Efficacy of Intra-articular Injection of a Newly Developed Plasma Rich in Growth Factor (PRGF) Versus Hyaluronic Acid on Pain and Function of Patients with Knee Osteoarthritis: A Single-Blinded Randomized Clinical Trial

Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders
Volume 10: 1–9

DOI: 10.1177/1179544117733452

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ABSTRACT

BACKGROUND AND OBJECTIVES: Knee osteoarthritis is the most common joint disease. We aimed to compare the efficacy and safety of intra-articular injection of a newly developed plasma rich in growth factor (PRGF) versus hyaluronic acid (HA) on pain and function of patients with knee osteoarthritis.

METHODS: In this single-blinded randomized clinical trial, patients with symptomatic osteoarthritis of knee were assigned to receive 2 intra-articular injections of our newly developed PRGF in 3 weeks or 3 weekly injections of HA. Our primary outcome was the mean change from baseline until 2 and 6 months post intervention in scores of visual analog scale, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Lequesne index. We used analysis of variance for repeated-measures statistical test.

RESULTS: A total of 69 patients entered final analysis. The mean age of patients was 58.2 ± 7.41 years and 81.2% were women. In particular, total WOMAC index decreased from 42.9 ± 13.51 to 26.8 ± 13.45 and 24.4 ± 16.54 at 2 and 6 months in the newly developed PRGF group (within subjects $P = .001$), and from 38.8 ± 12.62 to 27.8 ± 11.01 and 27.4 ± 11.38 at 2 and 6 months in the HA group (within subjects $P = .001$), respectively (between subjects $P = .631$). There was no significant difference between PRGF and HA groups in patients' satisfaction and minor complications of injection, whereas patients in HA group reported significantly lower injection-induced pain.

CONCLUSIONS: In 6 months follow up, our newly developed PRGF and HA, both are effective options to decrease pain and improvement of function in patients with symptomatic mild to moderate knee osteoarthritis.

KEYWORDS: Hyaluronic acid, intra-articular injections, platelet-rich plasma, growth factor, platelet, knee, osteoarthritis

RECEIVED: June 1, 2017. **ACCEPTED:** August 16, 2017.

PEER REVIEW: Three peer reviewers contributed to the peer review report. Reviewers' reports totaled 734 words, excluding any confidential comments to the academic editor.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

Osteoarthritis (OA) of the knee is a very common degenerative chronic disease which is responsible for debilitating pain and dependence in affected people.[1] The prevalence of OA is somewhat between 2.8% in Southeast Asia and 10% in the United States and as high as 19.3% in some rural areas of Iran.[2,3] In addition, in the middle-aged Iranian population, this figure reaches 43% based on the radiographic characteristics. This disease accompanies damaging effects on quality of life and may lead to loss of job or early retirement.[4] To overcome this morbid disease, various treatments have been proposed including patient education, physical agent modalities, as well as medical and surgical options. One of the less aggressive approaches that may postpone knee replacement in selected cases is intra-articular injection (IAI) through viscosupplementation products. Back in 1997, the United States Food and Drug Administration–approved hyaluronic acid (HA) for knee IAI for OA. After 3 years, the American College of Rheumatology (ACR) implemented this product in its released guideline.[5] In addition to viscoelasticity, exogenous HA may induce synthesis of endogenous HA and proteoglycans by chondrocytes, prevent cartilage destruction, and even stimulate its repair and reduce proinflammatory cytokines in synovial space.[6,7] Thus, by emergence of HA, we witnessed a new era for treatment of knee OA. At present, however, American Academy of Orthopaedic Surgeons cannot recommend use of IAI of HA, whereas ACR in its 2012 guideline has recommended this

approach for patients who are refractory to other modalities.[8,9] Investigators have been trying to find and test new biologic products to improve the efficacy of IAI.

Platelets and their various products' potentials in reducing inflammation and tissue repair have increasingly gained attention in regenerative medicine.[10,11] However, there is inconsistency about the efficacy and priority of use of these different platelet compounds in management of medical fields such as musculoskeletal disorders.[12] These compounds include platelet-rich plasma (PRP), plasma rich in growth factor (PRGF) and platelet-rich fibrin (PRF).[13] Recent studies emphasized on the role of PRP as a new and appropriate product for IAI. Platelet-rich plasma is a mixture of autologous platelet-released molecules such as transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), and insulinlike growth factor (IGF) that have been shown to induce injured cartilage repair.[6,14–18] Besides the above molecules, there are some bioactive molecules in plasma that may affect the OA pathophysiologic process.[19] The anticipated effect of PRP after injection depends largely on platelet activation and release in joint space.[20,21] To make sure only useful growth factors but not harmful inflammatory cytokines will reach chondrocytes, researchers developed a new product named PRGF. This product is free of white blood cells (WBCs) and is produced through in vitro activation of platelets with a fixed platelet and growth factor concentration. Soon after its introduction, this product showed promising effects in knee OA with less postinjection complications such as pain and swelling.[22] In contrast to PRP which has been compared with placebo or HA in many studies in the literature with conflicting results,[23–29] there is a scarcity of evidence which has employed PRGF for IAI.[30–32]

