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ORIGINAL ARTICLE

Comparison of hyaluronic acid and PRP intra-articular injection with combined intra-articular and intraosseous PRP injections to treat patients with knee osteoarthritis

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Abstract
The aim of this study was to evaluate the benefit provided by intraosseous infiltration combined with intra-articular injection of platelet-rich plasma to treat mild and moderate stages of knee joint degeneration (Kellgren-Lawrence score II–III) compared with other treatments, specifically intra-articular injection of PRP and of HA. Eighty-six patients with grade II to grade III knee OA according to the Kellgren-Lawrence classification were randomly assigned to intra-articular combined with intraosseous injection of PRP (group A), intra-articular PRP (group B), or intra-articular HA (group C). Patients in group A received intra-articular combined with intraosseous injection of PRP (administered twice, 2 weeks apart). Patients in group B received intra-articular injection of PRP every 14 days. Patients in group C received a series of five intra-articular injections of HA every 7 days. All patients were evaluated using the Visual Analogue Scale (VAS) and Western Ontario and McMaster Universities (WOMAC) score before the treatment and at 1, 3, 6, 12, and 18 months after treatment. There were significant improvements at the end of the 1st month. Notably, group A patients had significantly superior VAS and WOMAC scores than were observed in groups B and C. The VAS scores were similar in groups B and group C after the 6th month. Regarding the WOMAC scores, groups B and C differed at the 1st, 3rd, 6th, and 12th months; however, no significant difference was observed at the 18th month. The combination of intraosseous with intra-articular injections of PRP resulted in a significantly superior clinical outcome, with sustained lower VAS and WOMAC scores and improvement in quality of life within 18 months.

Keywords Hyaluronic acid · Intra-articular injection · Knee osteoarthritis · Platelet-rich plasma

Introduction

Knee osteoarthritis (OA) is a common form of arthritis, which is particularly observed with increasing age affecting the knee joints. The primary symptoms of knee OA are pain and physical limitations that affect an individual's quality of life [1]. Its pathological characteristics include osteophytes, articular cartilage lesions, synovitis, and subchondral sclerosis. Current drug therapies for knee OA are mainly based on the use of oral non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, glucosamine, and chondroitin. Biologic research is focused on the importance of growth factors in the maintenance of normal tissue structure and tissue lesion repair [2]. Intra-articular injections of hyaluronic acid (HA) and platelet-rich plasma (PRP) are used as other non-surgical treatment options. HA plays a role in the lubrication of the articular surface and transports nutritive substances derived from the synovium. Generally, however, HA in the synovial fluid of individuals with knee OA has been shown to be reduced [3, 4]. Various studies with conflicting results regarding the efficiency of HA in knee OA have been reported. A meta-analysis showed that HA injection in OA was associated with an increased risk for serious side effects and with slight and clinically irrelevant benefits [5].

PRP is an autologous biologic treatment made from the patient's own plasma, which contains growth factors released from platelets and endogenous fibrin scaffold. The rationale

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for the use of PRP is to stimulate the natural healing cascade and tissue regeneration by the platelet-derived factors directly at the site of treatment [6]. Evidence to support the use of PRP has been gathered from its role in tissue healing, cellular recruitment, growth, maturation, inflammation modulation, and joint functionality improvement [7]. As a minimally invasive treatment option, intra-articular injection of PRP has been widely used in clinical therapy, but the post-injection swelling and pain were more frequent with higher volume and multiple intra-articular injection of PRP. The subchondral bone has always been a factor in the cartilage repair process and OA. A new technique of PRP reported by Sanchez et al. [8] is intraosseous infiltration combined with intra-articular injection to approach severe OA; moreover, furthermore no adverse events were reported [9]. Whether this therapeutic strategy can be delivered at fewer intervals and at lower doses to relieve swelling and pain is not reported.

The hypothesis was that the addition of intraosseous injection of PRP into the subchondral bone to conventional intra-articular treatment of PRP or HA would achieve a positive effect to treat mild and moderate stages of knee joint degeneration (Kellgren-Lawrence score II–III).

Materials and methods

Patients and study design

The study protocol was reviewed and approved by the Reference Ethics Committee of Cangzhou Central Hospital. All patients signed the medical informed consent before the treatment. The study population consisted of 99 patients (62 females and 37 males) who presented at the outpatient department. Patients who appeared suitable for inclusion in the study were given appointments for evaluation by an experienced orthopedic physician regarding their suitability for inclusion according to the following criteria: (1) unilateral symptomatic knee with pain for at least 1 month or swelling, (2) radiographic findings of knee degeneration (Kellgren-Lawrence score of II–III) [10], (3) age 40–73 years, (4) body mass index (BMI) 18–32.5), and (5) knee stability without a severe trauma history. The exclusion criteria were as follows: (1) bilateral knee osteoarthritis indicative of treatment for both knees, (2) Kellgren-Lawrence score greater than III, (3) BMI > 32, (4) age > 73 years, (5) systemic autoimmune rheumatic diseases and blood disorders, (6) active immunosuppressive or anticoagulant therapy, and (7) intra-articular injection to the knee within the previous 1 year or previous joint infection, (8) use of corticosteroids for 3 weeks before the procedure, and (9) use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the 3 weeks before treatment.

