RESEARCH PAPER

Comparative Analysis of the Effectiveness of Intra-Articular Injection of Platelet-Rich Plasma versus Hyaluronic Acid for Knee Osteoarthritis: Results of an Open-Label Trial

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Abstract

Background: Platelet-rich plasma (PRP), an autologous source of growth factors, and hyaluronic acid (HA) are among the minimally invasive treatments for knee osteoarthritis (OA). This trial was designed to compare the effectiveness of intra-articular injection of PRP with HA (as one of the standard treatments) on mild to moderate knee OA.

Methods: In this phase I open-label clinical trial, 10 patients underwent intra-articular PRP injection and 10 others received HA injection. At baseline (pre-injection) visit and 1, 3, 6, and 12 months post-injection, clinical assessments were performed using visual analogue scale (VAS) and Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire. Physical examinations of the knee, including crepitation and range of motion (ROM) were performed at each visit. The follow-up responses were compared with the baseline visit.

Results: The PRP treatment was ascertained to be safe and caused no adverse effects. Significant improvements in the majority of KOOS subscales and VAS were found throughout the entire 12-month follow-up, following the PRP injections. HA injection, however, caused only one month significant improvement in the majority of patient-reported outcomes. In the majority of visits, the extent of improvements in the scores of KOOS subscales, as well as the extent of reduction in VAS were significantly greater in PRP recipients, compared to HA recipients. The ROM in both groups slightly increased after interventions. The frequency of coarse crepitation, which was detected in 100% of the patients in both groups at the baseline visit, decreased significantly to fine crepitation at the first follow-up visit in 80% and 40% of the PRP and HA recipients, respectively.

Conclusion: Intra-articular injection of PRP or HA alleviates symptoms and pain and improves functionality and physical examinations in patients with knee OA. However, PRP therapy produces greater and longer-lasting improvements in most of the outcome parameters compared to HA.

Level of evidence: II

Keywords: Comparative study, Hyaluronic acid, Intra-articular injections, Knee Osteoarthritis, Platelet-rich plasma

Introduction

Osteoarthritis (OA) is a chronic arthropathy characterized by loss of articular cartilage, inflammation, soft tissue damage, and subchondral bone remodeling. It is a major cause of pain and disability especially in the weight-bearing joints (1). The knee is the most common joint involved with OA in the Iranian

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population. The prevalence of knee OA in Iran has been estimated to be about 15.5% (2). The current treatment modalities for this chronic condition with no definitive cure have majorly focused on relieving pain, reducing related symptoms, and decelerating the articular degeneration. In this context, among the relatively new therapeutic approaches, platelet-rich plasma (PRP) has provided promising results mainly through the provision of protective effects against OA progression (3, 4).

PRP is an enriched extract of platelets derived from autologous peripheral blood, which contains high concentrations of multiple regenerative factors including platelet-derived growth factor, transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor, epidermal growth factor, and fibroblast growth factor (5-7). Other than these beneficial characteristics, PRP therapy has shown to be effective in soft-tissue and wound healing as well as potentiating the regeneration in articular and orthopedic disorders (3, 6, 8-10). Compared to current surgical approaches for OA, such as arthroplasty, lavage, debridement, subchondral stimulation, and tissue grafting (1, 11, 12); intra-articular injection of PRP can be placed among the minimally-invasive modalities, such as hyaluronic acid (hyaluronate, HA) injections, though its effectiveness is still under investigation.

HA is an anionic, non-sulfated glycosaminoglycan and an essential component of the extracellular matrix, especially in connective tissue. It is also known as a physiologic lubricant for synovial joints and a facilitator of cell migration and proliferation, anti-inflammation, and tissue regeneration (13, 14). Given these unique properties, some companies established platforms to purify HA from natural sources and manufacture HA-based products to be used for degenerative articular diseases. The intra-articular injection of HA achieved FDA approval for knee OA after providing relatively promising results in clinical trials in terms of reducing knee pain and improving daily activity. Although in the beginning, HA-based products have been promoted as potential structure-modifying agents, they have been later revealed to be mainly symptom-modifying, since they generally produce transient viscosupplementation that lack significant long-term benefit for the diseased cartilage (15, 16). In order to compare the effectiveness of intra-articular injection of PRP with hyaluronate (as one of the standard treatments for knee OA), this trial was carried out on a series of mild to moderate knee OA patients.

