

The efficiency of platelet-rich plasma treatment in patients with knee osteoarthritis

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Abstract.

OBJECTIVE: The aim of this study was to determine the effects of platelet-rich plasma (PRP) treatment on pain, functionality, quality of life, and cartilage thickness in patients with knee osteoarthritis (OA).

METHODS: Sixty patients with chronic knee pain were randomly separated into two groups. The first group was administered 4-ml PRP intra-articularly (IA) in three doses at one-week intervals, and the second group had only one dose of a 4-ml saline solution IA. The patients' pain was measured using the Visual Analogue Scale (VAS); functionality was measured using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC). The distal femur cartilage thickness was assessed using ultrasonography (USG).

RESULTS: All baseline parameters were similar ($p > 0.05$). In the first and sixth months after the treatment, the VAS scores of the PRP group were significantly low ($p < 0.001$). In the same group, only the pain sub-score was low in the WOMAC assessment in the first month after treatment. However, in the sixth month, all parameters of the WOMAC score were lower than those of the placebo group ($p < 0.05$). Cartilage thickness measurements were similar in the two groups ($p < 0.05$).

CONCLUSION: PRP treatment had positive effects on the pain, physical function, and quality of life of patients with knee OA, but it did not increase cartilage thickness.

Keywords: Osteoarthritis, platelet-rich plasma (PRP), cartilage thickness

1. Introduction

Osteoarthritis (OA) is the most commonly diagnosed disease of the joints around the world. OA particularly involves the knees, hips, hands, and spine. It is a major cause of disability in individuals aged 40 years and older. It also affects patients' activities and quality of life, and it incurs significant economic costs because it causes anxiety and depression in the later stages of the disease [1]. The most common symptoms of knee

OA are pain and the limitation of physical function. These symptoms affect the quality of life and the social activities of patients. The incidence of OA has increased because of longer life expectancy, the increasing elderly population, and the prevalence of obesity [2].

There is no definitive treatment for OA. Nationally and internationally published and generally accepted Knee OA treatment guidelines mainly include pharmacological treatment, non-pharmacological treatment and surgical treatment. Non-pharmacological treatment includes patient education, joint protective precautions, exercise, psychological support, diet, weight loss, physical treatment modalities, use of auxiliary devices, hydrotherapy and spa sessions [3–6]. Re-

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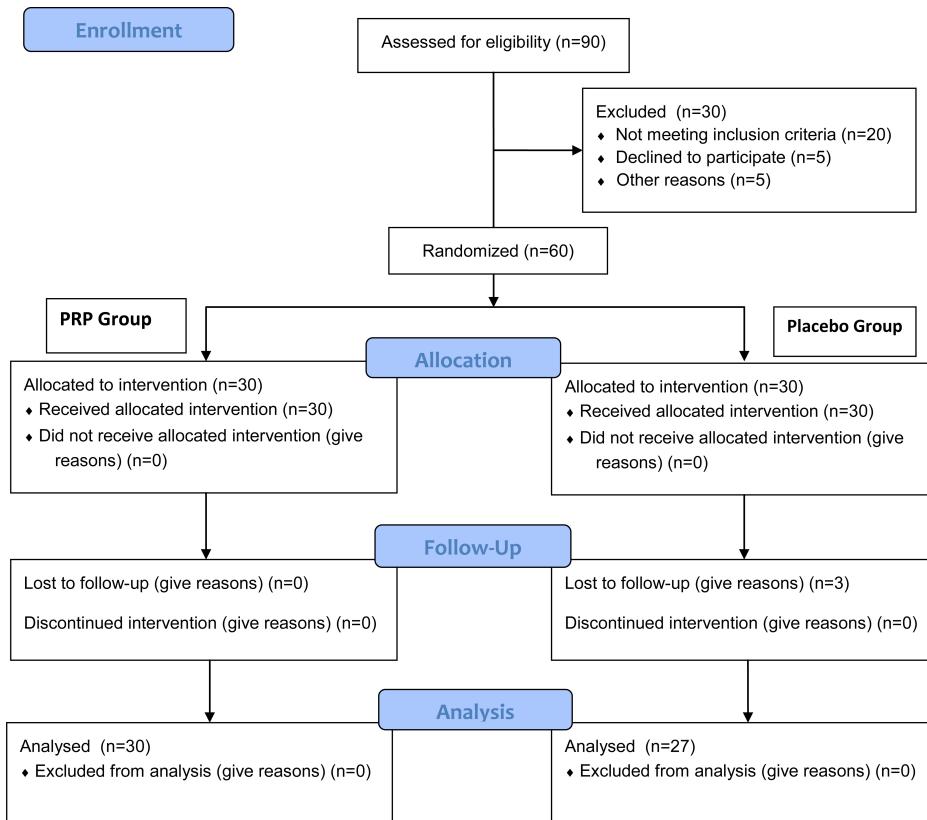


