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Original Article

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Analysis of IL-6 marker in synovial fluid of the knee joint in patients with osteoarthritis and rheumatoid arthritis before and after platelet-rich plasma administration

Masthan Basha Shaik*[1], Divya Vurundhur[2], Kiran Kumar Mallam[3], Mohammad Gulabi[4]

[1] *Associate professor, Department of orthopedics, ACSR Govt Medical College, Nelore, Andhra Pradesh, India*

[2] *Assistant professor, Department of orthopedics, ACSR Govt Medical College, Nelore, Andhra Pradesh, India*

[3] *Associate professor, Department of orthopedics, ACSR Govt Medical College, Nelore, Andhra Pradesh, India*

[4] *Senior Resident, Department of orthopedics, ACSR Govt Medical College, Nelore, Andhra Pradesh, India*

***Corresponding author:** Dr. Masthan Basha Shaik, **Address**: Department of Orthopaedics, ACSR Govt Medical College, Nelore, Andhra Pradesh, India, **Email**: bonesbasha@gmail.com, **Tel**: +918612319299

Abstract

***Background & Aims*:** Platelet-rich plasma (PRP) has emerged as a treatment for osteoarthritis (OA) and rheumatoid arthritis (RA). The aim of the current study is to assess the effect of PRP injection and its influence on interleukin-6 (IL-6) in the synovial fluid of the knee joint in OA and RA patients.

***Materials & Methods*:** A clinico-radiologically diagnosed case of OA and RA of 30 each was included during the study period of 6 months. 10 ml of patient blood was collected and PRP was prepared by the differential centrifugation method, and this freshly prepared PRP was injected into the affected knee joint slowly. IL-6 levels were assessed in synovial fluid at pre-PRP injection and at the 1st, 3rd and 6th months post-PRP Injection. Pain was measured using a Visual Analog Scale.

***Results*:** The mean age of the cases was 58.5 years for OA and 47.2 years for RA. Females were predominantly compared to males. Majority were Kellegren Lawrence radiological grade 3 in 15 (50%) cases, following grade 2 in 8 (26.67%) cases, grade 4 in 6(20%) cases and grade 1 in 1(3.33%) case. Mean IL-6 in OA cases was 89.5±24.5 pg/ml at pre PRP, and 66.48±23.1 pg/ml at post PRP ($p<0.001$, paired t-test). Mean IL-6 in RA was 97.5±18.9 pg/ml at pre-PRP injection, and 89.6±17.9 at post PRP ($p<0.001$, paired t-test). In OA cases, paired t-test between the mean pain scores (VAS) at follow-up depicts that the mean difference and standard error of difference between 6-month follow-up and one-month follow-up was -1.23 ± 0.20 statistically significant differences (95%CI: -1.71 to -0.73). In RA cases, paired t-test between the mean pain scores (VAS) at followup depicts that the mean difference and standard error of difference between 6-month follow-up and one-month follow-up was -1.38 ± 0.19 statistically significant differences (95% CI: -1.69 to -0.75). There was a positive correlation between levels of IL-6 and pain score ($r=0.309$, $p=0.004$).

***Conclusion*:** Study shows that anti-Inflammatory role of PRP, which was evidenced by the statistically significant difference in the IL-6 levels at follow-up. It may be beneficial to halt the progress in arthritic joints. This study successfully established an anti-inflammatory role of PRP in joint pathology by influencing the IL-6 levels.

Keywords: Interleukin-6, Osteoarthritis, Platelet-Rich Plasma, Rheumatoid Arthritis

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune condition in which alterations can spread to all tissues. Osteoarthritis (OA) is a slowly progressive synovial joint disease characterized pathologically by focal destruction of the articular cartilage, a hypertrophic response in neighboring bone which results in osteophyte formation and subchondral sclerosis, variable degrees of synovial inflammation, a thickening of the joint capsule, and damage to soft tissue structures such as ligaments and, in the knee, the meniscus (1). Rheumatic disorders encompass a wide range of conditions, including osteoarthritis (OA), rheumatoid arthritis (RA), autoimmune diseases such as systemic lupus erythematosus, osteoporosis, gout, fibromyalgia, back pain, and more (2).

Among chronic rheumatic illnesses, osteoarthritis and rheumatoid arthritis pose substantial public health issues and economic expenses in all nations, with an estimated worldwide burden of 3.8% for OA knee, and 1-2% for RA with a tendency more towards advancing age (3). India has a higher rate of OA proliferation than the rest of the world. OA is regarded as one of the most common musculoskeletal diseases worldwide (4,5). OA observed in the elderly with a prevalence of 22% - 39% in India. Prevalence of OA knee in rural and urban India is 3.9% and 5.5%, respectively (6).