A total of 86 of the 99 patients were randomly assigned into 3 groups using a random number table: group A received intra-articular injection of PRP 2 ml combined with medial tibial plateau and medial femoral condyle injection of PRP 2 ml; group B received intra-articular injection of PRP 6 ml; and group C received intra-articular injection of HA 2 ml. Two injections of PRP were administered every 14 days in groups A and B. The group C patients received five injections of HA, with 7-day intervals between each injection. Minus the lost patients, the final study sample consisted of 82 patients (Fig. 1) (ClinicalTrials.gov ID: NCT03329235).

PRP preparation

The PRP specimens were collected as described by Filardo et al. [11, 12]. A total 45-ml venous blood sample was drawn from the antecubital vein into 50-ml injectors containing 4 ml sodium citrate. Blood samples were centrifuged at 1480 rpm for 6 min at room temperature to separate the red blood cells from the buffy coat and the upper plasma layer. The buffy coat and the upper plasma layer were carefully collected using a serological pipette into a new centrifuge tube while attempting to not remove the erythrocyte layer, and centrifuged again at 3400 rpm for 15 min to obtain a two-part plasma, with the supernatant consisting of platelet-poor plasma and the lower part consisting of platelet-rich plasma. The upper three-quarter fraction of the plasma was discarded and of the remainder, which contained the approximately 7 ml of concentrated leukocyte-containing PRP, 1 ml was sent to the laboratory for quality analysis (platelet count and leukocyte count). To initiate the activation of platelet clotting, calcium chloride was added to the final liquid PRP immediately before injection. All the procedures were performed under sterile conditions.

Treatment

The patients in group A were positioned supine and the skin of the intra-articular injection site was prepared, and injection was performed through the lateral suprapatellar approach with 2 ml of PRP. Then, with the knee flexed 30°, the intraosseous puncture position was located at 1 cm proximal and 1 cm distal to the medial joint line using a C-arm. Subcutaneous and periosteal infiltration anesthesia was achieved by injecting 2 ml of 2% lidocaine hydrochloride into the medial femoral condyle and tibial plateau. Intraosseous puncture was performed using an epidural needle at an angle of 30–40° to the lower limb anatomical axis. The trocar tip was located at the midpoint of the femoral trochlea and the medial tibial intercondylar eminence on the anteroposterior view of the knee, and concomitantly directed into the midpoint of the anteroposterior diameter of the femoral condyle and tibial plateau on the lateral radiographic view. Once the trocars were placed in the proper position, the needle core was extracted and 2 ml of PRP was infiltrated into the subchondral bone of each structure (Fig. 2). Next, the needle was withdrawn and a sterile dressing was applied. The patients were advised to limit

