Biochemical markers were assessed before and six weeks after ACL repair. **Results:** Patients with ACL injuries exhibited significantly higher MCP-1 levels in comparison to both pre- and post-reconstruction levels ($P < 0.05$). Post-surgical MCP-1 levels remained significantly elevated compared to the control group ($P < 0.001$). IL-20 levels showed non-significant increases following surgery ($P > 0.05$); however, significant differences ($P < 0.001$) were observed between the patient and control groups. **Conclusion:** Elevated MCP-1 levels suggest its strong association with inflammation and the risk of ACL injuries. IL-20 appears to contribute to the production of pro-inflammatory cytokines, including IL-6 and MCP-1, which exacerbate joint inflammation and cartilage degradation. These findings indicate that IL-20 plays a critical role in the pathogenesis of knee osteoarthritis, particularly in patients with ACL injuries and the elderly, where it promotes cartilage destruction and joint degradation. Monitoring these biomarkers could aid in understanding disease progression and tailoring therapeutic interventions for ACL injury patients.

Keywords: injury ACL, Knee pathology, Inflammatory biomarkers, MCP-1, and IL-20.

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are among the most common and debilitating musculoskeletal injuries, particularly affecting athletes and physically active individuals [1]. The ACL plays a pivotal role in maintaining the stability of the knee joint, preventing anterior translation and rotational instability of the tibia in relation to the femur [2, 3]. Injuries to the ACL, whether partial or complete tears, are often accompanied by damage to other intra-articular structures such as the meniscus and collateral ligaments, resulting in complex joint pathologies that significantly impair knee function and mobility. If not adequately treated, ACL injuries can predispose patients to chronic joint instability, recurrent injuries, and early-onset osteoarthritis (OA). Beyond mechanical damage, ACL injuries initiate a cascade of biochemical events characterized by inflammation, immune cell infiltration, and the release of catabolic cytokines [4, 5]. These processes contribute to joint degeneration and cartilage degradation, ultimately influencing the long-term outcomes of ACL reconstruction and rehabilitation. Monitoring the biological response to ligament injury and repair is crucial for understanding disease progression and evaluating therapeutic efficacy [6]. In this context, biomarkers serve as valuable tools for detecting pathological changes at the molecular level [7]. Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, is a key pro-inflammatory chemokine involved in the recruitment of monocytes and other immune cells to

sites of tissue injury [8]. MCP-1 plays an important role in mediating inflammation and has been implicated in the pathogenesis of various joint disorders, including osteoarthritis and rheumatoid arthritis. Elevated levels of MCP-1 have been detected in synovial fluid and serum of patients with knee injuries, indicating its potential as a marker of cartilage turnover and joint inflammation [9]. Interleukin-20 (IL-20) is a member of the IL-10 cytokine family and has emerged as a significant mediator of tissue damage and inflammation in autoimmune and degenerative joint diseases [10, 11]. IL-20 promotes the expression of other inflammatory mediators such as IL-6 and MCP-1 and contributes to the destruction of articular cartilage by enhancing catabolic activity in chondrocytes and synoviocytes. Although IL-20 has been widely studied in the context of rheumatoid arthritis, its role in ACL injury-induced inflammation and cartilage turnover remains underexplored [12]. The interplay between these inflammatory markers and the structural integrity of the knee joint provides an opportunity to develop molecular diagnostics and targeted therapies. Understanding the temporal changes in MCP-1 and IL-20 levels following ACL injury and surgical intervention could offer insights into the biological processes governing healing and degeneration [13, 14]. Despite these supports, the knee is among the most susceptible joints in the body to stress and sports injuries because of its position between the tibia and the femur, the two longest bones [15, 16]. Cartilage

covers the articular surfaces of the knee joint. In a knee joint, you'll find articular cartilage, which covers the ends of bones, and meniscus, a wedge-shaped fibrotic cartilaginous tissue [17]. While walking, the knee joint bends to accommodate the wearer's weight and supports the body's weight in general [18]. One of the many chemokines that cause inflammation is monocyte chemoattractant protein-1 (MCP-1). It is very important for getting white blood cells to areas of tissue damage and inflammation [19]. When monocytes, memory T cells, macrophages, fibroblasts, endothelial cells, and mast cells bind to their cognate cell-surface receptor (CCR2), they release MCP-1. This causes responding leukocytes to move along a chemotactic gradient [20]. The receptor for CCL2 is CCR2, which is expressed in a variety of cell types of the immune system, including monocytes, activated T cells, NK cells, and DCs [21]. The location of the MCP-1 gene is on chromosome 1 at 11711q11.21-q21.1 using a three-exon and two-intron structure. and a 13-kDa molecule of human CCL2 is 22 kDa. The precursor molecule of MCP-1 is composed of a 23-amino-acid hydrophobic signal peptide. The monomeric polypeptide formed by the 76 amino acids that remain after the signal protein cleavage is known as the mature protein [22]. The synovial fibroblasts release the important chemokine MCP-1 when the inflammatory cytokines interleukin (IL)-1, tumor necrosis factor- α , and interferon- γ stimulate them [23]. Several clinical conditions rely on MCP-1, including cardiovascular disease, brain disease, bone and joint disorders, respiratory infections, cancer, endothelial dysfunction, and coronavirus disease (COVID-19). In addition, inflammation relies heavily on this chemokine [22]. Also, abnormal MCP-1 expression can speed up the progression of OA, which damages joints, helps monocytes become macrophages in the knee joint capsule, turns on osteoclasts to break down bone, causes inflammation, and more [24]. Interleukin-20 (IL-20), a member of the IL-10 family of cytokines that primarily originate from activated monocytes and dendritic cells and is known to promote inflammation [25, 26], is one of these cytokines. IL-20 heterodimeric receptors are found on phagocytes, endothelial cells, synovial fibroblasts, and osteoclasts, among other cell types. These receptors are what make the cytokine cause inflammation [27]. T-cells and monocytes produce the cytokine interleukin-20, which has 176 amino acids and is 20 kDa in size. A connection to Th-2-based immune responses has been suggested, and it has been associated with psoriasis. Like IL-19 and IL-24, it is located on chromosome 1 at the Q32 position. Its principal cells of distribution are keratinocytes and monocytes in the skin. Wolk recently verified the significance of maturing dendritic cells as an IL-20 secretory source [28]. In particular, IL-20 primarily targets keratinocytes, endothelial cells, and adipocytes [29]. There is evidence that IL-20's ability to cause inflammation is closely connected to the start and progression of several illnesses and diseases, such as RA, atherosclerosis, brain damage, ulcerative colitis, and renal failure [30]. It looks like IL-20 causes a lot of inflammation because it mainly affects the synovium and areas where bones are breaking down. IL-20 stimulates the production of several inflammatory cytokines, which exacerbates joint inflammation and damage [31].

