Section: Miscellaneous

Original Research Article

CLINICAL EVALUATION OF LEUKOCYTE-RICH PLATELET RICH PLASMA TREATMENT FOR OSTEOARTHRITIS

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ABSTRACT

Background: The regenerative effect of platelet rich plasma (PRP) is primarily linked to biomolecules in platelet α-granules, but a controlled inflammatory phase is also essential for healing. Leukocytes contribute to this phase by releasing both pro- and anti-inflammatory factors, supporting a process known as "inflammatory regeneration." Macrophage plasticity, particularly the shift from M1 to M2 phenotype, plays a key role in this transition. Therefore, PRP derived from the buffy coat, which includes leukocytes, may enhance regenerative outcomes and be more beneficial than previously thought. Hence we aimed to evaluate efficacy of leucocyte rich platelet rich plasma (LR-PRP) on outcomes of knee osteoarthritis.

Materials and Methods: Intra-articular LR-PRP injections were administered to 130 knees, with each patient receiving a total of six injections at 4-week intervals. Clinical outcomes were assessed using the Visual Analog Scale (VAS) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC score).

Results: Most patients experienced notable improvements in both VAS scores and WOMAC scores following treatment. The severity of the deformity did not appear to significantly influence treatment outcomes, and favourable results were observed even in cases with advanced K-L classification grades.

Conclusion: Based on the results of our study, we recommend considering LR-PRP as an adjunctive conservative treatment option in the non-operative management of osteoarthritis.

Keywords: knee osteoarthritis, LR-PRP, VAS score, WOMAC score.

INTRODUCTION

Osteoarthritis (OA) is a complex, long-term condition marked by the gradual breakdown of articular cartilage, ultimately resulting in reduced joint space. Researchers are actively exploring various methods to promote cartilage repair, particularly in cases of knee osteoarthritis (KOA). A range of therapeutic strategies have been investigated, including single-molecule drugs, hyaluronic acid injections, and corticosteroids, aiming to restore cartilage integrity and alleviate KOA-associated pain.

At present, there is growing interest in using immunomodulatory biological therapies to address cartilage damage and slow the progression of osteoarthritis (OA).[1] Research indicates that platelet-rich plasma (PRP) has the potential to enhance tissue healing and provide sustained relief from OA symptoms.[2] Platelet-rich plasma (PRP) injections quickly reduce inflammation in the synovial membrane, and this anti-inflammatory effect is sustained over time, contributing to the protection of cartilage. Further, there is increased interest towards knowing the cellular composition of PRP so as to know efficacy of these components in healing.

The concentration of leukocytes in PRP has attracted considerable attention. Elevated leukocyte levels have been associated with increased expression of catabolic pathways and pro-inflammatory cytokines, including interleukin-1 and tumour necrosis factor-alpha. Studies on cultured synoviocytes have shown that leukocyte-rich PRP (LR-PRP) can induce cell death and promote the release of various inflammatory mediators.[3,4] Furthermore, a prospective comparative study indicated that patients treated with LR-PRP were more likely to experience painful side effects.[5]

However, multiple injections of LR-PRP may produce fibrocartilaginous cover over inflamed cartilage over a period of six months.[6]

This study aimed to evaluate the effectiveness of multiple leukocyte-rich platelet-rich plasma (LR-PRP) injections in the treatment of knee osteoarthritis (KOA).

MATERIALS AND METHODS

A prospective study was conducted following approval from the local Ethics Committee. All participants provided informed consent after being notified that their data would be used for publication. Between January 2024 and August 2025, 170 patients with knee osteoarthritis (KOA) were assessed at our institute. Of these, 10 declined participations, 20 did not meet the inclusion criteria and 10 were lost to follow-up. Ultimately, 130 patients were included in the study. Patients in age group of 40-70years with BMI of 20-29.9 having chronic knee pain with radiological score of KL(Kellgren-Lawrence) classification 2-3 were included. Those patients with grade 4 knee osteoarthritis based on the K-L scale, history of femur or tibia fractures, previous knee surgeries such as arthroscopy, hyaluronic acid injections within the past six months, hemoglobin levels below 10 g/dL and history of oncohematological diseases, infections, or immunodeficiency were excluded.

All patients were clinically evaluated and investigated according to institutional protocol. All patients were evaluated by single pain physician having experience of more than 5 years. The evaluation was conducted at four time points: T0 (recruitment), T1 (one month after the final injection), T2 (three months post-injection), and T3 (six months post-injection). At each follow-up, patients were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Visual Analogue Scale (VAS). Additionally, MRI and X-ray images were obtained at baseline and six months and included in the analysis.

The LR-PRP concentrate used for injection was prepared at the Department of Pain medicine in our institute through apheresis of each patient's venous blood. Patients were instructed to fast for 10 hours prior to blood collection to minimize the impact of food intake on LR-PRP quality. Blood samples were processed at room temperature using Centrifuge System which separated the blood into plasma, buffy coat, and residual red blood cells.