Jain S *et al.* in a survey stated that "India is predicted as chronic disease capital by 2025 and expected to have 60 million people with arthritis" (7).

Various groups of cytokines were observed to be involved in the pathogenesis of rheumatic diseases, the most important of which are IL-1, tumor necrosis factor (TNF), IL-6, IL-15, IL-17, and IL-18 (8, 9,10).

In practice, the most commonly utilized intraarticular injections for OA knees were hydrocortisone, methylprednisolone, triamcinolone, and betamethasone. Tumor necrosis factor (TNF) inhibitors and Tocilizumab (an antibody that binds to the IL-6 receptor) were approved as a biological therapy for moderate to severe RA (11).

Platelet Rich Plasma, Mesenchymal Stem Cells treatments were reported to be safe and found to be superior in terms of pain relief (12). There is a need to identify prospective management to target numerous pathways involved in the pathophysiology of RA. Roux-Lombard et al. reported that in early RA, IL-6 and CRP levels correlated with proMMP-3, implying a relationship between IL-6 and proteinase activity (13). PRP is autologous blood derivatives that has platelet concentrations that are 3-5 times higher than normal (14). Several studies have aimed at the efficacy of PRP, but very few were found the correlation between PRP and inflammatory mediators.

The aim of the study is to assess the effect of PRP injection and its influence on IL-6 in the synovial fluid of the knee joint in osteoarthritis and rheumatoid arthritis patients.

Materials & Methods

Written and informed consent for surgery was taken from the patients before the surgery.

Study design: A prospective, interventional analytical study was conducted on clinico-radiologically diagnosed patients with osteoarthritis and rheumatoid arthritis. Study conducted for the duration of two years. A total of 30 Osteoarthritis and 30 RA patients were admitted at Department of Orthopedics, ACSR Govt. General Hospital, Nellore.

Inclusion criteria: Diagnosed case of osteoarthritis and rheumatoid arthritis in adult patients and patients who can give consent for the study.

Exclusion Criteria: Seronegative arthritis/connective tissue disorders, Septic arthritis, Gouty arthritis, Malignancy, Adjacent osteomyelitism, impending joint replacement surgery, Hemearthrosis, Infectious arthritis, joint prosthesis, Peri-articular cellulitis, uncontrolled diabetes, coagulopathy local joint diseases/systemic disease and congenital skeletal anomalies.

Procedure: After obtaining the informed consent, study participants were classified based on the clinic-radiological findings of the knee. Patient demographics, BMI, presenting complaints, past medical/surgical history, Allergy, and Bleeding tendencies were recorded. X-ray AP & lateral view (Kellegren Lawrence

grading), and platelet count were noted. Patients under study were given rescue medication (Tab. Paracetamol 1gr) for OA. Patients who are rheumatoid were advised to continue DMARDs.

PRP preparation: PRP was prepared using the differential centrifugation method. Briefly, centrifuged total of 10 ml of blood at 1500 rpm for 3 min. It is separated into 2 layers. The upper layer is made up of plasma, whereas the lower layer is made up of red blood cells. The top layer is removed and centrifuged at 2500 rpm for 3 min. Two layers were separated, with the upper layer containing platelet deficient plasma and the lower layer containing platelet rich plasma. The lower layer was collected and injected.

PRP injection: The PRP fluid after the centrifugation process was aspirated, and by using sterile aseptic conditions, the freshly prepared PRP was injected to the affected knee joint slowly.

Follow-up: Before PRP injection, under strict aseptic precautions, synovial fluid was aspirated from the affected knee joint using 10 ml syringe. It was transported in a sterile container to the lab, and centrifuged, a supernatant fluid subjected to IL-6 analysis. After 4 weeks of post PRP injection, the patient was reviewed for IL-6 analysis at 1st, 3rd and 6th month post-PRP injection.

Analysis of IL-6: IL-6 levels were measured using human IL-6 ELISA kit (Sigma-Aldrich Chemicals Private Limited, Bangalore, India) according to the manufacturer's instructions. Sample is added to the wells pre-coated with IL-6 monoclonal antibody. After incubation, a biotin-conjugated anti-human IL-6 antibody is added. After the incubation, unbound biotin-conjugated anti-human IL-6 antibody is washed away using washing step. Streptavidin-HRP is added. After incubation, unbound Streptavidin-HRP is washed away by another washing step. Substrate solution is then added and color develops in proportion to the amount of human IL-6. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm using Optical Densitometry (OD). By comparing the OD value of the samples to the standard curve, Human IL-6 levels were calculated.