Methods

Study Design

Expert doctors at Al-Furat Al-Awsat Private Hospital and Royal Private Hospital enrolled patients with suspected cases of cartilage

turnover in the anterior cruciate ligament as part of a case-control research design.

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 University of Al-Qadisiyah College of Medicine's Department of Medicinal Chemistry were the sites of all these experiments.

Sample Collections

Between November 2022 and November 2023, the research was ongoing. Individuals aged 18 to 60 years were included among the 60 patients who suffered an anterior cruciate ligament injury of the knee. Radiographic evidence (X-rays) and clinical observations form the basis of all diagnoses in bone and joint surgery. Preoperative magnetic resonance imaging (MRI) and postoperative computed tomography (CT) are both valid methods for analyzing the anterior cruciate ligament structure among the radiological testing options [32].

Depending on whether patients were classified as "preoperative" or "postoperative," two categories were created. Sixty healthy people were collected to serve as a control group. The ages of the participants ranged from twenty to seventy-five years on average. Appealingly healthy people were randomly selected for the study as controls. Osteoporosis and anterior cruciate ligament injuries are not among their medical conditions. Doctors in private clinics in Diwaniyah Governorate diagnosed the cases.

Blood Collection

Each subject underwent this twice. Before and six weeks after surgery, the second was taken. In this research, 5 ml was extracted intravenously from each patient and placed at room temperature for 15 minutes. Centrifuging the blood at 4000 rpm for five minutes yielded serum. A labeled Eppendorf tube was used to maintain the serum at -80°C until use.

Inclusion Criteria

The present study included the following: Male and female anterior cruciate ligament injury patients, ages ranging from 18-60 years, are diagnosed by Orthopedic and joint surgery specialists depending on clinical findings and are proven by radiological findings (x-ray) and preoperative MRI available for assessing the ACL injury.

Exclusion Criteria

The research excluded patients with acute or chronic liver disease, renal disease, thyroid disease, All types of diabetes mellitus, and autoimmune diseases. Due to the dysregulation of the immune system, the levels of cytokines are abnormal in these diseases and thus affect the diagnostic role of Interleukin 16, Interleukin 131, Interleukin 201, and Monocyte Chemotactic Protein 1.

Interleukin 20 and MCP-1 (monocyte chemotactic protein 1)

This ELISA kit uses the Sandwich-ELISA principle. The ELISA plate provided in this kit is pre-coated with an antibody specific for Human IL-20 and Human MCP-1. This ELISA kit applies to the in vitro quantitative determination of Human IL-20 and Human MCP-1 concentrations in serum, plasma and other biological fluids.

Table 1: Character

Item	
Sensitivity	37.50 pg/mL
Detection Range	62.50-4000 pg/mL
Specificity	This kit recognizes Human IL-20 in samples. No significant cross-reactivity or interference between Human IL-20 and analogues was observed
Repeatability	Coefficient of variation is < 10%

Assay Procedure

1. Find diluted standard, blank, and sample wells. Use 100 μ L of each dilution of standard, blank, and sample in the designated wells. Assay samples and standards in duplicate. Sample dilution ratios should be determined by preliminary tests or technical help. Seal the plate with the kit sealer. Incubate at 37°C for 90 minutes. Remember to apply solutions to the bottom of the micro ELISA plate to avoid foaming and touching the interior wall.

2. Pour out the contents of each well without washing. Straight away, fill up every well with 100 μ L of the Biotinylated Detection Ab working solution. Apply a fresh coat of sealant to the dish. Leave to incubate at 37°C for 1 hour.

3. Transfer 350 μ L of wash buffer to every well after removing the solution from each well. After soaking for one minute, remove excess solution from each well by aspirating or decanting it, and then pat dry using clean absorbent paper. Do this wash process three times. Please be aware that this and subsequent washing processes can be performed using a microplate washer. After washing, put the tested strips to use right away. Make sure that wells are never dry. 4. Add 100 μ L of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5. After collecting the solution from each well, proceed to wash the equipment five times, just as in step 3.

9. Fill up every well with 90 μ L of Substrate Reagent. Put a fresh coat of sealant on top of the plate. Let stand at 37°C for approximately 15 minutes. The plate should be shielded from light. Keep in mind that the actual color change will determine the reaction time, which can be either cut or extended up to 30 minutes. Before taking an optical density (OD) reading, allow the microplate reader to heat up for approximately 15 minutes.

7. Pour fifty microliters of Stop Solution into each well. It is important to keep in mind that the addition of the stop solution should be carried out in the same order as the addition of the substrate solution.

8. Using a microplate reader that is tuned to 450 nm, determine the optical density (OD value) of each well at the same time.

Statistical Evaluation

Graph Pad Prism 91 was used to analyze, summarize, and show the data. Categorical data were expressed as numbers, whereas numerical data were expressed as mean $\pm$ standard deviation. When analyzing data that was not regularly distributed, chi-square was utilized to assess the qualitative information, and one-way ANOVA and paired t-tests were employed to compare mean values across the various groups. Pearson's correlation coefficient was used to analyze the relationship between biological indicators. A P-value of $P < 0.05$ was deemed significant.