Materials and Methods

Study design and subjects

Patients enrolled in this open-label phase I clinical trial were diagnosed with primary knee OA based on ACR criteria (17) and were interviewed and examined in the Rheumatology Clinic. Inclusion criteria for the trial comprised age between 45 and 75 years and grade 2 (mild) or 3 (moderate) knee OA according to Kellgren and Lawrence grading scale (18). On the other hand, patients with inflammatory arthritis, concurrent endocrine disorders, secondary OA, major psychologic disorders, connective tissue disorder, any chronic debilitating disease requiring constant treatment (e.g. malignancies), history of allergy to biologic products, history of intra-articular injections (any form of treatments) during the past six months before the intervention, and those requiring constant treatment with anti-thrombotic medications were excluded from the study. The patients were randomized to receive either intra-articular PRP or HA injection and they were aware of the type of the treatment they received (no blinding was performed).

Ethics

The present study was approved by the Institutional Review Board of University and registered at the Iranian Registry of Clinical Trials, Tehran, Iran (IRCT2012080510507N1), where is a primary registry in the WHO Registry Network. The trial was performed according to the ethical standards of the Declaration of Helsinki. The written informed consent was obtained from the patients and they were all informed about the study objectives. Potential complications were explained to participants and they were free to withdraw from the study.

Treatments and follow-ups

The patients underwent intra-articular injection of 2 ml autologous PRP or 2 ml HA preparation (Hyalgan®, Fidia Pharma USA Inc., NJ) after local skin asepsis and antisepsis. The joint was passively mobilized to disseminate the fluid uniformly all over the joint after injection. For preparation of PRP, 60 ml peripheral venous blood was collected in a blood bag containing 9 ml clinical-grade citrate phosphate dextrose buffer, and the bag was then transferred to a clean room. Afterward, the blood underwent two subsequent centrifugation steps at 2000 g for 2 min and 4000 g for 8 min, and after removal of the supernatant plasma at each step, 2 ml PRP was prepared. The resultant PRP concentrate contains at least one million platelets per µl (4-5 times higher than the baseline blood) (19). The patients were evaluated prior to injection (baseline visit) as well as in four follow-up visits (1, 3, 6, and 12 months post-injection). The patients were allowed to consume a standard dosage of non-steroidal anti-inflammatory drugs as per the rheumatologist's prescription after injection and during the 12-month follow-up period; however, they were asked not to take corticosteroids or undergo any other intra-articular injections (e.g., dexamethasone, HA, or PRP). In addition, they were prohibited from extreme sport activities, intense physical loading, and kneeling and lifting. Patients who did not comply with this post-intervention care were excluded from the final analysis.

Outcomes measures

At each visit, the patients responded to the Persian version of a self-reported standardized questionnaire focused on knee and associated problems entitled "Knee injury and Osteoarthritis Outcome Score (KOOS)" (20, 21), and visual analogue scale for subjective measurement of pain (22). KOOS is a scoring system comprised of five separate subscales including (knee-

related) Symptoms, (knee-related) Pain, Function (activity) in daily living (ADL), Function in Sport and Recreation (Sport/Rec) and (knee-related) Quality of Life (QOL). The score for each subscale ranges from "0 to 100", with "0" and "100" representing utmost problems and no problem, respectively (20). In addition, in each visit, the patient's knee was examined by a rheumatologist in terms of crepitation, effusion, injuries in main knee ligaments (i.e., anterior and posterior cruciate ligaments, and medial and lateral collateral ligaments), and range of motion (ROM) in flexion and extension. Treatment-related complications and any other adverse events were recorded throughout the study. The follow-up responses were compared with the baseline visit and between treatment groups.

