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Effect of Dextrose Prolotherapy, Platelet Rich Plasma and Autologous Conditioned Serum on Knee Osteoarthritis: A Randomized Clinical Trial

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ABSTRACT

Knee osteoarthritis (OA) is one of the common degenerative articular disorders that are related to decreased quality of life. Currently, novel biologic therapeutic approaches are introduced in the literature for OA management. In this study, the clinical efficiency of Dextrose prolotherapy, platelet-rich plasma (PRP) and Autologous Conditioned Serum (ACS) injection on the level of pain and function in Knee OA were compared.

A randomized clinical trial was directed on 92 knee OA patients. Patients were randomly divided into three groups: 30 were received dextrose prolotherapy once in a week for three weeks, 30 received autologous PRP for two times with seven days interval, and in the remaining 32 patients 2ml of ACS were injected two times every seven days. Study participants were measured through the Western Ontario and McMaster Universities (WOMAC) score, the Visual Analogue Scale (VAS), at baseline, 1 and 6 months post-intervention.

Both ACS and PRP treated patients showed improvement in pain intensity and knee function during 1 and 6 months pursue; however, this progress was more significant in the ACS group. Dextrose prolotherapy showed no substantial changes in pain and function of the affected knee in treated patients.

Treatment of Knee OA with ACS and PRP injections are associated with pain reduction and knee function improvement. Not only, ACS therapy is more effective than that of PRP, but also due to its less variability in processing and less reported side effects, it could be considered as a safe and effective non-surgical alternative for OA management.

Keywords: Knee osteoarthritis; Platelet-rich plasma

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INTRODUCTION

Aging is usually associated with some musculoskeletal disorders leading to limited daily

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activities and decreased quality of life.[1] Deal with the growing pattern of the elderly population in any community, it is essential to recognize and prevent their musculoskeletal challenges in daily lifestyle.[2] In recent years, the increased elderly population has been associated with an increased number of people with osteoarthritis (OA).[3] OA is a degenerative, progressive, and chronic disorder of joint which involves the cartilage and its surrounded structures.[4] The most common characteristics of the OA are stiffness, pain, joint instability, swelling, and muscular weakness.[4] Knee OA is a pervasive disorder that is associated with weight-bearing and is responsible for activity of daily living (ADL) limitation in the elderly population.[5]

The global prevalence of people suffering from knee OA in 2010 was estimated to be 3.8% worldwide, and it is expected to become the fourth cause of disability in 2020.[6] Risk factors known for knee OA include genetics, female sex, history of trauma, advanced age, improper lifestyle habits, and obesity.[7,8] Whereas special treatment for OA disease is yet unavailable, current treatment modalities focus on symptom management and risk factor modification. Various knee OA treatment strategies include pharmacological (oral analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), intraarticular corticosteroids or hyaluronic acid injections), nonpharmacological (lifestyle modification, physical therapy, therapeutic exercises, orthosis) and surgical management have been proposed in the literature.[9-12]

Nowadays, biologic strategies mainly focus on OA pathology, and researchers are examining new ways to stimulate the regeneration of damaged cartilage.

Prolotherapy is one of the methods which can raise growth factor levels or increases growth factor efficiency to stimulate tissue regeneration.[13] Hackett first described this method in 1950, and various clinical studies have performed this method on humans and animals.[14-17] In dextrose prolotherapy, increased extracellular glucose has been revealed to increase the amount of numerous polypeptide growth factors in a diversity of human cells.[18-22] Additionally, contact of various human cells to a hypertonic dextrose solution may lead to an increase in levels of growth factors.[23,24] It is believed that prolotherapy may increase the levels of growth factors in target injected tissue, and improves the condition of vital cells in the joint like chondrocytes, fibroblasts, and osteocytes. So far, various studies have been done on dextrose prolotherapy; however, its therapeutic effects remain unclear. In one study, prolotherapy injection with 10% dextrose resulted in clinically and statistically significant improvements in knee osteoarthritis.[25] Also, Huang et al in an experimental study, confirmed that pain and function of chronic rotator cuff tendinosis of patients enhanced after hypertonic dextrose prolotherapy injection in a short-term period.[26]