Results

Serum Level of Monocyte Chemotactic Protein 1 (MCP-1) in Anterior Cruciate Ligament Injury Patients

The result of the present study showed an increase in the serum of Monocyte Chemotactic Protein 1 (MCP-1) after surgery as compared to before surgery in both before and after anterior cruciate ligament injury of the knee patients, at significant difference ($p < 0.05$). As shown in (Figure 1). Significant changes were indicated in the after-surgery group compared to the control ($P < 0.001$). NS non-significant changes were indicated between the before surgery and control ($p > 0.05$).

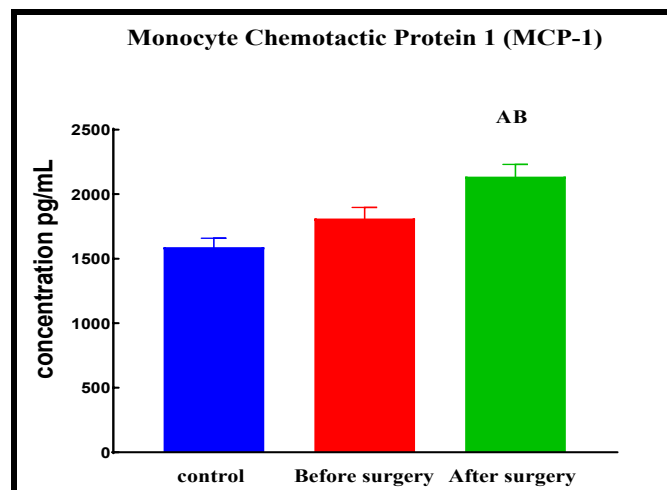


Figure 1: Estimation of Serum Concentrations of Monocyte Chemotactic Protein 1 [MCP-1 (pg/mL)].

compared to pre- and post-operative levels in individuals with ACL tears in the knee, the results, displayed as means $\pm$ SD, demonstrate a

notable increase in the serum level of Monocyte Chemotactic Protein 1 (MCP-1) from the pre- to post-operative times. A high increase

($P < 0.05$) was observed in the postoperative group, and there were significant changes ($P < 0.001$) compared to the control group. The results showed no statistically significant difference between the control and before surgery groups ($p > 0.051$). 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.

Comparison of Serum level of MCP-1 According to Age

A comparison of serum MCP-1 between the control group and the patients' group (before and after surgery) according to age intervals is

shown in Table 1 and Figure 2. In the age interval 20-39 years, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p = 0.0561$); 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 ($p = 0.067$). In the age interval of 40-59 years, as compared to the control group, the patient group's mean serum level was not significantly different before surgery ($p = 0.468$). However, after surgery, it became significantly higher ($p = 0.009$). Interestingly, there was no significant difference between the serum levels before and after surgery ($p = 0.160$).

Table 2: Comparison of Serum MCP-1 between the Control Group and Patients' Group (before and after treatment) according to Age Intervals

Age	Control Group	Patient Group Before	Patient Group After	P1	P2	P3
20-39	1589.8 ± 357.97	1839.8 ± 470.92	2117.0 ± 537.87	0.056	0.001***	0.067
n	20	25	25	NS		NS
40-59	1475.9 ± 361.33	1664.8 ± 511.34	2230.2 ± 454.04	0.468	0.009**	0.160
n	7	5	5	NS		NS

n: number of cases; data were presented as mean ± standard deviation; *p1*: comparison between control group and patients before surgery; *p2*: comparison between control group and patients after surgery; *p3*: comparison between patients' group before surgery and after surgery; *NS1*: not significant; *1: significant at $p < 0.05$; **1: significant at $p < 0.01$; ***1: significant at $p < 0.001$

Figure 2: Comparison of Serum MCP-1 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.

Comparison of Serum level of MCP-1 according to Sex

A comparison of serum MCP-1 between the control group and the patients' group (before and after surgery) according to sex is shown in Table 2 and Figure 3. In males, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p = 0.174$); however,

after surgery, it became significantly higher in the patient's group in comparison with the control group ($p = 0.002$); and, there was a significant difference in the serum level before surgery and after surgery ($p = 0.023$). In females, the mean serum level was not significantly different in the patient's group before surgery in comparison with the control group ($p = 0.146$); however, after surgery, it got significantly higher in the patient's group in comparison with the control group ($p = 0.029$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.675$).

Table 3: Comparison of serum MCP-1 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	1577.4 ± 333.09	1791.0 ± 483.91	2122.4 ± 515.84	0.174	0.002 **	0.023 *
N	12	27	27	NS		
Female	1597.3 ± 415.58	1987.3 ± 389.66	2256.7 ± 653.55	0.146	0.029 *	0.675
N	18	3	3	NS		NS

n: number of cases; data were presented as mean ± standard deviation; *p1*: comparison between control group and patients before surgery; *p2*: comparison between control group and patients after surgery; *p3*: comparison between patients' group before surgery and after surgery; *NS1*: not significant; *1: significant at $p < 0.05$; **1: significant at $p < 0.01$; ***1: significant at $p < 0.001$

Figure 3: Comparison of serum MCP-1 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.

Serum Level of Interleukin-20 in Anterior Cruciate Ligament Injury Patients

The result of the present study showed non-significant differences in the serum of interleukin-20 after surgery as compared

to before surgery in anterior cruciate ligament injury of the knee patients.

Indicating NS non-significant differences compared to the before surgery, p-value ($p > 0.05$). As shown in (Figure 4), a high increase was indicated in the after-surgery group compared to the control ($P < 0.001$).

Significant changes were indicated between the before-surgery and control ($P < 0.01$).