Recently, a new method has been developed for production of PRGF. In this method, the final product has a pool of growth factors with a specified concentration and without platelets. This product has been implemented extensively in dermatology, plastic surgery, and dentistry with a few applications for musculoskeletal disorders.[33–35] Given the repairing effect of newly developed PRGF, we aimed to compare its efficacy and safety with HA in IAI of patients with knee OA in a clinical trial setting.

Patients and Methods

This phase 2 randomized clinical trial (RCT) was conducted in 2016 in a referral university medical center. The design and protocol of this RCT was reviewed and approved by the institutional review board and then registered in the Iranian Registry of Clinical Trials (www.irct.ir) as a World Health Organization regulatory representative for clinical trials in Iran (IRCT2016071513442N11). This study was supervised by the institutional ethics committee to be in agreement with the Declaration of Helsinki.

Patient selection

We recruited 50- to 70-year-old patients with knee OA based on ACR criteria (Kellgren-Lawrence grade II to III) who complained of relevant symptoms for at least 6 months. The diagnosis and grading of OA were made after obtaining knee x-ray in anteroposterior weight-bearing and lateral views. Our exclusion criteria included presence of systemic diseases such as diabetes mellitus, immunodeficiency, and collagen vascular diseases; presence or history of malignancy; presence of autoimmune diseases or platelet disorders; body mass index more than 33 kg/m^2; taking nonsteroidal anti-inflammatory drugs (NSAIDs) during 2 days or aspirin during 7 days prior to injection; taking anticoagulant or antiplatelet medications during 10 days prior to injection; history of systemic steroids during 2 weeks prior to injection; any IAI during 3 months prior to enrollment; hemoglobin level below 12 g/dL or platelet count below 150 000/μL[36]; history of recent severe knee trauma; knee septic arthritis; presence of active ulcer or septic arthritis of knee; genu varum or valgum over 20°; hypersensitivity to egg and chicken proteins or HA; and consumption of ginger or turmeric during the week prior to injection.

Randomization

The design, objectives, benefits, and complications of study were disclosed with eligible patients by a physical medicine and rehabilitation specialist. After providing written informed consent, participants were allocated through permuted block randomization method into 2 groups. The recruitment and randomization were done by a resident assistant in physical medicine and rehabilitation who was not blinded to subject allocations. The first group received IAIs with PRGFs and the second with HA.

Interventions and product preparation

The first group of patients underwent two 3-week IAIs with a newly developed PRGF. For the process of PRGF preparation, in this new method, we needed to produce PRP first. Participants were referred to the hospital laboratory. Rooyagen Kit (Arya Mabna Tashkhis Corporation, RN: 312569) was opted for PRP processing. This kit uses a dual-spin system and is fully enclosed to maintain sterility all over the process. At first, 35 mL of blood was obtained to prepare PRP with concentrations of 4 to 6 times the average of normal values. Then, for anticoagulation, 5 mL of citric acid dextrose solution A was added. Complete blood count was assayed using 1 mL of this sample. After centrifuging for 15 minutes at 1600 rpm, 3 layers emerged, including red blood cells (lower), WBCs (middle), and plasma (upper). The 2 uppermost layers underwent another centrifuge for 7 minutes at 3500 rpm. Our product at this step consisted of 8 mL of plasma with 4.6 ± 0.7 times platelet concentration. At this step, we added 1.5 mL of platelet-activating factor (Rooyagen) consisting of epinephrine and calcium chloride 25 mmol/L and then stirred it. We then placed samples in a warm water device (T = 40°C ± 1°C) for 20 to 30 minutes to give the platelets the chance to release their factors. This release

is a consequence of platelet activation in which fibrin interacts with platelets via glycoprotein IIa/IIIb surface proteins. This interaction leads to secretion of growth factors by platelets. Thus, we have 2 phases in a plasma preparation. The resulting sample consisted of 2 phases: the supernatant liquid phase that was PRGF and the solid phase which was platelet clumps plus fibrin network. We performed a third centrifuge (4000 rpm for 4 minutes) to make platelets and attached fibrins stick to the bottom of the tubes. The remaining fluid (5 mL) was used for injection within 20 minutes of its preparation. We did not use any local anesthetics prior to injection of plasma product. Instead, patients were given a single dose of acetaminophen 2 hours before the injection. After applying proper disinfectants, the PRGF was injected by 21-G needles through the preferred classic approach which was lateral mid-patellar with extended knee. If this approach was not possible due to lack of adequate space, we used anteromedial approach with flexed knee (this happened for only 1 person). To distribute injected fluid all over the synovial space, we recommended active flexion and extension of knee after a 20-minute rest. The second injection was made after 3 weeks in similar settings. The number of injections was based on authors' experience considering no consensus on standard number of plasma products injection.

