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ARTHROSCOPY AND SPORTS MEDICINE

Multiple platelet-rich plasma injections are superior to single PRP injections or saline in osteoarthritis of the knee: the 2-year results of a randomized, double-blind, placebo-controlled clinical trial

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Abstract

Introduction The primary purposes of this study were to prove the efficacy of PRP injection therapy on knee pain and functions by comparing patients with mild to moderate OA with a placebo control group, and also to understand the effectiveness of multiple doses compared to a single dose. It was hypothesized that PRP would lead to more favorable results than the placebo at 1, 3, 6, 12 and 24 months after treatment.

Materials and methods 237 patients diagnosed with OA were randomly separated into 4 groups, who were administered the following: single dose of PRP (*n*: 62), single dose of sodium saline (NS) (*n*: 59), three doses of PRP (*n*: 63), and three doses of NS (*n*: 53). Clinical evaluations were made pre-treatment and at 1, 3, 6, 12 and 24 months post-treatment, using the Knee Injury and Osteoarthritis Result Score (KOOS), Kujala Patellofemoral Score, knee joint range of motion (ROM), measurements of knee circumference (KC), and mechanical axis angle (MAA) and a Visual Analog Scale (VAS) for the evaluation of pain.

Results The better score values in the groups were recorded at 3 and 6 months. Patients treated with PRP maintained better scores at 3, 6 and 12 months compared to the NS groups ($p < 0.05$). Multiple doses of PRP were seen to be more effective than single-dose PRP at 6 and 12 months ($p < 0.05$). At the end of 24 months, there was no significant score difference across all the groups. The most positive change in scores was found in stage 2 OA, and the most positive change in ROM was in stage 3 OA patients. In the PRP groups, KC decreased more at 1 and 6 months ($p < 0.05$). Compared to other age groups, patients aged 51–65 years scored better at 6 months ($p < 0.05$). A negative correlation was determined with MAA scores ($r = -0.508$, $p < 0.001$).

Conclusion In comparison to the placebo (NS), leukocyte-rich PRP treatment was determined to be effective in the treatment of OA. Multiple doses of PRP increase the treatment efficacy and duration. Of all the patients treated with PRP, the best results were obtained by patients aged 51–65 years, with lower MAA, and by K/L stage 2 OA patients.

Study design Randomized controlled trial; Level of evidence, 1.

Registration NCT04454164 (ClinicalTrials.gov identifier).

Keywords Osteoarthritis · Knee · Platelet-rich plasma · Intra-articular · Injections

What is known about the subject OA of knee is a major cause of chronic pain and musculoskeletal disability worldwide. OA causes pain and disability in patients and has a significant impact on society. Although there are many pharmacological and non-pharmacological treatment methods have been described in the treatment of OA, today, there is no definitive treatment that can prevent, stop or limit the progression of OA. PRP has recently been used by many physicians worldwide in the treatment of OA, and many trials have been conducted to understand the effectiveness of PRP treatment. However, these studies have not been standardized in terms of treatment approaches, evaluation scores, PRP preparation techniques, platelet counts, the use

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of anticoagulants, activating agents, separation techniques, injection numbers and frequency, and volume differences.

What this study adds to existing knowledge The results of this study showed that PRP has a positive effect on pain and clinical scores in the treatment of OA compared to a placebo, the efficacy duration of multiple doses in treatment is longer than a single dose, an increase in the MAA of the lower extremities has a negative effect on PRP treatment, and the patient group aged 51–65 years benefited more from PRP treatment. PRP treatment is not recommended for patients with advanced varus or valgus alignment, and surgical treatment options should be considered in patients with severe structural and anatomic changes in the knee joint.

There is no study in literature which has evaluated the relationship between PRP and the patellofemoral joint using the Kujala Patellofemoral Score. PRP treatment was seen to have a positive effect on the Kujala Patellofemoral Score, although no superiority of multiple doses over a single dose could be demonstrated on this score.

There is no clinical study in literature that has examined the relationship between PRP treatment and lower extremity MAA. PRP treatment affects the biochemical environment within the joint but cannot directly affect the structure of the joint. PRP treatment is not recommended for patients with advanced varus or valgus alignment. Surgical treatment options should be considered in patients with severe structural and anatomic changes in the knee joint.

The strengths of this study were the inclusion of a placebo control group, that patients were randomly grouped, it was double blinded with neither patient nor physician knowing the type of treatment applied, standard PRP preparation technique and outcome evaluation methods were used in all patients, multiple injection effectiveness was investigated in both the patient group and control group, the total number of patients is the highest number of subjects in the literature and the patient follow-up period is longer compared to the literature (2 years).

Introduction

Joint damage associated with OA should be considered as an organ failure similar to kidney or heart failure. Considering the pathophysiology of the disease, the products formed in anabolic and catabolic processes play a role in the development of the disease. At the end of this process, joint failure develops. For many years, the pathogenesis of OA is based on the thesis that cartilage degeneration develops as a result of mechanical loading on the joint for a long time, nowadays it is known that it is a disease that concerns the whole joint in which matrix proteases play a major role [1]. While growth factors, transforming growth factor (TGF-β) and chondrocytes repair the damage in cartilage tissue, matrix metalloproteinase (MMP)-1,3,13 and anti-aggregated enzymes—ADAM-4 and -5 damage cartilage tissue [2]. The balance between these anabolic and catabolic processes is disturbed. The chronicity of synovial inflammation in the joint causes macrophages to accelerate the catabolic process and this leads to the release of pro-inflammatory cytokines (IL-1β, IL-6, Tumor necrosis factor-alpha (TNF-α)) [3]. Besides these processes, pathological changes in the subchondral bone that can be seen in all stages of OA play an important role in the pathogenesis. In this pathological process, osteophyte formation, subchondral sclerosis, and chondral damage appear as radiological findings.

OA causes loss of joint cartilage resulting in a narrowing of the joint space. The treatment of cartilage disease is very difficult as cartilage tissue is isolated from the vascular, nervous and lymphatic systems, and so the potential for self-renewal of cartilage tissue is very low [4]. There are many studies conducted to stimulate cartilage tissue repair and to provide tissue formation to replace damaged cartilage tissue. In vitro and in vivo studies have shown that growth factors are effective in self-repair of cartilage tissue [5, 6]. Therefore, newly developed treatment methods have focused on stimulating the cartilage healing process and improving the damage that has already occurred. One of these treatment methods is platelet-rich plasma (PRP), a liquid rich in autologous growth factors [7] and rich in growth factors, cytokines, chemokines and other mediators [8]. PRP is obtained by centrifuging the patient's own blood, and the platelet rate in the obtained liquid is 4–5 times higher than in the blood. Growth factors revealed by the degranulation of platelets play an important role in the angiogenesis and proliferation of chondrogenic cells, secretion of the cartilaginous matrix, tissue remodeling and wound healing [9, 10].