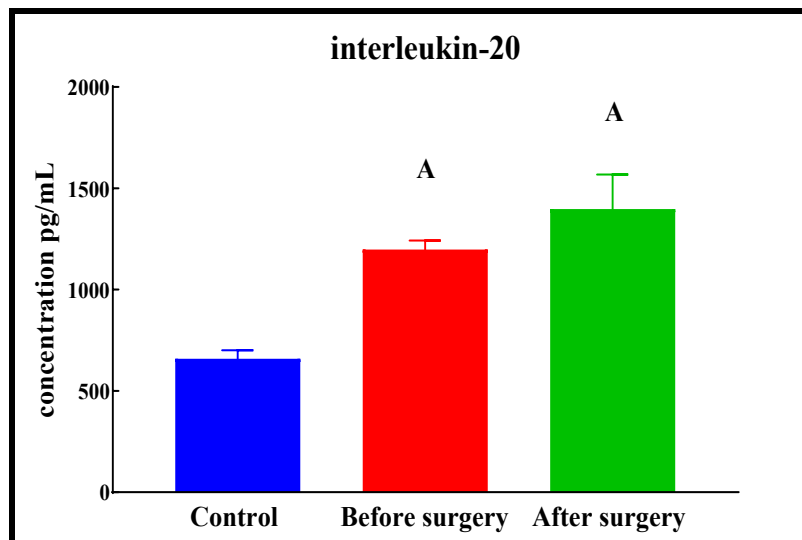


Figure 4: Estimation Serum Concentrations of Interleukin-20 [IL-20 (pg/mL)].

levels in anterior cruciate ligament injury of the knee patients with before-surgery and after-surgery groups and control. Data are expressed as means $\pm$ SD, indicating a significant change between patient groups and control ($P < 0.001$). Significant changes were indicated between the before-surgery and control ($P < 0.01$). NS non-significant change between patient groups. 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.

Comparison of Serum level of IL-20 according to Age

A comparison of serum IL-20 between the control group and the patients' group (before and after surgery) according to age intervals

is shown in Table 3 and Figure 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 ($1p < 0.001$), and after surgery, it became further significantly higher in the patient's group in comparison with the control group ($1p < 0.001$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.125$). 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 ($p = 0.075$), and after surgery, it was also not significantly different in the patient's group in comparison with the control group ($1p = 0.0861$); in addition, there was a significant difference in the serum level before surgery and after surgery ($1p = 0.6621$).

Table 4: Comparison of Serum IL-20 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	609.75 ± 85.43	1191.0 ± 182.53	1527.2 ± 1063.5	<0.001 ***	<0.001 ***	0.125
n	20	25	25			NS
40-59	795.17 ± 447.16	1296.5 ± 406.52	1191.5 ± 125.27	0.075	0.086	0.662
n	7	5	5	NS	NS	NS

n: number of cases; data were presented as mean ± standard deviation; *p1*: comparison between control group and patients before surgery; *p2*: comparison between control group and patients after surgery; *p3*: comparison between patients' group before surgery and after surgery; *NS*: not significant; *1: significant at $p < 0.05$; **1: significant at $p < 0.01$; ***1: significant at $p < 0.001$

Figure 5: Comparison of Serum IL-20 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.

Comparison of Serum level of IL-20 according to Sex

Comparison of serum IL-20 between the control group and the patients' group (before and after surgery) according to sex is shown in Table 4 and Figure 6. 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 ($p = 0.0031$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.1041$). In females, 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 got reduced but remained significantly higher in the patient's group in comparison with the control group ($p = 0.0171$); however, there was no significant difference in the serum level before surgery and after surgery ($p = 0.2031$).

Table 5: Comparison of Serum IL-20 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	574.47 ± 73.80	1176.2 ± 194.49	1507.2 ± 1025.19	< 0.001 ***	0.003 **	0.104
N	12	27	27			NS
Female	714.89 ± 280.81	1500.2 ± 346.51	1147.4 ± 22.63	< 0.001 ***	0.017 *	0.203
N	18	3	3			NS

n: number of cases; data were presented as mean ± standard deviation; *p1*: comparison between control group and patients before surgery; *p2*: comparison between control group and patients after surgery; *p3*: comparison between patients' group before surgery and after surgery; *NS*: not significant; *1: significant at $p < 0.05$; **1: significant at $p < 0.01$; ***1: significant at $p < 0.001$

Figure 6: Comparison of Serum IL-20 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.

Correlation Analysis

Correlations between serum markers of anterior cruciate ligament injury patients before and after surgery showed that before surgery, all serum markers showed positive correlations and after surgery, the same findings were observed positive correlations. As shown in Table 5 and Table 6.

Correlation between Monocyte Chemotactic Protein (MCP-1) and Other Biochemical Parameters in Anterior Cruciate Ligament Injury Patients before Surgery

Monocyte Chemotactic Protein 1 (MCP-1) was positively correlated with IL-6, IL-13, and IL-20 in anterior cruciate ligament

injury patients before surgery, there was a significant between MCP-1 and IL-6 ($P < 0.05$) as shown in Table 5). but non-significant weak positive correlation was shown between MCP-1 IL-13 and IL-20 ($P > 0.05$) in the pre-ACL-R patient group.

Correlation between Monocyte Chemotactic Protein (MCP-1) and other Biochemical Parameters in Anterior Cruciate Ligament Injury Patients after Surgery

Monocyte Chemotactic Protein 1 (MCP-1) was positively correlated with IL-6, IL-13, and IL-20 in anterior cruciate ligament injury patients after surgery, there was a significant between MCP-1 and IL-6 ($P < 0.05$) as shown in Table 6).

Also, high significance was shown between MCP-1 and IL-20 ($P < 0.001$), but a non-significant weak positive correlation was shown between MCP-1 and IL-13 ($P > 0.05$) in the post-ACL-R patient group.

Table 6: Correlation between Monocyte Chemotactic Protein 1 and other parameters in patients before surgery.