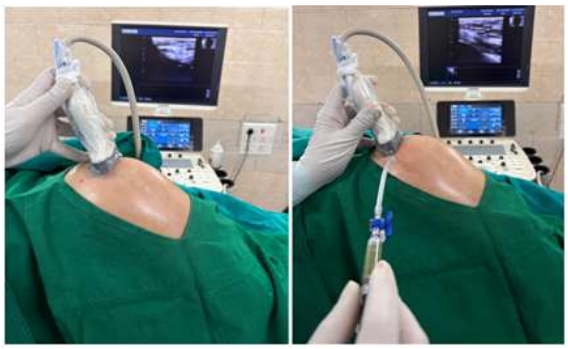

Figure 1 a and b: Technique of injection of LR-PRP using USG(MIPSI)

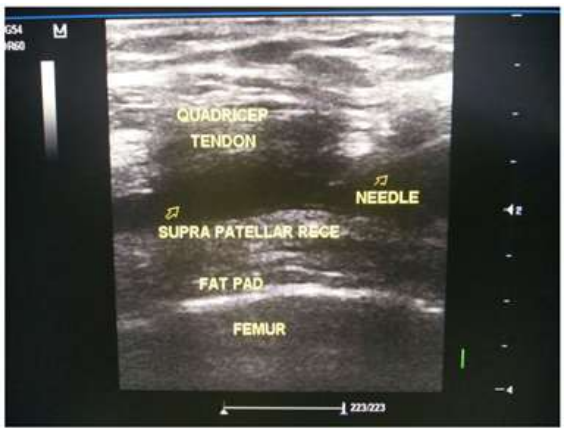

Figure 2: USG image of knee with needle in situ in suprapatellar space

We developed a modified PRP preparation protocol based on Shin et al.'s method to maximize platelet concentration. From each patient, 30 mL of whole blood was divided into two 10 mL glass tubes (9 mL blood + 1 mL sodium citrate per tube). The first centrifugation was performed at 1000 G for 5 minutes, separating the blood into red cells, buffy coat, and platelet-poor plasma (PPP). The PPP and buffy coat, including some red cells, were aspirated and transferred to a dry tube, then centrifuged at 1500 G for 15 minutes. After discarding the PPP, the remaining platelet-rich layer was gently mixed to produce ready-to-use PRP. Each preparation yielded approximately 2.4 mL of PRP per 30 mL of blood.

Minimally Invasive Pain and Spine Intervention (MIPSI):

2.4 ml of LR-PRP was administered after every 4 weeks (total 6 injections), beginning at the time of patient enrolment. Each procedure was performed with the patient lying in a supine position and the affected knee flexed at 90 degrees. All the procedures were carried out in an aseptic condition under ultrasound guidance in OT. A 22-gauge needle was inserted into the anterolateral aspect of the knee to deliver PRP into suprapatellar recess under ultrasound guidance [Figure 1 and 2]. Following the MIPSI, patients were monitored for 30 minutes and

were discharged if no complications arose. Post-mipsi care included a course of antibiotics, 24 to 48 hours of functional rest, paracetamol for managing any post-procedural discomfort, and the application of local cryotherapy. No adverse effects were reported in the treated cases.

The WOMAC index, which ranges from 0 to 96, includes five items assessing pain (0–20), two items for stiffness (0–8), and seventeen items evaluating functional limitations (0–68). A lower total WOMAC score indicates improvement following treatment.

This prospective clinical study involved data collection and analysis using SPSS software (version 23; IBM® Inc., Armonk, NY, USA). Descriptive statistics were calculated for the entire cohort, as well as by follow-up period and pathology type. Categorical variables were summarized as frequencies and percentages, while continuous variables were reported as medians with ranges if non-normally distributed, and as means with standard deviations if normally distributed. The Shapiro-Wilk test was employed to assess normality. For group comparisons across follow-ups and pathologies, Mann-Whitney U and Kruskal-Wallis tests were used due to the non-normal distribution of variables. A p-value less than 0.05 was considered statistically significant.

RESULTS

Table 1: Demographic and clinical parameters

Parameter	Mean±SD	Range
Age in years	61.7±7.64	40-70
Sex: Male n (%)	72(55.38)	
Female. n (%)	58(44.62)	
BMI(kg/m2)	24.05 ±2.28	22 to 29.9
Side of involvement		
Left n (%)	72(55.38)	
Right n (%)	47(36.15)	
Bilateral n(%)	11(8.46)	
K-L grade#		
Grade 2. n (%)	60(46.15)	
Grade 3. n (%)	70(53.84)	

Total 130 patient with severe knee arthritis were enrolled which included 72 males and 58 females with mean age of 61.7(40-70 years). BMI of these patients ranged between 22- 29.9 with mean of 24.05. [Table 1]

