Clinical Efficacy of a Single-dose of Platelet Rich Plasma in the Management of Early Knee Osteoarthritis: A Randomized Controlled Study With Mri Assessment and Evaluation of Optimal Dose

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Abstract

Background: Osteoarthritis (OA) causes substantial physical disability that limits a person's ability to indulge in daily activities. Non-steroidal anti-inflammatory drugs provide only minimal symptomatic benefit and are associated with mild to severe side effects, thus limiting its continuous use. This being the case, new alternate healing strategies with minimal adverse effects are being explored scientifically. In our study we have attempted to evaluate the clinical efficacy of a single dose of Platelet Rich Plasma (PRP) in treating osteoarthritis. Our primary objectives are:

i) To optimise the correct dose of PRP preparation to achieve therapeutic relief in knee OA

ii) To identify the therapeutic efficacy of PRP in selected patients suffering from symptomatic primary knee OA through Randomized Clinical Trials and analyse the possible outcome using MRI analysis.

Methods: A randomized, double-blind, 12-month, placebo-controlled study was conducted in 100 outpatients. PRP was prepared from the blood drawn from the subjects and administered intra-articularly guided by ultrasound. The degree of pain was assessed using WOMAC scores, the International Knee Documentation Committee score (IKDC) and six-minute pain free walking distance. The joint space width and articular cartilage thickness was evaluated through x-ray, ultrasonagraphy and MRI.

Result: PRP with absolute count of approximately 100 billion platelets brings out significant therapeutic relief. The WOMAC scores showed reduction from 54.7 at baseline to 28.8 at one month, and 29.9 at 3 months showing efficacy of PRP over placebo. Improvement was evident in one month scores of IKDC tests (Baseline- 53.6; 1 month - 76.9) and VAS (Baseline - 5.8; 1 month - 2.46) followed by slight decline at 3, 6-month and one-year scores. Similarly, the pain-free distance covered during a 6-min walk was significantly improved at one month in both the groups (PRP-146 ft.; placebo-122 ft; *p* value *<0.001*). No change is seen on MRI and Joint space width.

Conclusion: 7 times Concentration and absolute count of 100 billion platelets is crucial in a PRP formulation to be therapeutically effective in alleviating symptoms in moderate knee OA. The results indicate prevention of structural modification in the PRP group and better chondroprotective effects compared to placebo group.

Trial Registration: This study has been registered in the US Clinical Trial Registry (U.S. National Library of Medicine) with Trial registration no. 04198467. Date of registration: December 13, 2019. (Retrospectively registered)

URL - https://clinicaltrials.gov/ct2/show/NCT04198467?term=04198467&draw=2&rank=1

1. Background

Osteoarthritis (OA) is one of the most common causes of severe long-term pain and disability [1]. Characterized by the degeneration of the articular cartilage, OA eventually leads to the destruction of

joints. It is also said to be affecting millions of people worldwide, and this number is expected to multiply by 2020 [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the generally prescribed medication to treat the condition. But these groups of drugs only provide symptomatic relief rather than eradicating the disease. In addition, they pose an increased risk for cardiovascular diseases, stroke [3], gastrointestinal and renal complications [4]. In light of the complications associated with the use of NSAIDs that are prescribed to OA patients, it is imperative that disease-modifying treatment and better alternatives need to be explored to tackle this medical condition.

Extensive research on the possibilities of preventive interventions and therapeutic solutions has been carried out time and again. Some drug classes like structure-modifiers or chondroprotective agents (CPAs) not only delay the disease progression by promoting normalization of cartilage and synovial fluid but also slow down the degeneration of cartilage [5]. However, so far, we have only seen inconclusive results as far as the efficacy of CPAs is concerned. Some studies also showed that CPAs like glucosamine and chondroitin sulphate, when taken alone or in combination, did not effectively reduce the pain caused by OA [6]. Besides, chondroitin has been proven to produce only insignificant benefit in a meta-analysis of large-scale, methodologically sound chondroitin trials [7].

Alternative treatment options such as biological regenerative solutions and new tissue engineering-based strategies are considered potentially useful for patients affected by moderate OA [8, 9]. However, alternate options are yet to be explored and tested for cases where joint replacement is not a feasible option.

Platelet-rich plasma (PRP) classically described as “a volume of plasma that has a platelet count above baseline” indicates a concoction of plasma (the cellular, liquid portion of the blood containing proteins responsible for clotting and other bioactive molecules playing a significant role in wound repair) and platelets (and their associated growth factors and cytokines) [10]. Recently, the term PRP has been extended to include a mixture of final products based on their white cell and fibrin content namely, pure PRP, leukocyte-rich PRP, purified platelet-rich fibrin, and leukocyte - and platelet-rich fibrin [11].