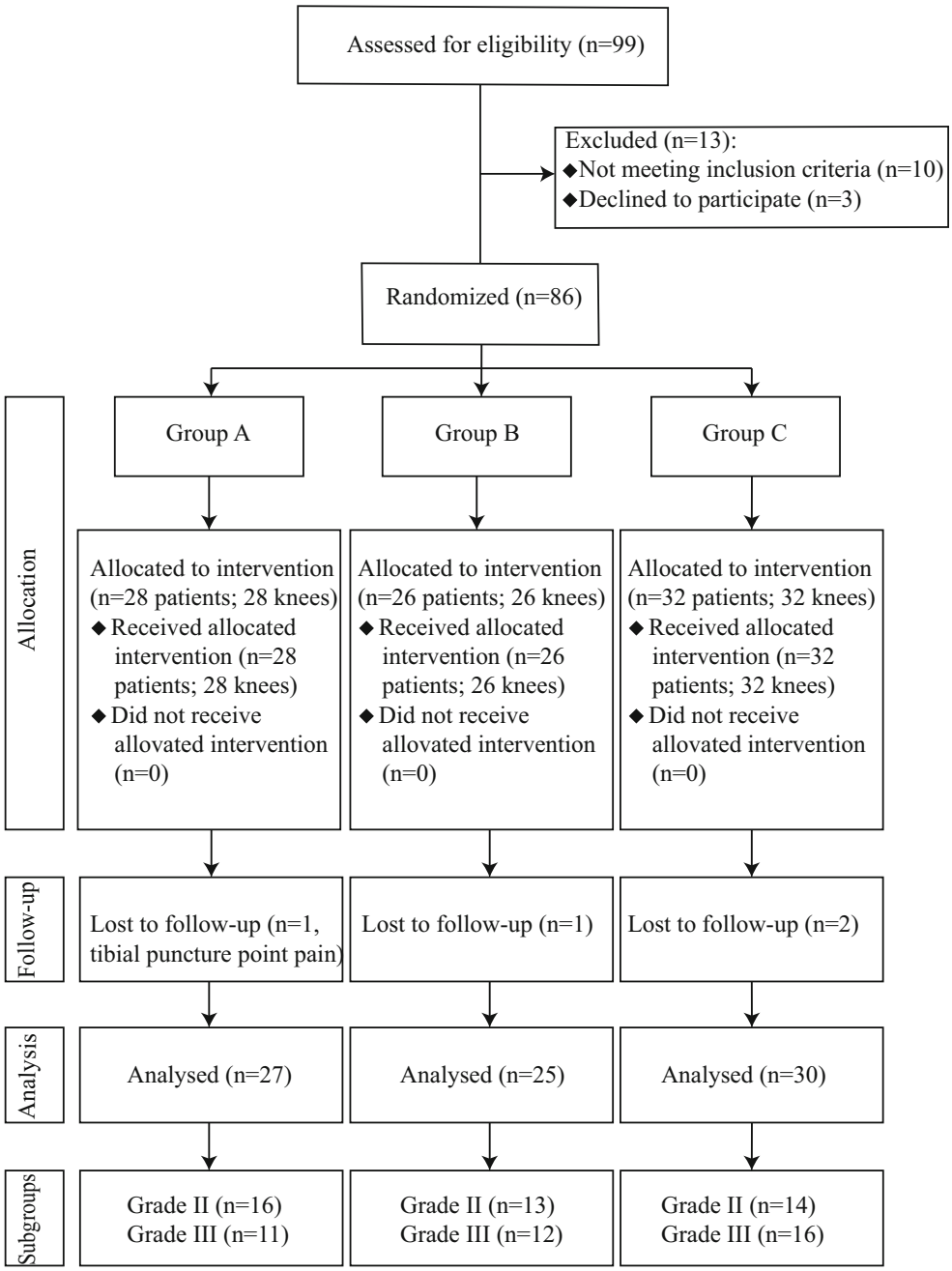

Fig. 1 Flow diagram of the study. *n*, number of patients

weight bearing for 24 h. This procedure was repeated 2 weeks later. During the follow-up period, nonsteroidal anti-inflammatory drugs were not allowed. Reduced joint motion and the application of local cold therapy three times a day were employed in case of discomfort.

The patients in group B received an intra-articular injection of PRP 6 ml using a lateral suprapatellar approach, which was repeated at an injection interval of 14 days. The weight-bearing activity was limited 24 h after surgery.

The patients in group C received 2 ml of HA (Freda, Shandong, China), which was a pre-filled syringe containing 20 mg of hyaluronic acid and the weight-average molecular weight was 0.6–1.5 Million Daltons. This 2 ml of HA was infiltrated intra-articularly through the lateral suprapatellar region in order to reach the joint space after lateralization of the patella. It was administered as 5 doses with 7-day intervals between each injection.

Data collection

General characteristics of the study patients, such as gender, mean age, BMI, and grade of knee OA, were recorded and blood tests were conducted before the first injection. The platelet count and PRP concentration factor were determined