Another reparative method to treat OA is platelet-rich plasma (PRP) treatment. PRP is defined as blood with concentrations of platelets above baseline levels that are obtained from the patient's blood.[27] As such, PRP is safe, cheap, and effective, and no additional procedures are required.[28] PRP contains at least seven growth factors that may enhance the cartilage defects healing.[13,29] Several studies have exposed the useful properties of RPR therapy in clinical trials. In a study, PRP injection into knee OA was ten times effective than that of the placebo group in pain reduction after six months follow up period.[30] However, Filardo et al in a randomized, double-blind prospective trial, contrasted the PRP and Hyaluronic Acid (HA) injections efficacy for the therapy of knee OA moderate signs in middle-aged patients. Data showed that PRP injection result was not better than the HA injection group.[31]

Autologous conditioned serum (ACS), is offered as a new method to manage OA and other musculoskeletal disorders.[32,33] ACS is a procedure, which stimulates the synthesis of interleukin 1 (IL-1) receptor antagonist (IL-1ra) and other anti-inflammatory cytokines in addition to multiple high concentrations of growth factors.[34] A study by Baltzer et al showed that ACS injection significantly recovers clinical signs of OA;[34] however, in another study assessing the *in vitro* effects of ACS and were observed no significant impact on cartilage proteoglycan (PG) metabolism compared to the controls.[35] Therefore, there is no explicit agreement about ACS efficacy in Knee OA management.

The main aim of this paper was a comparison of the effectiveness of three treatment approaches (dextrose prolotherapy, PRP, and ACS intraarticular injection) in knee OA patients for the first time.

MATERIALS AND METHODS

Study Design

A randomized clinical trial was done to assess the efficiency of dextrose prolotherapy, PRP, and

autologous conditioned serum (ACS) in reducing signs of OA in the knee (Clinical Trial Registry Number: IRCT20100720004422N6).

The study was performed in a 12-month period, from January of 2018 among the series of 92 knee OA patients (40-75 years), referred to the Physical Medicine and Rehabilitation Centers at Imam Reza Hospital of Tabriz University of Medical Sciences. The medical ethics committee of the university under No.IR.TBZMED.REC.1397.722 approved this study. The sample size was determined based on a cohen's criteria and G-power software. Considering the 5% false-positive rate (α=0.05), study power of 80% in two-way test and 1.15% possible follow-up loss, the final sample size was determined as 92 (dextrose=30, PRP=30, ACS=32).

The following inclusion criteria for patient selection were used: inflammation, pain, or any other symptom related to knee OA lasting at least three months; radiologic signs of grade II, III and IV knee OA and no use of NSAIDs. The exclusion criteria were as follows: rheumatic disease, any surgical intervention of the knee, infection, liver disease, diabetes, severe cardiovascular disease, coagulopathy, anticoagulant therapy, pregnancy.

After the diagnosis of knee OA by physical medicine specialist, patients who met the American College of Rheumatology criteria for OA were included in study.[7] Informed consent was obtained from all participants in the study, and the objectives of the study and methods were explained. Then, the patients were randomly assigned into three groups: dextrose prolotherapy (n=30), PRP (n=30) and, ACS (n=32) for three weeks. For the random allocation of patients in study groups, we used the RAS (Random) line. The ruler is typically attached by "no pain" (score of 0) and "greater pain intensity" (Score of 100). In this regard, patients were asked to place a mark on the VAS line at the point according to pain severity from the lowest to the highest.[36]

The Western Ontario MacMaster Osteoarthritis Index (WOMAC)

The WOMAC index, a self-administered and disease-specific form, was planned to measure pain, stiffness, and OA-related dysfunction. The person required just about 5 minutes to respond to the WOMAC scale items. In this paper, the Likert type of the Persian version of WOMAC was used. Persian version is a reliable and simple procedure and suggestions five responses, including none (0), mild (1), moderate (2), severe (3), and extreme (4). The higher scores represent the patients' more stiffness, more pain, and more functional restriction.[8]