There is no dearth of promising clinical results reported of PRP application in various musculoskeletal indications, including OA of the knee [12]. But the majority of the studies lack conclusive evidence of its efficacy and fail to focus on critical aspects such as the minimum volume of blood needed to procure PRP, the quantity (dose) required, and the concentration of platelets for a PRP injection to have the desired curative effect. Besides, they lacked proper standardisation and optimisation of effective PRP dose. Many of these studies nebulously report results of PRP application as derived from 20 to 100 ml of blood, or having a concentration of 2–10 million/ml and absolute platelet counts ranging from 5 million to 1 billion [13, 14]. Hence, suffice it to say that studies which reported a lack of evidence of PRP efficacy probably did not use the adequate dose and specific concentration. Our research focuses on such critical aspects hitherto not focused on by anyone previously.

This prospective placebo-controlled clinical study was primarily designed to evaluate the clinical effectiveness following a single intra-articular PRP injection on moderately affected joints with follow up of 12 months and compare the outcome with the patient group treated with placebo (sodium

hyaluronate). The secondary aim was to delve into any adverse events associated with the applied therapy.

2. Material And Methods

2.1. Patients

Outpatients who satisfied the clinical and radiological criteria set by the American College of Rheumatology for the diagnosis of symptomatic primary knee OA, with pain Visual Analog Scale (VAS) score of > 3 in the previous month were chosen for the study. In patients who showed symptoms in both the legs, the leg which was more painful was considered. The primary exclusion criteria included the following:

1. Presence of secondary knee OA due to injury, inflammatory or metabolic rheumatic illness, or osteonecrosis;
2. Prior intra-articular infusion of hyaluronic acid (HA), including lavage and corticosteroids within three months;
3. Acute osteoarthritic knee (JSW < 1 mm) requiring imminent surgery
4. Patients with other systemic co-morbidities.

This trial was ethically approved by the Institutional Committee for Stem Cell Research and Therapy, Anupam Hospital, Uttarkhand, India. Study protocol no. AAH-05-2014 Dated 18 May 2014. Informed Consent form was obtained from all the subjects before the study initiation. The entire trial was performed in accordance with the Declaration of Helsinki (1964) and its subsequent endorsements. The entire trial was conducted in Anupam Hospital, Rudrapur, Uttrakhand State of India.

2.2. Preparation of PRP

The following method was performed to obtain 10 ml of PRP.

Using a scalp vein set in a syringe having 6 ml, Acid Citrate Dextrose (ACD) Solution A as an anticoagulant, 60 ml blood sample was obtained from each patient's antecubital vein atraumatically to avoid the trauma of the platelets. Collected blood was transferred into four 15 ml falcon tubes and centrifuged at 600 × *g* for 10 min. The collected plasma fraction was centrifuged at 1000 × *g* for 5 min to obtain a platelet pellet. Supernatant platelet poor plasma (PPP) was then removed, leaving 3 ml plasma to resuspend the platelets.

This separated PPP was passed through one-micron special indigenous vial designed to flush back filter (mother cell) so that all the platelets present in PPP fraction are trapped in the filter. These trapped platelets are flushed back with 7 ml of PPP to retrieve most of the platelets. This 7 ml and 3 ml PRP obtained earlier is mixed together and then passed through a WBC filter (Terumo Imuguard) to filter off the leukocytes [15]. The final PRP concentrate (0.1 ml) was analysed for total leukocyte & platelet counts, under an inverted microscope with a hemocytometer for each sample. Five samples were selected

randomly (4, 9, 17, 29 and 42), and assessed for growth factors. The sample was treated with bovine thrombin reconstituted in 10% calcium chloride to assess the growth factors. The serum was collected by centrifugation. Platelet-derived growth factors (PDGF) and vascular endothelial growth factors (VEGF) were measured by ELISA.

2.3. Placebo control

Four ml of high-molecular-weight hyaluronic acid (HMWHA) with a concentration of 22 mg/ml was used for intra-articular administration in placebo group [16].

2.4. Study Design

This prospective, randomized (parallel design with allocation 1:1 ratio), double-blind, 12-month, placebo-controlled study in 100 outpatients was conducted following the 1964 Declaration of Helsinki and its subsequent endorsements [17]. All the patients were made to sign an informed consent form before the study commenced. The patients were randomly selected to receive one undistinguishable injection of PRP or placebo.

Blood was drawn from the patients in the study group, which was used for hematological examinations. Since the patients' eyes were closed with eye pads, none of them knew how much blood was being aspirated. Eyes were masked during intra-articular administration of PRP and placebo as well. Following skin disinfection of the knee joint, 9.9 ml from the total PRP procured (10 ml) was injected as soon as possible by an ultrasound-guided lateral approach, and the remaining 0.1 ml was sent for analysis. Patients received the placebo HA in a similar manner. Patients were advised to continue with usual quadriceps-strengthening exercises, routine precautions, physiotherapy and knee care.