The main goals in OA treatment are: to reduce joint pain and stiffness, increase functional capacity, reduce joint damage and increase quality of life. Many treatment options can be combined by considering the risk factors and symptoms of OA together. Among the several available guidelines for the management of knee OA, those from Osteoarthritis Research Society International (OARSI) and The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) were updated in 2019. Intra-articular PRP therapy has been strongly denied entry into the OARSI and ESCEO treatment guidelines for OA disease. Because the level of evidence of studies on the effectiveness of this treatment is very low and standards in the treatment protocol could not be achieved [11]. More studies with high evidence level are needed to prove the effectiveness of PRP treatment in terms of evidence-based science and to be included in OA treatment guidelines.

PRP treatment has been used by many physicians worldwide for the last three decades. PRP is rich in growth factors

and bioactive proteins. Thus, it stimulates recovery at the cell level. It is reported that PRP stimulates the healing, proliferation and differentiation involved in the regeneration of tissues [12, 13]. Many studies have been conducted on the use of PRP in OA treatment. Few of these studies are clinical trials with a control group [14–16]. Although it has been reported in many studies in the literature that PRP is effective in the treatment of OA [14–25], it has been reported that the effect of PRP is limited in the treatment of OA in some studies [26–29]. There are studies reporting that the success of PRP treatment is higher in patients with early-stage OA, young patients and low body mass index (BMI) [14–16]. Studies with high scientific evidence to show the effectiveness of PRP treatment in OA are lacking. In addition, the patient group that will benefit most from this treatment is not clear. Treatment approaches, evaluation scores, PRP preparation techniques, platelet counts, use of anticoagulants, activating agents, separation techniques, injection numbers and frequency, volume differences were not standardized in most studies [30, 31].

The primary purposes of this study were to prove the efficacy of PRP injection therapy on knee pain and functions by comparing patients with mild to moderate OA with a placebo control group, and also to understand the effectiveness of multiple doses compared to a single dose. It was hypothesized that PRP would lead to more favorable results than the placebo at 1, 3, 6, 12 and 24 months after treatment. A secondary purpose of this study was to understand the effect of lower limb mechanical axis angle (MAA) on PRP effectiveness. At the same time, we tried to understand the effect of PRP treatment on the patellofemoral joint using the Kujala Patellofemoral Score. It was also aimed to determine the indications for PRP treatment with this study by answering the question of which patient group benefits most from this treatment method. To provide a high level of scientific evidence, the PRP preparation and application techniques were standardized, high numbers of patients were followed prospectively for 2 years, patients were grouped with a randomization method, and a placebo was used as the control group.

Materials and methods

Study design

This prospective, randomized, placebo-controlled, double-blind study was conducted with the approval of the **Republic of Turkey** Ministry of Health, General Directorate of Health Services, Department of Blood, Organ and Tissue Transplant Services, Root Transplants Scientific Advisory Commission (Approval number: 56733164/203). All the details of this clinical study were explained to the patients, and all participated in the study voluntarily, providing written informed consent. All the study procedures complied with the principles of the Declaration of Helsinki.

Sample and sampling

The study included a total of 324 patients who presented at the Department of Orthopedics and Traumatology at **Ondokuz Mayıs** University Faculty of Medicine between 2018 and 2020. The patients included were those who were diagnosed according to the American College of Rheumatology criteria [32] and with stage 1–2–3 symptomatic OA according to Kellgren/Lawrence (K/L) staging [33], aged 18–80 years, and with a mean VAS pain score of > 4 of 10 (worst possible pain) over the course of 7 days during the previous month. Bilateral injection was not applied to any of the patients.

Exclusion criteria were OA secondary to joint inflammatory diseases, metabolic bone disease, coexisting backache, the presence of hematological disease (coagulopathy), bilateral symptomatic lesions, advanced stage OA (K/L grade 4), intra-articular injection made within the previous 3 months or arthroscopic lavage in the previous 1 year, the use of immunosuppressive drugs, current use of anticoagulant medications or non-steroidal anti-inflammatory drugs (NSAIDs) used in the 5 days before blood sampling, major axis deviation of the knee (> 15° varus or > 5° valgus deviation), hemoglobin level < 11.5 g/dL and platelet level < 100,000/μL or associated comorbidities, infection, tumor, crystal arthropathies, anemia, intense joint effusion, or known or possible pregnancy.

The power analysis of this study was calculated based on the sample size studies of previous studies. The difference of $d = 3$ units between the two means is to have a standard deviation of 6 units. A sample size of 200 was required for 95% power in a 95% confidence interval. All patients were selected according to pre-defined and established inclusion and exclusion criteria.

The patients included in the study were divided into 4 different groups using a computer-assisted randomization program (www.random.org/integers). The groups and treatments were as follows: Group A (*n*: 67) patients were administered a single injection of PRP, Group B (*n*: 69) a single injection of normal saline (NS: physiological control/placebo), Group C (*n*: 66) 3 injections of PRP, and Group D (*n*: 65) 3 injections of NS.

PRP preparation and implementation procedure

A total of 32 ml peripheral venous blood was taken from the antecubital vein. While taking blood, attention was paid to aseptic conditions and care was taken to be atraumatic in order not to damage the platelets. The blood was collected

in 8 sterile tubes of 4.5 mL with 3.2% sodium citrate as an anticoagulant (Fig. 1A). The tubes were then centrifuged once for 10 min at 1800 rpm.