In the second group, 3 weekly injections by HA (Hyalgan®, Fidia Farmaceutici S.p.A., Abano Terme, Italy) were given. Each prefilled syringe contained 20 mg of the active ingredient sodium hyaluronate in 2 mL of liquid with molecular weight of 500 to 730 kDa. Hyalgan syringes were injected using 21-G needles through similar above approach in sterile settings. At the end of the injections, patients were asked to flex and extend their knees several times. The second and third injections were administered at a 1-week interval with the same conditions as for the first injection. All the injections in both groups were made by the same skilled specialist in physical medicine and rehabilitation who was not blinded to subject allocations.

Follow-up after interventions

Subjects were discharged after a 10- to 15-minute rest. They were instructed to have only mild physical activities, limit weight-bearing over the affected lower limb, and to apply a 10-minute ice pack every 8 hours a day, for the first 2 days. In case of experiencing mild to moderate pain, up to four 500-mg tablets of acetaminophen were allowed, and if pain persisted, acetaminophen with codeine would be advised. Other drugs than these analgesics were prohibited up to 5 days postinjection. Routine recommended knee-oriented exercise therapy was instructed and encouraged by study authors similarly for both groups.

All study subjects were visited and interviewed at clinic 2 and 6 months after interventions by another resident assistant who was blinded. Patients were evaluated for the magnitude of pain, joint stiffness, injection site complications, and the amount of analgesic consumption. For any emergent condition, a 24-hour phone line was provided. Authors contacted subjects who did not attend their timely visits and asked the cause of their absence to investigate whether someone should be excluded from the study.

Outcome measures

Our main outcomes to measure were changes in patients' joint pain and function. To do so, we opted 3 widely accepted instruments including visual analog scale (VAS) to assess patient-reported pain in a quantitative fashion; the Persian, validated version of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC); and Persian version of Lequesne algofunctional index.[37] The latter 2 were filled at predetermined time intervals by the same interviewer who was a physical medicine and rehabilitation medicine resident. The WOMAC questionnaire consisted of 24 items (5 for pain, 2 for stiffness, and 17 for functional limitations) and is scored on a Likert scale. Less total scores mean lower symptoms and better function. However, Lequesne is made up of 11 items (5 for pain, 2 for maximal walking rate, and 4 for activities of daily life), in which less total scores again mean a better situation. These variables were measured at baseline (before intervention) as well as 2 and 6 months after completion of injections. Participants were told to discontinue their analgesics 48 hours before each evaluation. The secondary outcomes were the pain at injection site immediately after injection (scored by VAS) and patients' satisfaction (based on a 10 score visual scale from very good to very poor) after 6 months.

Sample size calculation

Based on a previous study[24] with a significant obvious mean difference in reduction in WOMAC pain scores between HA and PRGF-Endoret and the sample size calculation formula to compare 2 means, we needed 35 subjects in each group. With this figure, the power was 80% and the level of significance was .05.

Statistical analysis

Final data before and after the treatment were imported and analyzed by a medical statistics expert who was blinded to study grouping. The SPSS v.16 software was opted for analysis. Shapiro-Wilk or Kolmogorov-Smirnov tests were used to check normal distribution of variables. Independent samples *t* test was performed to compare mean values across 2 groups. To assess changes within and between groups, we conducted analysis of variance (ANOVA) for repeated measures.

Results

Patient characteristics

From December 2015 to November 2016, after primary evaluation of 310 patients with retractable knee OA, 221