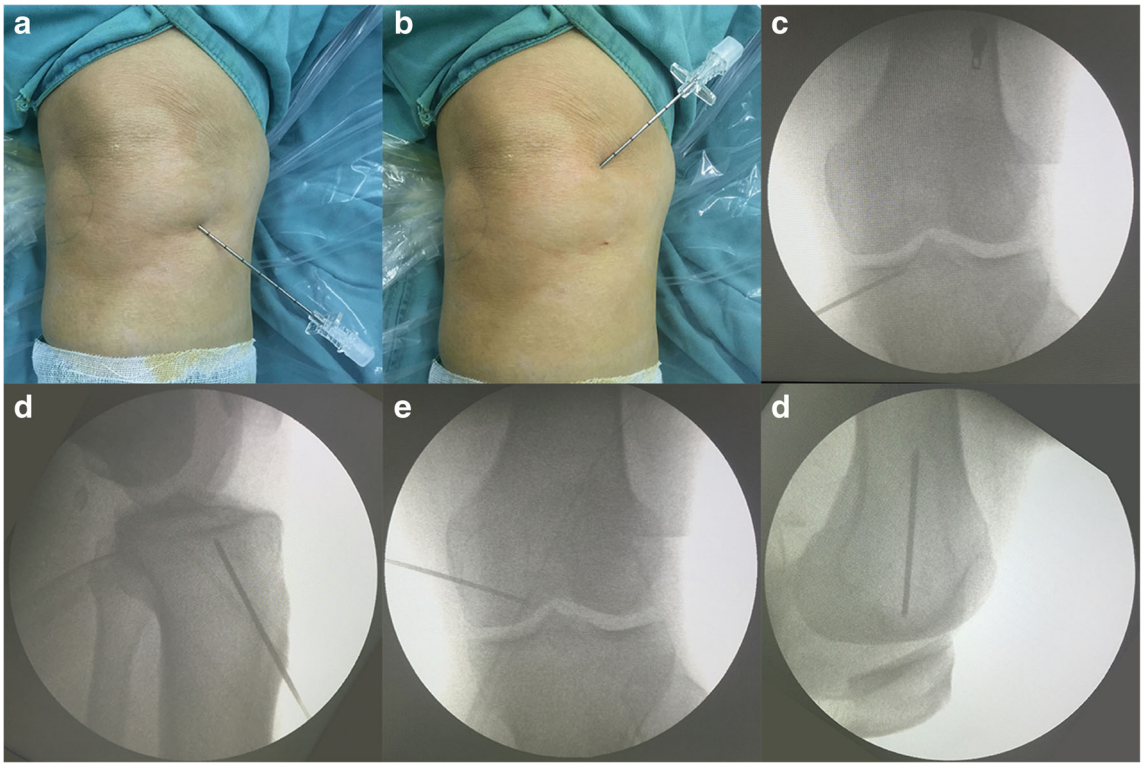

Fig. 2 A patient is positioned supine for intraosseous injection with C-arm guidance. **a**, **b** Intraosseous injection at the medial aspect of the tibial plateau and femoral condyle located 1 cm proximal and 1 cm distal to the medial joint line at an angle of 30–40° to the lower limb anatomical axis. **c**, **d** Trocar tip is located at the medial aspect of the tibial intercondylar eminence in the anteroposterior view and the midpoint of the anteroposterior diameter of the tibial plateau in the lateral view. **e**, **f** Trocar tip is located at the midpoint of the femoral trochlea in the anteroposterior view and the midpoint of the anteroposterior diameter of the femoral condyle in the lateral view

(concentration factor = platelet count in PRP/platelet count in whole blood). Feasibility was recorded using recruitment and retention rates, and safety was assessed by recording the number and nature of adverse events. Adverse events were recorded at all follow-up assessment visits. The primary outcome of the study was the Western Ontario and McMaster Universities (WOMAC) total and sub-scores. Firstly, knee pain was individually evaluated with pain VAS score (on a scale of 0–10, where 0 = no pain and 10 = worst pain), the patients drew a mark according their feeling. Secondary evaluation was performed with WOMAC, which were recorded on a five-point Likert scale scoring, with a response of "none" scored as 0, "mild" as 1, "moderate" as 2, "severe" as 3, and "extreme" as 4. The patients were evaluated before the treatment and at the 1st, 3rd, 6th, 12th, and 18th months from the last injection.

Statistical analysis

The sample size estimation was calculated using GPower software by considering an effect size among groups of more than 0.30 with a false-positive rate of 5% ($\alpha = 0.05$) and a power of at least 80% ($\beta = 0.20$), which required a theoretical minimum sample size of 66 patients. Considering the estimated 20% dropout rate, a total of 26 patients per group were required. Patient recruitment ceased when the minimum number of patients was reached using a random number table in all groups.

All categorical variables (gender, grade of knee OA) were expressed as frequency, and continuous variables (age, BMI, VAS scores, WOMAC scores) were expressed by the mean and standard deviation. The Pearson chi-squared test was used to compare gender and grade of knee OA. ANOVA was performed for age, BMI, initial VAS scores, and initial WOMAC total and sub-scores to compare the preoperative values, with analysis of variance for repeated measure used to compare the VAS scores, and WOMAC total and sub-scores at different follow-up periods. The least significant difference (LSD) test for post-hoc analysis was used to compare three independent groups at the same follow-up period. The statistical analyses were performed using the SPSS 17.0 software package. A p value < 0.05 was considered statistically significant, except for the test of heterogeneity for which $p < 0.10$ was used.

Results

Eighty-six of the 99 enrolled patients met the inclusion criteria. One participant withdrew from the trial due to minor pain of the medial tibial plateau region and swelling around

the knee joint, and another participant from group B was lost to follow-up for unexplained reasons. Two participants withdrew from group C due to individual-specific reasons (one sustained an acute myocardial infarction, the other sustained traffic accident injuries). Consequently, a total of 82 of the 86 participants were followed up at the end of 18 months. No significant differences were observed with respect to age, gender, osteoarthritis stage, BMI, and the preoperative values of the VAS and WOMAC scores (Table 1).

Eighteen adverse events were reported in 18 patients, 5 in the group A, 8 in the group B, and 5 in the group C (Table 2). Most of these adverse events (80% in the group A, 75% in the group B, and 60% in the group C) were not related to the type of treatment.

The mean platelet counts in the peripheral whole blood and PRP were $(140.73 \pm 11.26) \times 10^9$/L and $(789.68 \pm 17.80) \times 10^9$/L, with no significant differences between group A (concentration factor of 5.66 ± 0.41) and group B (concentration factor of 5.63 ± 0.46). The mean platelet concentration in PRP is 5.61-fold greater than that of whole blood. The mean leukocyte counts in the peripheral whole blood and PRP were $(5.25 \pm 0.49) \times 10^9$/L and $(29.92 \pm 1.54) \times 10^9$/L.

Patient pain recorded using the 100-mm scale VAS was reduced from pretreatment at the 1- and 3-month follow-up time points for all groups. Although there were different degrees of worsening from the 3- to 18-month periods, group A remained quite improved from pretreatment levels. Repeated measures ANOVA revealed significant differences in VAS pain for the 3 groups ($F = 722.457$, $p = 0.000$). Post-hoc tests showed the reduction in pain for group A was significantly greater than in groups B and C; however, no significant difference were found for groups B and C at the 1st, 6th, and 18th month ($p = 0.702$; $p = 0.192$; $p = 0.159$, respectively) (Table 3) (Fig. 3).

