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## ORIGINAL ARTICLE

# THE INTRA-ARTICULAR PURE-PLATELET RICH PLASMA AS A DISEASE-MODIFYING TREATMENT FOR PATIENTS SUFFERING FROM KNEE OSTEOARTHRITIS

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**Summary**

**Background:** The knee is the predominant weight-bearing joint affected by osteoarthritis, and knee osteoarthritis (KOA) is the 11th significant cause of disability. As life expectancy has increased, the need for knee replacement procedures has grown, putting a major financial burden on patients and healthcare systems. Intra-articular (IA) injection of platelet rich plasma (PRP) promises to be a potential method in research for the more preservative and biological strategies to treat this chronic degenerative condition. PRP has expected to contain a high number of growth factors and proteins involved in tissue repair mechanisms.
**Aim:** This study aimed to determine the effect of intra-articular injections (IAIs) pure-PRP and their dosage regimen efficacy in patients with mild and moderate knee OA.
**Material and method:** This study included 33 patients (11 male and 22 female). The patient groups included 16 with mild and 17 with moderate knee osteoarthritis. With ages ranged from 32 to 66 years old. These patients were divided into three groups based on dosage (12 received a single injection, 11 received a double injection, and 10 received a triple injection), with a two-week interval between injections. The outcome was measured using WOMAC index for pain, stiffness, and daily functional limitation, as well as laboratory testing for TNF-alpha using the Eliza technique. All the patients were assessed at baseline and after three months of injection. The study was designed as non-randomized controlled trial study during November 2021 to May 2022 at Al-imam Ali hospital, Babylon governorate, Iraq.
**Results:** Regarding severity, In both mild and moderate knee osteoarthritis, serum TNF-alpha was significantly lower after treatment ($p < 0.001$). All WOMAC scores were showed significantly lower levels after treatment with pure-PRP as compared with their levels before treatment ($p \leq 0.001$, Regarding the number of injections, all patients who received a single injection, double injections, and triple injections showed a significant decrease in the serum TNF-alpha and WOMAC scores (p 0.05) in both mild and moderate patients. The triple injections were more effective than double and single injections.
**Conclusions:** In conclusion, the treatment with pure-PRP was safe and satisfactory for patients in terms of relieving clinical symptoms. The WOMAC scores has indicated that improvement in pain relief for patients after injection and could act as an anti-inflammatory, as inflammatory marker TNF-alpha was reduced after injection, and there was a superior outcome to increasing dose numbers, as triple injection gave a positive outcome in both mild and moderate KOA when compared to single and double injection.

*Key words: knee OA; pure-PRP; TNF-alpha; WOMAC scores*

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## Introduction

The knee is the predominant weight-bearing joint predisposed to be affected by osteoarthritis (1). Many factors have been associated with the susceptibility and predisposition to knee arthritis, including excess weight, obesity, gender, and previous knee injuries, and Diabetes Mellitus is another factor that linked to the progression of OA in the knees. The common signs and symptoms of KOA are chronic pain, stiffness of the joints, extreme fatigue of the muscles, and inflammation resulting in swelling of the knee (2). Chronic pain is the most prevalent symptom of KOA and is exacerbated by weight-bearing activities and mobility of the affected joint (3).

The prevalence of KOA has increased significantly in recent decades and keep rising, due to the increase of obesity among society as well as other risk factors that associated with development (4). Knee OA affects more than 20% of people over the age of 45, and it is the second common reason that can cause loss of work performance following low back pain (5). More than 250 million older people (> 50 years) worldwide suffer from symptomatic KOA (6). Furthermore, KOA affects over one million people in Iraq, Saudi Arabia, Yemen, and Syria (7). KOA is appears to affect greater than 10% of the world's peoples, and the strong risk of developing KOA has risen to 45% after the age of 45 years (8). Knee OA is the 11$^{th}$ significant cause of functional limitation and disability (9), with 47% of women and 40% of men developing knee OA in their lifetimes (10). The prevalence of osteoarthritis musculoskeletal disease is higher around the world where KOA is the fourth cause of inability in women and the eighth cause of inability in men worldwide (11).

Inflammation recognizes as an important component of KOA. Local inflammation can occur within the synovial fluid as well as systemically, with inflammatory agents circulating in the blood. The severity and pattern of inflammation show significant variation as the disease progresses, with different cytokines present in the early and advanced stages (12). Furthermore, certain cytokines may cause damage to the extracellular matrix of joint tissue. As reflected in the pathogenetic cytokines, several of these cytokines may function as chemical indicators of the disease severity and pain of OA (13). Measuring the level of pro-inflammatory cytokines such as TNF-alpha can help to predict the possible consequences of disease, and besides that, the selected effectiveness of treatment modalities during the inflammatory phase can be investigated for each patient by testing plasma TNF-alpha levels (14). TNF-alpha is a member of the tumor necrotic factors superfamily (TNFSF) of proteins that play important roles in the biological activities in mammalian, such as cell growth and survival, apoptotic factors, immunological responses, and organogenesis in a variety of systems, including the immune, nervous, and ectodermal systems. TNF-alpha is a key inflammatory mediator that has functional effects on many cell types by stimulating internal signaling via its related cell receptors (15). There is a strong argument to be made that inflammatory mechanisms play a key role in the pathogenesis of OA (16). Additional to immune cells the articular macrophages, pro-inflammatory cytokines and chemokine such as IL-6 and TNF-alpha are produced by a major cell type in cartilage tissue chondrocytes, as well as by cells that line the joint cavity, synoviocytes (17). TNF-alpha concentration has found to be elevated within the synovial fluid of affected people with KOA and is involved in chondrocyte destruction. According to the evidence, the level of TNF-alpha in synovial fluid supernatants from cultured KOA synovial fluid were significantly higher than in non-arthritic (NA) synovial fluid (18).