Dextrose Prolotherapy Method

Dextrose prolotherapy solutions for maximum safety normally consist of sterile water, dextrose, and a small concentration of lidocaine. In this study, we used the combination of 50% dextrose (2 mL), bacteriostatic water (2 mL), and 2% lidocaine (1 mL). The small dose of lidocaine was used aimed at post-injection relief. Dextrose prolotherapy solutions were injected into the knee joint by a skilled specialist once a week for three weeks under the ultrasound guidance through the supra-lateral approach.

Platelet-rich Plasma Preparation

For PRP preparation, about 20 mL of venous blood was drained under aseptic precautions each time. Then, centrifuged two times, first at 1600 rpm for 13 minutes to separate erythrocytes, and a second at 3500 rpm for seven minutes to concentrate platelets to yield a PRP unit. The unit of PRP was contained 4X concentration of platelets and the lowest leukocyte. Platelet concentrate was injected into the knee joint by a skilled specialist under aseptic conditions 2 times every 7 days through the supra-lateral approach. The knees were immobilized for 10 minutes after injection.

Autologous Conditioned Serum (ACS) Preparation

20 mL of whole blood was taken from each patient under aseptic condition by sterile syringes containing glass beads. A combination of bioactive materials coated the glass beads, binder resin and additives to secure adhesion of the materials to the glass beads. Blood filled syringes incubated for 6-9 hours in 37°C to induce the production of IL-1Ra by white blood cells in whole blood. The blood-filled syringes were centrifuged after incubation, and the serum supernatant was separated. After aseptic harvesting, the serum was used. Autologous conditioned serum (2 mL) was injected into the knee joint by a skilled specialist under aseptic conditions two times every seven days through the supra-lateral approach.

Statistical Analysis

Analysis of the data was done using SPSS v.17

(SPSS Inc, Chicago, Illinois). All data were presented as mean±standard deviation (SD) for qualitative variables and frequency (percentage) for quantitative variables. The Kolmogorov-Smirnov test was used for the normality of quantifiable data. To compare means of the variable after the intervention, between intervention and control groups and measurement of variables fundamental used covariance analysis (ANCOVA). Mixed ANOVA was also used to compare the variation of quantitative variables before and after intervention in each group. Chi-square and independent t-test were used to analyze and measure the patient's baseline. A $p<0.05$ was considered as statistically significant.

RESULTS

Demographics and Baseline Characteristics

A total of 92 patients with knee OA were randomized to take dextrose, PRP, and ACS. No treatment complications and loss of follow-up were noted. Considering Table 1, baseline features of the dextrose, PRP, and ACS groups were similar. No substantial differences between the treatment groups in age, gender, or body mass index (BMI) before and during the study period. Additionally, the outcome measures of VAS and WOMAC total score were not significantly different in treatment groups at baseline assessment.

VAS Pain Scores

The intragroup analysis showed that pain intensity means in the dextrose prolotherapy group was not significantly different after one and six months of intervention ($p>0.999$). In the PRP group, one month after intervention, the mean of pain intensity was meaningfully decreased compared to the baseline level ($p=0.019$). However, this decrease was not sustainable in the long run period ($p=0.274$). In the ACS group, the mean pain intensity showed a significant decline than that of the baseline levels in the short term ($p=0.011$), and this decrease remained stable over the long time after six months ($p< 0.001$) (Table 2) (Figure 1).

The intergroup analysis of the groups showed that the pain intensity means at the beginning of the study were similar for all three groups ($p=0.120$). The pain intensity mean in the dextrose group did not differ significantly than the PRP group equally in the short term and the long run ($p=0.120$ and $p=0.891$). ACS was significantly able to control the pain intensity in a short time and long run compared to dextrose ($p=0.044$ and $p<0.001$, respectively) ($p<0.001$). However, during a month, there were no significant differences in pain intensity mean between ACS and PRP groups ($p>0.999$); however, the results showed that ACS was able to reduce pain in the long run ($p<0.001$) (Table 2) (Figure 1).