Regarding group A, significant improvements were found at the 3-month follow-up time point in WOMAC pain subscale; however, no significant difference were shown in groups B and C at the 18th month ($p = 0.118$). The WOMAC stiffness subscale indicated no significant difference in groups A and B at the 1st, 3rd, and 6th month ($p = 0.235$, $p = 0.773$, $p = 0.403$, respectively), the same as in groups B and C at the 1st and

Table 2 Adverse events

Group	Adverse events	Relation to the treatment	Outcome
Group A			
1	Ankle pain	Unrelated	Resolved
2	Nausea	Unrelated	Resolved
3	Knee pain and swelling	Highly likely	Withdrew
4	Numbness	Unrelated	Resolved
5	Cannot squat-thrusting	Unrelated	Resolved
Group B			
1	Low back pain	Unrelated	Resolved
2	Knee pain and swelling	Highly likely	Resolved
3	Knee swelling	Possible	Resolved
4	Upper respiratory infection	Unrelated	Resolved
5	Headache	Unrelated	Resolved
6	Other knee pain	Unrelated	Resolved
7	Low back pain	Unrelated	Resolved
8	Bronchitis	Unrelated	Resolved
Group C			
1	Knee swelling	Highly likely	Resolved
2	Cold	Unrelated	Resolved
3	Acute myocardial infarction at the 13th month	Unrelated	Withdrew
4	Traffic accident	Unrelated	Withdrew
5	Knee pain	Highly likely	Resolved

Table 1 Demographic distribution and comparison of the groups

	Group A ($n = 27$)	Group B ($n = 25$)	Group C ($n = 30$)	p value (between groups)
Age, (mean ± SD), (range), years	50.67 ± 8.70 (37–71)	54.16 ± 6.56 (37–71)	53.13 ± 6.41 (37–71)	> 0.05
Sex, (M:F), n	10:17	11:14	12:18	> 0.05
BMI, (mean ± SD), kg/m^2	28.19 ± 1.31	28.17 ± 1.43	28.69 ± 1.13	> 0.05
Kellgren-Lawrence, n				> 0.05
Grade II	16	13	14	
Grade III	11	12	16	
VAS score, (mean ± SD)	7.09 ± 0.31	7.02 ± 0.27	7.04 ± 0.33	> 0.05
WOMAC score, (mean ± SD)				
Pain	9.78 ± 1.09	9.57 ± 1.45	9.60 ± 1.19	> 0.05
Stiffness	5.04 ± 0.65	4.63 ± 0.56	4.72 ± 0.79	> 0.05
Physical function	36.22 ± 1.05	36.30 ± 1.26	35.56 ± 1.71	> 0.05
WOMAC total	50.15 ± 1.10	50.17 ± 1.60	49.88 ± 1.54	> 0.05

n number of patients, *M* male, *F* female, *BMI* body mass index, *VAS* Visual Analogue Scale, *WOMAC* Western Ontario and McMaster Universities Arthritis Index

Table 3 VAS scores for the three groups[a], M ± SD (95% CI)

	1st month	3rd month	6th month	12th month	18th month
Group A	3.24 ± 0.20	2.14 ± 0.19	2.18 ± 0.37	2.36 ± 0.41	3.78 ± 0.27
	(3.14–3.34)	(2.04–2.25)	(2.00–2.37)	(2.23–2.49)	(3.66–3.90)
Group B	3.46 ± 0.19 [b]	3.00 ± 0.27	4.26 ± 0.35 [b]	4.78 ± 0.19	6.66 ± 0.31 [b]
	(3.36–3.57)	(2.89–3.10)	(4.07–4.46)	(4.65–4.92)	(6.54–6.78)
Group C	3.44 ± 0.35 [b]	3.23 ± 0.31	4.44 ± 0.64 [b]	5.45 ± 0.38	6.54 ± 0.34[b]
	(3.34–3.53)	(3.14–3.33)	(4.26–4.61)	(5.32–5.57)	(6.43–6.65)
F (between groups)	5.769	131.519	183.596	628.523	740.149
p (between groups)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[a] *VAS* Visual Analogue Scale: values are reported as the mean ± SD (95% confidence interval); between-group values at the different months indicate significant differences ($p < 0.05$)

[b] Post-hoc analysis indicates no significant difference ($p > 0.05$)

18th month ($p = 0.113$, $p = 0.310$, respectively). Significant improvements were nevertheless found in the WOMAC physical function subscale in all groups. WOMAC total scores indicated significant improvements in group A at every follow-up time point, whereas those of groups B and C were not significant at the 18th month ($p = 0.100$) (Table 4) (Fig. 4).

Discussion

The most significant finding of this study was that the novel technique of intraosseous combined with intra-articular PRP injection was effective in relieving pain and improving the daily activities. The combination was more successful than intra-articular injection of PRP or of HA. The results demonstrate the feasibility of this technique in patients, with no serious adverse events reported. The clinical use of PRP is becoming more frequent in the treatment of mild and moderate OA. The main advantages of PRP are that it is obtained from the patient's own blood, avoids immune responses, and saves costs. The effective constituents of PRP are platelet-derived growth factor, vascular endothelial growth factor, insulin-like growth factor, and proteins such as fibrin, fibronectin, vitronectin, and thrombospondin that play a role in many stages of tissue