As life expectancy has increased, The demand for knee replacement surgeries has increased, putting a major financial burden on patients and healthcare systems (19). The injection of plasma rich in platelets (PRP) within the articular promises to be a potential method in research for the more preservative and biological strategies to treat this chronic degenerative condition. PRP is expected to contain a high number of factors and proteins involved in repair damaged tissue (20). Plasma-rich platelets (PRP) is a regenerative medicine product that has recently gained clinical interest in the professions of orthopedics medicine (21). PRP is an autologous blood product (plasma) that has been prepared to contain a greater concentration of platelets than that found in vivo blood (22). Intra-articular delivery of drugs has several benefits over systemic drug delivery, which include improved local bioavailability, lower exposure to the general system, fewer adverse effects, and lower costs (23, 24). PRP is prepared by separating patient-derived blood either in a one-spin or two-spin methods. Resulting in PRP that is either poor or rich in leukocytes (LP or LR). There is no clear direct benefit to use either LP- or LR-plasma rich platelets, but adverse events appear to be greater with LR-PRP (25). The LP-PRP support to be used in treatment of early KOA or progression-advanced OA might be due to the higher concentration of leukocytes in LR-PRP as well as the relatively strong inflammatory reactions induced (26). In synoviocyte culture, leukocyte-rich PRP stimulates

the production of several inflammatory markers and causes cell death (27). Furthermore, early evidence that LR-PRP causes much more painful reactions (28). High WBC concentrations have been shown to cause a considerable increase in the secretion of inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) and interleukin-1, as well as catabolic pathways. Based on this evidence, it has been proposed that PRP rich in leukocytes would be not suited for intra-articular injection (29).

**Patients and Methods**

This non-randomized controlled trial study was given its approval by the scientific Human Research Ethics Committee at the College of Medicine, Al-Qadisiyah University in Iraq. This study included 33 patients (11 male and 22 female). The patient groups included 16 with mild and 17 with moderate knee osteoarthritis. knee osteoarthritis classified Radiographically, depending on the Kellgren-Lawence (KL) 0–4 grading system was used to classify KOA severity depending on radiological findings by x-ray, where the x-rays of the affected joints are the main way osteoarthritis is identified (30, 31). With ages ranged from 32 to 66 years old. All patients were divided into three groups based on dosage (12 received a single injection, 11 received double injections, and 10 received triple injections), with two-week interval between injections.

**Sample collection:** Under aseptic environment from each patient 5 milliter of the blood sample collected at baseline and three months after injection with PRP. After that, the blood was withdraw from vein puncture stand for 15 minutes at room temperature. Then, the sample was centrifuge at 4000 rpm for 5 minutes to obtain serum for estimation the level of TNF-alpha.

**Pure-PRP preparation:** PRP can be prepared in a variety of ways (32). In this study, the PRP method of Buasset and his colleagues was used. Briefly, the first spin step was performed at 250 g for 15 minutes, separating the basic blood components, and the whole blood (WB) was separated into three layers: the lower was RBCs, the intermediate was buffy coats, and the upper was plasma. For the preparation of pure PRP, the plasma layer and superficial layer of buffy coats were transferred into a plain sterile tube (vacuum sterile plan tube; 191230, Al Malak Company/Iraq). The second spin step takes 15 minutes at 250 g. The upper portion of the separated plasma was discharged, and the lower portion was mixed and used for injection (33). The pure-PRP plasma results were produced a very low level in leukocytes, but rich in platelets. The blood components testing device (CBC system; B8823, Germany) was used to find out the numbers of platelets and leukocytes in the whole blood sample and in separated plasma before injection.

**Injection protocol:** Each patient was position in a supine with knee flexion position, and the injection site was prepared under sterile conditions using 70% alcohol and 4% povidine-iodine antiseptic disinfectant without the using of any local anesthesia at the injection site. The pure-PRP was injected with a sterile disposable syringe with a 21 G needle. The current study included patients with both unilateral and bilateral knee osteoarthritis. In the case of the patient who injected in both knees, the period between the injections was two weeks for each knee.

**Outcomes measures**

**Clinical outcomes:** For all patients, the clinical examinations including pain, stiffness, and function parameters) were performed by Western Ontario and McMaster Universities (WOMAC) index. The WOMAC score is a disease-specific and self-administered questionnaire that assesses three aspects of health status and outcome in patients with lower extremity (knee and/or hip) osteoarthritis are pain, stiffness, and physical function. It includes 24 questions about pain (5 items), stiffness (2 items), and function (17 items). Each question is associated with a Likert scale response ranging from 0 (best health state) to 4 (worst health state). The score for each sub-scale was computed summing the scores of each question in the sub-scale. The sub-scale of function ranges of 0–68, 0–20 for pain, and 0–8 for stiffness (34). The American Academy of Orthopedic Surgeons (AAOS) has chosen to approve the use of the WOMAC osteoarthritis index as part of the larger personal health tool assessment for lower extremity arthroplasty assessment (35).

**Estimation of TNF-alpha:** Estimation the concentration of TNF-alpha by sandwich Eliza technique using Elabscience® kit. As illustrated in Figure 1.