Table 1. Demographic characteristics of participants at the baseline level

Variable		Group			*p*-value
		Dextrose (n= 30)	PRP (n= 30)	ACS (n= 32)	
Female, n (%)		15 (50.0 %)	14 (46.7 %)	20 (62.5 %)	0.417 †
Age, yrs		57.90 ± 1.62	58.93 ± 1.71	61.28 ± 1.67	0.338 ‡
BMI, n (%)	Normal (18.50 – 25.00	9 (30.0)	5 (16.7%)	4 (12.5%)	
	Overweight (25.01 – 30.00)	14 (46.7%)	13 (43.3%)	11 (34.4)	0.150 ⁑
	Obese class I (> 30.01)	7 (23.3%)	12 (40.0%)	17 (53.1%)	
Grade, n (%)	II	7 (23.3%)	5 (16.7%)	6 (18.8 %)	
	III	12 (40.0%)	16 (53.3%)	9 (28.1%)	0.287†
	IV	11 (36.7%)	9 (30.0%)	17 (53.1%)	
VAS baseline (0-96)		67.00 ± 2.50	61.10 ± 1.21	61.25 ± 3.44	0.120 ‖
WOMAC total baseline (0-96)		65.93 ± 1.67	60.33 ± 3.70	56.28 ± 3.13	0.103 ‖

All values are mean ± S.E. or frequency (percentage); † Chi-square test; ‡ One-way Anova; ⁑ Exact test; ‖ Kruskal-Wallis test.

Table 2. Knee pain and function outcomes in participants

Variable	Dextrose (n= 30)	PRP (n= 30)	ACS (n= 32)	Difference between	Difference between	Difference between

				Dextrose and PRP	Dextrose and ACS	PRP and ACS
VAS† (0-96)						
Baseline	67.00 (2.50)	61.10 (1.21)	61.25 (3.44)	5.90 (2.78) > 0.999	5.75 (4.26) > 0.999	-0.15 (3.65) > 0.999
1 month	63.33 (2.47)	56.33 (1.021)	46.88 (4.45)	7.00 (2.67) 0.319	16.46 (5.09) 0.044	9.46 (4.57) > 0.999
6 months	63.30 (2.92)	55.00 (2.27)	35.00 (3.51)	8.30 (3.70) 0.891	28.30 (4.56) < 0.001	20.00 (4.18) < 0.001
WOMAC‡ (0-96)						
Baseline	65.93 (1.67)s	60.33 (3.70)	56.28 (3.13)	5.60 (4.05) > 0.999	9.65 (3.54) 0.231	4.05 (4.84) > 0.999
1 month	71.67 (2.95)	46.67 (4.30)	49.53 (3.67)	25.00 (5.22) < 0.001	22.14 (4.71) < 0.001	-2.86 (5.66) > 0.999
6 months	72.33 (2.57)	45.67 (3.82)	34.88 (3.35)	26.67 (4.60) < 0.001	37.46 (4.22) < 0.001	10.79 (5.08) > 0.999

†VAS: Visual analog Scale; ‡WOMAC: The Western Ontario MacMaster Osteoarthritis Index