Paracetamol (to a maximum of up to one gram) three times a day, was allowed to be used as a rescue drug. No systemic corticosteroid, CPA such as glucosamine or chondroitin, intra-articular treatment (lavage, HA, or corticosteroid), or potential symptom-modifying drug was allowed during the study.

2.5. Study assessments

Subjective assessments including the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [18], IKDC (the International Knee Documentation Committee) score [19], pain score (0–10VAS) and 6-min walking distance (6MWD) was performed at baseline and then at 1 month, 3 months, 6 months, and one year. The structural efficacy was evaluated by joint space width (JSW) on X-ray, articular cartilage thickness on ultrasonography, MRI at baseline and at 12 months.

The degree of pain, general debility and joint movement were assessed using WOMAC questionnaire test method. This questionnaire test typically contains 5, 2, and 17 questions respectively on pain, stiffness and physical function. The maximum achievable score is 96 and minimum, 0. IKDC scores were also chosen along with WOMAC to make the results comparable with other studies that use these parameters. The severity of pain was evaluated using Visual Analog Scoring Scale (VAS) 0–10. The patients were asked to score their pain on a scale of 0 (no pain) and 10 (worst pain). 6MWD was conducted by marking

off a 50-m distance in an interior hallway and asking the patients to walk as far as they could and as quickly as possible over 6 min. The total distance was measured and recorded.

Patients were asked to stand upright in a weight bearing position with fully extended joints at 1 m away from the X-ray source while being radiographed [20]. The width was gauged at the tapering point of the JSW [21]. Kellgren and Lawrence grade of the femoro-tibial joints and osteophytes were also assessed [22].

Having the patients sit with flexed knees, the articular cartilage thickness was measured by using an ultrasound. The linear transducer was placed perpendicular to the axial plane of the medial tibiofemoral joint in its long axis. The articular cartilage was estimated with calculating the initial point at the level of intercondylar indentation in the middle rim of the medial condyle. The articular cartilage thickness was measured as a distance perpendicular to the articular surface of the medial condyle at the level of which we have differentiated the cartilage well in the described anatomic location. Differentiated articular cartilage appeared as an isoechoic-mildly hyperechoic band-like structure bordered with the thin, medial condylar cortex. Since the majority of the patients had wide articular cartilage defects, the most appropriate tissue was used for measurement. Ultrasonographically, the articular cartilage on weight-bearing condyle appeared as a hypoechoic band with sharp anterior and posterior margins. It was found to be the thickest over the intercondylar area (8–10 mm) and thinnest over femoral condyles (average 4–5 mm) [23].

Radiological evaluation for each joint was done separately in the coronal, sagittal and transverse planes with 1.5 field strength MRI scans [24]. The posterior/meniscal and patellar cartilages were measured at the dense areas in the midsagittal plane through the medial condyle. The medial femoral cartilage of the affected knee was chosen for measurement. The following three regions of the medial femoral condyle were identified at the anterior patella, femoral, meniscal, and posterior condyle level [25]. The point with maximum thickness in the sagittal section passing through the middle was also identified for measurement as previously described by us earlier.

The patients were evaluated for any adverse effects and vital signs once a week for the first month and then once a month up to 6 months. Blood samples were collected at the time of entry into the study, at six months, and on a final visit to examine biological variables as well as liver and kidney functions. Tolerability was assessed and laboratory-based hematological and biochemical assays were performed. Adverse effects were categorized as isolated, intermittent, or continuous, and depending upon the interference with the patients' daily activities as: mild, moderate, or severe. A possible causal relationship with the mineral supplement was also assessed in terms of definite/possible/probable/non-assessable/none.

2.6. Statistical Analysis

All statistical analyses were done as an intention to treat (ITT), using all available data at each time point. The Bonferroni method was used to carry out multiple comparison tests [26]. ANOVA, paired and unpaired

t-tests, chi-squared test, Friedman's test, and Wilcoxon's signed rank test were also used wherever appropriate. Software programs used were SPSS 15.0 (SPSS Inc., Chicago, IL), SAS 9.1, and MS Excel. Quantitative data were defined as Mean ± Standard deviation. The Kolmogorov–Smirnov test was performed to determine the normal distribution of continuous variables [27]. The repeated variant analysis was performed to assess the time variance of the variables. Statistical significance was taken at 5% level of significance ($p < 0.05$).

3. Results

3.1. Patients screened for clinical trial

One hundred and eighty two subjects were initially screened out of which 100 subjects were considered eligible and were randomized (Table 1). The exclusion criteria included absence of radiological evidence of knee OA or absence of severe knee disease with JSW < 2 mm at the narrowest point. Among the 100 randomized clinical subjects, six patients in PRP group and 7 patients in placebo group withdrew from the study before completion. Reasons for withdrawal were personal, inefficacy, or an adverse event like increase in pain, or intake of NSAIDs in each treatment arm. One of the patients in the placebo group had acute synovial effusion as an adverse effect but returned to normal after aspiration and conservative treatment and so was removed from the study. None of the characteristics had any significant difference between groups (Table 1). P value for baseline characteristics between PRP and placebo group ranged between 0.1 to 0.99.