Parameter (Serum Biomarkers)	Monocyte Chemotactic Protein 1 (MCP-1)	
	pre-ACL-R (N=60)	
Interleukin-6 (IL-6)	r- value	0.414
	P-value	0.02 *
Interleukin-13 (IL-13)	r- value	0.277
	P-value	0.14 NS
Interleukin-20 (IL-20)	r- value	0.2
	P-value	0.36 NS

r: Correlation Value

Table 7: Correlation between Monocyte Chemotactic Protein 1 and other Parameters in Patients After Surgery

Parameter (Serum Biomarkers)	Monocyte Chemotactic Protein 1 (MCP-1)	
	post-ACL-R (n=60)	
Interleukin-6 (IL-6)	r- value	0.387
	P-value	0.03 *
Interleukin-13 (IL-13)	r- value	0.248
	P-value	0.19 NS
Interleukin-20 (IL-20)	r- value	0.619
	P-value	0.001 ***

r: Correlation Value

Discussion

The anterior cruciate ligament (ACL) is a common ligament injury in sports like soccer, football, and skiing, where players must be able to pivot and cut quickly [33]. When the anterior cruciate ligament (ACL) tears, the body reacts by producing more inflammatory cytokines and indicators of collagen breakdown in the injured joint. Patients with ACL tears often experience meniscus tears in addition to cartilage damage though the fact that ACL repair surgery might reduce cartilage damage in the future, it is evident that it adds to inflammation [34].

While anterior cruciate ligament repair cannot stop osteoarthritis from developing, it can enhance knee kinematics and lessen the likelihood of cartilage and meniscus injuries. Thanks to MRI's improved imaging capabilities, researchers can now see signs of knee degeneration earlier after an ACL injury, which has prompted them to investigate whether and how new treatments can slow or stop this progression [35].

Evaluation Concentrations of Pro-Inflammatory and Anti-Inflammatory Cytokine Levels of MCP-1 and IL-20 in Anterior Cruciate Ligament Injury Patients of the Knee Between Group Patients' (Before and After Surgery) Compared with Controls

Serum level of Monocyte Chemotactic Protein 1 (MCP-1)

On receptor binding of MCP-1 CCR2 promotes inflammation by activating cells like monocytes and other immune cells [36]. Multiple additional cytokines and factors influence the production of monocyte chemoattractant protein-1 by various cell types, including epithelial, endothelial, smooth muscle, fibroblast, astrocyte, and microglial cells [37]. In many diseases, they regulate the flow of immune cells to sites of injury or infection, including monocytes, microglia, and memory T lymphocytes. Multiple diseases, including those affecting the bones and joints, the lungs, the heart, and the brain, have been linked to MCP-1 [22]. High levels of MCP-1 have been observed in human investigations in chondrocytes and synovial fibroblasts from individuals suffering from knee osteoarthritis (OA).

Radiological findings and clinical inflammation in knee osteoarthritis are both correlated with MCP-1, suggesting that it reflects macrophage activity. Hence, MCP-1 could serve as a trustworthy measure of the seriousness of the condition [38]. All things considered, these results corroborate our findings that MCP-1 plays a role in the onset of knee osteoarthritis and could be useful in the diagnostic process. More severe knee osteoarthritis is linked to increased MCP-1 expression, which in turn correlates directly with grade. Hence, MCP-1 could be used as a biomarker to gauge the degree of knee osteoarthritis and as a therapeutic intervention target [39]. There was a significant difference ($P < 0.05$) in the levels of serum monocyte chemotactic protein-1 (MCP-1) before and after ACL reconstruction in knee patients, with an increase observed postoperatively compared to preoperatively. In comparison to the control group, the postoperative group likewise showed noticeably greater alterations ($P < 0.001$). Figure 1 illustrates this point. Consistent with a prior study, the present investigation found [37]. It has been demonstrated that there is a correlation between elevated levels of monocyte chemotactic protein-1 (MCP-1) and the proinflammatory cytokine monocyte chemoattractant protein-1 in cases of anterior cruciate ligament rupture. This finding suggests that bone bruising causes joint damage, which in turn increases inflammation following a tear and subsequent reconstruction of the ligament [40]. Patients who had anterior cruciate ligament injuries also had elevated MCP-1 levels, according to another study [41]. Immediate post-ACL damage and continuing for 12 months following ACLR, elevated serum MCP-1 and COMP concentrations are seen, which are linked to the onset of PTOA and other early unfavorable metabolic processes [23].

Serum Level of Interleukin-20

The family of interleukin 10 (IL-10) include IL-20, IL-19, IL-22, IL-24, and IL-26, among others [42]. The cytokine interleukin-20 (IL-20) has potent chemotactic and proinflammatory properties; it positively regulates angiogenesis and apoptosis, impacting keratinocytes, endothelial cells, and synovial membrane fibroblasts [43]. Mainly granulocytes, fibroblasts, and macrophages release IL-20 [44]. The inflammatory response is heightened by this interleukin, which causes neutrophils and T lymphocytes to migrate more rapidly [32]. TNF- α 1, IL-1 β 1, and metalloproteinase 11 and 13 (MMP1-1 and MMP1-13) are all enzymes that are secreted by osteoarthritic synovial fibroblasts (OASFs) when they are stimulated by IL-20, as stated in a study [45]. Not only does IL-20 affect chondrocytes, but it also enhances the activity of matrix metalloproteinase (MMP) and interleukin 6 (IL-6). At the same time, it suppresses the expression of transforming growth factor β (TGF- β 1), bone morphogenetic protein 2 (BMP1-2), and the ability to make aggrecan [46]. Recent research has shown that IL-20 plays a role as a proinflammatory cytokine in several inflammatory diseases [47]. IL-20 to the development of knee OA-related inflammation and cartilage degradation [48]. In patients with anterior cruciate ligament injuries to the knee, the current investigation found no statistically significant changes in interleukin-20 serum levels between pre- and post-operative periods ($p > 0.05$). Figure 4 demonstrates this. In comparison to the control group, the after-surgery group showed a significant increase ($P < 0.001$). Significant alterations were also noted between the control group and the group that was examined before surgery ($P < 0.01$). This is the first study to examine IL-20 as a biomarker in individuals who have ruptured the anterior cruciate

ligament of the knee, as no prior research has focused on this particular lesion.