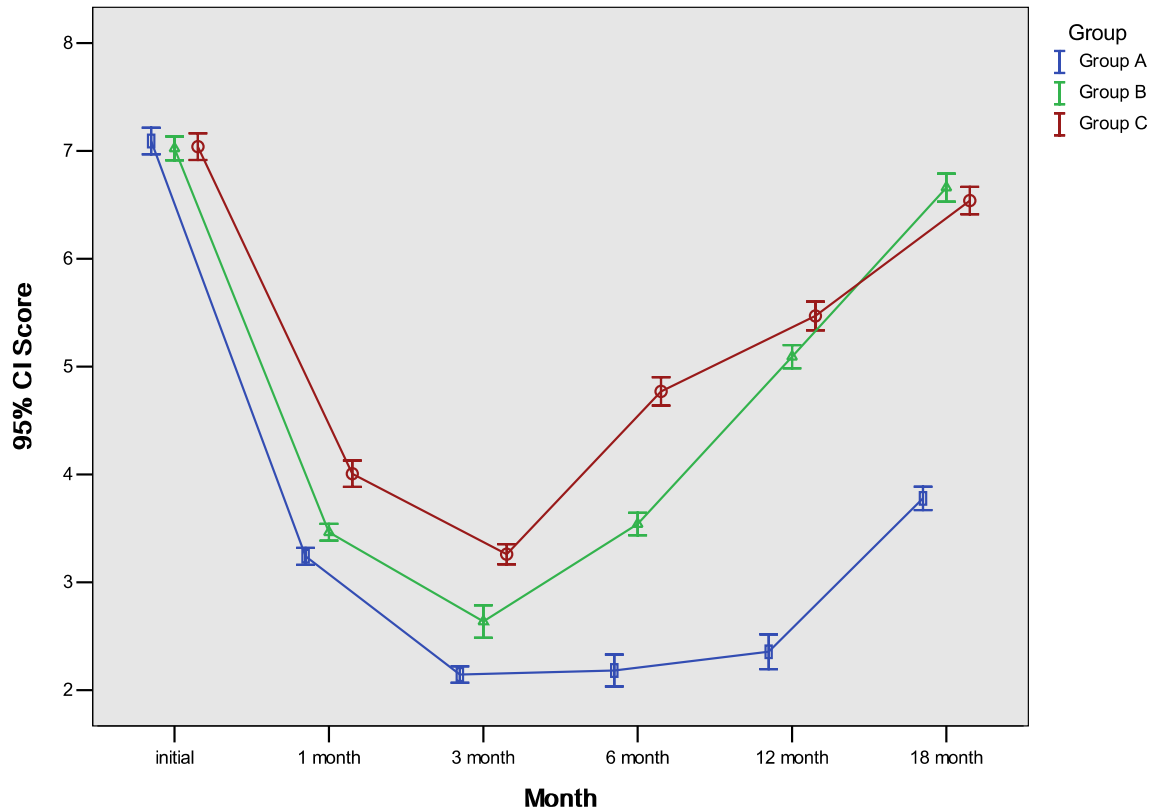

Fig. 3 VAS pain scores, comparison between the three groups (0–10)

Table 4 WOMAC scores for the three groups[a], M ± SD (95% CI)

	1st month	3rd month	6th month	12th month	18th month
Pain					
Group A	4.26 ± 0.76[b]	3.74 ± 0.66	3.78 ± 0.64	5.19 ± 0.62	6.15 ± 0.60
	(3.95–4.57)	(3.47–4.02)	(3.50–4.05)	(4.94–5.43)	(5.93–6.37)
Group B	4.23 ± 0.90[b]	4.20 ± 0.81[b]	4.70 ± 0.70	6.43 ± 0.57	8.57 ± 0.50[b]
	(3.94–4.53)	(3.94–4.46)	(4.44–4.96)	(6.20–6.67)	(8.36–8.78)
Group C	4.40 ± 0.76[b]	4.12 ± 0.67[b]	5.56 ± 0.82	7.24 ± 0.72	8.32 ± 0.63[b]
	(4.08–4.73)	(3.84–4.01)	(5.27–5.85)	(6.99–7.49)	(8.09–8.55)
p	> 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Stiffness					
Group A	2.56 ± 0.64[c]	2.52 ± 0.58[b]	2.67 ± 0.62[b]	3.04 ± 0.65	3.41 ± 0.50
	(2.30–2.81)	(2.28–2.76)	(2.44–2.90)	(2.77–3.29)	(3.17–3.65)
Group B	2.77 ± 0.68[b,c]	2.57 ± 0.57[b]	2.80 ± 0.55[b]	3.60 ± 0.62	4.07 ± 0.69[b]
	(2.53–3.01)	(2.34–2.80)	(2.58–3.02)	(3.36–3.84)	(3.84–4.29)
Group C	3.04 ± 0.68[b]	3.04 ± 0.73	3.32 ± 0.63	4.08 ± 0.70	4.24 ± 0.66[b]
	(2.78–3.31)	(2.79–3.29)	(3.08–3.56)	(3.82–4.34)	(3.99–4.49)
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Function					
Group A	22.07 ± 1.24	21.11 ± 1.48	23.89 ± 1.22	27.70 ± 2.02	27.93 ± 1.88
	(21.61–22.54)	(20.60–21.62)	(23.36–24.41)	(26.77–28.64)	(26.95–28.90)
Group B	22.80 ± 1.32	23.00 ± 1.41	28.10 ± 1.42	31.17 ± 2.68	33.63 ± 2.75
	(22.36–23.24)	(22.52–23.48)	(27.60–28.60)	(30.28–32.05)	(32.71–34.56)
Group C	24.16 ± 1.07	24.88 ± 1.01	29.84 ± 1.46	33.72 ± 2.56	35.84 ± 2.90
	(23.67–24.65)	(24.35–25.41)	(29.29–30.39)	(32.75–34.69)	(34.83–36.85)
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Total					
Group A	29.22 ± 1.45	24.89 ± 0.89	28.74 ± 1.56	33.70 ± 1.20	36.41 ± 1.74
	(28.57–29.87)	(24.34–25.43)	(28.18–29.30)	(32.82–34.59)	(35.40–37.42)
Group B	30.63 ± 1.73	31.20 ± 1.73	34.37 ± 1.22	39.97 ± 2.93	48.07 ± 1.9[b]
	(30.02–31.25)	(30.68–31.72)	(33.84–34.90)	(39.13–40.80)	(47.11–49.02)
,Group C	31.68 ± 1.89	32.48 ± 1.48	38.84 ± 1.60	43.40 ± 2.35	46.88 ± 3.8[b]
	(31.01–32.36)	(31.91–33.05)	(38.26–39.42)	(42.48–44.32)	(45.83–47.93)
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[a] *WOMAC* Western Ontario and McMaster Universities Arthritis Index: values are reported as the mean ± SD (95% confidence interval); between-group values at the different months indicate significant differences ($p < 0.05$)

