

ORIGINAL ARTICLE

# Interleukin-4 Enhances the Production of Anti-Inflammatory Macrophages That Inhibits the Growth of Osteoclasts to Improve Knee Functionality

Haider Ali Yousif<sup>1, </sup>, Ajil A. Alzamily<sup>1, \*</sup>, and Ihsan Abdulabbas Alsalman<sup>1, </sup>

<sup>1</sup>College of Medicine, Al Qadisiyah University, Diwaniya, Iraq.

## Abstract

### Corresponding author:

ajil.alzamily@qu.edu.iq

<sup>1</sup>College of Medicine,  
Al Qadisiyah University,  
Diwaniya, Iraq.

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**Background** : The knee is one of the largest and most complicated joints in the body. Knee osteoarthritis is a common disease for many people in many countries, especially those who have an unbalanced and unhealthy lifestyle, where they suffer from pain and difficulty in movement. Given that treatments and medicines generally have side effects, it was possible to search for an alternative treatment that is safer and it has the greatest effect. Platelet-rich plasma therapy is an important and useful treatment by injecting it locally into the knee joint, which allows for repairing the damage in the joint and rebuilding it at the level of the microenvironment. Significant disappearance of symptoms the innate immune system stimulates interleukin-4, an anti-inflammatory cytokine secreted by the body in response to the inflammation that occurs in the affected area of the body, where it works to stimulate the repair of damage.

**Patients and procedure** This non-randomized controlled pilot study included 32 patients (18 females and 14 males) with knee osteoarthritis and 16 healthy subjects (5 females and 11 male) from November 2021 to June 2022. All KOA patients diagnosed by orthopedic specialists were based on clinical treatment and radiological findings (X-rays). They were divided into two groups based on the type of PRP; they received: 21 patients received a single injection of pure-PRP in the knee joint and 11 patients received a single injection of LR-PRP in the knee joint. The PRP were received by patients was 4 ml in the case of both groups. The ages of the participants (patients and control group) ranged between 35-75 years old. Outcomes were measured at two points at baseline and six months after injection with IL-4 as an indicator.

**Results** The results of this study were showed that the concentration of IL-4 was significantly higher in KOA patients as compared to control subjects, and both the pure-PRP injected group and the LR-PRP injected group showed a significant decrease in the concentration of IL-4 after six weeks of intervention, but the pure-PRP injected group showed a higher reduction in the serum level of IL-4 as compared to the LR-PRP injected group.

**Conclusions** In conclusion, pure-PRP and LR-PRP showed benefits in terms of inflammation reduction. In both groups, there was a significant reduction in the concentration of IL-4, but the pure-PRP gave a superior outcome as compared to the LR-PRP.

**Keywords:** Pure-PRP, Leukocyte rich-PRP, KOA, IL-4

# 1 Introduction

The knee is one of the largest and most complicated joints in the body. The knee joint is a synovial joint that links the tibia, patella, and femur. It consists of two articulations: the tibiofemoral and patellofemoral joints. whereas the patellofemoral joint is an articulation between the patella and the femur, the tibiofemoral joint is an articulation between the tibia and the femur [1]. Under different stresses, the knee keeps its control and stability. Most of the body's weight is held up by two bone joints. The joint between the patella and the femur allows forces from the quadriceps femoris to pass over the knee without causing friction [2]. The knee's medial ligament complex is made up of one large ligament, a group of thickenings and tendon attachments on the kneecap. The tibial collateral ligament is another name for the superficial medial collateral ligament. The mid-third medial capsular ligament is another name for the deep medial collateral ligament [3]. Knee osteoarthritis affects all three compartments of the knee joint (the medial, lateral, and patellofemoral joints), typically progressing slowly over the course of 10 to 15 years and making it difficult to participate in day-to-day activities [4]. The causes of knee osteoarthritis (OA) can be broken down into two categories: those that cannot be changed and those that can be changed. Hereditary and congenital variables both fall into the category of non-modifiable risk factors. Treatment can be directed toward modifiable risk factors if necessary [5]. Platelets are non-nucleated cell elements that are found in human blood. Platelets possess hemostatic, immunological, and inflammatory capabilities [6]. They include alpha, dense, and lysosomal types of granules, all of which contribute more than a thousand distinct biomolecules called growth factors relevant to hemostasis, inflammation, regeneration, and immunological function. They are contained within them [7]. Platelet-rich plasma, also known as PRP, is a type of autologous blood product that can be injected into injured areas to give platelet-derived growth factors (GFs) and aid healing [8]. PRP is a potentially beneficial therapeutic option because of its autologous nature, which in theory lowers the risk of any adverse effects and makes it an appealing choice [9]. Platelet granule secretion is one of the most unpredictable variables in platelet rich plasma (PRP) injection. Platelet granules are responsible for the release of numerous growth factors (GFs), some of which include platelet derived GF, insulin-like GF-1, transforming GF beta, and epidermal GF. It is possible for the concentrations of these GFs to differ between patients as well as at different times within the same patient [10]. In addition, platelet granules store chemicals that are thought to play a complex role in

