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Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial

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Abstract
Purpose To compare the effectiveness of intraarticular (IA) multiple and single platelet-rich plasma (PRP) injections as well as hyaluronic acid (HA) injections in different stages of osteoarthritis (OA) of the knee.
Methods A total of 162 patients with different stages of knee OA were randomly divided into four groups receiving 3 IA doses of PRP, one dose of PRP, one dose of HA or a saline injection (control). Then, each group was subdivided into two groups: early OA (Kellgren–Lawrence grade 0 with cartilage degeneration or grade I–III) and advanced OA (Kellgren–Lawrence grade IV). The patients were evaluated before the injection and at the 6-month follow-ups using the EuroQol visual analogue scale (EQ-VAS) and International Knee Documentation Committee (IKDC) subjective scores. Adverse events and patient satisfaction were recorded.
Results There was a statistically significant improvement in the IKDC and EQ-VAS scores in all the treatment groups compared with the control group. The knee scores of patients treated with three PRP injections were significantly better than those patients of the other groups. There was no significant difference in the scores of patients injected with one dose of PRP or HA. In the early OA subgroups, significantly better clinical results were achieved in the patients treated with three PRP injections, but there was no significant difference in the clinical results of patients with advanced OA among the treatment groups.
Conclusion The clinical results of this study suggest IA PRP and HA treatment for all stages of knee OA. For patients with early OA, multiple (3) PRP injections are useful in achieving better clinical results. For patients with advanced OA, multiple injections do not significantly improve the results of patients in any group.
Level of evidence I.

Keywords Hyaluronic acid · Intraarticular injection · Knee osteoarthritis · Platelet-rich plasma

Introduction

The incidence of articular cartilage pathology is increasing because of the increase in sports activities and the prominence of physical activities in all age groups [6, 31]. Because of the continued increase in the mean age of the active population, OA is the most common degenerative joint disorder found in elderly individuals, and it has a significant effect on society [3, 9].

OA is a major cause of pain and disability and is detrimental to quality of life. Many non-invasive treatment options have been recommended to relieve symptoms and extend the quality of life and years of athletic activity for those with OA [13]. The treatment usually begins with non-steroidal anti-inflammatory drugs (NSAIDs), which have potential side effects that limit their use and lack clear data about their clinical therapeutic potency [5, 33]. Topical agents are widely used clinically for short-term use and are

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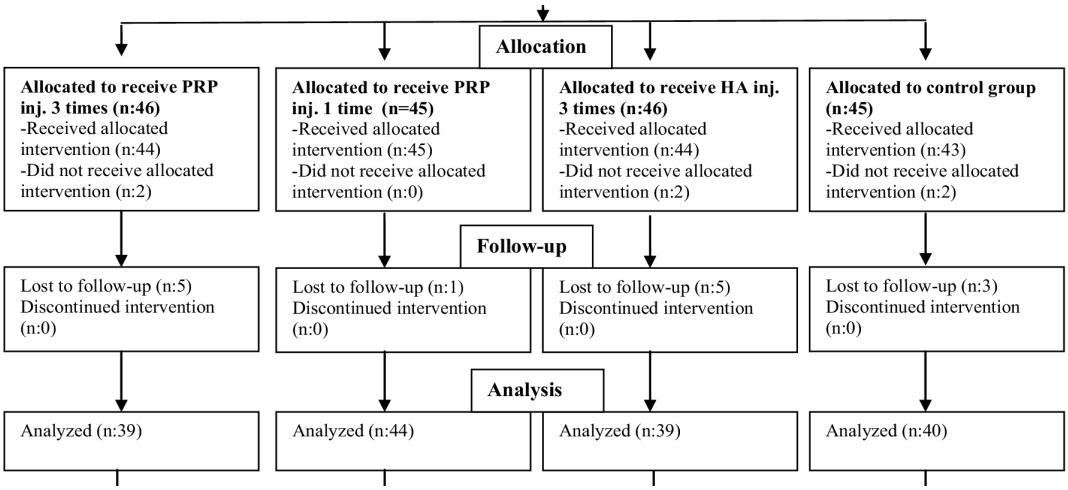
Allocation
Allocated to receive PRP inj. 3 times (n:46)
-Received allocated intervention (n:44)
-Did not receive allocated intervention (n:2)
Allocated to receive PRP inj. 1 time (n=45)
-Received allocated intervention (n:45)
-Did not receive allocated intervention (n:0)
Allocated to receive HA inj. 3 times (n:46)
-Received allocated intervention (n:44)
-Did not receive allocated intervention (n:2)
Allocated to control group (n:45)
-Received allocated intervention (n:43)
-Did not receive allocated intervention (n:2)
Follow-up
Lost to follow-up (n:5)
Discontinued intervention (n:0)
Lost to follow-up (n:1)
Discontinued intervention (n:0)
Lost to follow-up (n:5)
Discontinued intervention (n:0)
Lost to follow-up (n:3)
Discontinued intervention (n:0)
Analysis
Analyzed (n:39)
Analyzed (n:44)
Analyzed (n:39)
Analyzed (n:40)