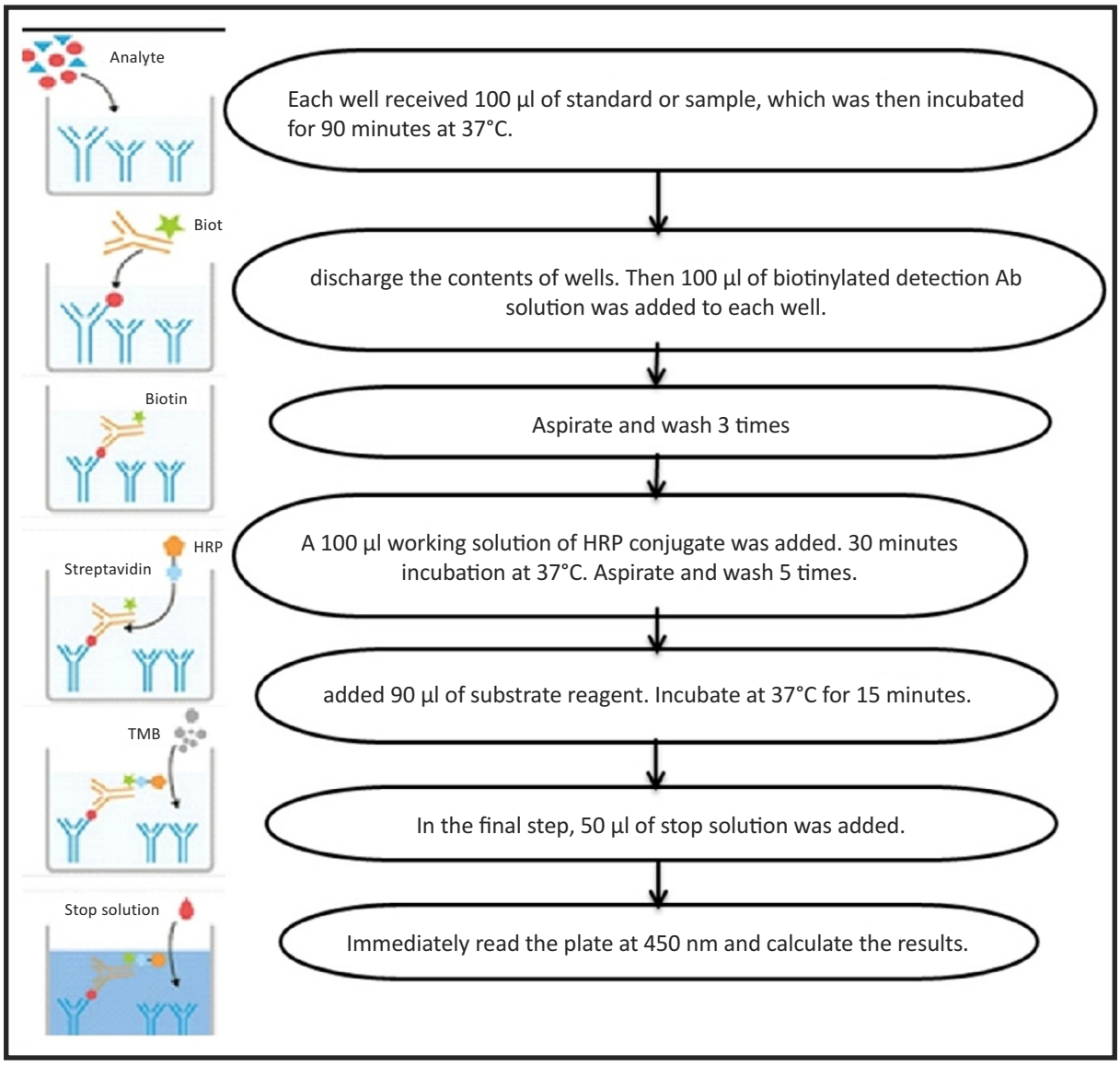


**Figure 1.** Steps of assay procedure of TNF-alpha by using Eliza technique.

**Inclusion criteria:** Patients with ( grade 2) and moderate (grade 3) knee OA.

**Exclusion criteria:** The study excluded all patients with following criteria:

- Co-morbidity with chronic disease, infectious disease, hematological disease.
- Advance (grade-4) KOA.
- Non-steroid anti-inflammatory drugs at least 2 weeks prior the study and any IAIs in preceding 3 months.

**Statistical analysis:** Statistical summarization, analysis, presentation of data was performed using SPSS version 21 (Statistical Package for the Social Sciences) and Microsoft Office Excel 2010. Categorical variables were used as numbers and percentages, whereas numerical data was represented as mean ± standard deviation. Chi-square test was used to compare qualitative data. Independent-samples t-test and Paired sample t-test were applied to compare quantitative variable to calculate the degree of significant among groups. Bivariate correlation was carried by using Pearson correlation coefficients. ($p \leq 0.05$) was a considered as significant.

**Results:**

The comparison of demographic characteristics between mild and moderate disease as shown in Table-1. The mean age show no significant differences between patients with mild disease and patients with moderate disease, 54.38 ±7.97 years versus 51.82 ±10.44 years, respectively ($p$ = 0.438). Also the mean BMI show no significantly different, 25.06 ±2.43 kg/m$^2$ versus 27.57 ±4.97 kg/m$^2$, respectively ($p$ = 0.078). Patients with moderate disease included more females than males, 14 (82.4%) versus 3 (17.6%), respectively, while patients with mild disease included equal proportions of males and females 8 (50.0%) versus 8 (50.0%), respectively and the difference in gender proportions between mild and moderate disease was significant ($p$ = 0.049).

**Table 1.** Demographic characteristics.

| Characteristic | Mild (*n* = 16) | Moderate (*n* = 17) | *P.value* |
|---|---|---|---|
| **Age (years)** | | | |
| Mean ±SD | 54.38 ±7.97 | 51.82 ±10.44 | 0.438 I NS |
| Range | 38 - 65 | 32 - 66 | |
| **BMI (kg/m$^2$)** | | | |
| Mean ±SD | 25.06 ±2.43 | 27.57 ±4.97 | 0.078 I NS |
| Range | 21.26 - 30.85 | 20.2 - 37.13 | |
| **BMI (kg/m$^2$)** | | | |
| Normal weight, *n* (%) | 8 (50.0 %) | 5 (29.4 %) | 0.189 C NS |
| Overweight, *n* (%) | 7 (43.8 %) | 7 (41.2 %) | |
| Obese, *n* (%) | 1 (6.3 %) | 5 (29.4 %) | |
| **Gender** | | | |
| Male, *n* (%) | 8 (50.0 %) | 3 (17.6 %) | 0.049 C * |
| Female, *n* (%) | 8 (50.0 %) | 14 (82.4 %) | |

***n***: number of cases; **SD**: standard deviation; **I**: independent samples *t*-test; **C**: chi-square test; **NS**: not significant; *: significant at $p \leq 0.05$.