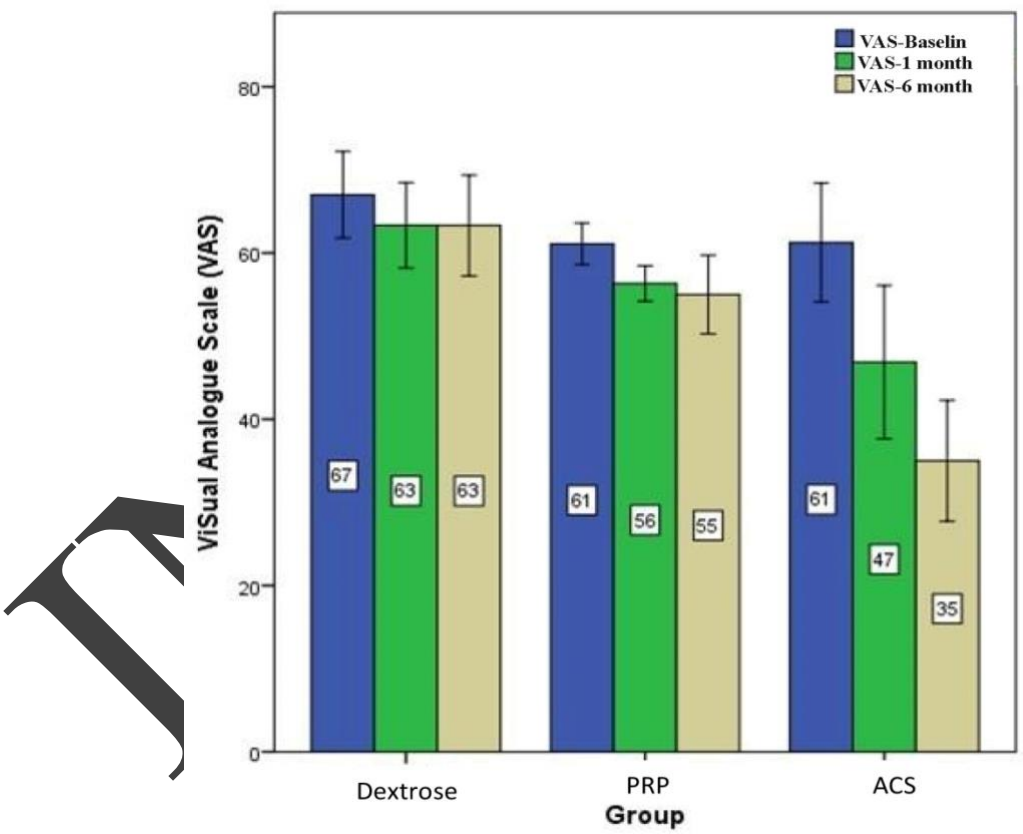

Figure 1. Knee pain outcome in participants. VAS: Visual analog Scale

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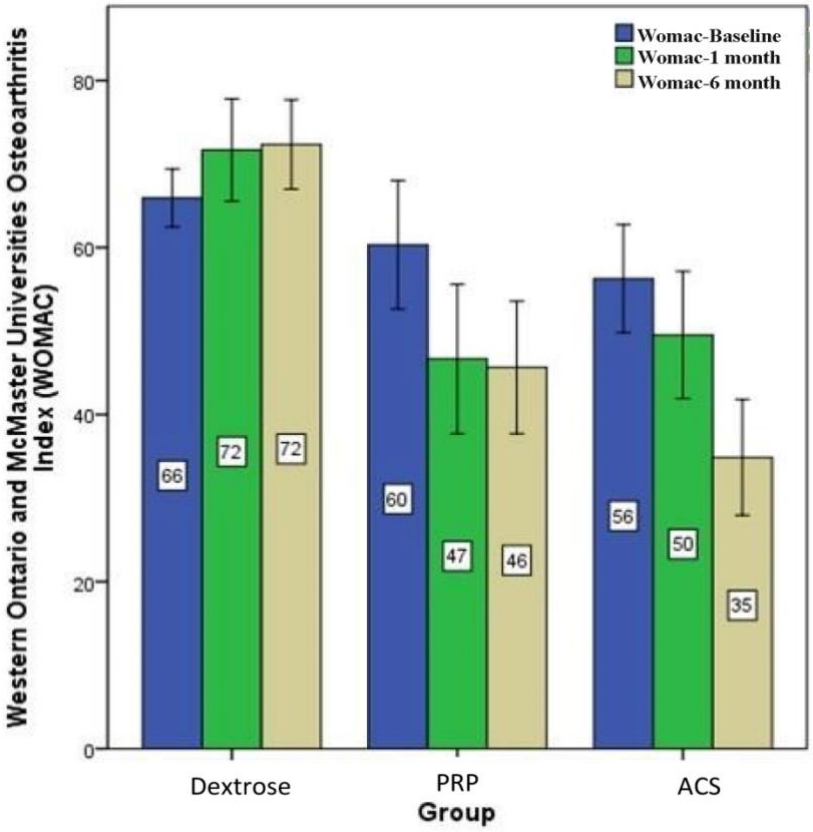