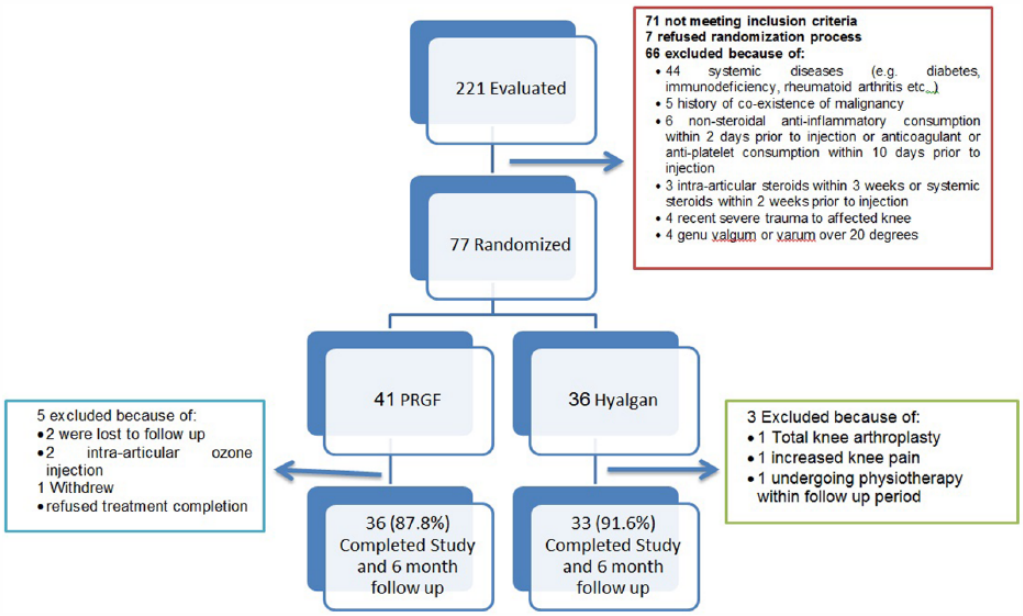

Figure 1. Enrollment and allocation diagram.

Table 1. Baseline characteristics.

	HA	PRGF	*P* VALUE	TOTAL	STATISTICAL TEST
Age, y	59.5 ± 7.54	57.0 ± 7.18	.303	58.2 ± 7.41	*t* test
Male:female	6:27	7:29	.999	13:56	χ^2
BMI, kg/m^2	27.5 ± 2.9	28.6 ± 2.82	.102	28.0 ± 2.88	*t* test
Duration of pain, y	6.2 ± 3.9	7.4 ± 5.22	.417	6.8 ± 4.63	*t* test
Right:left	23:13	16:17	.231	39:30	χ^2
Grade II:III	15:18	15:21	.811	30:39	χ^2
History of previous physiotherapy	19 (57.6%)	29 (80.6%)	.066	48 (69.6%)	χ^2
History of previous injection	13 (39.4%)	18 (50%)	.469	31 (44.9%)	χ^2

Abbreviations: BMI, body mass index; HA, hyaluronic acid; PRGF, plasma rich in growth factor.

were candidates for IAI. Of these individuals, after providing informed consent, 76 subjects who met our inclusion and exclusion criteria were randomized into 2 treatment groups. Finally, 69 completed the study protocol and follow-up who were qualified for intention-to-treat analysis, including 36 in PRGF and 33 in Hyalgan groups (Figure 1). Among all subjects, mean age was 58.25 ± 7.41 years (43-70), 81.2% were women, and 21 (73.9%) were obese or overweight. Baseline characteristics of study subjects are depicted in Table 1. As evident, subjects were matched statistically between both groups.

PRGF specifications

A total of 10 volunteers from PRGF group were selected, and the concentration of different growth factors in their final PRGF product was evaluated. The result was as follows: vascular endothelial growth factor = 298 ± 38.5 ng/mL, PDGF = 311 ± 62.5 ng/mL, IGF = 1.4 ± 0.2 ng/mL, TGF-β1 = 1.8 ± 0.2 ng/mL, and TGF-β 2 = 1.3 ± 0.1 ng/mL. Our product did not have any platelet or WBCs. The concentration of hepatocyte growth factor was 307 pg/mL measured by enzyme-linked immunosorbent assay method.

Outcomes

The VAS for pain, WOMAC, and Lequesne instrument mean scores acquired by subjects at baseline and 2 and 6 months after injection have been shown in Table 2 and Figures 2 to 5. The %change from baseline was calculated with the following formula: ([Baseline score − 6th month score/Baseline score]*100). Based on ANOVA for repeated measures, the decrescendo pattern observed in WOMAC and VAS was statistically significant within each group during time, but the difference between

Table 2. WOMAC and VAS mean scores at baseline and 2 and 6 months after injection.