Pain score: All patients who received PRP injections were assessed at 1st, 3rd and 6th month post PRP Injection using Visual Analog Scale (VAS) (Edek, et al.).

Statistical analysis: A one-way repeated measures ANOVA was conducted to identify the difference in the mean pain score across the follow-up. The mean difference levels of IL-6 and their standard error of difference between the follow-up were analyzed using paired t-test. Chi-square test was used to assess the association between the 2 categorical variables. Pearson's correlation test analyzed to identify the relation between IL-6 levels and pain score. Statistical analysis performed using statistical package for Social Science Program Version 25.0 (SPSS, IBM, US) for Windows. P value is significant if less than 0.05.

Results

This study was conducted in 30 OA cases, in which 5 cases had bilateral osteoarthritis of knee, 15 cases had unilateral osteoarthritis of knee, 8 cases with bilateral rheumatoid arthritis of knee and 2 cases of unilateral rheumatoid arthritis of knee.

Baseline characteristics: The mean age of the cases was 57.5 years (45-79 yrs) with a standard error of 2.2 years. The median age was 56 years with inter-quartile range of 49 - 64.5 years. The mean age of the cases was 58.5 years for OA and 47.2 years for RA. Females were predominant compared to males. Female were found to be 76.67% and 80% in OA and RA cases respectively. The BMI of the cases ranged between 23.5 to 36.5 kg/m^2. 18 (30%) of study population was found to be overweight and 8 (13.33%) was found to be obese.

Kellegren Lawrence radiological grading was ranged from 1-4, and the majority were grade 3 was seen in 15 (50%), following grade 2 in 8 (26.67%) cases, grade 4 in 6(20%) cases and grade 1 in 1(3.33%) case respectively.

Changes in IL-6 levels pre & post PRP: In Osteoarthritis cases, mean IL-6 levels in OA cases were 89.5±24.5 with maximum IL-6 level being 139.5 pg/ml at pre PRP. While the mean levels of IL-6 had reduced to 66.48±23.1 at post PRP. There is a decrease in IL-6

levels. This difference was found to be statistically significant ($p<0.001$, paired t-test). In rheumatoid arthritis cases, mean IL-6 level was 97.5±18.9 with maximum IL-6 level being 129.5.7pg/ml at pre PRP. While the mean IL-6 level was reduced to 89.6±17.9 at post PRP. There is a decrease in IL-6 levels. This difference was found to be statistically significant ($p<0.001$, paired t-test) (Table 1) (Figure 1) (Figure 2).

Table 1. Comparison of IL-6 levels in pre- and post PRP in osteoarthritis and RA cases

	IL-6 levels		Mean difference ±	95%Confidence Interval	p valve
	Pre PRP	Post PRP	SE	of Difference	
Osteoarthritis cases (N=30)	89.5±24.5	66.48±23.1	-23.02 ± 1.4	-26.4 to -14.8	<0.001
RA (n=30)	97.5±18.9	89.6±17.9	-7.9± 1.0	-10.1 to -3.9	<0.001

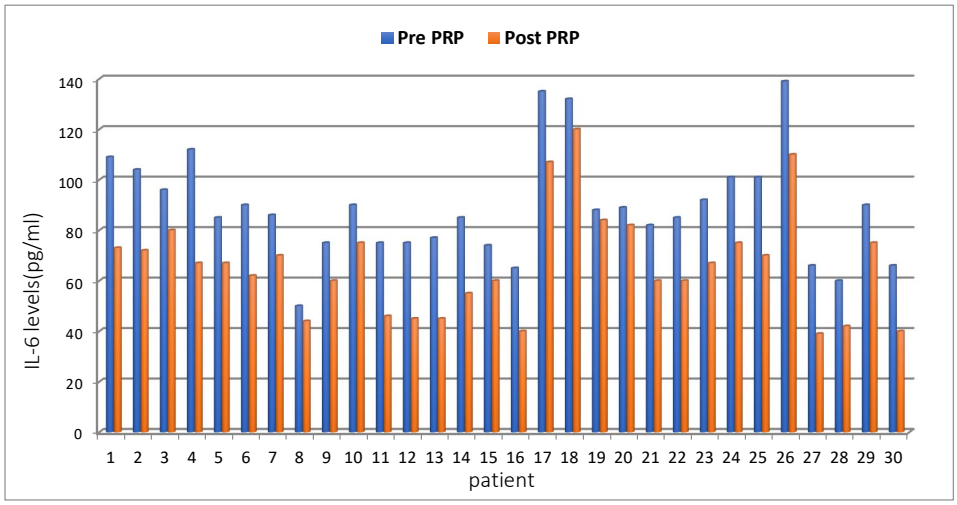

Fig. 1. Change in the IL-6 values before and after PRP in patients with osteoarthritis