Statistical analyses

Data were analyzed using SPSS® software (Version 20; IBM, USA). The normality of quantitative data was assessed using the Shapiro-Wilk test. Accordingly, all quantitative variables had normal distribution and were reported with mean and standard error of the mean (SEM). Qualitative variables were presented with frequency and percentage. Paired samples t-test and chi-squared test were used to analyze the change of a quantitative parameter and frequency distribution in two visits, respectively. In addition, the student t-test and chi-squared test were used to evaluate the between-group difference of quantitative and qualitative parameters at each visit, respectively. P-values less than 0.05 were considered statistically significant.

Results

General profile of the patients

In total, 26 patients met the inclusion criteria, of which 12 patients entered the PRP group and the other 14 patients entered the HA group. Two patients in the PRP group were finally excluded from the analyses owing to non-compliance with post-intervention care (one due to missing scheduled follow-up visits and one due to an episode of acute coronary syndrome two months after the injection, which required the patient to limit her physical activity for a couple of months) which could confound the reliable self-reported outcomes on daily and physical activities. In the HA group, four patients were excluded from the final analyses owing to undergoing intra-articular injection(s) of dexamethasone and/or HA in another clinic a few months after inclusion in the study. Table 1 summarizes and compares demographics and baseline clinical characteristics of the patients included in the analyses; and shows that the baseline parameters were not significantly different between the two cohorts.

Treatment outcomes

Subjective outcomes: PRP treatment was found to be safe and produced no adverse effects and complications. HA injections were also associated with no adverse events. Considering the KOOS subscales, PRP treatment resulted in significant improvement of symptoms, pain, and ADL throughout the entire 12 months follow-up, compared to the baseline visit. However, the improvement in Sport/Rec was only significant for the first three months. Moreover, the improvement in QOL began to be significant from the 6th-month visit. The highest scores were recorded in the 3rd month for symptoms, pain, ADL, and Sport/Rec and in the 6th month for QOL (Figure 1). On the other hand, HA injection caused only one month of significant improvement in symptoms, pain and Sport/Rec and a three-month improvement in ADL, though did not lead to any significant change in QOL. Given the subjective measurement of pain intensity using VAS, PRP therapy resulted in significant decrease in pain in all visits compared to the baseline, with the lowest record at the 6th-month visit; whereas, HA caused only a one-month reduction in pain with a gradual increase in the rest of the visits (Figure 1). Despite no significant between-group difference at the baseline visit, the patients who received PRP achieved significantly higher KOOS scores in pain, ADL, and QOL at the 6th and 12th months follow-ups compared with HA recipients, and at the same time points, they reported significantly lower pain intensity (VAS).