Figure 2. Knee function outcome in participants. WOMAC: The Western Ontario MacMaster Osteoarthritis Index

WOAMC Total Score

The intragroup analysis of the WOAMC score in intervention groups showed that the WOAMC score means in the dextrose group was not significantly different after one month (p>0.999). Moreover, after six months, the WOAMC score mean in this group showed no significant changes in comparison to baseline levels (p>0.999). In the PRP group, in the short term, the WOAMC score mean was not also significantly different compared to baseline levels (p=0.108); however, after six months, this score was significantly decreased in this group (p=0.037). Likewise, in ACS group the WOAMC score means showed no significant changes after one month, (p>0.999), while after six months this score showed a significant decrease (p<0.001) (Table 2) (Figure 2).

The intergroup analysis of the WOAMC score in intervention groups displayed that the WOAMC score means at the beginning of the study was not significantly different among groups (p=0.103). In contrast, the WOAMC score say was significantly higher in the dextrose group than that of the PRP group in the short and long term (p<0.001 and p<0.001). ACS compared to dextrose also significantly controlled the WOAMC score in a short time and long run (p<0.001, p<0.001, respectively). However, no significant differences were observed in the WOAMC score mean between ACS and PRP groups in the short and long term (p=0.999 and p=0.999) (Table 2) (Figure 2).

DISCUSSION

Osteoarthritis (OA) is a common progressive, chronic disorder of the articular cartilage associated with pain, disability, and eventually deformity of the affected joint.[37,38] Numerous studies have stated that the prevalence of Knee OA is high in older people, mainly older women, and this disease is one of the key reasons for musculoskeletal disability among this population.[33,34,37,39] Pain and loss of function are the key clinical features leading to physical disability and reduced quality of life in patients with knee OA.[40]

Growth factors and cytokines excreted into the intraarticular environment are responsible for cartilage degradation in OA and are more secreted by the articular cartilage itself, activated immune cells and synoviocytes.[41] Therapies may decrease symptoms and prevent further functional impairment, and the patient's quality of life improves; however, no definite approaches have been introduced to stop the progression of this disease, yet.[39,42-44] Recently, new methods, including prolotherapy, platelet-rich plasma (PRP) treatment, and autologous conditioned serum (ACS), are getting clinical attention to treat knee OA and cartilage lesions.

Prolotherapy is a complementary injection therapy (Hypertonic Dextrose) for knee OA, which has been promoted tissue repair or growth by increasing growth factor levels in the target tissue.[25,45,46] Rabago et al in an uncontrolled pilot study, reported that pain, function, and stiffness of patients with Knee OA are significantly improved at 52 weeks after treatment with dextrose prolotherapy.[46] Additionally, this group in 2013 compared dextrose prolotherapy with saline injections and at-home exercise groups in adults with symptomatic Knee OA and observed important signs of progress in composite WOMAC scores at 26 and 52 weeks in dextrose prolotherapy group.[45] Another study was characterized the morphological, biological, and mechanical healing responses of the rat with stretch-injured medial collateral ligaments (MCLs) treated with prolotherapy and showed that MCLs of rat in dextrose group were meaningfully larger than those in the saline solution control group and uninjured ligaments. Still, changes in laxity and strength properties (biomechanical outcomes) were not seen.[47] By contrast, in our study, there was no significant progress in WOMAC overall score after dextrose prolotherapy in Knee OA patients. Furthermore, in the current study, the pain intensity means in Knee OA patients after dextrose prolotherapy was not significantly different compared to baseline levels. This may be because ofthe small sample size (only 30 patients) and a short follow-up period.