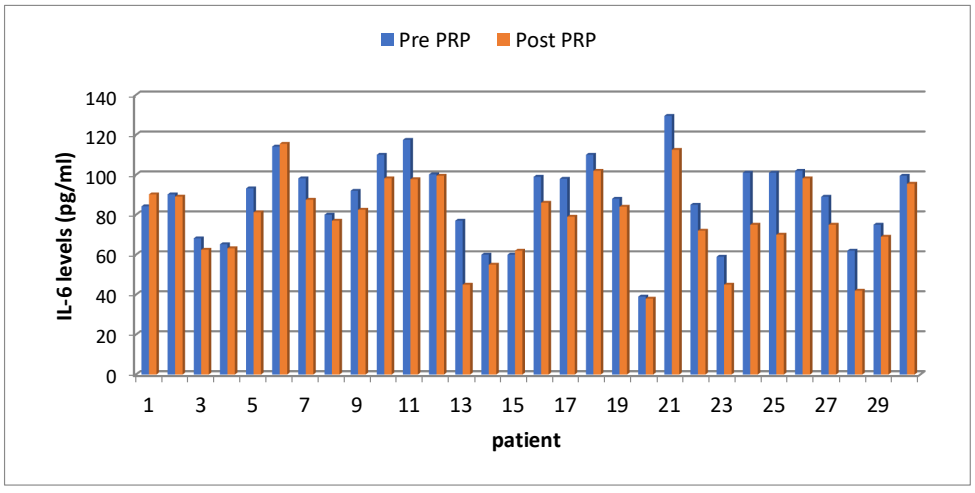

Fig. 2. Change in the IL-6 values before and after PRP in patients with in RA

Comparison of pain score: The pain scores were measured at the end of 1 month, 3 months and 6 months. Improvement in pain score was experienced by the patient as early as one month of follow-up and was maximum by the third month, but then there is not much decrease in the pain score at 6-month follow-up.

Pain score in Osteoarthritis: A one-way repeated measures analysis of variance was conducted to identify the difference in mean score across follow-up. The ANOVA indicated a significant time effect, Wilks' Lambda = 0.39; F=13.2, p<0.001. By comparing the mean score results shows that there is a decrease in mean pain score as the follow-up period progresses.

Table 2. Pain score assessment during the follow-up in patients with osteoarthritis

Pain Score	Mean ± SD	Mean difference ± SE	95% Confidence Interval of Difference	p valve
Post PRP 1 month	5.66 ± 1.25	-1.11 ± 0.15	-1.22 to -0.69	<0.001
Post PRP 3 months	4.55 ± 1.29			
Post PRP 3 months	4.55 ± 1.29	-0.12 ± 0.19	-0.45 to 0.49	0.45
Post PRP 6 months	4.43 ± 1.55			
Post PRP 1 month	5.66 ± 1.25	-1.23 ± 0.09	1.71 to -0.73	<0.001
Post PRP 6 months	4.43 ± 1.55			

In OA cases, t-test between the mean pain scores (VAS)at followup depicts that the mean difference and standard error of difference between 3-month follow-up and one-month follow-up was -1.11 ± 0.15, this difference was statistically significant with the mean decrease 95% CI ranging between -1.22 to -0.69. The mean difference and standard error of difference between 6-month follow-up and one-month follow-up was -1.23 ± 0.20, this difference was statistically significant with the mean decrease 95%CI ranging between -1.71 to -0.73. The mean difference and standard error of difference between 6-month follow-up and 3-month follow-up was -0.12 ± 0.19, this difference was not statistically significant (Table 2).

In rheumatoid arthritis cases, one-way repeated measures analysis of variance indicated a significant time effect, Wilks' Lambda = 0.29; F=23.6 p<0.001. By comparing the mean score results shows that there is a decrease in mean pain score as the follow-up period progresses.

In RA cases, upon analysis using paired t-test between the mean pain scores (VAS)at followup depicts that the mean difference and standard error of difference between 3-month follow-up and one-month follow-up was -1.34 ±0.14, this difference was statistically significant with the mean decrease 95%CI ranging of 1.55 to -0.72. The mean difference and standard error of difference between 6-month follow-up and one-month follow-up was -1.38 ± 0.19, this difference was statistically significant with the mean decrease 95% CI range of -1.69 to -0.75. The mean difference and standard error of difference between 6-month follow-up and 3-month follow-up was -0.04 ± 0.12, without statistically significant (Table 3).