Comparison of all Marker Concentrations in the Patient's Anterior Cruciate Ligament Injury of the Knee between the Control Group and the patients' Group (Before and After Surgery) According to Age Intervals

Serum Monocyte Chemotactic Protein 1 (MCP-1) Concentration

In all age groups, our study found that monocyte chemotactic protein-1 (MCP-1) concentrations only rise after ACL repair surgery and in healthy people, and there is no discernible difference in these levels between the two patient groups (pre- and post-surgery). Adhitya IPGS et al. found similar results [43, 49], which are consistent with the current study's findings. Twelve months following anterior cruciate ligament repair surgery, elevated MCP-1 serum concentrations continue [50].

Serum Interleukin-20 (IL-20) Concentration

Among different age groups, serum IL-20 concentration study showed that it increased within age interval [20, 32] years, serum level was significantly higher in the patients group before and after surgery in comparison with the control group however, there was no significant difference in the serum level of IL-20 concentration before surgery and after surgery in the age period 20-39 years. On the other hand, there is a substantial variation in the blood level of IL-20 concentration before surgery and after surgery in the age range of 40-59 years. However, there is no difference between the patient groups and the control group in this age range. One of the reasons for this is that IL-20 has the potential to play a significant part in the aetiopathogenesis of knee osteoarthritis. In patients with osteoarthritis (OA) and the elderly, IL-20 is responsible for the inflammation and breakdown of joint cartilage. It also contributes to the progress of knee joint degeneration.

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

Serum Monocyte Chemotactic Protein 1 (MCP-1) concentration

In males, serum MCP-1 was not significantly different before surgery compared to the control group, but after surgery, it was significantly higher. Serum MCP-1 levels were significantly different post-ACLR surgery. In females, serum MCP-1 did not change before and after ACL replacement surgery. This study agrees with Lisee C and Nissman D, et al. [43] results.

Serum Interleukin-20 (IL-20) Concentration

The patient group, regardless of gender, had substantially greater concentrations of serum IL-20 both before and after surgery compared to the control group. Still, the blood IL-20 concentration levels of the two groups of patients (male and female) did not alter significantly between the pre- and post-ACLR repair surgery periods. This is the first study to examine IL-20 as a biomarker in individuals who have ruptured the anterior cruciate ligament of the knee, as no prior research has focused on this particular lesion. The systemic inflammatory reaction may be mainly caused by IL-20. These findings further support the idea that IL-20 and MMP-3 can serve as a sensitive diagnostic tool for osteoarthritis. Serum IL-18 and IL-20 levels were shown to be higher in the study group and

were smaller than in the 1control 1group, according to Waszczkowski et al. [45].

Conclusion

The findings of this study highlight the significant involvement of inflammatory mediators, particularly monocyte chemoattractant protein-1 (MCP-1) and interleukin-20 (IL-20), in the pathological processes associated with anterior cruciate ligament (ACL) injuries. Elevated serum levels of MCP-1 in patients with ACL injuries, both before and after surgical intervention, suggest that this chemokine plays a critical role in initiating and sustaining inflammation in the knee joint. MCP-1 likely contributes to the recruitment of immune cells to the injury site, thereby amplifying the inflammatory response and promoting cartilage degradation, which could predispose patients to early-onset knee osteoarthritis (OA). Although IL-20 levels did not show significant postoperative changes, their consistently higher expression in ACL-injured patients compared to healthy controls underscores their involvement in the chronic inflammatory environment of the injured joint. IL-20 may exert its effects by promoting the secretion of other pro-inflammatory cytokines, such as IL-6 and MCP-1, thereby contributing to joint tissue catabolism and cartilage matrix breakdown. Its role as a potential upstream regulator in the inflammatory cascade warrants further investigation, especially in the context of long-term joint remodelling and OA development. Importantly, the persistent elevation of MCP-1 and the notable difference in IL-20 levels between patients and controls suggest that

these markers may serve as valuable diagnostic and prognostic tools in the management of ACL injuries. Their measurement could provide clinicians with insights into the degree of inflammation, the status of cartilage turnover, and possibly the effectiveness of surgical or rehabilitative interventions. Moreover, they offer potential therapeutic targets for modulating the inflammatory response and preserving joint integrity post-injury. In conclusion, this study supports the utility of MCP-1 and IL-20 as biochemical indicators of cartilage turnover and inflammation in ACL injuries. Integrating such biomarkers into clinical practice could advance precision medicine approaches in orthopaedic care by enabling early detection of degenerative changes and personalized treatment strategies. Future research should focus on longitudinal assessments of these markers in larger cohorts and investigate their interaction with other cytokines and structural changes through advanced imaging and molecular analysis. This will further elucidate their role in the pathophysiology of ACL-related joint damage and potentially contribute to novel interventions aimed at improving patient outcomes and preserving knee function.

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Adherence to Ethical Standards

The Ethics Committee at Al-Qadisiyah University approved the study (registration code CMUQ 3544 dated 12/15/2020).