Table 1
Baseline Characteristics of 100 Randomized and Treated Patients with Medial Knee Osteoarthritis Treatment Group

Baseline Characteristics	**PRP (*n* = 50)**	**Placebo (*n* = 50)**
Demographic characteristics	54.4	55.8
Age (years; mean)		
Sex (*n* = male)	30	28
Weight (kg; mean)	70.6	71.2
Height (cm; mean)	168.4	167.8
Right knee (*n*)	28	24
IKDC score	53.6	54.2
Pain score 0–100 VAS (mean)	5.8	5.9
WOMAC score (total)	52–66	50–68
	Mean = 54.7	**Mean = 54.4**
6MWD	1224–1488	1190–1520
	Mean = 1320	**Mean = 1386**
Cartilage thickness (mm; MRI)	4.48–4.98	4.43–5.00
	Mean = 4.60	**Mean = 4.64**
JSW (mm)	3.42–4.68	3.48–4.72
	Mean = 3.81	**Mean = 3.78**
Osteophyte score (4 grade) no. (%)		
0	1	2
1	21	22
2	26	23
3	2	3
Kellgren and Lawrence score (5 grades) no. (%)		
0	0	0
1	1	2
2	6	8

6MWD – 6-min walking distance; PRP - Platelet-rich plasma; IKDC - International Knee Documentation Committee; JSW, joint space width; VAS, Visual Analogue Score; WOMAC - IKDC.

Baseline Characteristics	PRP (*n* = 50)	Placebo (*n* = 50)
3	42	40
4	1	0

6MWD – 6-min walking distance; PRP - Platelet-rich plasma; IKDC - International Knee Documentation Committee; JSW, joint space width; VAS, Visual Analogue Score; WOMAC - IKDC.

3.2. PRP Analysis

The baseline platelet count ranged from 180 to 350 million/ml, with a mean value of 230 million/ml. The PRP concentrate had a platelet count ranging from 1080 to 1850 million/ml, with a mean value of 1270 million/ml, and a mean recovery of 78% with the average platelet concentration of 6.8. The total leukocyte count was zero in our PRP analysis. PDGF values ranged from 50,781 to 76,886 pg/ml, with a mean value of 64,862 pg/ml. VEGF values ranged from 134 to 2429 pg/ml, with a mean value of 789 pg/ml.

3.3. Patient Evaluation

Symptomatic outcome measures showed improvement from baseline in both PRP and placebo groups at one month. The composite WOMAC score of the PRP group improved from 54.7 at baseline to 28.85 at one month, as compared to 40.2 from baseline of 54.4 at 1 month for placebo ($p < 0.05$). This improvement marginally declined at 3- and 6-month follow-up, and reasonably at 1-year follow-up *($p < 0.001$)*. However, the scores were still better at one year as compared to those at baseline *($p < 0.001$)*. The decline was more profound in the placebo group where patients reached baseline status by six months, and scores were inferior to baseline at one year with statistical significance *($p < 0.02$)*.

3.4. Pain score

The mean pain sub score decreased from baseline to one month, followed by a slight increase in pain at 3, 6- and 12-month follow-up. However, the mean pain at 12 months was still less than pain score observed at baseline *($p < 0.05$)*.

The pain sub-score also showed significant improvement *($p < 0.05$)* in the placebo group at one month but it could not be maintained at 3, 6, and 12-month follow-up and scores were inferior to baseline at one year with statistical significance *($p < 0.01$)*. One-year significant difference was noted between PRP and Placebo group for WOMAC, composite pain, stiffness and physical function ($p < 0.0001$).

3.5. Evaluation tests

The trend of other secondary WOMAC parameters such as stiffness and physical function was similar to pain and composite score pattern. Details are provided in Table 2.

No correlation of changes in scores of all WOMAC parameters to age, sex, or BMI in either group was observed.

Table 2
WOMAC Scores of Patients in PRP and Placebo Groups Over 1 Year

Duration	Treatment	Composite	Pain	Stiffness	Physical Function
Baseline	PRP	54.7	11.4	4.1	39.2
	Placebo	54.4	11.2	4.4	38.8
1 Month	PRP	28.8	6.1	1.7	21
	Placebo	40.2	7.6	3.4	29.2
3 Months	PRP	29.9	6.3	1.8	21.8
	Placebo	43.8	8.2	3.6	32
6 Months	PRP	33.5	7.1	2.3	25.1
	Placebo	54.8	10.8	4.6	39.4
12 Months	PRP	41.8	7.9	2.5	31.4
	Placebo	61.1	12.1	4.8	44.2

PRP - platelet-rich plasma; WOMAC - Western Ontario and McMaster Universities Osteoarthritis Index.