the process of tissue regeneration. These compounds include adenosine diphosphate, adenosine triphosphate, histamine, mdopamine, serotonin, cathepsin D, cathepsin E, elastases, and hydrolases [11]. However, the efficacy of the specific type of PRP treatment may vary depending on the stage of the healing process and the nature of the damage [12]. Interleukin (IL)-4 is a well-known cytokine that is mostly involved in the anti-inflammatory process; however, new research have revealed that IL-4 also plays a part in the differentiation process of a variety of cells [13]. T lymphocytes, basophils, and mast cells can all release IL-4, which regulates B cell and macrophage proliferation and affects the particular immunological activity of hematopoietic cells [14]. These cells migrate to damaged tissues and aid in the repair process there by secreting cytokines; these cytokines also raise the expression of anti-inflammatory and chondroprotective proteins such as IL-4 and IL-10 [15]. IL-4 is able to prevent the release of mediators that are connected to pain, inflammation, and catabolism [16], capable of inhibiting the production of tumor necrosis factor- $\alpha$  and interleukin-1  $\alpha$  by human monocytes that have been stimulated by lipopolysaccharide (LPS) [17]. The anti-inflammatory property is directly dependent on the inflammatory status of chondrocytes or synoviocytes, as well as the surrounding milieu, operating on molecules and pathways downstream from those cells [18]. It is necessary for there to be cytokine receptors present on chondrocytes in order for IL-4 to be able to remodel the extracellular matrix of the articular cartilage. Chondrocytes are responsible for the production of interleukin 4, and the cellular surface of these cells expresses cytokine receptors [19].

## 2 Methods

### 2.1 Subject

This non-randomized controlled pilot study included 32 patients (18 female and 14 male) with knee osteoarthritis and 16 healthy subjects (5 female and 11 male) from November 2021 to June 2022. All KOA patients diagnosed by orthopedic specialists are based on clinical treatment and radiological findings (X-rays) they are divided into two groups based on the type of PRP they received: 21 patients received a single injection of pure-PRP in the knee joint and 11 patients received a single injection of LR-PRP in the knee joint. The PRP dose received by patients was 4 ml in the case of both groups. The ages of the participants (patients and control group) ranged between 35-75 years old. Outcomes were measured at two points at baseline and six months after injection with IL-4 as an indicator. The Human Medical Research

Ethics Committee at Al-Qadisiyah University / College of Medicine approved this study, which was designed in Al-Diwaniyah and Babilon Governorate in Iraq country.

### 2.1.1 Sample collection

Collect 5 ml of the patient's whole blood by puncturing a vein and drawing it out. After that, the blood was held at a temperature that was constant at room temperature for 10 to 15 minutes while it was contained in a gel tube. The blood was separated by spinning it in a centrifuge at a speed of 4000 revolutions / minute for 5 minutes. The serum was placed in an identified Eppendrofe tube and stored at a temperature of  $-80^{\circ}\text{C}$  until it was required.

### 2.1.2 Estimation the concentration of biomarker

the serum concentration of IL-4, was measure by ELISA system by ELISA sandwich technique using Elabscience kits.

### 2.1.3 Statistical analysis

The data of the current study was analyzed using graph pad Prism 9.2.1 and Microsoft excel version 19. Presented as mean  $\pm$  standard deviation. T-test, one-way ANOVA and correlation analysis were used to measure the degree of significance.