		WOMAC SCORES				VAS
		PAIN	FUNCTION	STIFFNESS	TOTAL	
PRGF	Baseline	9.2±2.97	30.6±10.09	3.0±2.01	42.9±13.51	7.8±1.78
	At 2 mo	5.8±2.96	19.5±9.79	1.6±1.66	26.8±13.45	4.9±2.21
	At 6 mo	5.3±3.60	17.6±11.70	1.5±1.84	24.4±16.54	4.6±2.78
	Mean difference[a]	−3.9±4.06	−12.9±9.40	−1.6±1.98	−18.5±14.08	−3.2±2.56
	% change from baseline[b]	38±47.0	44±27.2	54±50.4	44±28.1	42.2±29.81
HA	Baseline	8.7±3.01	27.8±9.62	2.3±1.64	38.8±12.62	7.4±1.48
	At 2 mo	5.9±2.65	20.6±8.04	1.2±1.39	27.8±11.01	4.8±1.80
	At 6 mo	5.9±2.79	20.1±7.77	1.3±1.48	27.4±11.38	4.8±2.39
	Mean difference	−2.8±1.75	−7.6±5.81	−1.0±1.76	−11.5±7.66	−2.7±2.01
	% change from baseline	32±25.1	26±28.5	40±70.7	30±19.1	37.1±29.09
P value between groups		.847	.894	.189	.985	.648

Abbreviations: HA, hyaluronic acid; PRGF, plasma rich in growth factor; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[a]6th month − baseline.
[b]([Baseline − 6th month]/Baseline)*100.

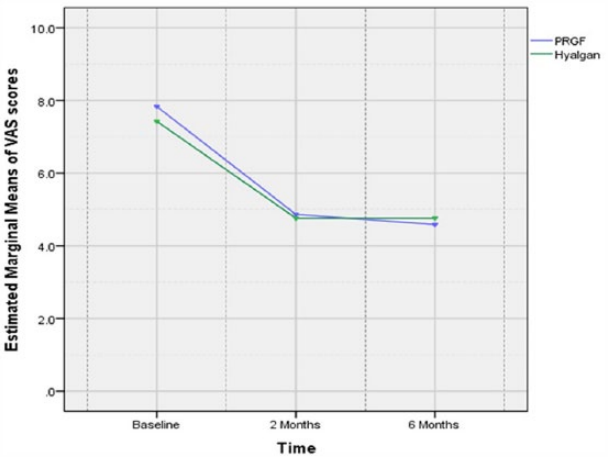

Figure 2. Means of VAS at baseline and 2 and 6 months after injection. PRGF indicates plasma rich in growth factor; VAS, visual analog scale.

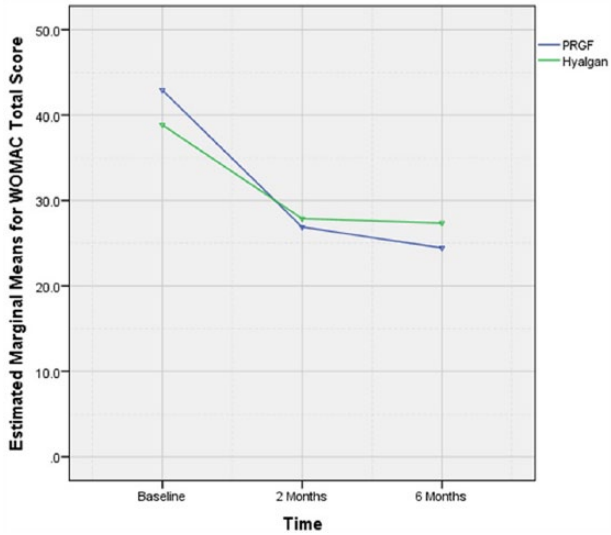

Figure 3. Means of WOMAC total scores at baseline and 2 and 6 months after injection. PRGF indicates plasma rich in growth factor; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

2 study groups was not significant. We defined success rates of both interventions as any decrease in about 30% or higher from baseline scores at third assessment 6 months after intervention. According to this definition, success rates for each scale were as follows (PRGF vs Hyalgan): WOMAC total 61.1% vs 54.5% (P=.631), WOMAC pain 61.1% vs 63.6% (P=.999), WOMAC stiffness 58.3% vs 54.5% (P=.811), and WOMAC function 63.9% vs 45.5% (P=.151). Considering P values, none of the differences in success rates were significant. Table 2 shows WOMAC subscale scores before, after 2, and 6 months of intervention in both groups.

According to the Lequesne scale scores, the success rates were as follows based on various subscales (PRGF vs HA): 52.8% vs 33.3% for total (P=.145), 19.4% vs 6.1% for pain (P=.154), 34.4% vs 25.8% (P=.585) for walk, and 36.1% vs 15.2% for activities of daily life (P=.059).

The rates of satisfaction, complications, and injection-induced pain are presented in Tables 3 and 4. The mean injection-induced pain score was 3.8±2.92 in PRGF and 1.9±1.49 in Hyalgan group (P=.016). Minor complications due to injection occurred in 7 (19.4%) and 2 (6.1%) of the subjects in PRGF and Hyalgan groups, respectively (P=.154). These complications included swelling (one in PRGF vs none in

Hyalgan) or stiffness and heaviness of injection site (other cases). At the end of the follow-up, the mean satisfaction score within a range of 0 to 4 was 2.7 ± 1.47 and 2.7 ± 1.18 in PRGF and Hyalgan group, respectively (P = .726). In both study groups, 66.7% of subjects have good or very good satisfaction with the intervention they have undergone.