After centrifugation, whole blood is divided into 3 layers according to gravity: layer 1—plasma (top layer), layer 2—platelets and leukocytes (middle layer, "buffy coat"), layer 3—erythrocytes (bottom layer). After the centrifugation processes, there was approximately 4 ml of blood; 2 ml of plasma at the top, 0.2 ml of buffy coat in the middle and 1.8 ml of erythrocyte layer at the bottom. These values are approximate because the layers are measured differently in each patient (different level of erythrocyte volume). With this technique, the Anitua method, which is frequently preferred in the separation process, was modified [34]. To standardize the separation process, the entire middle layer (0.2 ml) of each patient and the first layer of 0.8 ml, rich in platelets, just above the middle layer were collected. Thus, it was attempted to obtain approximately the same layers from each patient, although the layers differ between patients (Fig. 1B). The 1.2 ml portion of the top plasma and 1.8 ml of erythrocyte layer were removed and discarded. The remaining 0.8 ml plasma and 0.2 ml buffy coat were transferred to

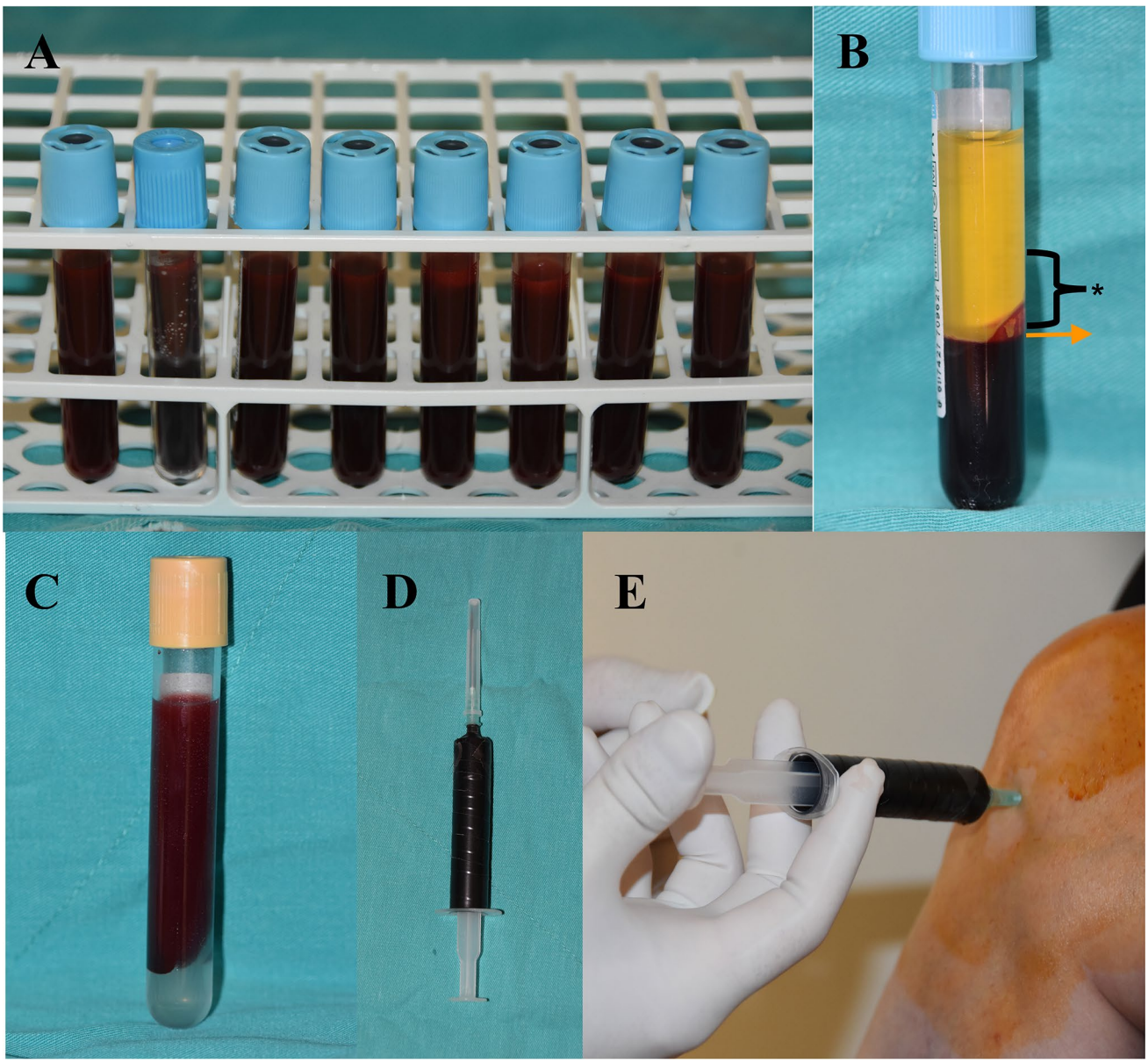

Fig. 1 **A** 8 tubes of a total of 32 ml blood prepared for centrifugation. **B** The appearance and layers formed after centrifugation. The orange arrow indicates the buffy coat layer (0.2 ml). *The platelet-rich layer (0.8 ml) on the buffy coat collected from each patient is shown. **C** In total, 8 ml of PRP was obtained. **D** The solution ready for injection was transferred to the syringe covered with black sterile tape. **E** Injection administration (anterolateral and inferior portal)

a separate sterile tube. A total of 8 ml of PRP was collected, 1 ml from each tube. This procedure was performed by the same study nurse for each patient. Of the 8 ml final PRP obtained, 5 ml was injected into the patient's knee through the anterolateral portal under sterile conditions (Fig. 1C, E), and the remaining 3 ml PRP was sent to the laboratory for platelet and leukocyte measurement. For platelet activation, 5.5% calcium chloride (CaCl2) (50 µl CaCl2 in 1 ml PRP) was used. The separation procedure was completely performed inside the biosafety cabinet**.** PRP with high concentration leukocytes (9000–11,000 leukocytes/µL) as leukocyte-rich PRP. The platelet count was measured to average 128×10^5/µl (> 5 × patients' baseline). The product is type 2A as per the Mishra classification [35]. Following the injection, the patients were observed for an average of 15–20 min until it was ensured that no side effects had occurred.

Blood collection, centrifugation, and PRP preparation were performed by a separate blinded study group and took a total of 30 min for each patient. Blinded study groups: it consists of 4 healthcare professionals who take blood from patients, 1 working nurse who performs PRP separation, 1 physician who administers the injection, and 3 physicians who interview patients. Since these groups were independent from each other, study blindness was achieved. The blinded study group prepared either PRP or NS according to the study randomization list. Following the necessary preparation procedures, the solution prepared for injection (PRP or NS) was covered with sterile black tape and delivered to the physician who would perform the injection (Fig. 1D). Thus, both patient and physician who evaluated the patient were blinded to the groups. The PRP or NS was injected into the knee joint with an anterolateral approach. Multiple injections of PRP or NS were completed with three doses at one-month intervals.

During the follow-up period (especially in the first 6 months), NSAIDs were not allowed, and paracetamol (dosage, 500 mg tds) was prescribed in case of discomfort; all patients were advised not to use anti-inflammatory or anti-analgesic drugs as much as possible.