## 3 Results

### 3.1 Demographic characteristics

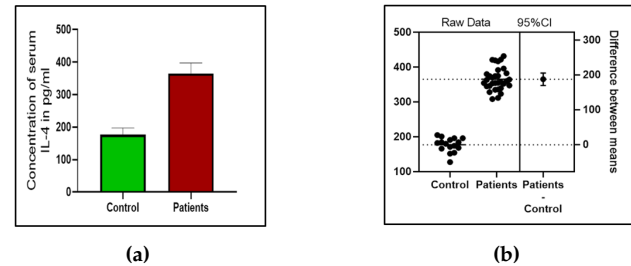
It has been observed that the percentage of males between the ages of 35 to 39 was 50%, whereas the percentage of females between the ages of 50 to 59 was 61%, which was significantly ( $P < 0.0001$ ) greater than the percentage of males, which was 22%. The female prevalence rate was 28% between the ages of 69 to 70, which was substantially ( $P < 0.05$ ) greater than the male prevalence rate of 14%. It was observed that there was no consistent association between age and Body Mass Index (BMI).

**Table 1:** Demographic parameters of studied patients.

	Control		Patients		P
	Male	Female	Male	Female	
Number	11	5	14	18	
	Age				
30-39 year	6(55%)	2(40%)	7(50%)	/	/
40-49 year	2(18%)	2(40%)	2(14%)	2(11%)	NS
50-59 year	2(27%)	1(20%)	3(22%)	11(61%)	$P < 0.0001$
60-70 year	/	/	2(14%)	5(28%)	$P < 0.05$
	BMI				
30-39 year	20.05-36.33	27.28-28.73	18.10-25.1	/	NS
40-49 year	23.25-27.13	26.11-35.52	22.75-24.34	22.02-24.34	$P < 0.001$
50-59 year	23.97-37.04	27.10	25.06-25.31	20.20-38.64	$P < 0.001$
60-70 year	/	/	28.70-29.41	25.46-30.85	NS

### 3.2 Comparison between patients and control group

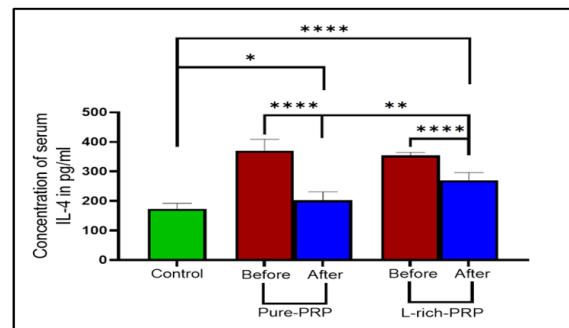
There is a rise in the level of IL-4 concentration in patients compared with control (Figure 1 A), the evaluation plot demonstrates the presence of a significant rise in the level of IL-4 in the group of patients as compared to the group that served as the control (Figure 1 B).



**Figure 1:** Estimation of serum IL-4. (A) a comparison between control and patients' group, (B) an estimation plot that illustrates the presence of a significant increase in the level of IL-4 in the patients' group as compared to control.

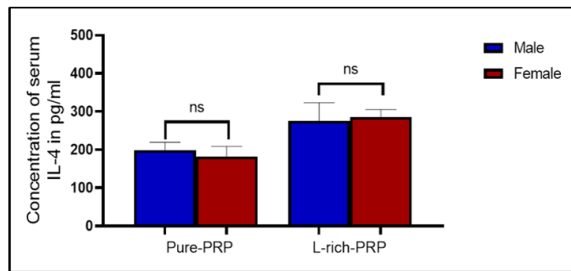
### 3.3 Comparison between Pure-PRP and L-PRP

The level of IL-4 concentration in the serum of patients before the injection of pure-PRP and L-PRP was increase more than control. A significant decrease in the serum concentration of IL-4 after the injection. However, the concentration was noticeably higher when compared to the control, as shown in the Figure 2.



**Figure 2:** Estimation of IL-4 serum concentration after the injection of Pure-PRP and L-rich-PRP. A significant decrease ( $P < 0.0001$ ) in the serum concentration of IL-4 as compared to before injection. However, the concentration significantly ( $P < 0.5$ -  $P < 0.0001$ ) higher than control.

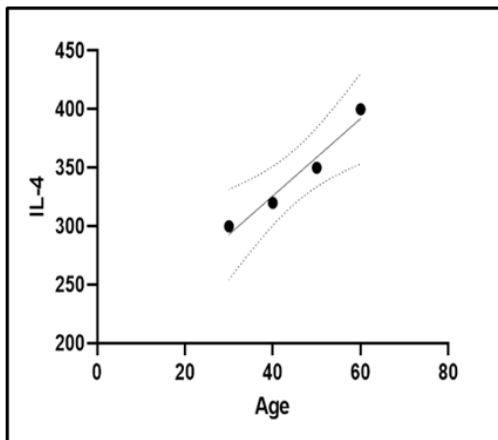
When comparing the level of the serum of IL-4 concentration between female and male after the injection of pure-PRP and L-PRP there is no significant between them before injection, as shown in the Figure 3.