Fig. 1. Flow diagram of the study. *n* Number of patients, PRP Platelet-rich plasma.

garding pharmacological treatment, non-steroidal anti-inflammatory drugs (NSAID) can be effective in the short term, but because of their potential side effects, their use is limited. Orally administered disease-modifying drugs, such as chondroitin and glucosamine, can be listed among the treatment options, but there is not enough evidence for their effectiveness [7]. Although intra-articular (IA) steroid therapy has been proven effective in the short term, it has been shown to negatively affect cartilage structure and is therefore recommended for use in OA patients in advanced stages [7].

Platelet-rich plasma (PRP), which is obtained by centrifuging the whole blood, is the plasma component that includes a higher concentration of thrombocytes than the whole blood. Since it contains numerous growth factors, PRP injections are used in the treatment of various musculoskeletal diseases. PRP treatment is minimally invasive and reliable, and is reported to be effective when administered according to appropriate indications [8]. In recent years, *in vitro* and *in vivo* animal experiments have shown positive advancements regarding the effect of PRP on the release

of many anti-inflammatory and anti-catabolic mediators, which has attracted interest in this treatment. Although there have been many previous studies on PRP practices, there is a limited number of randomized, placebo-controlled studies. Moreover, a complete consensus of the effectiveness and mode of application of this treatment method has not yet been conducted.

In this study, we aimed to assess the effects of PRP treatment on pain, functionality, quality of life, and cartilage thickness in knee OA patients. To the best of our knowledge, our study is the first in the literature to apply a double-blind, prospective, randomized, placebo-controlled design.

2. Materials and methods

2.1. Participants

This study was designed as a double-blind, random-



Fig. 2. Tube after the first centrifuge.

ized, placebo-controlled trial. A total of 60 patients who presented to our outpatient clinic with knee OA were included in the study. The OA diagnosis was made clinically and radiologically based on the criteria used by the American College of Rheumatology (ACR). Approval to conduct the study was obtained from the Şişli Hamidiye Etfal Research and Training Hospital Ethics Committee (no. 1139). All participating patients were informed about the study, and their written consent was obtained.

2.2. Inclusion criteria

Patients between 50–75 years old who had knee pain during the previous year (Visual Analogue Scale (VAS) > 4, grade 1–3 OA according to the Kellgren-Lawrence classification and no pathologies in the laboratory and coagulation parameters) were included in the study.

2.3. Exclusion criteria

Patients were excluded when any one of the following criteria was present: 1) rheumatological disease other than OA; 2) systemic active infectious disease or tumor; 3) IA injection to the knee and physical treatment practices in the last three months; 4) NSAID usage in the last seven days, which would disrupt thrombocyte metabolism; 5) previous history of knee joint surgery; 6) severe mental retardation; 7) blood thrombocyte count equal to or lower than 150,000/micro-liters before the treatment and/or bleeding disorder; 8) Hepatitis B, C, or HIV; 9) previous history of traumatic knee cartilage injury.

2.4. PRP preparation

The system we used included biphasic centrifuga-



Fig. 3. Tube after the second centrifuge.

tion; two tubes, A and B, were used for each patient. First, to obtain PRP from the patient, 10 cc of blood was collected into tube A with sodium (Na)-citrate using a secure holter. After the patient's blood was collected in the tubes with Na-citrate, the tubes were inverted slowly to ensure homogenization. Two A tubes from two patients were placed opposite in the centrifuge (Revmed, VS-5000 i2, Korea) and were then centrifuged for 10 minutes at 2,000 rpm. After the sound indicating that the centrifugation was complete, the tubes were removed from the centrifuge. After centrifugation, three layers were obtained. The bottom layer was rich in erythrocytes, the middle layer was the buffy coat (leukocyte + thrombocyte), and the top layer comprised plasma (Fig. 2). The plasma and the buffy coat were obtained after the centrifuge were transferred to tube B using a 3-cc injector. Tube B was inverted, and the activation was achieved by the 10% calcium chloride (CaCl_2) found in the tube. In the second step, two B tubes were placed in the centrifuge and spun for five minutes at 4,000 rpm. After the sound indicating that the centrifugation was complete, the tubes were removed from the centrifuge. At the end of the second procedure, the bottom layer had a small amount of clotting, and the top layer had approximately 4 ml of PRP (Fig. 3).