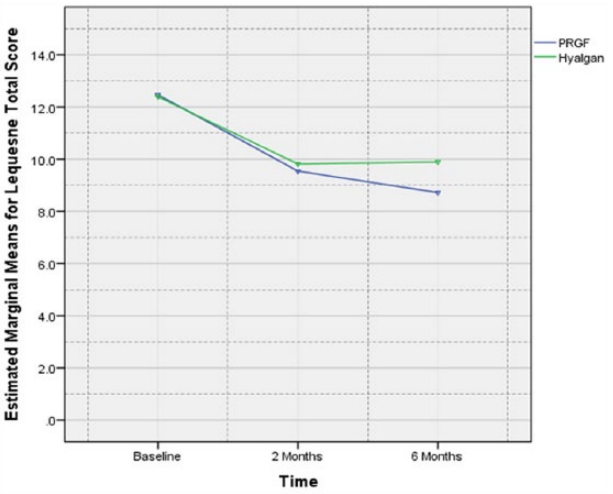

Figure 4. Mean values of Lequesne total at baseline and 2 and 6 months after injection. PRGF indicates plasma rich in growth factor.

Discussion

In brief, our study showed that both PRGF and HA yielded significant decline in pain measured by VAS and significant improvement in scores calculated by WOMAC and Lequesne instruments. The improvement in WOMAC scores from baseline to 6 months postinjection was 44% and 30% in PRGF and HA groups, respectively. In addition, a similar trend was observed in pain reduction by VAS criteria. Although higher rate of success and also complications were recorded in PRGF compared with HA group, no significant differences were seen. An interesting point was that about two-thirds of patients in both groups were satisfied by injection therapies. Although PRGF injection was significantly more painful than HA.

Thus, to explain our results, HA itself is a component of normal joint synovial fluid and cartilage, which is produced by chondrocytes. This product is responsible for viscoelasticity of joint fluid and provides a lubricated surface over joint cartilages to move softer.[38] After injection of HA, this "lubrication" effect