References

- [1] A. Albdeery and A. Alzamily, "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-1 β in serum," *Journal of Biomedicine and Biochemistry*, vol. 1, no. 3, pp. 39-49, 2022.
- [2] R. Bammidi, M. Jakka, V. R. Balaga, N. Barla, V. S. Vittanala, and S. J. S. Pampana, "Design and analysis of knee cap," *International Journal of Medical Reviews and Case Reports*, vol. 4, no. 6, p. 41, 2020.
- [3] P. Juneja, A. Munjal, and J. B. Hubbard, "Anatomy, joints," in *StatPearls* StatPearls Publishing, 2023.
- [4] E. Vaienti, G. Scita, F. Ceccarelli, and F. Pogliacomi, "Understanding the human knee and its relationship to total knee replacement," *Acta Bio Medica: Atenei Parmensis*, vol. 88, no. Suppl 2, p. 6, 2017.
- [5] J. F. Abulhasan and M. J. Grey, "Anatomy and physiology of knee stability," *Journal of Functional Morphology and kinesiology*, vol. 2, no. 4, p. 34, 2017.
- [6] A. A. Alzamily and W. M. Jifeel, "The use of collagen type-II as an indicator for assessment regeneration effect post intra-articular pure-PRP injection in KOA patient," *Journal of Biomedicine and Biochemistry*, vol. 1, no. 2, pp. 7-14, 2022.
- [7] Y. L. Chng, "Biomarkers of anterior cruciate ligament injury and recovery," Queensland University of Technology, 2019.
- [8] J. Guyot, *Atlas of human limb joints*. Springer Science & Business Media, 2013.
- [9] A. Kositsky, "Semitendinosus structure and function in healthy and anterior cruciate ligament reconstructed individuals," Itä-Suomen yliopisto, 2023.
- [10] M. G. Wheatley, D. G. Thelen, K. J. Deluzio, and M. J. Rainbow, "Knee extension moment arm variations relate to mechanical function in walking and running," *Journal of the Royal Society Interface*, vol. 18, no. 181, p. 20210326, 2021.
- [11] H. A. Yousif, A. A. Alzamily, and I. A. Alsaman, "Interleukin-4 enhances the production of antiinflammatory macrophages that inhibits the growth of osteoclasts to improve knee functionality," *Journal of Biomedicine and Biochemistry*, vol. 1, no. 3, pp. 50-56, 2022.
- [12] A. Mohamadi et al., "Evolution of knowledge on meniscal biomechanics: a 40 year perspective," *BMC Musculoskeletal Disorders*, vol. 22, pp. 1-13, 2021.
- [13] J. Prathap Kumar, M. Arun Kumar, and D. Venkatesh, "Healthy gait: Review of anatomy and physiology of knee joint," *International Journal of Current Research and Review*, vol. 12, no. 6, pp. 1-8, 2020.
- [14] Y. Ragab et al., "Bilateral extensive steroid-associated osteonecrosis (SAON) of femur, tibia and patella: Successful early management with combined antiresorptive and anabolic bone agents," *The Egyptian Rheumatologist*, vol. 45, no. 4, pp. 324-328, 2023.
- [15] F. V. Sciarretta and J. G. Lane, "Anatomy of the Knee," in *The Art of the Musculoskeletal Physical Exam*. Springer, 2023, pp. 393-402.
- [16] J. Webster, "The Influence of Soft Tissue Balancing on Postoperative In Vivo Tibiofemoral Contact Kinematics in Total Knee Arthroplasty," The University of Western Ontario (Canada), 2018.
- [17] A. J. Fox, A. Bedi, and S. A. Rodeo, "The basic science of human knee menisci: structure, composition, and function," *Sports health*, vol. 4, no. 4, pp. 340-351, 2012.
- [18] X. Wu, W. Cao, H. Yu, Z. Zhang, Y. Leng, and M. Zhang, "Generating electricity during locomotion modes dominated by negative work via a knee energy-harvesting exoskeleton," *IEEE/ASME Transactions on Mechatronics*, vol. 27, no. 6, pp. 4451-4461, 2022.
- [19] S. She et al., "Functional roles of chemokine receptor CCR2 and its ligands in liver disease," *Frontiers in immunology*, vol. 13, p. 812431, 2022.
- [20] R. Bartolini, "Investigating the orchestration of the inflammatory response: a role for chemokine receptors CCR1, CCR2, CCR3 and CCR5," University of Glasgow, 2019.
- [21] M. A. Mir, U. Jan, and Ishaq, "CCL2-CCR2 Signaling Axis in Cancer," in *Cytokine and Chemokine Networks in Cancer*. Springer, 2023, pp. 241-270.
- [22] S. Singh, D. Anshita, and V. Ravichandiran, "MCP-1: Function, regulation, and involvement in disease," *International immunopharmacology*, vol. 101, p. 107598, 2021.
- [23] A. Mahdavi Gorabi et al., "The role of mesenchymal stem cells in atherosclerosis: prospects for therapy via the modulation of inflammatory milieu," *Journal of Clinical Medicine*, vol. 8, no. 9, p. 1413, 2019.