PRP is another alternative method in OA treatment. PRP mainly contains concentrated platelets and growth factors that could regulate the inflammatory response and cause curing over a long period.[48,49] The chief advantages of PRP are a low expense, simple preparation, and easily obtainable from the patient’s blood.[28] Chang et al in 2014 studied and compared the effects of intra-articular PRP and HA injection in knee OA in a systematic review. The study verified that PRP results in significant functional progress in patients with knee cartilage pathology, whose effects last at least 12 months. Patients in the PRP group, compared to patients receiving HA, had further and longer improvement. Also, there were better improvements among those patients with milder forms of OA than advanced ones.[50] Many studies reported that PRP treatment had significantly decreased the total WOMAC score of patients with knee OA and improvement of the quality of life.[51,52] Similar outcomes were obtained in another study; Ahmad et al compared the effectiveness of PRP and Hyaluronic acid (HA) intra-articular injections for primary Knee OA and showed better development in pain and functional status in PRP received patients compared to HA injection.[34] Rendering to this work and similar studies, considering the side effects of anti-inflammatory and pain-relieving treatments, PRP therapy can be considered as a beneficial and safe healing choice in selected patients with OA who fail to respond to new treatments including ADL modification, physical modalities, and therapeutic exercise. In the present study, there was a significant decrease in the total WOMAC score of patients with knee OA in the PRP group after six months. Moreover, PRP reduced pain intensity; however, this decrease was not sustainable in the long run.

Autologous conditioned serum (ACS) is considered as another therapeutic method and plays an important role in Knee OA improvement by numerous cytokines up-regulation, including IL-1Ra.[53,54] ACS usage might have a positive result on the pain and function of Knee OA. Recent reports demonstrated that ACS injection was associated with reduced pain, improved joint function, and enhanced quality of life in knee OA patients.[55,56] García-Escudero & Trillos found that mixture therapy of ACS and physiotherapy significantly decreased pain, which this decline was sustained after two years of the study and also showed a large development in WOMAC scores at two years. Therefore, ACS pooled with physiotherapy was an efficient treatment for Knee OA.[57] By contrast, Zarringam et alstudied the long-term result of ACS injection treatment and they presented that the medical usage of ACS has no preventive or delaying effect on surgical treatment for end-stage knee OA, compared to placebo after ten years treatment.[58] The present study displayed a decrease in knee pain intensity score in the ACS intervention group, in which this decline was stable

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in the long-term. Likewise, there was a significant decrease in the total WOMAC score of patients with knee OA in the ACS group after six months. Therefore, the outcome of this study was well-matched with García-Escudero & Trillo's literature. It is believed that ACS may subside OA symptoms by two mechanisms. Firstly, since ACS (unlike PRP) contains IL-1Ra as an important anti-inflammatory molecule, it can block interleukin one receptors, which are presented on the surface of chondrocytes. This blockade may result in the prevention of chondrocyte apoptosis and cartilage degradation. Secondly, ACS has high concentrations of autologous growth factors such as Epidermal Growth Factor, Fibroblast Growth Factor, Tissue Growth Factor-β, Vascular Endothelial Growth Factor, Insulin-like Growth Factor, and Platelet-derived Growth Factor which are responsible for cartilage repair and regeneration in joint. In summary, ACS has both anti-inflammatory (which is not presented in PRP) and regenerative properties.

There were limitations to this study. First of all, due to the limited budget, we were not able to have a long-term follow-up period for 12 or 24 months. Secondly, due to the different characteristics of injected materials (color and viscosity), it was not possible to design a double-blinded study. Finally, our results indicate that ACS and PRP treatment are more effective than that of dextrose in terms of VAS and WOMAC scores for the management of knee OA symptoms. Therefore significant pain relief, stiffness reduction, and improvement in function can be reached with ACS and PRP in knee OA patients. The authors consider that the ACS and PRP can increase the quality of life in knee OA patients in the long term and may delay joint surgery.

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