Advanced OA	13 (35.5 %)	14 (37.8 %)	14 (38.8 %)	13 (32.5 %)	n.s.

The groups were homogeneous

OA Osteoarthritis, *n.s.* non-significant

bacteriological tests, 1 unit was used for injection within 2 h, and the remaining 2 units were stored at −30 °C.

There was no significant difference in the total number of platelets per millilitre between the PRP groups (n.s.) (PRP_3 group: concentration factor of 5.2× (1118,000 µL); PRP_1 group: concentration factor of 5.3× (1152,000 µL); n.s.).

The injections were administered every 7 days in all the groups. In the PRP_3 and PRP_1 groups, 1 mL of $CaCl_2$ was added to activate the platelets. For the second and third injections in the PRP_3 group, the samples were thawed in a dry thermostat at 37 °C for 30 min before administration.

In the HA group, 39 patients were treated with a high molecular weight HA preparation [30 mg/2 mL, Orthovisc (Anika Therapeutics Inc, Woburn, Massachusetts, USA)]. The treatment consisted of 3 injections of 2 mL once weekly.

Treatment procedure and follow-up

The skin was sterilely dressed, and each injection was administered by an unblinded physician using the superolateral approach with a 22-g needle. The knee was immobilized for 10 min after the injection, and the patient was discharged after a 1-h observation with instructions to use cold therapy on the affected area for pain relief. Physical activity was not limited; however, NSAIDs were not allowed during the follow-up period. Paracetamol was prescribed for discomfort.

The patients were evaluated before the injection and at the 6-week, 3-month and 6-month follow-ups by the clinician who was blinded to the patients and content of the injections. The EQ-VAS (as recommended by the ICRS evaluation package) and IKDC subjective scores were used for the clinical evaluation. Adverse effects were recorded, and patient satisfaction (satisfied, partially satisfied, not satisfied) at the end of 6 months was evaluated.

All the participants provided written informed consent, and the study was approved by the Inonu University, Turgut Ozal Medical School, Malatya, Turkey Ethics Committee (2013-171).

Statistical analysis

GPower software was used for the sample size estimation. A sample size of 140 individuals in total (35 per arm) was proposed to give 80 % power to detect an effect size of 0.8 (one-tail) between groups for continuous outcome variables. Anticipating protocol violators and early discontinuations of 25 %, it was projected that 175 patients should be included in the study.

The data were reported as the means ± standard deviations (SDs) or frequencies. Normality was confirmed using the Shapiro–Wilk test. The quantitative data were compared by one-way analysis of variance (ANOVA) followed by the Bonferroni test when the variances were homogenous or the Tamhane T2 test when the variances were non-homogenous. The qualitative data were analysed using Pearson's Chi-square test. In each group, the knee scores at the 6-week, 3-month and 6-month follow-ups were compared with repeated measures one-way ANOVA followed by the Bonferroni test, and $p < 0.05$ values were considered significant. All the analyses were conducted with IBM SPSS software, v. 22.0 for Windows.