Outcome measures

The pain complaints of the patients were evaluated with the Visual Analog Scale (VAS) and Knee Injury and Osteoarthritis Result Score (KOOS) pain subscale scoring system. The patients were administered a satisfaction assessment questionnaire at 1, 3, 6, 12 and 24 months after the treatment. In the clinical evaluations of the patients, pain, joint effusion, movement, stiffness and other symptoms, function in daily living (ADL), function in sport and recreation (Sport/Rec) and knee related quality of life (QOL) were evaluated with the 5 subscales of the KOOS scoring system. The Kujala Patellofemoral Score (KUJALA) was used to understand the effect of the PRP on patellofemoral joint function. A long-leg standing radiograph was taken of each patient, and this was used for the measurement of the MAA of the lower limbs. Knee joint range of motion (ROM) was measured using a goniometer at each outpatient follow-up examination. Knee circumference (KC) was measured midpatellar technique using a tape measure. All parameters were measured before the injection and at 1, 3, 6, 12 and 24 months after injection by a blinded observer. Any side-effects that may have been caused by the treatment were noted during the follow-up examinations.

Statistical analysis

The data obtained were evaluated using SPSS v.20 software (Statistical Package for Social Sciences). A value of $p < 0.05$ was considered statistically significant. In the comparison of two independent groups showing normal distribution, two Independent t-tests were performed, and One-way Analysis of Variance (One-Way Anova) was used to compare more than two groups. The Kruskal–Wallis H test was performed to investigate differences between more than two independent groups that did not conform to normal distribution. When there was a difference between the groups, to determine from which group or groups this difference originated, the Mann–Whitney U test was used to compare the two groups. A new limit level was calculated by dividing the 0.05 value of Type-1 error limit level to the number of comparisons. A new p value was obtained using post hoc Bonferroni correction.

Results

The follow-up of 237 patients was completed and statistical analysis was performed (Fig. 2). The average follow-up time in all groups is 24 months. There were no significant differences between the 4 groups according to age, BMI, MAA, baseline scores of VAS, KOOS and KUJALA ($p > 0.05$) (Table 1).

The most common side effect was an increase in knee pain after 10 days of the injection (16%) followed by dizziness (11.8%), tachycardia (10.9%), sweating (9.2%), knee swelling (7.2%), headache (5.1%), syncope (2.9%), and gastritis (0.8%). Statistically significantly more side effects were observed in the group A, compared to the group B ($p = 0.006$) and in the group C, compared to the group D ($p = 0.019$) (Table 2). There was no statistically significant difference between the two PRP groups in respect of side effects ($p > 0.05$). No signs of infection were observed in any patient.

The satisfaction rates of all the groups at 1, 3, 6, 12 and 24 months were noted as follows respectively; 74.2%,

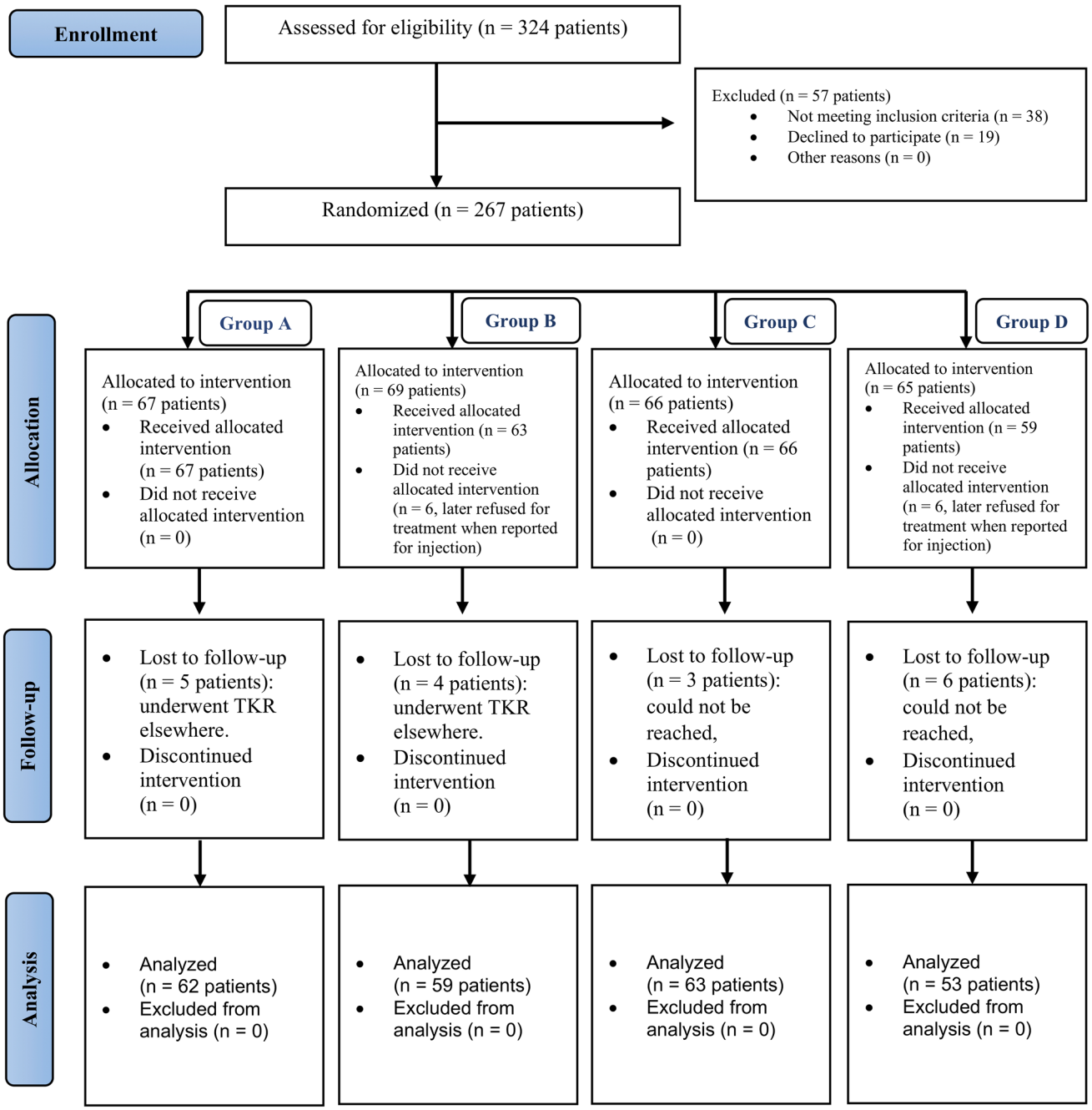

Fig. 2 Consolidated Standards of Reporting Trials (CONSORT) flow diagram used in the design of the trial. **TKR* Total Knee Replacement