**Figure 3:** Estimation of IL-4 serum concentration after the injection of Pure-PRP and L-rich-PRP. No significant difference finds between male and female after the injection.

### 3.4 Estimation of serum level for the marker in relation to age

The relationship between the marker and age for all age groups is shown a simple liner regression, a strong correlation between interlukine-4 and levels with age Figure 4.



**Figure 4:** Estimation of serum level for the three markers in relation to age. simple linear regression showing a strong correlation between IL-4 level and age ( $r=0.8$ ,  $P<0.05$ ).

## 4 Discussion

This study applied a new biological treatment for knee osteoarthritis (OA). Autologous blood products can supply cellular and humeral mediators (blood growth factors) for tissue healing. In this study, the injection of both types of platelet-rich plasma (pure-PRP and L-PRP) were compared with healthy persons. This study consists of two primary groups, the first of which comprises of patients whose knee joints were injected with pure-PRP. In addition to a group of healthy individuals, the second group consists of patients whose knee joints were injected with L-PRP. Regarding on the number of patients, indicated this study that women are more susceptible to knee os-

teoarthritis than men.

Osteoarthritis is caused by a series of complex biological events that involve cartilage, bone, synovium, ligaments, periarticular fat, the meniscus, and muscle [20]. Synovitis is one that typically manifests in OA joints [21]. This is because the immune response is turned on low grade of inflammation in affected joints. This is likely cause damage to joint tissues that creates an environment like a chronic wound [22]. In vivo studies support the notion that LR-PRP generates a stronger inflammatory response than Pure-PRP. It has been found, for instance, that LR- PRP causes a larger acute inflammatory response than Pure- PRP [23]. This finding has been validated by studies of intra-articular PRP injections in patients with arthritic knees [24]. It is interesting to note that there is evidence that LR-PRP possesses both qualities that promote inflammation and those that reduce inflammation [25]. In one study that compared LR-PRP and LP-PRP, both types of PRP were shown to be equally effective; however, the LR-PRP group reported a higher rate of post-injection discomfort [26]. Concerning leukocyte subtypes, there is evidence that polymorphonuclear neutrophils (PMNs) may be detrimental to cartilage [27].

Interlukine-4 is one of the primary cytokines that are characteristic of Th2 cells [28]. IL-4 therapy suppresses the expression of inflammatory genes caused by lipopolysaccharide (LPS) in chondrocyte cells. To confirm IL-4's anti-inflammatory effects on disc cells, LPS-treated cells were treated with or without IL-4. IL-4 significantly decreased LPS-induced expression of inflammatory genes [29]. The results of this study showed that the concentration of IL-4 was significantly higher in KOA patients as compared to control subjects. The results of the present study agree with (Ericka P. et al 2021) analysis of the effects of IL-4 on human synovium and joint tissue in vitro indicated that IL-4 inhibits osteoarthritis-related phenotypes [30], and agree with (GIESECK et al 2018), IL-4 improves macrophage phagocytosis of cartilage debris, a potential way to control joint inflammation. IL-4 induces macrophage polarization via the type I receptor. Although the exact polarization status of macrophages in an osteoarthritis joint is unknown, IL-4's effects on macrophages may be critical for maintaining tissue homeostasis. Interleukin-4 rises in response to tissue injury [31]. Regarding platelets rich plasma injection both the pure-PRP injected group and the LR-PRP injected group showed a significant decrease in the concentration of IL-4 after six weeks of intervention, but the pure-PRP injected group showed a higher reduction in the serum level of IL-4 as compared to the LR-PRP injected group.

## 5 Conclusion

In conclusion, pure-PRP and LR-PRP showed benefits in terms of inflammation reduction. In both groups, there was a significant reduction in the concentration of IL-4, but the pure-PRP gave a superior outcome as compared to the LR-PRP.

**Conflict of Interest:** No conflicts of interest exist between the authors and the publication of this work.

**Ethical consideration:** The ethical committee approved the study at the University of Al-Qadisiyah.

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