Statistically significant improvements in IKDC scores *($p < 0.001$)* were noted during one-month post-injection evaluation, with a mean value of 76.9 at 1 month versus 53.6 at baseline in patients in the PRP group. The placebo group also showed improvement at 1 month from 54.2 at baseline to 68.4 *($p < 0.05$)* (Table 3).

Table 3
IKDC and VAS Scores of Patients in PRP and Placebo Groups over 1 Year

Duration	Treatment	IKDC	VAS
Baseline	PRP	53.6	5.8
	Placebo	54.2	5.9
1 Month	PRP	76.9	2.46
	Placebo	68.4	3.8
3 Months	PRP	76	2.5
	Placebo	62	4.1
6 Months	PRP	68.9	2.92
	Placebo	54.8	5.6
12 Months	PRP	62.8	3.4
	Placebo	50.4	6.3

IKDC - International Knee Documentation Committee; PRP - platelet-rich plasma; VAS – Visual Analog Score.

The improvement was more apparent during 1-month post-injection evaluation *($p < 0.001$)* followed by a slight decline in scores at 3, 6-month and one-year follow-up. However, the IKDC score was significantly better than at baseline *($p < 0.05$)*.

Statistical significance *($p < 0.001$)* was seen in IKDC score at one month in placebo group from baseline of 54.2 to 68.4, but it could not be maintained at 3, 6, and 12-month follow-up. Additionally, the scores were inferior to baseline at one year with statistical significance *($p < 0.03$)*. One-year significant difference was noted between PRP and Placebo group for IKDC *($p < 0.0001$)*.

Also, the progress was evident on the VAS scale in terms of decrement in the severity of pain in both the groups at one month (*p* value *0.001* for both groups) (Table 3).

The PRP group showed statistically significant improved outcome in the long run because better pain control was achieved during the 3-and 6-month follow-up. During the one-year follow-up, the placebo group demonstrated a decline with a mean score of 6.3, while the PRP group showed better control with a mean score of 3.4, as shown in Table 3. At one year significant statistical difference was noted between PRP and Placebo group for VAS (*$p < 0.0001$*).

The pain-free distance covered during a 6-min walk was significantly improved at one month in both the groups (PRP-146 ft.; placebo-122 ft; *p* value *< 0.001*). However, the placebo group could not sustain the improvement at six months and one year, even though scores were a little better than baseline at one year

with no statistical significance *(p < 0.14)* (Table 4). At one year significant statistical difference was noted between PRP and Placebo group for 6MWD *(p < 0.005)*.

Table 4
Pain-Free Distance Covered During 6MWD Test in PRP and Placebo Groups Over 1 Year

Treatment	Baseline	1 Month	3 Months	6 Months	12 Months
PRP Group	1320	+ 146	+ 140	+ 136	+ 120
Placebo Group	1336	+ 122	+ 68	+ 52	+ 16
6MWD – 6-minute walking distance; PRP - platelet-rich plasma.					

In the PRP group, 26% and 24% of patients showed an improvement of covering 100 ft distance in one month and three months, respectively, as compared to 20% and 11% patients in the placebo group.

At one-year rescue medication at least once a week (paracetamol, with dosing limited to 4 × 500 mg/day) was required by 24 patients in the PRP group, as compared to 36 patients in the placebo group *(p < 0.005)*. Furthermore, there was a 26% reduction in the use of paracetamol in the PRP group, as compared to that in the placebo group.

There was no marked increase in JSW, rather both the groups had deterioration *(p < 0.05)*. But it was better maintained in the PRP group though *p* value was statistically insignificant (< 0.18) (Table 5). One year insignificant statistical difference was noted between PRP and Placebo group for JSW *(p < 0.18*).

Table 5
Joint Space Width as Measured on Standing X-ray

Study Group	Baseline	1 Year
PRP Group	3.81	3.77
Placebo Group	3.78	3.68
PRP - Platelet-rich Plasma.		

The percentage of patients with JSN > 0.2 mm was 4.5% (*n* = 2; and 11.6% (*n* = 5) in PRP treated and placebo groups, respectively.

The MRI evaluation demonstrated that the cartilage thickness improved in none (Fig. 1). In the PRP group, it remained unchanged in 42 patients at one year *(p < 0.152)* and in the placebo group, (n = 5) patients lost thickness by at least 0.2 mm *(p < 0.198*). One year difference between PRP and placebo group was *(p < 0.6)*. This indicates chondroprotective and anti-inflammatory disease stabilization effect of PRP, although insignificant statistically.

The overall difference between the PRP and Control group at one year highly suggests the efficacy of the treatment (Table 6).