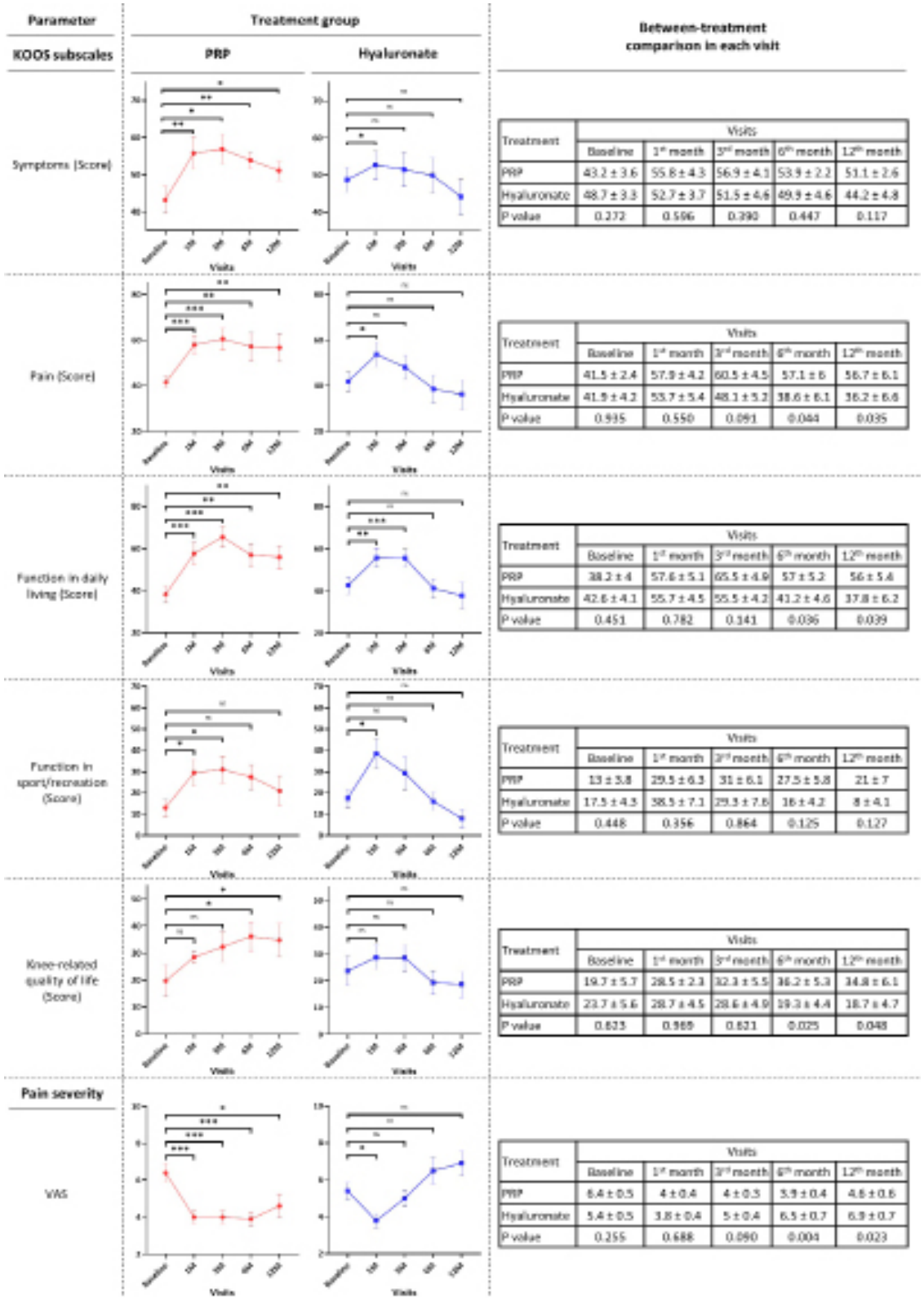
The between-group difference of "extent of change" for each parameter at each visit is shown in Table 2. In the majority of visits (especially the two last follow-up visits), the extent of improvement in scores of KOOS

Table 1. Demographics and baseline clinical features of patients included in the study

Parameters, unit	Grouping		*P value*
	PRP	Hyaluronate	
Number of patients	10	10	---
Age, yr, mean ± SEM	52.2 ± 1.9	55.1 ± 2.5	0.355
Female gender, n (%)	9 (90)	10 (100)	0.305
Osteoarthritis severity*, n (%)			
Grade 2	4 (40)	5 (50)	0.653
Grade 3	6 (60)	5 (50)	
On-admission knee pain severity, VAS¶, mean ± SEM	6.4 ± 0.5	5.4 ± 0.5	0.255

*Based on Kellgren and Lawrence grading

¶ Visual analogue scale



Symptoms (Score)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	43.2 ± 3.6	55.8 ± 4.3	56.9 ± 4.1	53.9 ± 2.2	51.1 ± 2.6
Hyaluronate	48.7 ± 3.3	52.7 ± 3.7	51.5 ± 4.6	49.9 ± 4.6	44.2 ± 4.8
P value	0.272	0.596	0.390	0.447	0.117