Cell counting was conducted to determine whether the platelet count was sufficient using the PRPs of some participants in the biochemistry clinic in our hospital. The average thrombocyte count was four to six times higher than the blood thrombocyte count (900,000–1,100,000 per mm) (3). The average leukocyte count was approximately as high as the number of leukocytes in the patient's blood ((1.0x) 5,000–10,000/mm³).

2.5. Treatment schedule and follow-up

The study population consisted of 90 consecutive patients who presented at the outpatient clinic with a

one-year history of pain that worsened in weight bearing (VAS score ≥ 4). The study sample consisted of 60 participants who met the inclusion criteria. Based on a random numbers table, these patients were randomly divided into two groups of 30 patients each (Fig. 1):

PRP group: Injection of 4 ml of PRP in three sessions at one-week intervals and an exercise program.

Placebo group: Injection of 4 ml of saline (physiological serum) in a single session and an exercise program.

The injections were performed laterally when the patient was in a seated position, and the knee was in a flexed position at 90 degrees. A 21-gage needle was used for the injections. Immediately after the injection, the patient's knees were flexed-extended to ensure that the solution was well distributed, and the patient was kept at rest in the supine position for 15 minutes. All injections were prepared and performed by a physician (H.E.) who was not blinded to the patients. In the triple injection protocol, injections were performed at one-week intervals. In each injection, the patient's blood was collected and processed as described above.

Similar to the first group, blood was collected from the patients in the second group to determine the placebo effect. The collected blood was tested to eliminate the infected samples. Using the same technique, 4 ml of 0.9% saline solution was injected into the patients.

To ensure that the patients were blinded to the treatment, injectors containing PRP and saline were covered with a blank piece of barcode paper on which the patient's name was written. Hence, the patients in both groups did not have any knowledge about the drug administered to them.

The patients were asked to rest their knees and to refrain from activities that could cause pain for two days after the injection. Patients were allowed to use 3 gr/day of paracetamol if needed, and they were forbidden to use NSAIDs (e.g., ibuprofen, diclofenac, etodolac, meloxicam, etc.). The patients were informed that it was necessary to receive as little medical treatment as possible during the six-month follow-up period.

Two days after the injections were administered, the patients were prescribed a rehabilitation program. In the exercise program, first, joint mobility range (ROM) exercises and stretching exercises, which included the hamstring, rectus femoris, and gastrocnemius muscles, were performed. Exercises for strengthening the

quadriceps femoris muscle were initiated after the third dose was administered to the patients in the first group and after the single injection was administered to the patients in the second group. The exercise program was taught to the participants, and a six-month exercise protocol was used. The patients were asked whether they performed their exercises during each control period. Except this additional follow-up was done.

The VAS was used to assess the patient's pain. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess the functional state, and the Short Form-36 (SF-36) was used to assess quality of life. All patients were evaluated three times: at the beginning of the treatment and at the first and sixth months following the completion of the therapy.

The WOMAC OA index, which is used to assess pain, stiffness, and physical function using a Likert pain scale, is composed of three sections and 24 questions. Higher WOMAC scores show increased pain and stiffness as well as impaired physical function.

The SF36 is a short questionnaire consisting of 36 items that are used to measure eight multi-item variables: physical functioning (10 items), social functioning (2 items), role limitations due to physical problems (4 items), role limitations due to emotional problems (3 items), mental health (5 items), energy and vitality (4 items), pain (2 items), and general perception of health (5 items). For each variable, the item scores are coded, summed, and transformed on a scale from 0 (worst possible health state measured by the questionnaire) to 100 (best possible health state).

The femoral cartilage thickness was measured ultrasonographically (US) at the beginning of the treatment and at the sixth-month control. Moreover, in each control, the injection site was checked for the presence of pain, increases in temperature, swelling, redness, or any other side effect.

Using USG, the evaluation of the parameters and the measurement of the cartilage thickness were performed by a physician (B.D.) who was blinded to the treatment of the patients.

2.6. Ultrasonographic assessment

Using ultrasonography, the femoral cartilage measurements were taken using a linear probe of 7–12 MHz (Esaote MyLab Five).

Application technique: Horizontal imaging was performed at the suprapatellar region when the patient was in the supine position, and the knees were at

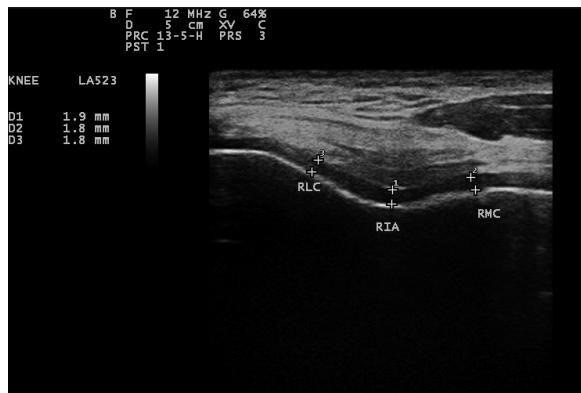


Fig. 4. Right femur cartilage thickness measurement (RLC: Right lateral condyle, RMC: Right medial condyle, RIA: Right intercondylar area).

maximum flexion. The femoral cartilage thickness was measured in three different places: medial, mid, and lateral (Fig. 4).