[b,c] Post-hoc analysis indicates no significant difference ($p > 0.05$)

recovery [13]. The most common methods of using PRP are by intra-articular injection; however, this form of injection does not reach the deeper layers of the cartilage, thereby limiting its therapeutic potential, and with only short-term effectiveness observed over placebo for relieving pain and stiffness and improving knee function in early knee OA [14–16]. PRP's main mechanisms of action on degenerative OA are on recurring inflammation and angiogenesis through its proteins and growth factors when delivered intra-articularly. However, subchondral bone also may be a tissue target for infiltration, as examined in a thorough review of those research findings by Sanchez et al. [8], which described the role of subchondral bone in OA pathophysiology and its relationship with clinical symptoms. Consequently, we endeavored to compare the combination of intra-articular and intraosseous injections of PRP with the intra-articular injection of PRP and of HA.

Previous RCTs have investigated the efficacy of PRP compared to HA or saline [17, 18]. Many of those reports suggested that PRP injections offered a significant clinical improvement at up to 1 year of follow-up [19, 20]. Gobbi et al. [21] reported that the clinical effectiveness continued for 2 years. In our study, although the PRP group showed significantly greater improvement in pain, stiffness and lower limb physical function after 3 months compared to HA, at the end of the 18th month, the clinical efficacy was decreased; however, PRP still appeared effective compared with the initial values, and the combination