Pain (Score)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	41.5 ± 2.4	57.9 ± 4.2	60.5 ± 4.5	57.1 ± 6	56.7 ± 6.1
Hyaluronate	41.9 ± 4.2	55.7 ± 5.4	48.1 ± 5.2	38.6 ± 6.1	36.2 ± 6.6
P value	0.935	0.550	0.091	0.044	0.035

Function in daily living (Score)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	38.2 ± 4	57.6 ± 5.1	65.5 ± 4.9	57 ± 5.2	56 ± 5.4
Hyaluronate	42.6 ± 4.1	55.7 ± 4.5	55.5 ± 4.2	41.2 ± 4.6	37.8 ± 6.2
P value	0.451	0.782	0.141	0.036	0.039

Function in sport/recreation (Score)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	13 ± 3.8	29.5 ± 6.3	31 ± 6.1	27.5 ± 5.8	21 ± 7
Hyaluronate	17.5 ± 4.3	38.5 ± 7.1	29.3 ± 7.6	16 ± 4.2	8 ± 4.1
P value	0.448	0.356	0.864	0.125	0.127

Knee-related quality of life (Score)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	19.7 ± 5.7	28.5 ± 2.3	32.3 ± 5.5	36.2 ± 5.3	34.8 ± 6.1
Hyaluronate	23.7 ± 5.6	28.7 ± 4.5	28.6 ± 4.9	19.3 ± 4.4	18.7 ± 4.7
P value	0.623	0.969	0.621	0.025	0.048

VAS	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	6.4 ± 0.5	4 ± 0.4	4 ± 0.5	5.9 ± 0.4	4.8 ± 0.6
Hyaluronate	5.4 ± 0.5	3.8 ± 0.4	5 ± 0.4	6.5 ± 0.7	6.9 ± 0.7
P value	0.255	0.688	0.090	0.004	0.023

Figure 1. Between-visit comparison based on the treatment groups, and between-group comparison of patient-reported outcomes (subjective outcomes) at each visit. Markers and whiskers in graphs are representatives of mean and SEM, respectively.

subscales was significantly higher in the PRP recipients, compared to that in the HA recipients. The extent of VAS reduction was also significantly greater in PRP recipients than that in the HA recipients.

Objective Outcomes: In physical examinations, the ROM in flexion and extension slightly increased after interventions in both PRP and HA groups, though the difference was not statistically significant. All patients in both treatment groups had coarse crepitation at baseline visit which improved to fine crepitation at the first follow-up visit in 80% and 40% of the PRP and HA recipients, respectively. The between-visit comparison of the frequency distribution of coarse crepitation was found to be significant after intervention until the 6th month and 3rd month in the PRP and HA recipients, respectively [Figure 2]. It is worth mentioning that the

Table 2. Comparison of between-visit change (extent of change) in patient-reported outcome parameters based on the treatment groups