Results

Six patients left during the first treatment because they were unable to tolerate injection therapy and were therefore excluded from the study for not beginning the treatment protocol. There was a statistically significant improvement in the IKDC and EQ-VAS scores in all the treatment

Table 2 EQ-VAS scores and IKDC subjective scores at basal and 6-month evaluations

	PRP_3	PRP_1	HA	Control
EQ-VAS				
Basal	50.3 ± 5.2^a	50.3 ± 5.8^a	50.5 ± 4.6^a	50.2 ± 4.5^a
6 months	71.4 ± 10.8^b	62 ± 6.3^c	60.8 ± 7.2^d	48 ± 5.1
IKDC				
Basal	40.4 ± 5^a	41.2 ± 6.1^a	40.6 ± 4.5^a	40.4 ± 4.3
6 months	60.8 ± 9.8^b	50.2 ± 6.7^c	48.4 ± 6.2^d	36.5 ± 4.8

All groups achieved significantly better clinical scores when com-

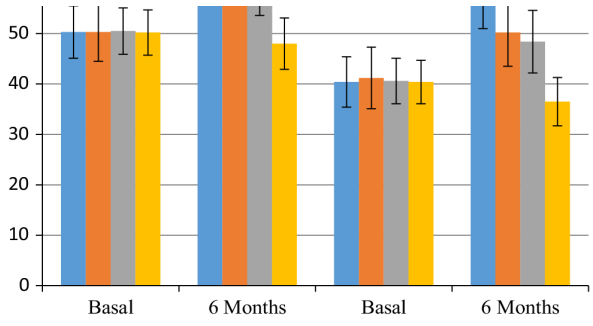

Fig. 2 All treatment groups had significantly higher results than control group ($p < 0.05$). PRP_3 group had significantly better results at 6-month evaluation. No difference was found between PRP_1 and HA group at follow-up results ($p > 0.005$)

the control group ($p < 0.005$). PRP_3 showed a significant improvement compared with the PRP_1 and HA groups ($p = 0.001$), and there were no significant differences between the PRP_1 and HA groups (n.s.) (Tables 3, 4).

For the advanced OA subgroups, significantly better results were achieved for all the treatment groups compared with the control group ($p < 0.05$). However, there were no significant differences in the knee scores between the advanced OA subgroups of the PRP_3, PRP_1 and HA groups at the 6-month follow-up (n.s.) (Tables 3, 4).

At the end of 6 months, 76.9 % of the patients were sat-

The most important finding of the present study was that multiple PRP injections resulted in better clinical results, particularly in patients with early OA. However, there was no difference in the results between treatment methods in the patients with advanced OA. One of the important results of this study was that a single dose of PRP or HA did not have a superior effect on the patients with early or advanced OA.

Synovial fluid viscoelasticity that results from HA is essential for normal joint function and acts as a lubricant and shock absorber [1]. Some clinical studies and meta-analyses have demonstrated satisfactory results with IA HA treatment, whereas others found no differences compared with placebo groups [2, 10, 14, 30]. In this study, significant improvements were observed in the HA group, suggesting that IA HA treatment is an effective treatment for

have shown different PRP treatment results. In a study that utilized an anterior cruciate ligament-transected rabbit model, the authors reported that PRP-treated rabbits had significantly decreased progression of OA [26].

A few prospective studies have been designed to evaluate the effectiveness of PRP on knee degeneration and have obtained statistically significant improvements in all the clinical scores at the end of therapy [7]. However, a limitation of these studies was the lack of a control group. In contrast to improved results, some prospective studies have concluded that PRP does not affect outcomes [12, 22]. In a randomized, double-blind study with a control group, Patel et al. [25] concluded that single or double PRP injections in knees with mild or moderate OA produced improved scores compared with those resulting from saline injections. However, the patients treated with two injections may have known that they were not in the control group. We tried to avoid such bias by administering an equal number of injections to all the patients. To our knowledge, only a few prospective studies were designed to evaluate the effectiveness of PRP and the superiority of HA and PRP treatment for knee degeneration [4, 13, 27]. These well-designed studies concluded that PRP injections showed better clinical results than HA injections; however, there are limitations in the studies. These studies did not have control groups and did not include randomization, except the study by Cerza et al. [4]. To address these limitations, a control group was included into the study. Injections were postponed, and patients were randomly divided into groups prior to injection therapy; then, patients were asked to return for injection therapy.