Table 6
Comparison of outcome between PRP and control group at one year

S. No	Test type	PRP	Placebo	*p* value
1	Composite	41.8	61.1	0.0001
2	Pain	7.9	12.1	0.0001
3	Stiffness	2.5	4.8	0.0001
4	Physical function	31.4	44.2	0.0001
5	IKDC	62.8	50.4	0.0001
6	VAS	3.4	6.3	0.0001
7	6MWD	120	16	0.0001
8	Rescue med	24	36	0.05
9	JSW	3.77	3.68	0.18
10	Unchanged cartilage thickness (MRI)	42	38	0.6

6MWD – 6-min walking distance; PRP - Platelet-rich plasma; IKDC - International Knee Documentation Committee; JSW, joint space width; VAS, Visual Analogue Score; WOMAC - IKDC.

3.6. Ultrasonography

Ultrasonographically, no change was noticed in either group at six months and one year.

3.7. Adverse effects

The number of patients experiencing adverse effects was not very dissimilar. In the PRP group, eight patients out of 44 (18%) experienced side-effects; while in the placebo group, ten patients out of 43 (23%) experienced side-effects. Synovitis was detected as an adverse effect in two patients from the PRP group and five patients from the placebo group, which resolved within seven days.

In the PRP group, 22 patients developed a temporary, mild to moderate pain following the injection. Two patients experienced severe pain, which resolved in two days following the injection. In the placebo group, an equal number of patients complained of mild pain. Five patients experienced moderate pain and stiffness after injection for seven days corresponding to synovitis.

Transient adverse effects such as giddiness, headache, nausea, palpitation, sweating, increased BP, respiration and pulse rates were seen in 18 and 20 patients in the PRP and placebo groups, respectively. Hematological and biochemical safety evaluation showed that PRP is safe to use.

4. Discussion

Surgeons have been using PRP to treat several orthopaedic conditions for many years [28]. However, in the recent years it is used in the treatment of symptomatic knee OA by enhancing the regeneration of articular cartilage [29].

Our study showed the potency of a single injection of PRP on functional outcomes and cartilage repair in knee OA. The results of WOMAC, IKDC, VAS, and 6MWD proved significant improvement in the first-month itself compared to the baseline. These results were significant in the symptomatic and functional recovery for 6-months following PRP application. In the PRP group, all scores improved significantly from baseline to 1st month, followed by slight worsening (insignificant) at 3rd and 6th -month follow-up, and reasonable worsening *(p < 0.002)* at 12th month follow-up. However, the mean scores at 12 months were still significantly better than that at baseline *(p < 0.002)*.

In the placebo group, although the improvement was noted at the 1st month, the values were not significant (*p < 0.0354*), and it could not be maintained at 3, 6, and 12-month follow-up. As compared to placebo, there were significantly evident benefits of PRP from the 1st month onward. Further significant differences between the two groups were noted after the study. This improvement was not significant (9% and 1%, respectively), over their baseline walking distances at 12 months in PRP and placebo group. The need for rescue medication was reduced by 54% (n = 24), in the PRP group and 83% (n = 36), in the placebo group.

Our study demonstrated no structural efficacy of PRP compared to placebo. Although it showed lower JSN in the PRP group, it would be safe to say that the PRP group had chondroprotective structural benefits in terms of better maintenance of JSW as an outcome measure.

Comparative analysis with related studies

We observed that the PRP application improved pain and clinical outcomes, which correlates with the results from other studies [30, 31, 32]. However, direct comparisons were difficult to make because of differences in platelet-separation techniques, the volume of blood used to obtain PRP, concentration factor, the absolute number of platelets injected, outcome scoring systems, and no standard structural efficacy criteria. Our study was unique as it addressed the adequate dose.

In a similar randomized study with PRP and HA treatment arms (49 patients and 50 patients in each arm respectively), IKDC score was significantly higher in the PRP group compared to the placebo group at 24 and 52 weeks (*p = 0.003*) but statistically lower VAS score in the PRP group than in the placebo group at 24 weeks *(p < 0.0096)* and 52 weeks *(p < 0.0039)* [33].

In a study carried out by Paterson on 23 patients, minor pain and swelling during the injection period were reported by two patients from the photo-activated PRP (PA-PRP) group. Significant improvements were

demonstrated by the PA-PRP group in the VAS *(p < 0.01)* and KOOS (Knee injury and Osteoarthritis Outcome Score) scores *(p < 0.05)* at 12 weeks [34].

A similar study indicated a non-significant reduction in IKDC scores from 2 to 6 months, which significantly decreased at 12 months and then further decreased at 24 months. Although the absolute number of platelets injected was very small (6.5 million/knee), the response rate was good probably because of the patients being males with low BMI [35].