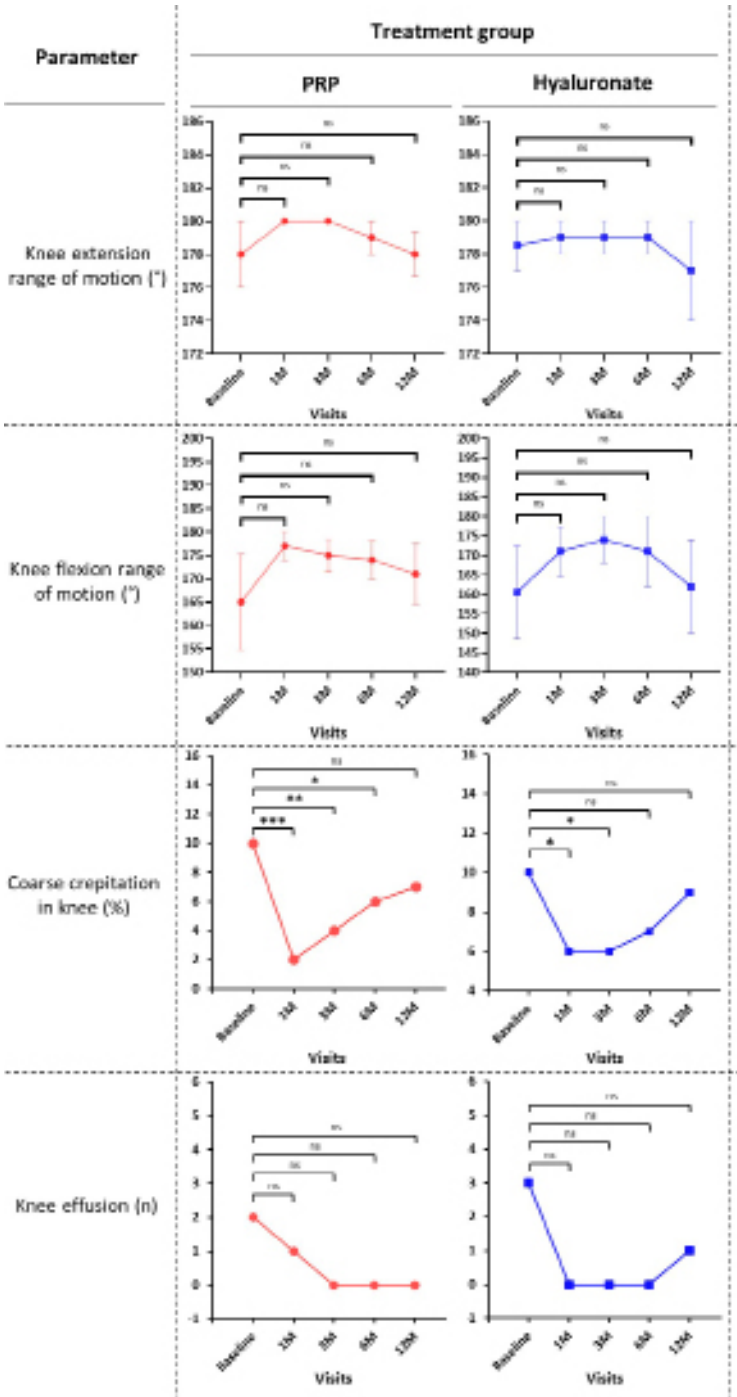
Parameter	Subsequent visit minus baseline visit	Treatment		*P value*†
		PRP	Hyaluronate	
KOOS subscales				
Between-visit change in symptoms, score; mean ± SEM	1st mo.* - baseline	12.6 ± 3.5	4 ± 1.4	0.035
	3rd mo. - baseline	13.7 ± 5.2	2.8 ± 3.7	0.016
	6th mo. - baseline	10.7 ± 3.3	1.2 ± 3.2	0.046
	12th mo. - baseline	7.9 ± 3.3	(-)4.5¶ ± 3.3	0.015
Between-visit change in pain, score; mean ± SEM	1st mo. - baseline	16.4 ± 3.6	11.8 ± 5	0.465
	3rd mo. - baseline	19 ± 4.2	6.2 ± 3.2	0.026
	6th mo. - baseline	15.6 ± 4.6	(-)3.3 ± 3.7	0.005
	12th mo. - baseline	15.2 ± 4.8	(-)5.7 ± 4.9	0.007
Between-visit change in function in daily living, score; mean ± SEM	1st mo. - baseline	19.4 ± 2.2	13.1 ± 4	0.182
	3rd mo. - baseline	27.3 ± 4	12.9 ± 1.7	0.004
	6th mo. - baseline	18.8 ± 4.2	(-)1.4 ± 4.5	0.004
	12th mo. - baseline	17.8 ± 4.4	(-)4.8 ± 6.3	0.009
Between-visit change in sports/recreation, score; mean ± SEM	1st mo. - baseline	16.5 ± 7.4	21 ± 7.5	0.674
	3rd mo. - baseline	18 ± 7.4	11.8 ± 6.7	0.542
	6th mo. - baseline	14.5 ± 6.8	(-)1.5 ± 4.8	0.036
	12th mo. - baseline	8 ± 8	(-)9.5 ± 4.9	0.049
Between-visit change in quality of life, score; mean ± SEM	1st mo. - baseline	8.8 ± 4.7	5 ± 6.5	0.641
	3rd mo. - baseline	12.6 ± 5.9	4.9 ± 3.2	0.266
	6th mo. - baseline	16.5 ± 6	(-)4.4 ± 4.2	0.010
	12th mo. - baseline	15.1 ± 6.3	(-)5 ± 4.2	0.017
Pain severity				
Between-visit change in VAS, score; mean ± SEM	1st mo. - baseline	(-)2.4 ± 0.4	(-)1.6 ± 0.8	0.113
	3rd mo. - baseline	(-)2.4 ± 0.3	(-)0.4 ± 0.7	0.011
	6th mo. - baseline	(-)2.5 ± 0.4	1.1 ± 0.8	0.001
	12th mo. - baseline	(-)1.8 ± 0.7	1.5 ± 0.7	0.003