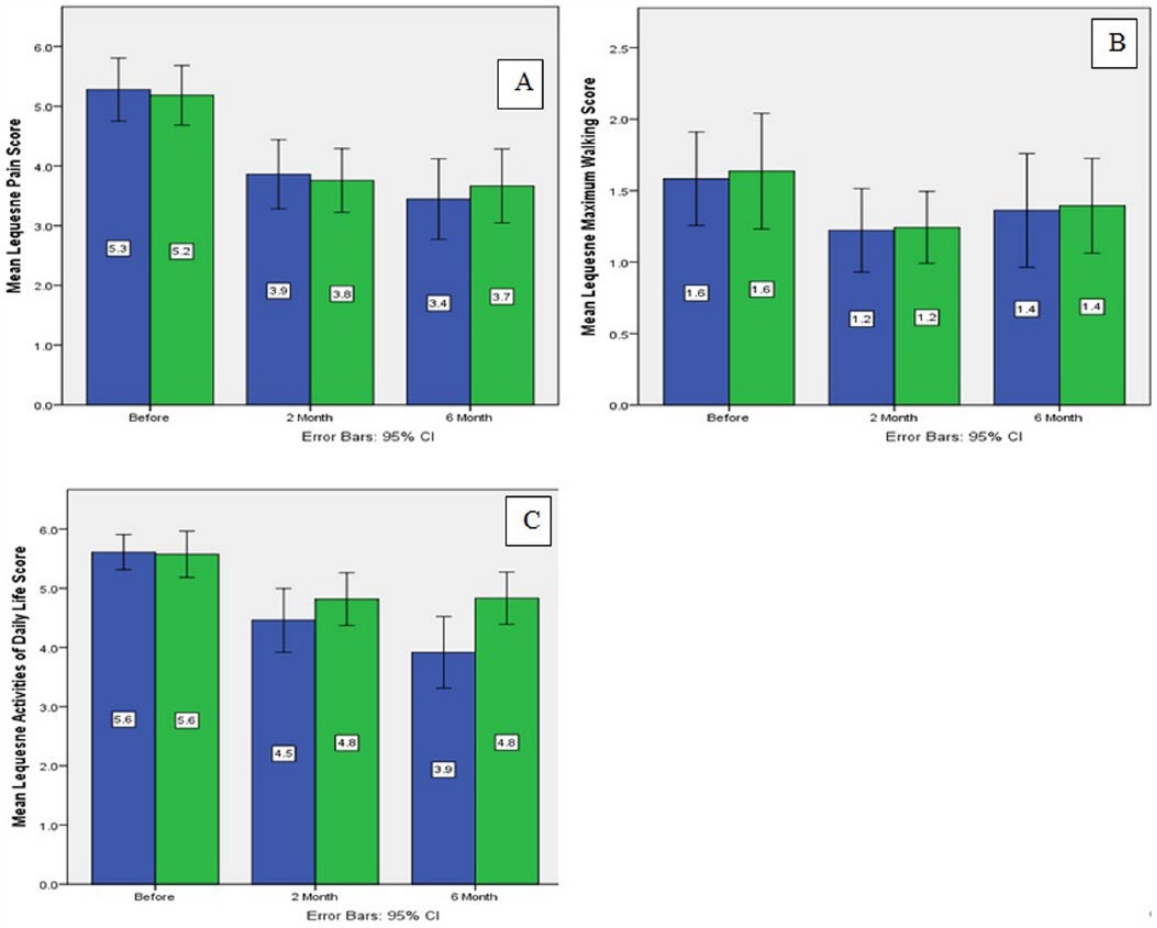

Figure 5. Mean Lequesne index scores at baseline and 2 and 6 months postinjection in PRGF and HA groups. (a) pain subscale, (b) maximum walking subscale, and (c) activities of daily life subscale. Blue: PRGF, green: HA. 95% CI indicates 95% confidence interval; HA, hyaluronic acid; PRGF, plasma rich in growth factor.

Table 3. Rate of patient satisfaction 6 months postinjection.

	PRGF	HA	TOTAL	*P* VALUE	STATISTICAL TEST
Poor	8 (22.2%)	6 (18.2%)	14 (20.3%)	.626	Mann-Whitney *U* test
Regular	4 (11.1%)	5 (15.2%)	9 (13.0%)		
Good	10 (27.8%)	13 (39.4%)	23 (33.3%)		
very good	14 (38.9%)	9 (27.3%)	23 (33.3%)		

Abbreviations: HA, hyaluronic acid; PRGF, plasma rich in growth factor.

Table 4. The rate of minor injection complications and injection-induced pain score in study groups.

	PRGF	HA	TOTAL	*P* VALUE	STATISTICAL TEST
Minor complications due to injection	7 (19.4%)	2 (6.1%)	9 (13.0%)	.154	χ^2
Injection-induced pain score	3.8 ± 2.92	1.9 ± 1.49	2.9 ± 2.50	.016	Mann-Whitney *U* test

Abbreviations: HA, hyaluronic acid; PRGF, plasma rich in growth factor.

normally fades away within a few days at the site of injection and is transient. It seems that it has some role in endogenous HA synthesis, stimulation of chondrocyte metabolism, and inhibition of chondrodegenerative enzymes, as well as inflammatory process. Earlier studies have shown superiority of a combination of NSAIDs and HA over NSAIDs alone after 6 months consumption.[39] Second, PRGF by its pool of growth factor causes repair or regeneration of injured cartilage. There is an anabolic effect of PRGF on chondrocytes and synoviocytes leading to increased cell proliferation and matrix production. In addition, PRGF compounds contain signals that mediate anti-inflammatory effects.[40] Platelet-derived growth factors help chondrocytes to proliferate and maintain their hyaline like phenotype. Insulin like growth factor stimulates proteoglycan synthesis. Transforming growth factor-β has an essential role in differentiation of mesenchymal stem cells and deposition of cartilaginous matrix. In addition, PRGF is a rich source of active biologic compounds that suppress the inflammatory catabolic environment in the joint. The last but exclusive cause of PRGF efficacy lies in its supernatant containing autologous proteins that inhibit the destructive impacts of interleukin 1 on proteoglycan synthesis.[14–19,41,42]

Some studies have reported contrary results to ours. Vaquerizo et al[24] showed superiority of 3 weekly injections of PRGF-Endoret over one injection of long-acting HA (DUROLANE ®, 60mg/3ml, high molecular weight, stabilized gel produced using a unique, proprietary technology, NASHA®) after 24 and 48 weeks. Despite this superiority in controlling pain and improving function, the complications did not differ between tested products.[24] In their retrospective study, Sanchez et al[32] reported advantages of PRGF over HA (5 weekly injections) based on WOMAC scores. However, later, their RCT showed that 3 weekly injections of PRGF-Endoret was associated with considerably more reductions in VAS scores than 3 weekly HA injections ($P < .05$). In contrast, no statistically significant difference was reported in WOMAC scores between 2 groups.[31] In agreement with these findings, Raeissadat et al documented the superiority of plasma products such as PRP over HA using instruments of physical function and quality of life.[23]