67.7%, 61.3%, 32.3% and 16.1% in group A; 64.4%, 33.9%, 16.9%, 8.5% and 5.1% in group B; 85.7%, 81%, 73%, 61.9% and 38.1% in group C; 75.5%, 52.8%, 39.6%, 24.5% and 7.5% in group D. The proportion of patients satisfied with their treatment showed a significant decrease after 6 months in group A, after 12 months in group C, after 1 month in group B and after 3 months in group D ($p < 0.05$). No statistically significant difference was determined between the PRP groups and NS groups at the end of the first month ($p > 0.05$), and there was a statistically significant difference at 3, 6, 12 and 24 months ($p < 0.001$). No statistically significant difference was found between the group A and group C in terms of patient satisfaction in the first 6 months ($p > 0.05$), and a statistically significant difference was found at 12 and 24 months ($p < 0.001$).

Table 1 Baseline characteristics of the 4 groups

	Group A $n=62$	Group B $n=59$	Group C $n=63$	Group D $n=53$	P Value (Between Groups)
Age, mean ± SD (range), years	53.29 ± 12.97	56.29 ± 10.53	57.38 ± 8.78	53.47 ± 11.31	0.177
Sex, M: F, *n*	41:21	48:11	54:9	35:18	0.019*
BMI, mean ± SD (range)	31.09 ± 5.52	30.67 ± 4.51	30.68 ± 4.63	29.22 ± 4.79	0.188
K/L grade, *n* (%)					
1	7(11.3)	3(5.1)	2(3.2)	3(5.7)	
2	43(69.4)	46(78)	38(60.3)	44(83)	
3	12(19.4)	10(16.9)	23(36.5)	6(11.3)	
VAS score, mean ± SD	6.98 ± 1.68	6.95 ± 1.69	7.10 ± 1.41	6.77 ± 1.75	0.754
KOOS score, mean ± SD	64.5 ± 15.8	63.3 ± 16.9	59.9 ± 17.8	66.4 ± 14.6	0.151
Symptom	48.7 ± 16.7	47.6 ± 16.4	42.4 ± 14.1	49.6 ± 15.1	0.079
Pain	50.7 ± 17.9	46.6 ± 16.6	45.7 ± 14.3	53.7 ± 17.6	0.059
ADL	33.1 ± 21.8	29.4 ± 20.8	26.7 ± 16.9	35.8 ± 18.2	0.057
Sport/Rec	36.4 ± 17.6	33.2 ± 20.1	30.5 ± 13.9	32.9 ± 16.1	0.362
QOL					
Kujala Score, mean ± SD	55.9 ± 15.8	55.5 ± 14.3	52.9 ± 15.0	58.6 ± 13.1	0.231
MAA, mean ± SD, degree	6 ± 2.82	5.49 ± 2.55	5.56 ± 3.08	4.91 ± 2.56	0.177
ROM, mean ± SD, degree	120.6 ± 9.5	121.3 ± 9.8	114.4 ± 10.6	123.7 ± 11	0.000**

*$p<0.05$

**$p<0.001$

Table 2 Distribution of patients with side effects by groups

	Groups		*P* Value (between groups)
Patient with side effects *n* (%)	Group A ($n=62$) 12 (19,4)	Group B ($n=59$) 2 (3,4)	0.006*
	Group C ($n=63$) 20 (31,7)	Group D ($n=53$) 7 (13,2)	0.019*

*$p<0.05$

VAS pain scores

The VAS scores of all patients were noted prior to injection, and at 1, 3, 6, 12 and 24 months. There were significant differences between pre-treatment and post-treatment results in all groups during the 24-month follow-up period ($p<0.001$) and the better VAS values were recorded at 3 and 6 months in all 4 groups. When the VAS scores of the groups were compared, no statistically significant difference was determined between the groups in respect of the baseline and 1-month VAS values ($p>0.05$). There was a statistically significant difference in the scores of 3, 6, 12 and 24 months between the groups ($p<0.05$). The VAS scores of the PRP groups (A and C) were better than those of the NS groups (B and D) at 3, 6 and 12 months ($p<0.001$). The VAS scores of the group C (4.16 ± 2.13) were better than those of the group A (5.79 ± 1.99) at 12 months ($p<0.001$). There was no statistically significant difference between the groups in the VAS scores at the end of the 24 months (Fig. 3).

Clinical outcomes

KOOS scoring system

In all subscales of KOOS [symptom (KOOS.S), pain (KOOS.P), activity daily living function (KOOS.ADL), sports and leisure evaluation (KOOS.SP) and quality of life (KOOS.QL)], statistically significant differences was seen between the pre-treatment and post-treatment results of each group according to the months ($p<0.001$). Groups treated with PRP and NS were compared in all parameters of KOOS. The superiority of PRP treatment in groups receiving the same amount of dose was demonstrated after 3 months. Compared to the groups treated with NS at 3, 6, and 12 months, the PRP treatment groups had higher KOOS values ($p<0.05$). Multiple-dose patient groups and single-dose patient groups were compared. In patients receiving the same treatment, the superiority of the dose was shown after 6 months. Compared to the groups treated with a single dose, the three-dose treatment groups had higher KOOS