*mo. = month

¶Negative figures show "decrease" and positive figures denote "increase"

†*P values* are computed using Student's t-test analyzing the difference between the two treatments for each unique parameter.

absence of crepitation was not detected in any patient at any of the visits after both interventions; therefore, improvements in crepitation were only in the context of transition from coarse to fine in this study. In the first-month follow-up, the reduction in the frequency of coarse crepitation was much greater in the PRP group than that in the HA group, though the difference was only close to the level of significance (P=0.068) [Figure 2]. Mild



Knee extension range of motion (°)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	178 ± 2	180 ± 0	180 ± 0	179 ± 1	178 ± 1.3
Hyaluronate	178.5 ± 1.5	179 ± 1	179 ± 1	179 ± 1	177 ± 3
P value	0.844	0.331	0.331	1	0.764

Knee flexion range of motion (°)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	165 ± 10.2	177 ± 3	175 ± 10.8	174 ± 4	171 ± 6.4
Hyaluronate	160.5 ± 11.8	171 ± 6.4	174 ± 6	171 ± 9	162 ± 12
P value	0.777	0.407	0.886	0.764	0.517

Coarse crepitation in knee (%)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	100%	20%	40%	60%	70%
Hyaluronate	100%	60%	60%	70%	90%
P value	1	0.068	0.371	0.639	0.274

Knee effusion (n)	Visits				
Treatment	Baseline	1st month	3rd month	6th month	12th month
PRP	20%	10%	0%	0%	0%
Hyaluronate	30%	0%	0%	0%	10%
P value	0.606	0.305	1	1	0.305

Figure 2. Between-visit comparison based on the treatment groups, and between-group comparison of knee physical examination (objective outcomes) at each visit. In graphs illustrating the range of motion in flexion and extension, markers and whiskers are representatives of mean and SEM, respectively. In graphs illustrating coarse crepitation and mild knee effusion, markers show frequency.

effusion was detected in two PRP recipients and three HA recipients at the baseline visit, which disappeared after both interventions, though the reductions in this parameter were not significantly different (perhaps owing to the low number of cases in this trial). The four main ligaments of the knee were found to be normal in all patients (both treatment groups) at baseline and all follow-up visits. Multivariable analyses showed no significant impact in terms of age and OA severity grading on the clinical response in each of the study parameters (in all time points).

Discussion

In this trial, the safety and efficacy of PRP therapy for knee OA was ascertained, and it was found that the improvements in patient-reported outcomes and physical examinations of the knee following intra-articular PRP injections were superior to HA injections. Moreover, the efficacy of PRP therapy was more durable than HA injections, since the differences of study parameters in the follow-up visits compared with baseline visit remained significant until 12 months in PRP recipients (for the majority of parameters); whereas, they were significant for only one month for the majority of parameters and three months for only two parameters in HA recipients.