The classification of patients with KOA according to severity by radiological finding (x-ray) based on the Kellgren-Lawrence (KL) 0–4 grading system is used to classify KOA severity to mild (grade-2) and moderate (grade-3). Patients were categorized into 48.5% with mild disease and 51.5% with moderate disease as shown in Figure-2.

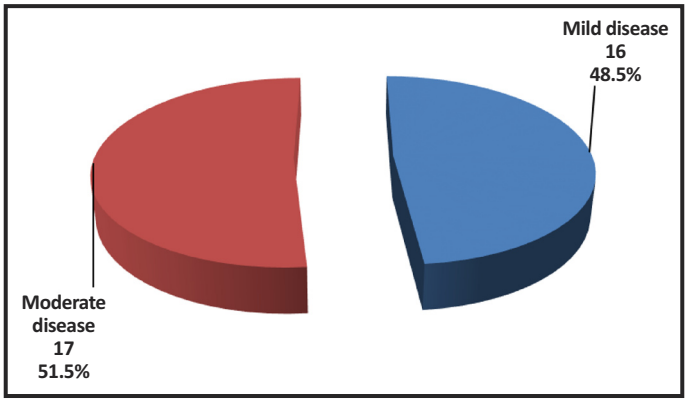


**Figure 2.** A pie chart shows the classification of KOA patients based on disease severity.

The comparison of serum marker and WOMAC scores before and after treatment in mild disease as shown in Table-2. Serum TNF-alpha level was decrease significantly after treatment ($p < 0.001$). All WOMAC scores were showed significantly lower levels after treatment in comparison with their levels before treatment ($p \leq 0.001$).

**Table 2.** A Comparison of serum marker and WOMAC score before and after treatment in mild disease.

| Characteristic | Before (*n* = 16) | After (*n* = 16) | *P.value* |
|---|---|---|---|
| TNF-alpha | 22.59 ±2.12 | 15.89 ±2.73 | < 0.001 P *** |
| Pain | 8.56 ±0.96 | 4.94 ±1.84 | 0.001 P *** |
| Stiffness | 4.25 ±1.44 | 1.62 ±1.15 | < 0.001 P *** |
| Physical function | 26.44 ±5.34 | 14.06 ±5.05 | 0.001 P *** |
| Total Score | 39.50 ±5.75 | 20.62 ±7.49 | 0.001 P *** |

TNF-alpha: *tumor necrosis factor-alpha*; **n**: number of cases; **SD**: standard deviation; **P**: Paired sample *t*-test;
***: significant at $p \leq 0.001$.

The comparison of serum markers and WOMAC score before and after treatment in moderate disease as shown in Table-3. Serum TNF-alpha show significant reduce in the level after 3 month treatment in compare to baseline ($p < 0.001$). All WOMAC scores were showed significantly decrease in the levels after 3 month in comparison with their levels before treatment ($p \leq 0.001$).

**Table 3.** A Comparison of serum marker and WOMAC score before and after treatment in moderate disease.

| Characteristic | Before (*n* = 17) | After (*n* = 17) | *P.value* |
|---|---|---|---|
| TNF-alpha | 30.48 ±3.39 | 23.49 ±4.80 | < 0.001 P *** |
| Pain | 12.82 ±1.63 | 7.65 ±2.74 | < 0.001 P *** |
| Stiffness | 6.12 ±1.36 | 3.41 ±1.70 | 0.001 P *** |
| Physical function | 33.06 ±6.53 | 21.65 ±8.71 | 0.001 P *** |
| Total Score | 52.00 ±8.35 | 32.71 ±12.56 | < 0.001 P *** |

TNF-alpha: *tumor necrosis factor-alpha*; **n**: number of cases; **SD**: standard deviation; **P**: Paired sample *t*-test;
***: significant at $p \leq 0.001$.

## A Comparison of serum marker and WOMAC according to severity of disease and number of injections

The comparisons of serum marker and WOMAC before treatment and after treatment in mild disease according number of injections as shown in Table-4. Single injection, double injections, and triple injections resulted in significant reduction in TNF-alpha and WOMAC scores ($p < 0.05$).

**Table 4.** A Comparison of serum marker and WOMAC before treatment and after treatment in mild disease according number of injections.

| Characteristic | Single injection | | | Double injection | | | Triple injection | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before *n* = 5 | After *n* = 5 | *P –value* | Before *n* = 6 | After *n* = 6 | *P –value* | Before n = 5 | After n = 5 | *P –value* |
| TNF-alpha | 22.42 ± 2.39 | 17.96 ± 3.02 | 0.043 P * | 21.61 ± 1.66 | 16.13 ± 1.62 | 0.028 P * | 23.95 ± 1.99 | 13.53 ± 1.80 | 0.003 P ** |
| Pain | 8.60 ± 0.89 | 5.60 ± 2.19 | 0.042 P * | 8.60 ± 0.89 | 5.50 ± 1.87 | 0.043 P * | 9.20 ± 0.84 | 3.60 ± 0.55 | 0.004 P ** |
| Stiffness | 3.60 ± 1.14 | 1.60 ± 1.34 | 0.041 P * | 4.50 ± 1.38 | 2.00 ± 1.41 | 0.027 P * | 4.60 ± 1.82 | 1.20 ± 0.45 | 0.002 P ** |
| Physical function | 25.20 ± 3.19 | 15.40 ± 2.61 | 0.042 P * | 28.17 ± 8.42 | 17.17± 5.38 | 0.046 P * | 25.60 ± 0.89 | 9.00 ± 2.00 | 0.003 P ** |
| Total Score | 38.20 ± 3.11 | 22.60 ± 5.86 | 0.043 P * | 40.67 ± 9.25 | 24.67 ± 8.17 | 0.046 P * | 39.40 ± 1.82 | 13.80 ± 2.28 | 0.003 P ** |

TNF-alpha: *tumor necrosis factor-alpha*; **n**: number of cases; **SD**: standard deviation; **P**: Paired samples *t*-test; **NS**: not significant;
*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

The comparisons of serum marker and WOMAC before treatment and after treatment in moderate disease according number of injections as shown in Table-5. Single injection, double injections and triple injections resulted in significant reduction in TNF-alpha and WOMAC scores ($p < 0.05$).