The total WOMAC scores demonstrated a statistically significant decrease at multiple time points. Specifically, there was a notable reduction from T0 to T1(28.50±15.50 Vs 20.40±11.30 $p < 0.0001$) from T1 to T2(20.40±11.30 Vs 16.00 ±08.10$p < 0.0001$) from T2 to T3(16.00 ±08.10 Vs 09.50±6.53 $p < 0.0001$) and overall from T0 to T3(28.50±15.50 Vs 09.50±6.53 $p < 0.0001$)

There was significant improvement in VAS score too at multiple time points. Specifically, there was a notable reduction from T0 to T1(7.0±0.50 Vs 6.0±0.29 $p < 0.0001$) from T1 to T2(6.0±0.29 Vs 4.00 ±0.29$p < 0.0001$) from T2 to T3(4.00 ±0.29 Vs 02.50±0,29 $p < 0.0001$) and overall from T0 to T3(07.50±0.50 Vs 02.50±0.29 $p < 0.0001$).

DISCUSSION

Most patients experienced notable improvements in both VAS scores and WOMAC scores following treatment. The severity of the deformity did not appear to significantly influence treatment outcomes, and favourable results were observed even in cases with advanced K-L classification grades. Using our standardized preparation and administration protocol for leukocyte-rich platelet-rich plasma (LR-PRP), the treatment appears to offer a promising short-term option for managing symptoms in individuals with knee osteoarthritis (KOA). It is important to note that existing literature shows considerable variability in PRP injection dosing schedules for KOA treatment.[7-9]

Kenmochi et al,[10] observed in their study, seven months after treatment with LR-PRP and PRF, cartilage defects were covered by a substantial layer of fibrocartilage, as seen through arthroscopy. They hypothesized that high concentrations of LR-PRP might promote the formation of fibrocartilage over damaged cartilage areas. Furthermore, frequent administration over a longer period could potentially be beneficial in managing advanced knee osteoarthritis (KOA), similar to its observed effects on localized cartilage defects. These findings appear to align with the observations reported by Fang et al.[11]

Reports on PRP treatment outcomes vary significantly, largely due to differences in the cellular composition of PRP preparations. The presence of various cell types, particularly leukocytes, has been a topic of ongoing debate concerning the optimal cellular makeup for therapeutic effectiveness. There is currently no clear consensus, as leukocyte-rich PRP has demonstrated both beneficial and adverse effects depending on the clinical context. Some researchers advocate for the exclusion of neutrophils or a controlled presence of these cells to minimize the production of reactive oxygen species (ROS) and matrix metalloproteinases, which may negatively impact tissue healing.12 In manually prepared PRP

(in-house settings), separating neutrophils from the buffy coat remains challenging. However, advancements in commercial PRP systems have led to improved technologies that allow for more precise cell separation.

Recent studies have highlighted a shifting perspective on the role of neutrophils in PRP therapy. Traditionally viewed as potentially harmful due to their pro-inflammatory effects, emerging evidence now suggests that the interaction between neutrophils and activated platelets can also produce anti-inflammatory responses. Parrish et al,[13] (2017) reported that such interactions contribute positively to the healing process by demonstrating the anti-inflammatory potential of platelet-neutrophil interplay. Initially, activated platelets release arachidonic acid, which neutrophils convert into pro-inflammatory mediators like leukotrienes and prostaglandins.[14] However, when platelets interact with neutrophils, they can further convert these leukotrienes into lipoxins—a class of anti-inflammatory molecules. Lipoxins play a crucial role in limiting neutrophil activation, preventing excessive immune cell migration (diapedesis), and promoting the resolution phase of inflammation. Notably, this conversion process depends on the leukotrienes generated by neutrophils, indicating that neutrophils are essential for the subsequent anti-inflammatory action. This ability to shift from pro-inflammatory to anti-inflammatory signalling helps regulate neutrophil activity and supports tissue healing.[15]

Hence to summarize, leukocytes play a vital dual role in both initiating inflammation and guiding cellular communication, making them essential contributors to tissue regeneration. Neutrophils, for instance, release cytokines that attract macrophages—cells known for their remarkable plasticity, which enables them to modulate the inflammatory environment and kick-start the healing process. This orchestrated response, described by few authors,[16] as "regenerative inflammation," highlights the necessity of a controlled inflammatory phase to facilitate subsequent tissue repair. Macrophages are also critical during the remodelling and repair stages, further emphasizing their importance. Additionally, platelet granule release serves as a signalling mechanism for leukocytes, reinforcing the interconnectedness of these cell types. Therefore, including leukocytes in PRP preparations—particularly through buffy coat harvesting—may enhance therapeutic outcomes, as their presence seems to support rather than hinder regeneration. The authors propose that macrophages function as key coordinators within the healing process. Ultimately, the success of PRP therapy may depend not only on the stage of healing and type of injury but also on the specific leukocyte profile within the PRP formulation.

CONCLUSION

Including leukocytes in PRP preparations—particularly through buffy coat harvesting—may enhance therapeutic outcomes, as their presence seems to support rather than hinder regeneration.

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