A single dose of PRP administered in 22 patients between the ages of 30 and 70 with grade 0–3 early knee OA was reported to have reduced pain score with high six months and one year functional and clinical scores from baseline. No conspicuous changes in MRI were found in at least 73% of the patients at one year [36]. In comparison with this study, our study that included patients with advanced OA yielded better results.

A study reported by Patel et al. had the patient number and demography very similar to that in our study. They used 100 ml of blood to achieve platelet counts of 310,000/µl. The absolute count of 2385.6 million platelets injected per knee (variable and low compared to our study) demonstrated an equal benefit with single and double injections of WBC-filtered PRP. There was a significant improvement in all WOMAC scores within 2–3 weeks, with slight worsening at 6-month follow-up, without any influence of age, sex, and weight [37].

In six level I and II studies, four randomized controlled trials, and two prospective non-randomized studies (n = 577; mean age = 56.1 years) by Khoshbin et al., WOMAC and IKDC scores were shown to be significantly better with PRP than HA or NS injections *(p < 0.001)*. Frequent side effects were reported in patients in the PRP group than in the HA group *(p = .002)*. In our study, local undesirable effects were more in the placebo group [38].

Majority of the studies on the treatment of human degenerative cartilage lesions with PRP showed improved pain, stiffness, functional state, and no change in radiological outcomes. Among various biological treatment options being explored, PRP was selected because of being autologous, ease of processing, and being in extensive research for 20 years. An additional advantage of autologous venous blood over synthetic chemicals is that it eliminates the risk of allergic reactions and possible transmission of infections [39].

Clinically effective concentration of platelets was injected (approximately 1 million/µl) for an appropriate therapeutic result [40], that was achieved using various manual centrifugation techniques. Standardization and optimization are crucial for the preparation of PRP, failing of which could cause inconclusive therapeutic results. Platelet loss in the supernatant was minimized during standardization.

Growth factors – the healing promoters

The growth factors that are secreted by the platelets (within a time span of 10 min to 5–10 days) assist in various stages of the repair process [41]. These growth factors have a direct effect on the physical and biomechanical properties of the joint, cartilage biosynthesis and degradation. In addition, they possess anti-inflammatory effects and a direct analgesic effect related to interaction with pain receptors [42]. They enhance the synthesis of type II collagen and chondrocytes by stimulating the proliferation of chondrocytes and pluripotent mesenchymal stem cells. Also, they suppress inflammatory mediators such as interleukin-1, encourage matrix deposition, and slow down degeneration [43]. The migratory ability of the proliferative cells is increased, which leads to better regenerating capability and slows down the natural progression of the disease. Hence, growth factors help stabilize cartilage homeostasis and promote the healing potential in the degenerating articular cartilage. They aid in articular cartilage repair and halt the degeneration process [39].

The placebo group also showed improvements over time in the treatment of pain, activities, and composite scores, but they were not significant due to the lubricating and shock-absorbing properties of HA [44]. HA also downregulates the gene expression of OA-associated cytokines and regulates the suppressor T-cells for cell proliferation.

We preferred to use inactivated PRP since it increases proliferation of the mesenchymal stem cells fivefold [45], improves cartilage, and aids in bone formation. Activated PRP may inhibit chondrogenesis and osteogenesis *in vivo* and *in vitro* [46].

Use of leukocyte-depleted PRP, with less pro-inflammatory cytokines, avoids the activation of the NF-κB pathway, promotes growth and chondrogenesis *in vivo*, and yields better cartilage repair compared with PRP with leukocytes [47]. The functional outcomes of leukocyte-poor PRP were better in comparison with PRP rich in leucocytes [48].

Our study delivers standard PRP processing with little variation. High level of consistency in absolute platelet counts would help in standardizing a dose for treatment. The study duration based on the previous personal data might have been too short of demonstrating any structural change in the knee. The significance of radiographic joint space narrowing measurements after only 2 or 3 years of follow-up is unclear in many study participants who have typical, slow-progressing disease.

MRI Assessment

MRI evaluation should have been more extensive with three-dimensional MOCART (magnetic resonance observation of cartilage repair tissue) to quantify the regeneration in cartilage following the treatment. Further MRI changes of cartilage 0.2 mm are too small to be consistently and reliably picked up. Also, a huge placebo response might operate quite independently and be sufficient to skew the results. Patients in study groups were adapted to improved diet and a healthy lifestyle, since good habits aid in overall improvement and quick convalescence. Patients' expectation that all potential treatments in the randomized protocol provide benefits may have resulted in placebo response. Although the use of rescue

medication was greater in placebo and mineral groups during the study period, this may have masked the differences between positive benefits related to the treatment and placebo groups.

Since OA is a joint failure and not just cartilage tissue disorder, disease-modifying agents in OA treatment would more likely to succeed if they focused primarily on correcting the abnormal mechanics and then addressing the cartilage loss if needed [49]. Moreover, since articular cartilage is not innervated, approximately 50% of the patients with radiological changes of OA are symptomatic; therefore, the treatment is based on symptoms rather than radiological changes. Even a successful CPA might have a minimal effect on symptoms, leading to patients being reluctant to adhere to a therapy that does not improve their symptoms [50].