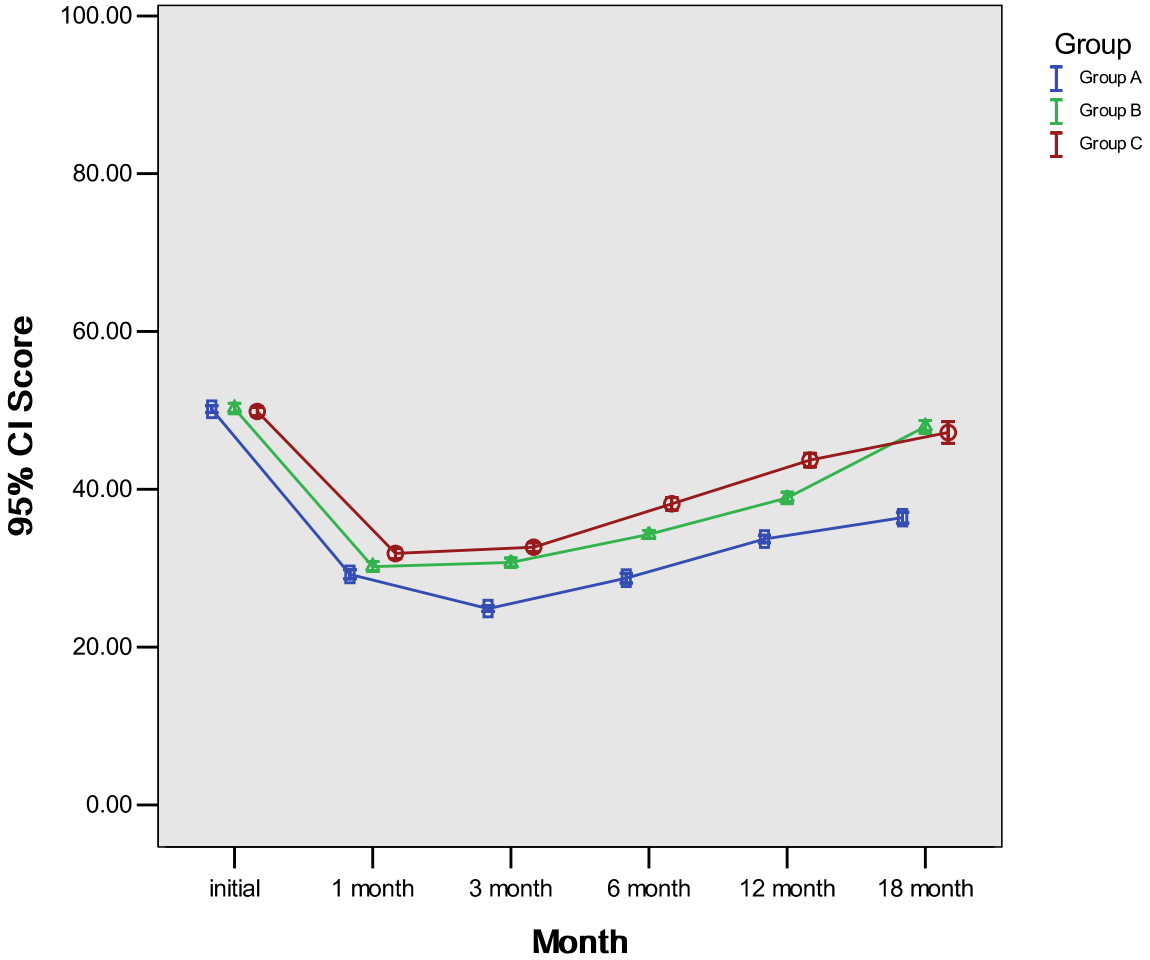

Fig. 4 Total WOMAC scores, comparison between the three groups (0–96)

injection PRP group was also significantly superior to HA. It is possible that the intraosseous infiltrations combined with intra-articular injections [22] may have additive effects. The VAS and WOMAC pain subscales showed that the combined injections were more effective than HA injection: in the HA group, worsened VAS and WOMAC parameters were observed at the second follow-up compared with our first follow-up, indicating no sustained long-term effects and the beginning of waning therapeutic benefits, whereas slight worsening was observed in the combination injection PRP group after 1 year. Additionally, for continued treatment effectiveness and to achieve prolonged clinical relief, we repeated the injection at two-week intervals, which demonstrated that multiple PRP injections using our new technique are useful to achieve improved clinical results.

In our experience, we used PRP from which leukocytes were not extracted overall; rather, we attempted to remove the platelet-poor plasma in the supernatant and the erythrocytes in the lower layer. Previous studies reported that the presence of leukocytes in joints might generate a negative proinflammatory environment in OA cartilage [23], which includes an increase in inflammatory IL-1 activity, activation of NF-kB and COX-2 expression, and promotion of catabolic pathways [24, 25]. Recently, however, Riboh et al. [26] concluded that leukocyte-poor PRP and leukocyte-rich PRP had similar safety profiles, and that the adverse reactions to PRP might not be directly related to leukocyte concentration. Because most studies have demonstrated improved results with leukocyte-containing PRP [27–29], we used LR-PRP as our study material, which resulted in improved outcome scores compared with HA to treat knee OA.

In our study, the selection criteria established according to the Kellgren-Lawrence Classification were grades II to III. Specifically, patients with Kellgren-Lawrence IV osteoarthritis were excluded because it has been shown that, in such cases, intra-articular treatment is less effective [30]. Regarding the PRP preparation, some authors have questioned the rpm of centrifugation [16, 22, 31]. In our preliminary experiment, we demonstrated that two centrifugations, one at an initial 1480 rpm for 6 min and a second at 3400 rpm for 10 min, separated the erythrocytes suitably and extracted the PRP from the buffy coat as much as possible. Our injection protocol was done in accordance with Sánchez et al. [22] who infiltrated the PRP intraosseous or intra-articular with the same dosages of 5 ml. But in our preliminary experiment, we found it was hard

to infiltrate the PRP more than 2 ml into the subchondral bone because of the over-large resistance, which may be because the needle tip was too close to the subchondral bone. So we reduced the dosage to 2 ml intraosseous injection.

Limitations of our study are the open-label trial in which both the researchers and participants know which treatment is being administered, and results may be biased by treatment allocation. Second, despite its wide application in clinical practice and the positive findings reported, almost all the studies have used questionnaires and were based on subjective findings. No objective findings were reported in those trials. In the follow-up study, we will attempt to investigate objective function using two index measures of lower extremity function (maximum single leg hop and number of knee bends in 30 s) [32]. We also intend to assess the pathology and morphology of the cartilage and subchondral bone with intraosseous injection of PRP in a rabbit OA model. Third, the lack of a prior registration to regulatory authorities may occur in the publication bias. Thus, in future studies, a prior registration to provide more definitive conclusions and a large clinical trial is needed to confirm our results. And the use of post-hoc test including the LSD that may have potentially exaggerated the statistical significance. Furthermore, because of the subchondral bone injection, this technique requires the proper administration of local anesthesia, which itself requires the use of a C-arm.

Conclusion

Our study provides some evidence that the combination of intraosseous with intra-articular injection of PRP improves self-reported pain and subscales of WOMAC in patients with knee OA (Kellgren-Lawrence grade II to III); moreover, it is superior to the intra-articular injection of PRP or HA at 18 months at minimum and an encouraging treatment option.

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Compliance with ethical standards The study protocol was reviewed and approved by the Reference Ethics Committee of Cangzhou Central Hospital. All patients signed the medical informed consent before the treatment.

Disclosures None.

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