For knee OA patients, minimally invasive and less-expensive treatments, such as PRP and HA may be more attractive options compared to surgical modalities. The PRP therapy might also be capable of reversing cartilage degeneration (5, 9, 23). However, questions remain that how much these treatments are effective and for how long they produce a clinical response. The enriched content of growth factors and bioactive proteins in PRP products facilitates (1) activation and proliferation of local cells, especially chondrocytes, (2) increased angiogenesis (3), enhanced expression of extracellular matrix, and reduced inflammation in an OA joint (3, 24, 25). In this context, a recent study conducted by Mousaei Ghasroldasht et al. demonstrated that following PRP therapy, upregulations of IGF-1 and bone morphogenetic protein 2 (BMP-2) gene expression occur, and both events are effective in enhanced cartilage matrix biosynthesis (26). Moreover, PRP-derived growth factors can promote regenerative capacity and chondrogenic differentiation of synovial stem cells, which are originally mesenchymal stem/stromal cells and are several folds more frequent in an OA joint compared to a healthy joint (27, 28) to repair cartilage defects (5, 25, 29, 30). On the other hand, although HA can decrease friction and reduce articular inflammation, it is not effective enough in arresting the progression of underlying degenerative processes (31). Hence, through the stimulation of cartilage regeneration in mild to moderate OA, PRP injection is able to delay the need for last-line therapeutic choice (i.e., surgical modalities), even so, its long-term efficacy requires further investigations.

Several studies have shown that the peak effectiveness of HA can be reached between one and two months after injection and residual effects last up to six months (32, 33), which are in agreement with the findings of the present study, though with poorer durability of the therapeutic response, since we found deteriorations in most of the study parameters in three months post-injection. Conversely, the peak effectiveness of PRP therapy was achieved in the 3rd month after the injection and the improvements in the majority of subjective and objective outcomes persisted up to 12 months after injection, which was consistent with several other studies (23, 34-36). Moreover, as shown in the present study, the extent of improvements in clinical parameters was greater after PRP injection compared to that following HA injections. Based on these results, the PRP injections produce superior and longer-lasting clinical responses compared to HA injections. This is in line with two recent meta-analyses comparing HA with PRP injection for knee OA established the superiority of PRP over HA (37, 38).

Intra-articular injection of PRP and HA alleviates symptoms and pain and improves physical functionality in patients with knee OA. Both products are effective as symptom-modifying agents for patients with mild to moderate knee OA. However, PRP therapy with a favorable safety profile produces greater and longer-lasting improvements in symptoms, quality of life, and daily function of knee OA patients, compared with the HA (as an approved treatment for the disease). Nonetheless, in order to obtain compelling evidence on the potential superiority of the efficacy of PRP over HA for knee OA, large-scale double-blind randomized trials should be performed.

Limitations

This was an open-label non-blinded randomized phase I trial. Hence, to rule out the placebo effects of the injections, the next phase of the trial requires blinding of the participants and a placebo-controlled arm. Moreover, a limited number of patients were included based on the phase of this trial and this may affect the statistical inferences. This trial was designed to evaluate the clinical data and patient-based assessments of health quality and symptoms (via a self-report questionnaire) after the therapies. Although not imperative, some studies have recommended assessing potentially unwanted hypertrophic and angiogenic effects of PRP therapy using different markers, such as tumor necrosis factor-α, VEGF, TGF-β (7, 39, 40). This may be considered as a limitation of this study. Some scientists have argued that the potential of batch-to-batch variations based on the patient's physiologic background can compromise the homogeneity of PRP products. To overcome this limitation, a standard protocol for extraction of PRP from the patients was used in this study, and additionally, a cut-off of at least one million platelets per µl was set to make the formulations injected for the PRP recipients more unified. Furthermore, only the effect of single-injection was evaluated for both arms in this trial. Some studies have shown that double or multiple injections may produce more prolonged efficacy (34, 36). However, since we aimed to appraise the treatment durability of a single dose of the treatments, the results provided a basis for the next phase of the trial to compare single infusion with double/additional infusions.

Conflicts of interest: The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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