A part of available literature is in line with our findings. Some studies compared similar products with our newly developed PRGF (eg, PRP or PRGF-Endoret) with HA and reported no significant difference in pain, physical function, and performance up to 6 months after injection but a change in favor of PRP in the 12th month.[27–29] Nevertheless, Filardo et al[26] found similar results even after 12 months of injection. Both PRGF and PRP are autologous products from the patient's body itself with promising long-term effects compared with HA. Platelet-rich plasma has been had a painful injection, whereas analgesics as effective as NSAIDs are not allowed. We assumed that PRGF has the same benefits as PRP without its drawbacks due to the lack of platelet debris and WBC. Also, there is no contraindication for postinjection NSAIDs use with our newly developed PRGF. We studied these objectives but arrived at similar outcomes in 6-month follow-up, but the study is still ongoing until 12-month follow-up.

There are some explanations for such inconsistent findings when comparing HA and plasma-derived products. There are a number of ways to extract plasma products such as PRP and PRGF. One of the explanations for such inconsistency is producing compounds with different methods and concentrations, yielding different end products despite similar names.[43] Mostly, this difference is related to the concentration of platelets, leukocytes, and growth factors which are the very causes of efficacy in transforming a joint destructive environment into a repairing or regenerating status. We used a new way for production of PRGF that consists of triple centrifuges to clear the end product of any cell or material except growth factors. Aside

from plasma products, different preparations of HA have also been employed. One of these products is long-acting Durolane and another one is short-acting Hyalgan which were used in our study. Another explanation might be the frequency of injections throughout literature. In various studies, there has been a range of single to multiple injections with weekly to every 3- or 4-week schedules.[25,44] As seen in various studies, there is no consensus among investigators on standard frequency of injections. Thus, based on our previous experiences with plasma products[20,21] and trying to make a balance between 2 groups in terms of costs as well, we selected twice-injection schedule. Duration of follow-up is another factor of difference among studies. Several investigators claim that there is no difference between HA and plasma products until 2 and 6 months after injection, but the major difference would emerge at the 12th month postinjection. Thus, our results may have differed if we reported the 12-month follow-up.

Previous studies have emphasized on the superior efficacy of PRP in younger patients[26] with earlier degrees of OA.[45] In various investigations, different patient populations have been recruited with various ages. Our patients were evenly distributed in both groups with similar proportions of grades 2 and 3 of OA. The last cause of inconsistent findings would be different tools that have been used by authors to assess the efficacy of tested compounds on pain and function. These include VAS, WOMAC, Lequesne, SF-36, IKDC (International Knee Documentation Committee), Tegner, and transpatellar circumference. All these have their specific subscales. However, the effect of various treatments is different on each tool and their subscales. We tried to select 3 assessment tools with the highest popularity in this field of research among investigators. The difference although nonsignificant was more prominent in Lequesne compared with WOMAC so that in one subscale called "activities of daily life" it was near significance level ($P = .059$) in favor of PRGF.

One of major limitations of our study was a relatively short follow-up period. Some studies showed that the interval between 6 and 12 months after injection is the very important period in which the difference between HA and PRGF will show up. In other words, the effects of HA may gradually fade away after 6 months of its injection, whereas the effect of plasma products might be durable until 1 year. Failure to blind subjects was another limitation that was due to the extraction of PRGF from patient blood samples and different injection schedules. It was possible but not ethical to draw blood from patients and then to draw away the collected blood in HA group. In fact, the ethics committee insisted on not to collect blood from subjects in HA group. However, the data analyst and the physician responsible for follow-up interviews and visits were kept blinded to allocation.

In summary, our study showed that IAI with both PRGF and HA is somehow equally effective for reducing pain and joint symptoms and improving function in patients with mild to moderate knee OA. The positive effect of both products lasted up to 6 months postinjection with no significant difference. None of the tested products had superiority over one another in terms of both the magnitude and duration of symptom relief as well as function improvement. Longer follow-up might be needed to observe a difference between PRGF and HA.

Acknowledgements

The authors would like to thank their dear colleagues from Physical Medicine and Rehabilitation Research Center and Clinical Research Developmental Center of Shahid Moddares Hospital, Maryam Karimian, Javad Vafipoor, Leila Salehifard, Mehrnaz Mehrabi, Mehrnoush Moeinfard, and Dr Mohammadhossein Mohammadi for their cooperation in data gathering. This article has been extracted from the thesis written by Mrs. Gharooee Ahangar in School of Medicine Shahid Beheshti University of Medical Sciences. (Registration No: 433)

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