**Table 5.** A Comparison of serum marker and WOMAC before treatment and after treatment in moderate disease according number of injections.

| Characteristic | Single injection | | | Double injection | | | Triple injection | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before *n* = 7 | After *n* = 7 | *P –value* Pa | Before *n* = 5 | After *n* = 5 | *P –value* Pa | Before n = 5 | After n = 5 | *P –value* Pa |
| TNF-alpha | 30.09 ± 2.45 | 27.62± 2.02 | 0.043 P * | 29.82 ± 3.09 | 23.07 ± 3.53 | 0.028 P * | 31.69 ± 4.99 | 18.13 ± 2.76 | 0.003 P ** |
| Pain | 12.29 ± 0.95 | 9.43 ± 2.76 | 0.042 P * | 12.80 ± 0.84 | 6.80 ± 2.49 | 0.027 P * | 13.60 ± 2.70 | 6.00 ± 1.58 | 0.003 P ** |
| Stiffness | 5.71 ± 1.38 | 4.57 ± 1.90 | 0.071 P NS | 5.60 ± 0.55 | 2.40 ± 1.14 | 0.038 P * | 7.20 ± 1.48 | 2.80 ± 0.84 | 0.001 P ** |
| Physical function | 32.71 ± 6.18 | 27.00 ± 9.33 | 0.116 P NS | 31.20 ± 7.19 | 19.20 ± 7.92 | 0.043 P * | 35.40 ± 7.09 | 16.60 ± 4.56 | 0.003 P ** |
| Total Score | 50.71 ± 8.34 | 41.00 ± 13.27 | 0.062 P NS | 49.60 ± 8.20 | 28.40 ± 11.17 | 0.042 P * | 56.20 ± 8.67 | 25.40 ± 5.68 | 0.003 P ** |

TNF-alpha: *tumor necrosis factor-alpha*; **n**: number of cases; **SD**: standard deviation; **P**: Paired samples *t*-test; **NS**: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

The correlations between serum TNF-alpha and WOMAC scores before and after treatment as shown in Table-6 and -7, respectively. Before treatment serum marker showed positive correlations to WOMAC scores ($p < 0.05$) and after treatment the same findings were observed ($p < 0.05$).

**Table 6.** Correlations between serum marker and WOMAC sores before treatment.

| Characteristic | TNF-alpha | |
|---|---|---|
| | R | P |
| Pain | 0.724 | < 0.001 *** |
| Stiffness | 0.654 | < 0.001 *** |
| Physical function | 0.373 | 0.033 * |
| Total Score | 0.548 | 0.001*** |

**Table 7.** Correlations between serum marker and WOMAC scores after treatment.

| Characteristic | TNF-alpha | |
|---|---|---|
| | R | P |
| Pain | 0.463 | 0.007 ** |
| Stiffness | 0.455 | 0.008 ** |
| Physical function | 0.452 | 0.008 ** |
| Total Score | 0.473 | 0.005 ** |

## Discussion

The current study along with studies that increasing interest on using PRP as treatment option for knee osteoarthritis that can reducing joint pain and improve daily limitation (36). In the discussion about the utilization of the leukocytes in PRP, the present study was based on the use of pure-PRP in which the leukocyte concentration is very low or not present, as these cells can produce higher concentrations of cytokines and catabolic markers that can result in greater synoviocyte death (37). It has thought that neutrophils reveal metalloproteinase and free radicals that damage the extracellular matrix and increasing inflammatory activity in the joint. Some evidences have showed that low concentration of leukocytes leads to greater ability of repair (38). Many studies on the influence of leukocytes have been proved that pure-PRP and LP-PRP are better than LR-PRP in terms of decreasing the level of the main inflammatory cytokines such as TNF-alpha, IL-1B, and prostaglandin-E2 that induce the inflammatory pathway and cause pain in KOA (39–41). In this study, leukocyte-pure PRP was used to avoid leukocytes from producing joint injury or excessive inflammatory reactions.

The current study compares the clinical outcomes of pure-PRP injection in within knee joint affected by KOA. The assessment of clinical outcome was made by using the WOMAC index for pain, stiffness, and daily activity scores. All the variable of subjective outcome assessment at two points pre-intervention and 3 months after intervention. Despite the fact that most studies have revealed a positive effect of pure-PRP on knee osteoarthritis,