Although a significant decrease in knee scores was recorded within 6 months following the treatment, it has been hypothesized that IA PRP and HA treatment would be beneficial in patients with all stages of OA. In patients with early OA, significantly better clinical results were obtained with multiple PRP injections; it is hypothesized that multiple PRP injections for these patients would yield an effective treatment method. Unlike previous studies [13, 25], this study has revealed no significant difference between single PRP injections and HA injections in patients with early OA.

Patel et al. [25] concluded that a single dose of PRP is as effective as a double dose. Our study confirms this conclusion only for patients with advanced OA, as no difference between treatment methods has been found. Multiple injections are unnecessary for patients with advanced OA. These results may provide guidance with respect to treatment protocols because there is currently no consensus regarding treatment methods.

The better clinical results observed in patients with a lower degree of cartilage injury could be explained by a high response to GFs by less degenerated joints with a higher percentage of living and vital cells. Despite the significantly better results in the advanced OA group compared with the control group, the lack of a significant difference between the treatment options for this group supports this theory. In this study, patient scores in the control group worsened over time, indicating that patients with OA need to be treated with effective methods to avoid discomfort and further disability.

PRP and HA may influence joint homoeostasis by reducing synovial membrane hyperplasia and modulating the cytokine level. This mechanism temporarily leads to an improved clinical outcome without affecting the cartilage tissue structure [19]. Despite declining clinical outcomes, the significantly better results associated with multiple IA PRP injections in patients with early OA suggest that further experimental studies should be conducted on this issue. The differences between multiple and single PRP injections with respect to the effects on cellular mechanism should be clearly explored. Even if multiple injections showed no different effects from those of single injections at the cellular level, the clinical efficacy of multiple injections is obvious. Determining the most appropriate time for additional injections is important in the planning of future treatments.

this study is that image guidance was not used to ensure the location of the needle in the knee joint. Ideally, the present investigation would have been conducted through a multicentre study; however, it has been predicted that it would be difficult to optimize the treatment protocols for all the centres. Not optimizing the treatment protocols would affect the reliability of the study and would have decreased the value of the study; thus, we performed a single-centre study.

Conclusion

The clinical results indicate that IA PRP and HA treatment is suggested for all stages of knee OA. For patients with early OA, multiple (3) PRP injections are useful in achieving better clinical results. For patients with advanced OA, multiple injections are unnecessary and do not significantly affect patient knee scores.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

1. Balazs EA, Denlinger JL (1993) Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol Suppl 39:3–9
2. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G (2006) Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev 2:CD005321
3. Busija L, Bridgett L, Williams SR, Osborne RH, Buchbinder R, March L, Fransen M (2010) Osteoarthritis. Best Pract Res Clin Rheumatol 24:757–768

chi A, Fornasari PM, Marcacci M (2012) Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. BMC Musculoskelet Disord 13:229
9. Grogan SP, D'Lima DD (2010) Joint aging and chondrocyte cell death. Int J Clin Rheumtol 5:199–214
10. Housman L, Arden N, Schnitzer TJ, Birbara C, Conrozier T, Skrepnik N, Wei N, Bockow B, Waddell D, Tahir H, Hammond A, Goupille P, Sanson BJ, Elkins C, Bailleul F (2014) Intra-articular hylastan versus steroid for knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc 22(7):1684–1692