The limitations of this study were its short duration (one year), lack of assessment for remnant effects after discontinuation of treatment, and limited sample size (50 patients/treatment arm). A study with longer treatment duration on a greater number of patients would be helpful to verify the treatment effect and explore the lack of significance.

5. Conclusion

WBC-filtered PRP in concentration of seven times and absolute counts of approximately 100 billion cells are effective in alleviating symptoms in early knee OA. PRP in knee OA has the benefit of preventing structural modifications and infers chondroprotective effect compared to placebo. Further studies are needed to validate the place of these treatments in the management of knee OA.

Abbreviations

6-MWD - 6-Minute Walking Distance

ACD - Acid Citrate Dextrose

BMI – Body Mass Index

CPA – Chondroprotective Agent

HMWHA - High-Molecular-Weight Hyaluronic Acid

IKDC - International Knee Documentation Committee

ITT - Intention to Treat

JSW - Joint Space Width

MOCART - Magnetic Resonance Observation of Cartilage Repair Tissue

NS – Normal Saline

NSAID - Non-steroidal anti-inflammatory drug

OA - Osteoarthritis

PGDF - Platelet-derived growth factors

PPP - platelet poor plasma

PRP - Platelet Rich Plasma

VAS – Visual Analogue Score

VEGF - Vascular Endothelial Growth Factors

WOMAC - Western Ontario and McMaster Universities Osteoarthritis Index

Declarations

Ethics Approval and Consent to participate

Ethical approval was obtained by the Institutional Committee for Stem Cell Research and

Therapy, Anupam Hospital, Rudrapur, Uttarkhand, India. All patients were recruited following written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable

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Author’s contributions

HB - designed and conducted the study

JL - Drafted the study protocol

JLP DC, DAW - Assisted in designing the protocol, supervising the study and reviewing the results

AB - Supervised the entire study and treated the selected patient population

IP - Helped in drawing study protocol and registering in trial registry

SM ST - Analyzed the results and was a major contributor in writing the manuscript

All authors read and approved the final manuscript

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Figures

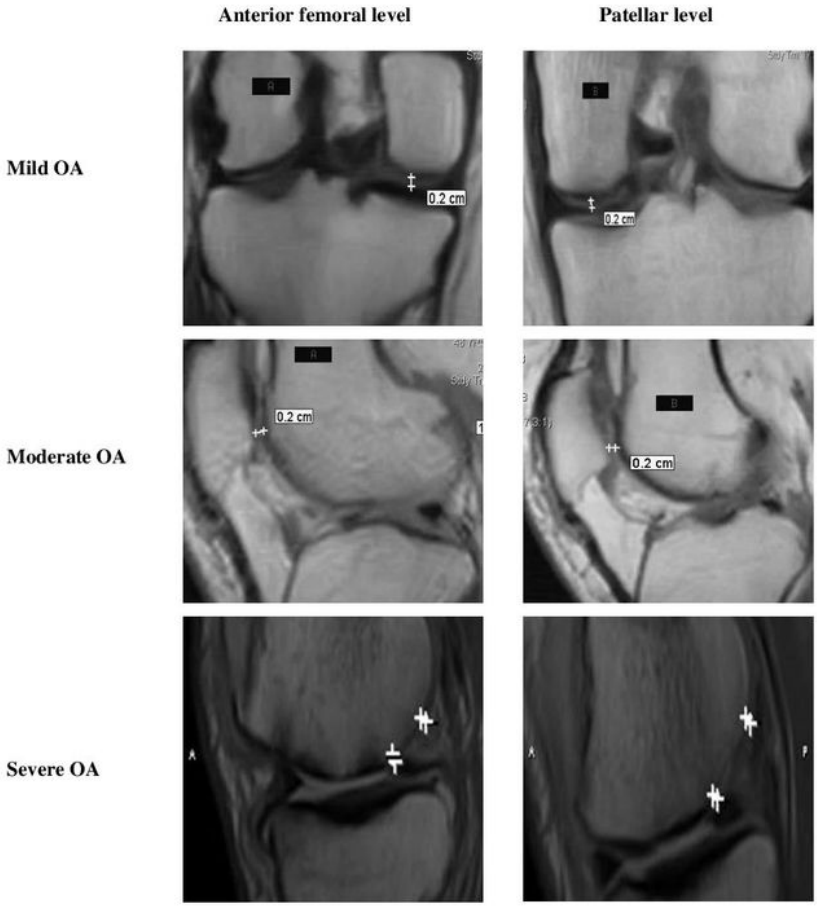

Figure 1

Evaluation of articular cartilage thickness using MRI after one year of PRP treatment