- [24] F. Ni, Y. Zhang, X. Peng, and J. Li, "Correlation between osteoarthritis and monocyte chemoattractant protein-1 expression: a meta-analysis," *Journal of Orthopaedic Surgery and Research*, vol. 15, pp. 1-9, 2020.
- [25] M. Kutwin and A. Woźniacka, "Interleukins 20 and 8—less widely known cytokines in psoriasis," *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, vol. 40, no. 2, pp. 194-203, 2023.
- [26] H. Haybar, B. Bandar, E. Torfi, A. Mohebbi, and N. Saki, "Cytokines and their role in cardiovascular diseases," *Cytokine*, vol. 169, p. 156261, 2023.
- [27] U. M. Wegenka, "IL-20: biological functions mediated through two types of receptor complexes," *Cytokine & growth factor reviews*, vol. 21, no. 5, pp. 353-363, 2010.
- [28] R.-X. Leng, H.-F. Pan, J.-H. Tao, and D.-Q. Ye, "IL-19, IL-20 and IL-24: potential therapeutic targets for autoimmune diseases," *Expert opinion on therapeutic targets*, vol. 15, no. 2, pp. 119-126, 2011.
- [29] M.-C. W. Hsieh *et al.*, "Stem cell-based therapeutic strategies in diabetic wound healing," *Biomedicines*, vol. 10, no. 9, p. 2085, 2022.
- [30] A. E. A. Surace and C. M. Hedrich, "The role of epigenetics in autoimmune/inflammatory disease," *Frontiers in immunology*, vol. 10, p. 1525, 2019.
- [31] H.-H. Wang, Y.-H. Hsu, and M.-S. Chang, "IL-20 bone diseases involvement and therapeutic target potential," *Journal of biomedical science*, vol. 25, pp. 1-10, 2018.
- [32] A. Ahmed, M. A. Razaque, M. Kaleem, A. U. Zaman, R. Akram, and S. Javed, "Diagnostic accuracy of magnetic resonance imaging in detecting anterior cruciate ligament injuries," *Medical journal of Indonesia*, vol. 26, no. 3, pp. 218-23, 2017.
- [33] V. S. Gill, S. V. Tummala, S. P. Boddu, J. C. Brinkman, K. S. McQuivey, and A. Chhabra, "Biomechanics and situational patterns associated with anterior cruciate ligament injuries in the National Basketball Association (NBA)," *British Journal of Sports Medicine*, vol. 57, no. 21, pp. 1395-1399, 2023.
- [34] E. R. Hunt, C. A. Jacobs, C. E. W. Conley, M. L. Ireland, D. L. Johnson, and C. Lattermann, "Anterior cruciate ligament reconstruction reinitiates an inflammatory and chondrodegenerative process in the knee joint," *Journal of Orthopaedic Research*, vol. 39, no. 6, pp. 1281-1288, 2021.
- [35] E. R. Hunt, "Anterior Cruciate Ligament Injury-induced Alterations in Inflammation and Muscle Physiology," 2020.
- [36] E. C. Cheung, M. DiLallo, B. T. Feeley, and D. A. Lansdown, "Osteoarthritis and ACL reconstruction—myths and risks," *Current reviews in musculoskeletal medicine*, vol. 13, pp. 115-122, 2020.
- [37] V. Molnar *et al.*, "Cytokines and chemokines involved in osteoarthritis pathogenesis," *International journal of molecular sciences*, vol. 22, no. 17, p. 9208, 2021.
- [38] T. L. Cranford *et al.*, "Loss of monocyte chemoattractant protein-1 expression delays mammary tumorigenesis and reduces localized inflammation in the C3 (1)/SV40Tag triple negative breast cancer model," *Cancer Biology & Therapy*, vol. 18, no. 2, pp. 85-93, 2017.
- [39] Y. Yamanaka, A. Gingery, G. Oki, T.-H. Yang, C. Zhao, and P. C. Amadio, "Effect of a monocyte chemoattractant protein-1 synthesis inhibitor on fibroblasts from patients with carpal tunnel syndrome," *Journal of Orthopaedic Science*, vol. 26, no. 2, pp. 295-299, 2021.
- [40] D. Loukov, S. Karampatos, M. Maly, and D. Bowdish, "Monocyte activation is elevated in women with knee-osteoarthritis and associated with inflammation, BMI and pain," *Osteoarthritis and Cartilage*, vol. 26, no. 2, pp. 255-263, 2018.
- [41] F. Preda *et al.*, "Deep convolutional neural network-based automated segmentation of the maxillofacial complex from cone-beam computed tomography: A validation study," *Journal of Dentistry*, vol. 124, p. 104238, 2022.
- [42] L. G. Keil *et al.*, "Bone bruising severity after anterior cruciate ligament rupture predicts elevation of chemokine MCP-1 associated with osteoarthritis," *Journal of Experimental Orthopaedics*, vol. 9, no. 1, p. 37, 2022.
- [43] C. Lisee *et al.*, "Tibiofemoral articular cartilage composition differs based on serum biochemical profiles following anterior cruciate ligament reconstruction," *Osteoarthritis and cartilage*, vol. 29, no. 12, pp. 1732-1740, 2021.
- [44] Q. Zeng, N. Hu, Z. Li, Y. Yang, and Z. Zhou, "Evolutionarily conserved IL-20 enhances the response of JAK1/STAT3 axis in *Carassius cuvieri* × *Carassius auratus* red var," *Reproduction and Breeding*, vol. 3, no. 2, pp. 72-81, 2023.
- [45] M. Waszczykowski, A. Fabiś-Strobin, I. Bednarski, J. Narbutt, and J. Fabiś, "Serum and synovial fluid concentrations of interleukin-18 and interleukin-20 in patients with osteoarthritis of the knee and their correlation with other markers of inflammation and turnover of joint cartilage," *Archives of Medical Science: AMS*, vol. 18, no. 2, p. 448, 2022.
- [46] S. Rutz, X. Wang, and W. Ouyang, "The IL-20 subfamily of cytokines—from host defence to tissue homeostasis," *Nature Reviews Immunology*, vol. 14, no. 12, pp. 783-795, 2014.
- [47] Y.-H. Hsu *et al.*, "Anti-IL-20 monoclonal antibody inhibited inflammation and protected against cartilage destruction in murine models of osteoarthritis," *PLoS One*, vol. 12, no. 4, p. e0175802, 2017.
- [48] G. Li and Y. Song, "Cinnamaldehyde Induces the Expression of MicroRNA-1285-5p and MicroRNA-140-5p in Chondrocytes to Ameliorate the Apoptosis and Inflammatory Response," *Cartilage*, vol. 14, no. 3, pp. 375-385, 2023.
- [49] I. P. G. S. Adhitya, I. Kurniawati, R. Sawa, T. F. Wijaya, and N. P. A. C. Dewi, "The risk factors and preventive strategies of poor knee functions and osteoarthritis after anterior cruciate ligament reconstruction: a narrative review," *Physical Therapy Research*, vol. 26, no. 3, pp. 78-88, 2023.
- [50] L. Longobardi *et al.*, "Longitudinal analysis of serum biochemical changes following anterior cruciate ligament injury and reconstruction: a matched comparison-control analysis," *Orthopaedic Journal of Sports Medicine*, vol. 8, no. 7_suppl6, p. 2325967120S00354, 2020.



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