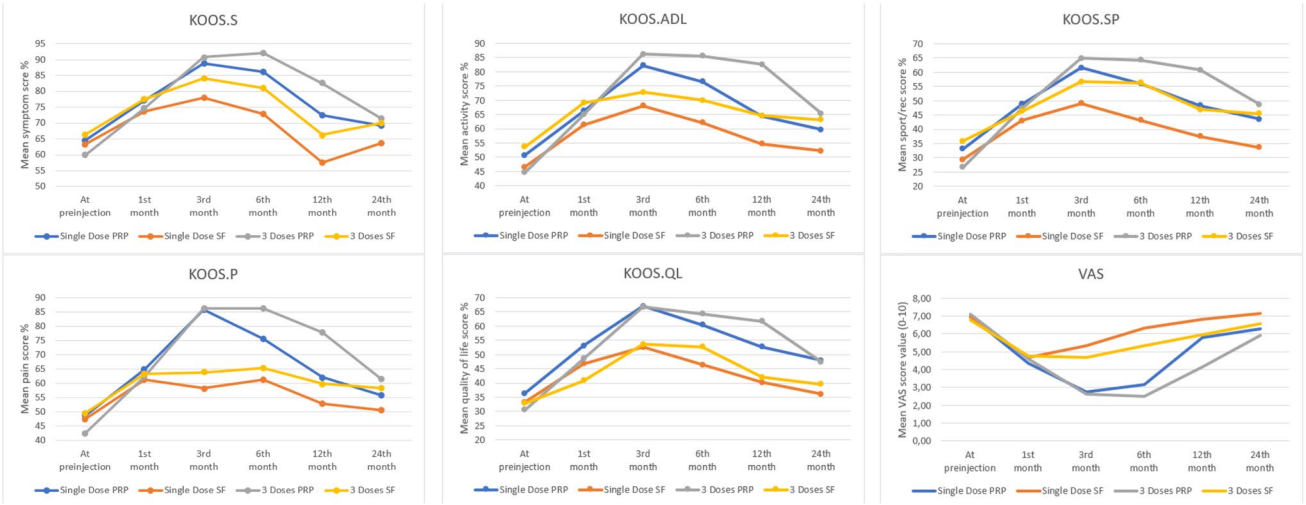

Fig. 3 The changes in the KOOS subscales and VAS scores of the groups observed by months

Table 3 Comparison of Kujala score differences by months

	Mean ± standard deviation		*P*
	Group A	Group B	
6–0 months	14.9 ± 11.9	10.5 ± 13.7	0.007***
12–0 months	10.6 ± 10.9	3.3 ± 11	0.000**
24–0 months	7.3 ± 9.8	-2.1 ± 8	0.000**
	Group C	Group D	
6–0 months	18.1 ± 12.7	13.2 ± 10.9	0.027 0.001***
12–0 months	14.1 ± 11.7	7.3 ± 10.1	
24–0 months	9.2 ± 11.1	2.1 ± 8.7	0.001***
	Group A	Group C	
6–0 months	14.9 ± 11.9	18.1 ± 12.7	0.187
12–0 months	10.6 ± 10.9	14.1 ± 11.7	0.108
24–0 months	7.3 ± 9.8	9.2 ± 11.1	0.423
	Group B	Group D	
6–0 months	10.5 ± 13.7	13.2 ± 10.9	0.049
12–0 months	3.3 ± 11	7.3 ± 10.1	0.006***
24–0 months	− 2.1 ± 8	2.1 ± 8.7	0.001***

**($p < 0.001$)

***($p < 0.008$)

values at 6 and 12 months ($p < 0.05$). There was no statistically significant difference between the groups in the KOOS score values at the end of the 24 months (Fig. 3).

Kujala patellofemoral scoring system

The difference in the Kujala Score values of the groups was compared at the different time points (Table 3). The differences in Kujala Scores at 0–1, 0–3, 0–6, 0–12 and 0–24 months, showed no significant difference between the groups in the early months. The difference between 0–6, 0–12 and 0–24 months was statistically significant ($p < 0.001$). The difference in Kujala Score in 0–6, 0–12 and 0–24 months was found to be higher in patients administered PRP compared to patients administered NS ($p < 0.05$). The differences in Kujala Score was not found to be statistically significant in the group C compared to the group A at all months ($p > 0.05$).

To understand the effect of age and grading on treatment, the 125 patients administered PRP were separated into three age groups: 18–50 (*n*: 33), 51–65 (*n*: 74), 66–80 (*n*: 18) years groups and were also separated into 3 groups according to the OA stage. The difference in the VAS, KOOS total (KOOS.T) and ROM score values of the groups before injection and at 6th month was compared. The score differences of patients aged 51–65 years were found to be statistically significant compared to the other age groups ($p < 0.001$). Details are given in Fig. 4. In the 125 patients receiving PRP therapy, the effect of the OA grade on therapy was investigated. The VAS score change was not statistically significant ($p < 0.05$). A statistically significant difference was determined in the KOOS.T score change in OA grade 2 and in ROM in OA grade 3 ($p < 0.05$) (Fig. 4).

There was no correlation of the mean scores of all the KOOS parameters with sex and BMI in all groups ($p > 0.05$), indicating that all patients irrespective of sex and BMI had equal benefit from the procedure.

A statistically significant decrease was observed in knee circumference (KC) in all groups at 0, 1 and 6 months ($p < 0.001$). The difference in KC values of the groups between the 0, 1 and 6 months was compared. A statistically significant difference was observed between the groups in respect of the difference in KC values at 0–1 and 0–6 months ($p < 0.05$). The KC difference in patients treated with PRP

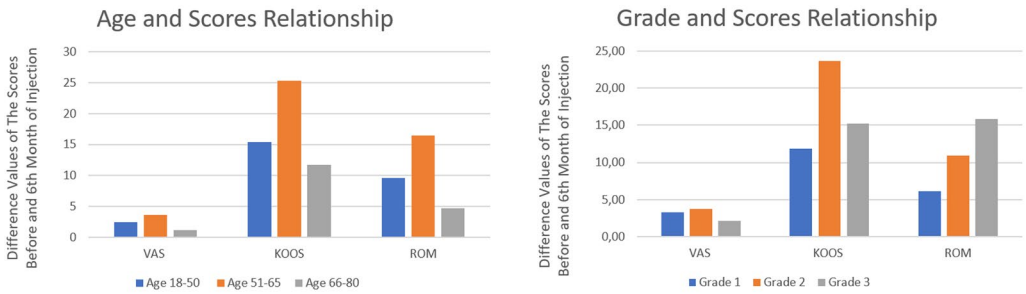

Fig. 4 The scores of 125 patients treated with PRP by age and grade of groups

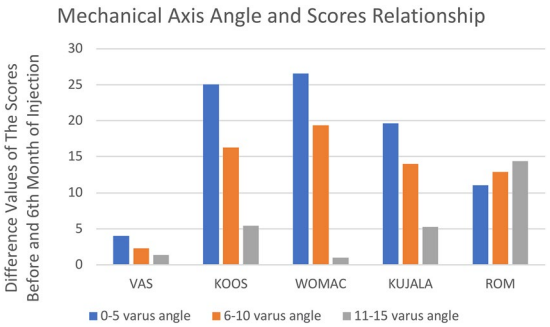

Fig. 5 Comparison of the difference in score values between 0 and 6 months of patients who underwent PRP grouped according to the MAA values

was higher at 0–1 and 0–6 months compared to patients treated with NS ($p < 0.001$). The decrease in the KC measurements of the PRP groups was more significant than in the NS groups ($p < 0.05$). No association with multiple doses was established ($p > 0.05$). As a notable exception, only the 0–6 months KC difference was found to be statistically significant in the group C compared to the group A ($p = 0.007$).