Table 3. Difference in VAS-pain score across follow-up in rheumatoid arthritis cases

Pain Score	Mean ± SD	Mean difference ± SE	95% Confidence Interval of Difference	p-value
Post PRP 1 month	6.99 ± 0.55	-1.34 ± 0.14	-1.55 to -0.72	<0.001
Post PRP 3 months	5.65 ± 0.99			
Post PRP 3 months	5.65±0.99	-0.04 ± 0.12	-0.29 to 0.28	0.89
Post PRP 6 months	5.61± 1.12			
Post PRP 1 month	6.99 ±0.55	-1.38 ± 0.19	-1.69 to -0.75	<0.001
Post PRP 6 months	5.61± 1.12			

Pearson's correlation test shows that there was a positive relation between the decrease in the IL-6 levels and decrease in pain score (r=0.309, p=0.004).

Discussion

An interventional study analyzed the effect of PRP on knee joint inflammation using IL-6 as an inflammatory marker in synovial fluid in 30 OA and 30 RA patients.

Basic Characteristics:

The mean age of the cases was 58.5 years for OA and 47.2 years for RA. Females outnumbered males. Obesity is a significant risk factor in the pathophysiology of OA. In our study, 18 (30%) population were overweight, and 8 (13.33%) were obese.

KL grading is divided into 4 grades based on the radiographic findings. In our study, grade III was seen in 15 (50%), grade II in 8 (26.67%), grade IV in 6 (20%), and grade I in 1 (3.33%).

According to Brandt KD et al., the severity of symptoms and KL grade are unrelated. There has been no direct correlation between KL grading and IL-6 (15).

IL-6 & PRP:

When compared to other cytokines, the precise involvement of IL-6 in inflammation and OA is less known. The concentration of IL-6 appears to decrease with increasing severity of OA (16). In contrast to the previous notion, IL-6 modulates the severity of inflammation through the modulation of other pro-inflammatory cytokines TNF- and IL-ß (17).

There is no unanimity in this area of PRP yet. Initially, research employed 3 injections at 3-week intervals, identical to hyaluronic acid without any rationale (14).

Changes in IL-6 levels- pre- & post PRP:

Before PRP injection, the mean IL-6 among the OA cases was 89.5±24.5 pg/ml with maximum IL-6 level being 139.5 pg/ml. While the mean levels of IL-6 had been reduced to 66.48±23.1 at post PRP. Before PRP injection, the mean IL-6 in RA cases was 97.5±18.9 with maximum IL-6 level being 129.5.7pg/ml. While the mean IL-6 level was reduced to 89.6±17.9 at post PRP. There is a decrease in IL-6 levels. This difference was found to be statistically significant (p<0.001, paired t-test).

Observations of our study extend the results with previous studies showing the increased levels of IL-6 in synovial fluid from patients with RA.

Madhok et al. studied 93 patients with RA and confirmed that serum IL-6 values are increased in RA, and independent of age, duration of RA, and patient gender. No associations were noted with duration of morning stiffness and VAS pain score (18).

Houssiau et al. study shows that IL-6 levels were considerably elevated in the synovial fluid of patients with various inflammatory arthritis (19).

Pain score assessment:

Pain scores were measured at the end of one month, 3 months and 6 months using VAS post PRP. There is decrease in mean VAS score as the follow-up period increases. Improvement in pain score was experienced by the patient as early as one month of follow-up and

tends to be maximum by 3rd month, but then there is not much decrease in the pain score at 6-month follow-up which indirectly signifies the anti-inflammatory role could be the reason for clinical improvement, as for cartilage regeneration and remodeling would have required much more time and have given sustained results.

In the last 25 yrs, various researchers have attempted to establish the role of cytokines in arthritic joints and the superiority of PRP over other intra-articular therapies individually. Till date very few studies compared the effect of PRP in joint inflammation using IL-6 as a biomarker.

This study successfully established an anti-inflammatory role of PRP in joint pathology by influencing the IL-6 biomarker levels.

Conclusion

Study supports the evidence that established the effective role of IL-6 in the inflammatory joint disease. Study also highlighted the present state of knowledge in the novel platelet products substitutes and understood that PRP is not merely just platelet alone, and it is a biological milieu of bioactive factors. This study shows the anti-Inflammatory role of PRP, which was evidenced by a statistically significant difference in the IL-6 levels.

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Nil.

Conflict of interests

The authors declare that they have no conflicts of interest.

Funding/Support

No funding was required for the study.

Data Availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

Ethical Statement

Study protocol has been conducted after approval by the ethical committee. Written and informed consent for surgery was taken from the patients before the surgery.

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