it poses a challenge for the clinical trial because of the lack of standardization, including variation in dosage regimen, separation technique, and concentration of platelets (42). However, there is no consensus on the efficiency of PRP therapy, although recent trials have revealed it to be clinically helpful, especially in early stage knee OA (43, 44). The current study described platelet-rich plasma as a safe treatment with no serious complications reported after injection. The results of the current study showed that patients gave better WOMAC scores post-intervention with pure-PRP, where significant improvement was identified in all scores in both mild and moderate KOA at high significant improvement. The WOMAC scores for total patients after 3 months of PRP injection show there is a statistically significant reduction in the total WOMAC score and sub-scores. The current result is consistent with studies that demonstrate the clinical benefit of PRP according to the WOMAC scores, including Kuculmez and colleagues have been showed that a significant changes in WOMAC scores at three and six months after injection with PRP (45). In patients with mild to moderate knee osteoarthritis, intra-articular injections of leukocyte-poor PRP can give clinically significant functional improvement in WOMAC scores for at least a year (15% at first, 21% at second, 18% at sixth , and 21% at twelve months) (46). Güvendi and colleagues have proved that the IAIs of PRP are a safe therapeutic option that is effective in controlling KOA symptoms for up to 6 months following administration. WOMAC sub-scores, and total WOMAC score significantly decreased at the second and sixth months when compared to baseline, and no significant differences was present between single and triple injections. There was no variation in scores between the sixth and second months (47). Regarding the dosage regimen in both mild and moderate KOA groups, all of the single, double, and triple injections in mild KOA showed positive outcomes in improvements at all scores. While the moderate KOA group, all groups injected showed the positive improvements, although, stiffness, physical function, and the WOMAC score showed no significant improvement at single injection, which is consistent with the finding of Kavadar and his colleagues. They have been showed that IAIs of PRP can be used as a reliable and effective therapy for patients with moderate KOA and at least with second or third injections (48). The key finding was that triple PRP injections were superior to double and single injections in terms of effectiveness. This finding, which is consistent with Turgut and colleagues have showed that the WOMAC scores improved significantly after the second and sixth months, demonstrating that multiple intra-articular PRP results in better clinical improvement as compared to a single injection. This therapeutic injectable approach may be an option for patients who are averse to surgical interference or at risk of anesthesia (49).

The current study showed a significant efficacy of injection of pure-PRP in lowering the inflammation degree of affected joints, where there is a highly significant decrease in the level of TNF-alpha, which reflects a better improvement in the inflammation of the joint in knee osteoarthritis patients. The results of the present study agree with the findings of Lippross *et al.*, have found that PRP significantly reduces inflammation where the level of TNF-alpha reduced in KOA (50). Along with the evidences have provided by previous studies such as Penninx BW *et al*. and Stannus OP *et al*. (51, 52), the higher levels of TNF-alpha in KOA patients support the presence of inflammation reactions in the pathophysiology of KOA, and the decrease in TNF-alpha concentration after PRP injection supports one of the treatment mechanisms reflected by IAIs of PRP, which is its anti-inflammatory action, which is consistent with the evidence obtained by several studies (36, 53, 54). Regarding the dosage regimen in both mild and moderate KOA groups, all single, double, and triple injections in mild and moderate KOA have showed the positive outcomes in reduction in the serum levels of TNF-alpha. Although all groups showed reduction in the levels of TNF-alpha, the multiple PRP injections showed more reduction than double and single injections whereas no significant have been found between double and single. The present result consistent with previous studies that have been found the inflammation and other symptom improvements showed a positive correlation with an increase in the dose number of PRP injection, despite there is differences in methodology, duration, volume, interval and frequency of injection. Tavassoli *et al*. (55), have showed after three months of follow-up that two doses were more effective than a single dose. Gormeli *et al*. (56), have found that three doses of PRP are more effective than two or one, while having no effect on severe KOA patients. Huang *et al*. (57), have showed that the three doses of PRP produce significantly more improvements than the double or single dose. Chouha *et al*. (58), have showed the triple dose of PRP results in a long-term anti-inflammatory effect, whereas a short-term anti-inflammatory effect occurs after only a single injection on the affected joint. The current results disagree with studies by Patel *et al*. (59) and Guvedi *et al*. (47), were both have showed there is no statistically difference between single and three PRP injection in KOA patients.

As a limitation of the study, in addition to the relatively small sample size used, this study relied on short-term follow-up and did not include a placebo or reference group. The recommendations of this study incorporated a large sample of participants and a lengthy period of follow-up to evaluate the long-term effects of PRP.

## Conclusions

In conclusion, the treatment with pure-PRP was safe and satisfactory for patients in terms of relieving clinical symptoms. The WOMAC scores has indicated that improvement in pain relief for patients after injection and could act as an anti-inflammatory, as inflammatory marker TNF-alpha was reduced after injection, and there was a superior outcome to increasing dose numbers, as triple injection gave a positive outcome in both mild and moderate KOA when compared to single and double injection.

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## Competing Interests

There are no conflicts of interest associated with the publishing of this paper.

## Adherence to Ethical Standards

The ethical committee approved the study at the University of Al-Qadisiyah (registration code CMUQ 11/1000 on 8/3/2022).