A moderate negative correlation was found between lower extremity MAA and the differences in the 0–24 months VAS, KOOS, WOMAC and KUJALA scores of the 125 PRP patients (rho: − 0.51, − 0.45, − 0.50, − 0.46, respectively). As the MAA increased, a negative change was observed in pain and clinical scores except ROM (Fig. 5).

Discussion

The main finding of this study is showed that PRP improved the pain score and improved knee function. The KOOS scores of the PRP patient groups showed a worsening trend after the 3rd month in the single-dose group and after the 6th month in the 3-dose group. The effectiveness of PRP treatment according to the initial KOOS values was seen to continue for 6 months with a single dose and for 12 months with three doses which was consistent with the findings of studies by Kon et al. [21] and Patel et al. [15]. There was no statistically difference between the values at 24 months and baseline values in all the groups in this study. Although single-dose PRP is as effective as three-dose PRP, the effect was seen to decrease significantly after 6 months. Therefore, it can be recommended that PRP injections are repeated at 6-month intervals. Jang et al. [36] reported that the duration of the effect of a single dose of PRP on VAS pain score is expected to continue for 8.8 months.

Although PRP has been reported to be effective in literature, there is a lack of studies of a high scientific evidence level. There are few published studies with placebo-controlled, double-blind and randomized designs [14–16, 37]. PRP-related studies in the current literature are conflicting due to a lack of standardization of study protocols, platelet separation techniques and outcome measurement methods. In addition, it is not clear which group of patients will benefit most from this treatment. These gray zones in PRP treatment could be clarified by randomized, placebo-controlled studies with many subjects.

In many clinical studies comparing PRP and NS, a similar computer-assisted randomization program has been used [14, 15]. However, there are serious differences between the blinding methods [14–16, 37, 38]. In this study, the syringe was covered with a black sterile band by a separate team and delivered to the physician applying the injection. Thus, both the patient and the physician were blinded to the treatment applied. With this randomization and blinding, it was aimed to eliminate any potential selection bias of the researchers and the possibility that a possible difference between the treatments during the analysis phase was due to the bias in selection or distribution of the treatment groups.

Many different techniques can be applied during PRP preparation, resulting in different platelet concentrations in PRP treatments [39]. As the scientific proof of bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/µl, it is this concentration of platelets in a 5-ml volume of plasma which is the current working definition of PRP. Lower concentrations cannot be relied upon to enhance wound healing, and higher concentrations

have not yet been shown to further enhance wound healing [40]. In this study, a new technique was developed based on Annitua's PRP preparation technique. PRP prepared with this modified technique has been tried to be standardized in all patients. Type 2A PRP was applied to all patients in the PRP groups according to the Mishra classification.[35]. The number of platelets injected in this series was an average of 128×10^5 compared with 6.5 million used by Kon et al. [21] (higher than this work) and 238.56×10^7 used by Patel et al. [15] (lower than this work).

The increase in pain complaints seen in the first 10 days after injection in 38 patients (16%) was the most common side effect observed in groups with PRP. When the frequency of side effects in the groups was compared, more side effects were seen in the PRP patients compared to patients who received placebo, whereas multiple-dose injections were not determined to affect the frequency of side-effects. In the occurrence of side effects, platelet count and CaCl2 used as an activator are known to have a contributing role [15].

Patient satisfaction has not been considered in clinical studies comparing PRP and NS in literature [14–16, 37, 38]. In this study, it was observed that subjective patient satisfaction was higher and lasted longer in PRP and multiple-dose groups. It should also be emphasized that subjective patient satisfaction was seen to be in parallel with the VAS and KOOS scores, which can be considered an important finding of the study.

The patellofemoral joint should be evaluated before injection in patients with PRP injection. In the literature, there is only one clinical trial which has examined the relationship between PRP and the patellofemoral joint [36]. To the best of our knowledge, there is no study in literature which has evaluated the relationship between PRP and the patellofemoral joint using the KUJALA scoring system. Jang et al., stated that the results of PRP treatment were negatively affected in the presence of patellofemoral joint degeneration [36]. In this study, PRP treatment was seen to have a positive effect on the KUJALA score, although no superiority of multiple doses over a single dose could be demonstrated on this score. In a recent study, it has been shown by magnetic resonance imaging (MRI) that PRP treatment has a significant positive effect on the cartilage volume of the patellofemoral joint [41]. This recently published double-blind randomized clinical trial parallels the relationship between PRP and KUJALA score in our study.

In a 6-month randomized, placebo-controlled study by Görmeli et al., it was stated that in the early stages of OA, multiple PRP injections were more effective than a single injection. In that study, the injection amounts applied in the PRP (5 ml), HA (2 ml) and placebo (NS not specified) groups were not the same [20], which may have added bias to the treatment effect and blinding method. In this study, three doses of PRP and three doses of NS were seen to be more effective in treatment compared to a single dose of PRP and the duration of action was longer ($p < 0.05$). The result that multiple-dose PRP is effective is consistent with findings in the literature [20, 42, 43]. Patel et al., reported that single-dose PRP is as effective as multiple-dose PRP [15]. However, the patient follow-up period was limited to 6 months in that study. The results of this study showed that multi-dose PRP was superior to single-dose PRP in all the scores, except for the KUJALA score.

Wu et al. [16] found that intra-articular injections of PRP or NS improved pain, stiffness, disability, and knee strength in patients with mild to moderate knee OA. Recent studies in literature have reported that placebo has a therapeutic effect and has therapeutic potential [44]. In this study, NS used as placebo was determined to have a positive effect on pain and clinical scores. Important placebo effects have been observed in almost every knee injection study. However, while placebo plays an important role in PRP results, as demonstrated by the similar outcome compared to saline up to 6 months, the PRP benefit exceeds the mere placebo effect [19]. PRP effect over time shows superiority over placebo. Therefore, to prove the effectiveness of a treatment, this treatment should show a greater effect than the placebo, which is believed to have therapeutic potential. The gold standard method of demonstrating the effectiveness of treatments is placebo-controlled studies.