11. Irrgang JJ, Anderson AF, Boland AL, Harner CD, Kurosaka M, Neyret P et al (2001) Development and validation of the International Knee Documentation Committee subjective knee form. Am J Sports Med 29:600–613
12. Kesikburun S, Tan AK, Yilmaz B, Yaşar E, Yazicioğlu K (2013) Platelet-rich plasma injections in the treatment of chronic rotator cuff tendinopathy: a randomized controlled trialwith 1-year follow-up. Am J Sports Med 41:2609–2616
13. Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, Fornasari PM, Giannini S, Marcacci M (2011) Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy 27:1490–1501
14. Lo GH, LaValley M, McAlindon T, Felson DT (2003) Intra-articular hyaluronic acid in treatment of knee osteoarthritis: a meta-analysis. JAMA 290:3115–3121
15. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH (2010) The use of platelet rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy 26:269–278
16. McCarrel T, Fortier L (2009) Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. J Orthop Res 27:1033–1042
17. Mehta V (2010) Platelet-rich plasma: a review of the science and possible clinical applications. Orthopedics 33:111
18. Mei-Dan O, Carmont MR, Laver L, Mann G, Maffulli N, Nyska M (2012) Platelet-rich plasma or hyaluronate in the management of osteochondral lesions of the talus. Am J Sports Med 40:534–541
19. Mix KS, Sporn MB, Brinckerhoff CE, Eyre D, Schurman DJ (2004) Novel inhibitors of matrix metalloproteinase gene expression as potential therapies for arthritis. Clin Orthop Relat Res 427:S129–S137
20. Mladenovic Z, Saurel AS, Berenbaum F, Jacques C (2014) Potential role of hyaluronic acid on bone in osteoarthritis: matrix metalloproteinases, aggrecanases, and RANKL expression are partially prevented by hyaluronic acid in interleukin 1-stimulated osteoblasts. J Rheumatol 41:945–954
21. Mongkhon JM, Thach M, Shi Q, Fernandes JC, Fahmi H, Benderdour M (2014) Sorbitol-modified hyaluronic acid reduces oxidative stress, apoptosis and mediators of inflammation and catabolism in human osteoarthritic chondrocytes. Inflamm Res 63:691–701
22. Morishita M, Ishida K, Matsumoto T, Kuroda R, Kurosaka M, Tsumura N (2014) Intraoperative platelet-rich plasma does not ımprove outcomes of total knee arthroplasty. J Arthroplasty 29(12):2337–2341
23. Nakazawa F, Matsuno H, Yudoh K, Watanabe Y, Katayama R, Kimura T (2002) Corticosteroid treatment induces chondrocyte apoptosis in an experimental arthritis model and in chondrocyte cultures. Clin Exp Rheumatol 20:773–781
24. Ostergaard M, Halberg P (1998) Intra-articular corticosteroids in arthritic disease: a guide to treatment. BioDrugs 9:95–103
25. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A (2013) Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med 41:356–364
26. Saito M, Takahashi KA, Arai Y, Inoue A, Sakao K, Tonomura H, Honjo K, Nakagawa S, Inoue H, Tabata Y, Kubo T (2009) Intraarticular administration of platelet- rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee. Clin Exp Rheumatol 27:201–207
27. Say F, Gürler D, Yener K, Bülbül M, Malkoc M (2013) Platelet-rich plasma injection is more effective than hyaluronic acid in the treatment of knee osteoarthritis. Acta Chir Orthop Traumatol Cech 80:278–283
28. Sundman EA, Cole BJ, Fortier LA (2011) Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 39:2135–2140
29. van Buul G, Koevoet WL, Kops N, Bos PK, Verhaar JA, Weinans H, Bernsen MR, van Osch G (2011) Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. Am J Sports Med 39:2362–2370
30. van der Weegen W, Wullems JA, Bos E, Noten H, van Drumpt RA (2015) No difference between intra-articular injection of hyaluronic Acid and placebo for mild to moderate knee osteoarthritis: a randomized, controlled, double-blind trial. J Arthroplasty 30(5):754–757
31. Widuchowski W, Widuchowski J, Trzaska T (2007) Articular cartilage defects: study of 25,124 knee arthroscopies. Knee 14:177–182
32. Xie X, Wang Y, Zhao C, Guo S, Liu S, Jia W, Tuan RS, Zhang C (2012) Comparative evaluation of MSCs from bone marrow and adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. Biomaterials 33:7008–7018
33. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P (2008) OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 16:137–162