## References

1. Tiulpin A, Thevenot J, Rahtu E, et al. Automatic knee osteoarthritis diagnosis from plain radiographs: a deep learning-based approach. Scientific reports. 2018 Jan 29;8(1):1-0.
2. Frankel L, Sanmartin C, Hawker G, et al. Perspectives of orthopaedic surgeons on patients' appropriateness for total joint arthroplasty: a qualitative study. Journal of evaluation in clinical practice. 2016 Apr;22(2):164-170.
3. Primorac D, Molnar V, Rod E, et al. Knee osteoarthritis: a review of pathogenesis and state-of-the-art non-operative therapeutic considerations. Genes. 2020 Jul 26;11(8):854.
4. Bany Muhammad M, Yeasin M. Interpretable and parameter optimized ensemble model for knee osteoarthritis assessment using radiographs. Scientific Reports. 2021 Jul 12;11(1):1-1.
5. Stewart WF, Ricci JA, Chee E, et al. Lost productive time and cost due to common pain conditions in the US workforce. Jama. 2003 Nov 12;290(18):2443-2454.
6. Srilekha C, Kumar CP. The study on prevalence and management of osteoarthritis in South India. International Journal of Orthopaedics Sciences. 2019;5(4):112-117.
7. Shang Y, Liu T, Wei Y, et al. Scoring systems for predicting mortality for severe patients with COVID-19. EClinicalMedicine. 2020 Jul 1;24:100426.
8. Thati S. Gender differences in osteoarthritis of knee: An Indian perspective. Journal of Mid-life Health. 2021 Jan;12(1):16.
9. Musumeci G, Aiello FC, Szychlinska MA, et al. Osteoarthritis in the XXIst century: risk factors and behaviours that influence disease onset and progression. International journal of molecular sciences. 2015 Mar 16;16(3):6093-6112.
10. Katz JN, Neogi T, Callahan LF, et al. Disease modification in osteoarthritis; pathways to drug approval. Osteoarthritis and Cartilage Open. 2020 Jun 1;2(2):100059.
11. Arden N, Blanco F, Cooper C, et al. Atlas of osteoarthritis. Springer Healthcare Limited; 2014.
12. Mabey T, Honsawek S, Tanavalee A, et al. Plasma and synovial fluid inflammatory cytokine profiles in primary knee osteoarthritis. Biomarkers. 2016 Oct 2;21(7):639-644.
13. Imamura M, Ezquerro F, Marcon Alfieri F, et al. Serum levels of proinflammatory cytokines in painful knee osteoarthritis and sensitization. International journal of inflammation. 2015 Oct;2015.
14. Aktas E, Sener E, Zengin O, et al. Serum TNF-alpha levels: potential use to indicate osteoarthritis progression in a mechanically induced model. European Journal of Orthopaedic Surgery & Traumatology. 2012 Feb;22(2):119-122.
15. Lim H, Lee SH, Lee HT, et al. Structural biology of the TNFα antagonists used in the treatment of rheumatoid arthritis. International journal of molecular sciences. 2018 Mar 7;19(3):768.

16. Gundogdu G, Gundogdu K. A novel biomarker in patients with knee osteoarthritis: adropin. Clinical rheumatology. 2018 Aug;37(8):2179-2186.
17. Hou SM, Hou CH, Liu JF. CX3CL1 promotes MMP-3 production via the CX3CR1, c-Raf, MEK, ERK, and NF-κB signaling pathway in osteoarthritis synovial fibroblasts. Arthritis Research & Therapy. 2017 Dec;19(1):1-2.
18. Wei Y, Bai L. Recent advances in the understanding of molecular mechanisms of cartilage degeneration, synovitis and subchondral bone changes in osteoarthritis. Connective tissue research. 2016 Jul 3;57(4):245-261.
19. De La Mata J. Platelet rich plasma. A new treatment tool for the rheumatologist?. Reumatología Clínica (English Edition). 2013 May 1;9(3):166-171.
20. Delanois RE, Etcheson JI, Sodhi N, et al. Biologic therapies for the treatment of knee osteoarthritis. The Journal of Arthroplasty. 2019 Apr 1;34(4):801-813.
21. Evans CH, Kraus VB, Setton LA. Progress in intra-articular therapy. Nature Reviews Rheumatology. 2014 Jan;10(1):11-22.
22. Emami A, Tepper J, Short B, et al. Toxicology evaluation of drugs administered via uncommon routes: intranasal, intraocular, intrathecal/intraspinal, and intra-articular. International journal of toxicology. 2018 Jan;37(1):4-27.
23. Anitua E, Sánchez M, Orive G, et al. A biological therapy to osteoarthritis treatment using platelet-rich plasma. Expert Opinion on Biological Therapy. 2013 Dec 1;13(8):1161-1172.
24. Andia I, Maffulli N. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. Nature Reviews Rheumatology. 2013 Dec;9(12):721-730.
25. Braun HJ, Kim HJ, Chu CR, et al. The effect of platelet-rich plasma formulations and blood products on human synoviocytes: implications for intra-articular injury and therapy. The American journal of sports medicine. 2014 May;42(5):1204-10.
26. Frizziero A, Giannotti E, Ferraro C, et al. Platelet rich plasma intra-articular injections: a new therapeutic strategy for the treatment of knee osteoarthritis in sport rehabilitation. A systematic review. Sport Sciences for Health. 2012 Jul;8(1):15-22.
27. McCarrel TM, Minas T, Fortier LA. Optimization of leukocyte concentration in platelet-rich plasma for the treatment of tendinopathy. JBJS. 2012 Oct 3;94(19):e143.
28. Riboh JC, Saltzman BM, Yanke AB, et al. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. The American journal of sports medicine. 2016 Mar;44(3):792-800.
29. López-Cotarelo P, Gómez-Moreira C, Criado-García O, et al. Beyond chemoattraction: multifunctionality of chemokine receptors in leukocytes. Trends in immunology. 2017 Dec 1;38(12):927-941.
30. Saleem M, Farid MS, Saleem S, et al. X-ray image analysis for automated knee osteoarthritis detection. Signal, Image and Video Processing. 2020 Sep;14(6):1079-1087.
31. Shamir L, Ling SM, Scott WW, et al. Knee x-ray image analysis method for automated detection of osteoarthritis. IEEE Transactions on Biomedical Engineering. 2008 Oct 7;56(2):407-415.
32. Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. Journal of cutaneous and aesthetic surgery. 2014 Oct;7(4):189.
33. Bausset O, Giraudo L, Veran J, et al. Formulation and storage of platelet-rich plasma homemade product. BioResearch open access. 2012 Jun 1;1(3):115-123.
34. Longo UG, Ciuffreda M, Candela V, et al. Hip scores: A current concept review. British Medical Bulletin. 2019 Sep 1;131(1).
35. Johanson NA, Liang MH, Daltroy L, et al. American Academy of Orthopaedic Surgeons lower limb outcomes assessment instruments: reliability, validity, and sensitivity to change. JBJS. 2004 May 1;86(5):902-909.
36. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surgery, Sports Traumatology, Arthroscopy. 2010 Apr;18(4):472-479.
37. Assirelli E, Filardo G, Mariani E, et al. Effect of two different preparations of platelet-rich plasma on synoviocytes. Knee Surgery, Sports Traumatology, Arthroscopy. 2015 Sep;23(9):2690-2703.
38. Cavallo C, Filardo G, Mariani E, et al. Comparison of platelet-rich plasma formulations for cartilage healing: an *in vitro* study. JBJS. 2014 Mar 5;96(5):423-429.
39. Araya N, Miyatake K, Tsuji K, et al. Intra-articular injection of pure platelet-rich plasma is the most effective treatment for joint pain by modulating synovial inflammation and calcitonin gene-related peptide expression in a rat arthritis model. The American Journal of Sports Medicine. 2020 Jul;48(8):2004-2012.
40. Jayaram P, Liu C, Dawson B, et al. Leukocyte-dependent effects of platelet-rich plasma on cartilage loss and thermal hyperalgesia in a mouse model of post-traumatic osteoarthritis. Osteoarthritis and cartilage. 2020 Oct 1;28(10):1385-1393.