This study results demonstrated a definite relationship with Kellgren/Lawrence staging in the 125 patients who were administered PRP. While the VAS score change was not statistically significant, the changes in KOOS score in grade 2 OA and ROM in grade 3 OA were determined to be statistically significant. Cole et al., reported that the group that benefited the most from PRP treatment was K/L OA grade 1 according to the WOMAC score but not on the VAS score [26]. Kon et al. also found that patients with cartilage lesions and early OA showed superior results when treated with PRP compared to HA [22]. In this study, K/L OA grade 2 patients were seen to benefit most from PRP treatment. Since the initial KOOS values of grade 1 patient was already high, the difference from the 6th month values were less. Grade 2 patients had lower baseline KOOS, so the difference from baseline to 6th month values was greater. Moreover, the number of grade 1 patients (n: 9) was very low in this study. After PRP treatment, improvement in ROM was detected in all patients. However, since the initial ROM values of advanced K/L grade patients were low, the difference in ROM values after PRP treatment was found to be greater. After PRP treatment, the increase in ROM in K/L grade 3 OA patients was higher than at other grades.

There are studies in literature reporting that PRP treatment is more effective at a younger age[14, 22], and there are others which state that age has no effect on clinical results [15, 25, 45]. Kon et al., found a negative correlation between

age and clinical outcomes [21]. However, in this study, a significant improvement was found in the KOOS scores of patients aged 51–65 years, compared to the other age groups. The concentration of PDGF and ILGF-1 in the PRP solution negatively correlates with age [46]. In another study, it was stated that the amount of release of PDGF, TGF-β1, IGF-1, and EGF from platelets is higher in young patients [47]. Consequently, the amount of tissue growth factors that play a role in tissue healing, angiogenesis, anti-inflammation and cell migration decreases with advancing age. This situation impairs the effectiveness of PRP treatment. In this study, that the patients who benefitted most from PRP treatment were aged 51–65 years can be attributed to both the lower baseline score values and the higher growth factor release of the younger age group compared to the older age group. Since the baseline score values of the 18–50 years age group were high, the increase to the post-treatment values were lower. The low baseline score values of the 66–80 years group and the lack of this difference in the post-treatment values can be attributed to the current knowledge regarding the physiological and pathological changes at cellular and molecular levels that can correlate with aging in the joint and that can contribute to the development and progression of OA [48]. However, this difference may also be due to the non-homogeneous distribution of patients and patient numbers in the groups. Therefore, to be able to better clarify the relationship between age and PRP, there is a need for further studies with more homogeneous patient groups and a higher number of patients.

There are few clinical studies in the literature which have examined the effect of PRP treatment on KC [28]. Filardo et al., recommended KC measurement follow-up in patients who underwent PRP [49], and reported in another study that both PRP and HA treatment decreased KC (trans patellar measurement), with no advantage of either of these two treatments over the other [28]. Cole et al. [26] stated that the concentration of pro-inflammatory cytokine IL-1β and TNF-α in the knee shows a decreasing trend. This finding demonstrates that the anti-inflammatory effect of PRP treatment contributes to the improvement of clinical symptoms in OA treatment. The content of PRP includes abundant growth factors, and analyses have shown that the TGF-B level is 7 times, the PDGF level is 30 times, and the EGF level is 10 times that of whole blood [50]. In this study, the decrease in KC measurement in patients administered PRP was thought to be due to the anti-inflammatory effect of PRP, and the support of the biochemical environment in the joint in favor of the anabolic process.

There is a significant relationship between the frontal alignment of the lower extremity and the pathogenesis of OA [51, 52]. The relationship between lower extremity malalignment and the development of OA and the progression of the process has been shown in many studies [51–56]. In this study, the lower extremity MAA of the 125 PRP patients was measured and compared with the pain and clinical scores. A moderate negative correlation was found between the difference in 0–6 month scores. As the MAA increased, a negative change was observed in pain and clinical scores. To the best of our knowledge, there is no clinical study in literature that has examined the relationship between PRP treatment and lower extremity MAA. PRP treatment affects the biochemical environment within the joint but cannot directly affect the structure of the joint. PRP treatment is not recommended for patients with advanced varus or valgus alignment. Surgical treatment options should be considered in patients with severe structural and anatomic changes in the knee joint.

Filardo et al. reported that treatment with PRP injections can reduce pain and improve knee function and quality of life with short-term efficacy [18]. Although the symptomatic benefits of PRP have been demonstrated, there are few evidence-based scientific studies in the literature [14–16, 21, 57]. Further, scientific, evidence-based studies are needed to better understand the PRP mechanism of action, standardize PRP preparation, classification and application techniques, determine PRP treatment indications and determine whether the duration of action is temporary.

The strengths of this study were the inclusion of a placebo control group, that patients were randomly grouped, it was double blinded with neither patient nor physician knowing the type of treatment applied, standard PRP preparation technique and outcome evaluation methods were used in all patients, multiple injection effectiveness was investigated in both the patient group and control group, the total number of patients is the highest number of subjects in the literature and the patient follow-up period is long compared to the literature (2 years).

Study limitations

The OA K/L stage and age distribution of the patients included in the study was not homogeneous. As there were no grade 4 patients in the study, the effects of PRP on advanced grade OA patients are not known.

Only direct radiographs were used as the imaging method in the follow-up of the patients in this study. Imaging with MRI would have been better to view changes in articular cartilage. Cartilage in the joint could be mapped with MRI and the effect of PRP could be shown more objectively.

There was no biological analysis of the joint in this study. Possible changes in intra-articular biological analysis to be performed before and after injections could be seen objectively.

Conclusion

The results of this study showed that PRP has a positive effect on pain and clinical scores in the treatment of OA compared to a placebo, the efficacy duration of multiple doses in treatment is longer than a single dose, an increase in the MAA of the lower extremities has a negative effect on PRP treatment, and the patient group aged 51–65 years benefited more from PRP treatment. PRP treatment is not recommended for patients with advanced varus or valgus alignment, and surgical treatment options should be considered in patients with severe structural and anatomic changes in the knee joint. There is a need for further clinical studies with a larger patient population, including all stages of OA, and imaging of articular cartilage, biological analysis of the joint and longer follow-up to clarify these findings.

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Availability of data and material If requested, all data can be transmitted to the journal.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval This prospective, randomized, placebo-controlled, double-blind study was conducted with the approval of the Republic of Turkey Ministry of Health, General Directorate of Health Services, Department of Blood, Organ and Tissue Transplant Services, Root Transplants Scientific Advisory Commission (Approval number: 56733164/203). All the details of this clinical study were explained to the patients, and all participated in the study voluntarily, providing written informed consent. All the study procedures complied with the principles of the Declaration of Helsinki.

Consent to participate The informed consent form, permission to participate in this clinical study and the publication of the data of this study in any journal were obtained from all participants included in this study.

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