41. Yin W, Qi X, Zhang Y, et al. Advantages of pure platelet-rich plasma compared with leukocyte-and platelet-rich plasma in promoting repair of bone defects. Journal of translational medicine. 2016 Dec;14(1):1-9.
42. Castillo TN, Pouliot MA, Kim HJ, et al. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. The American journal of sports medicine. 2011 Feb;39(2):266-271.
43. Chang KV, Hung CY, Aliwarga F, et al. Comparative effectiveness of platelet-rich plasma injections for treating knee joint cartilage degenerative pathology: a systematic review and meta-analysis. Archives of physical medicine and rehabilitation. 2014 Mar 1;95(3):562-575.
44. Lai LP, Stitik TP, Foye PM, et al. Use of platelet-rich plasma in intra-articular knee injections for osteoarthritis: a systematic review. PM&R. 2015 Jun 1;7(6):637-648.
45. Kuculmez O, Sirin FB, Sarikaya NO, et al. Effect of platelet-rich plasma on serum and urine biomarkers in patients with knee osteoarthritis. Int. J. Clin. Exp. Med. 2020 Jan 1;13:5040-5049.
46. Lin KY, Yang CC, Hsu CJ, et al. Intra-articular injection of platelet-rich plasma is superior to hyaluronic acid or saline solution in the treatment of mild to moderate knee osteoarthritis: a randomized, double-blind, triple-parallel, placebo-controlled clinical trial. Arthroscopy: The Journal of Arthroscopic & Related Surgery. 2019 Jan 1;35(1):106-117.
47. Güvendi EU, Aşkin A, Güvendi G, et al. Comparison of efficiency between corticosteroid and platelet rich plasma injection therapies in patients with knee osteoarthritis. Archives of Rheumatology. 2018 Sep;33(3):273.
48. Kavadar G, Demircioglu DT, Celik MY, et al. Effectiveness of platelet-rich plasma in the treatment of moderate knee osteoarthritis: a randomized prospective study. Journal of Physical Therapy Science. 2015;27(12):3863-3867.
49. Turgut MC, Ayas MS, Erhan OK, et al. Multiple Injections of PRP/Steroid Combination Result in Better Clinical Outcames in Advanced Osteoarthritis: A Prospective Randomized Study. New Trends in Medicine Sciences.;3(1):36-42.
50. Lippross S, Moeller B, Haas H, et al. Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint. Arthritis & rheumatism. 2011 Nov;63(11):3344-3353.
51. Penninx BW, Abbas H, Ambrosius W, et al. Inflammatory markers and physical function among older adults with knee osteoarthritis. The Journal of rheumatology. 2004 Oct 1;31(10):2027-2031.
52. Stannus OP, Jones G, Blizzard L, et al. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. Annals of the rheumatic diseases. 2013 Apr 1;72(4):535-540.
53. Marmotti A, Rossi R, Castoldi F, et al. PRP and articular cartilage: a clinical update. BioMed Research International. 2015 May 5;2015.
54. Hassan MH, Abuhamdah S, Saleem TH, et al. Assessment of therapeutic effects of platelet-rich plasma in knee osteoarthritis: possible role of inflammatory cytokines.
55. Tavassoli M, Janmohammadi N, Hosseini A, et al. Single-and double-dose of platelet-rich plasma versus hyaluronic acid for treatment of knee osteoarthritis: A randomized controlled trial. World Journal of Orthopedics. 2019 Sep 9;10(9):310.
56. Görmeli G, Görmeli CA, Ataoglu B, et al. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. Knee Surgery, Sports Traumatology, Arthroscopy. 2017 Mar;25(3):958-965.
57. Huang PH, Wang CJ, Chou WY, et al. Short-term clinical results of intra-articular PRP injections for early osteoarthritis of the knee. International Journal of Surgery. 2017 Jun 1;42:117-122.
58. Chouhan DK, Dhillon MS, Patel S, et al. Multiple platelet-rich plasma injections versus single platelet-rich plasma injection in early osteoarthritis of the knee: an experimental study in a guinea pig model of early knee osteoarthritis. The American Journal of Sports Medicine. 2019 Aug;47(10):2300-2307.
59. Patel S, Dhillon MS, Aggarwal S, et al. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. The American journal of sports medicine. 2013 Feb;41(2):356-364.
60. Merkhan MM, Shephard MT, Forsyth NR. Physoxia alters human mesenchymal stem cell secretome. Journal of Tissue Engineering. 2021 Oct;12:20417314211056132.
61. Forsyth NR, Steeg R, Ahmad M, et al. Mimicking Physiological Oxygen in Cell Cultures. Cell Culture Technology 2018:129-137. Springer, Cham.
62. Narayanasamy KK, Price JC, Merkhan M, et al. Effect of PEI-coated MNPs on the Regulation of Cellular Focal Adhesions and Actin Stress Fibres. bioRxiv. 2019 Jan 1:617480.