2.7. Statistical analysis

The study data were uploaded to a computer environment and analyzed using Statistical Package for Social Sciences (SPSS) for Windows 22.00 (SPSS Inc., Chicago, IL, USA). The descriptive statistics were presented as mean \pm standard deviations, distributions of the frequencies, and percentages. The Pearson chi-square test and Fisher's exact test were used to evaluate the categorical variables. Visual (a histogram and probability graphs) and analytical methods (the Shapiro-Wilk test) were used to determine whether the data followed a normal distribution. On the data that followed a normal distribution, Student's t-test was used to determine the statistical significance between two independent groups. The paired sample t-test was used to determine the statistical significance between two dependent groups. On the data that did not follow a normal distribution, the Mann-Whitney U test was used to determine the statistical significance between two independent groups. The Wilcoxon signed rank test was used to determine the statistical significance between two dependent groups; and the Friedman Test was used to determine the statistical significance between three dependent groups. If a significant difference was detected between three dependent groups, a post-hoc Bonferroni correction was used to identify the source of the difference. A p -value < 0.05 was considered statistically significant.

In our study, the sample size was determined as at least 50 individuals using PASS 11 (Power and Sam-

Table 1
Distribution of some of the descriptive and clinical characteristics of the study groups

	PRP (n = 30)	Placebo (n = 27)	<i>p</i>
Age (years), $\bar{X} \pm S$	61.30 ± 7.91	60.19 ± 6.80	0.573 ^a
Gender <i>n</i> (%)			
Male	1 (3.3)	3 (11.1)	0.336 ^b
Female	29 (96.7)	24 (88.9)	
BMI (kg/m^2), $\bar{X} \pm S$	30.37 ± 4.47	30.70 ± 3.97	0.766 ^a
Any additional diseases* <i>n</i> (%)			
None	6 (20.0)	7 (25.9)	0.594 ^c
Yes	24 (80.0)	20 (74.1)	
Injected knee <i>n</i> (%)			
Right	18 (60.0)	18 (66.7)	0.602 ^c
Left	12 (40.0)	9 (33.3)	
Stage of osteoarthritis of the injected knee <i>n</i> (%)			
I	2 (6.6)	3 (11.1)	0.802 ^c
II	14 (46.7)	13 (48.1)	
III	14 (46.7)	11 (40.7)	

n: Number of patients; %: Percentage; \bar{X} : Mean; S: Standard deviation; BMI: Body Mass Index; *CVS disease, Lumbar disc hernia, Kidney disease; a: Student's T Test; b: Fisher's Exact Test; c: Chi-Square Test.

ple Size, version 11, for Windows) according to the WOMAC score calculated in previous similar studies in the literature. The power of the test is expected to be approximately 80.62% in this condition.

3. Results

Of the 60 patients who participated in the study, three were excluded because they could not come to the control visits. Therefore, the final sample consisted of 57 patients. In this sample, 7% of the patients were male and 93% were female; the mean age was 60.77 ± 7.36 years (min: 50, max: 75). There were no statistically significant differences between the patients in the PRP group and the patients in the placebo group before the treatment in terms of age, gender, body mass index (BMI), additional diseases, and the stage of osteoarthritis in the injected knee ($p > 0.05$). The demographic data and comorbid diseases of the patients participating in the study are shown in Table 1.

3.1. Pain scores

There were no differences between the two groups in terms of their VAS scores before the treatment ($p > 0.05$) (Table 2). In both groups, statistically significant recovery of the VAS scores in the first and sixth months after the treatment was detected compared with the scores before the treatment. This recovery was more pronounced in the PRP group ($p < 0.05$) (Fig. 5).

Table 2
Distribution of the VAS scores between the study groups and within each study group before and 1st and 6th months after the treatment

VAS		Before treatment	1st month	6th month	<i>p</i> *
		$\bar{X} \pm S$	$\bar{X} \pm S$	$\bar{X} \pm S$	
Rest	PRP (<i>n</i> = 30)	3.87 ± 2.14 ^{bc}	1.80 ± 1.67	1.20 ± 1.56	< 0.001
	Placebo (<i>n</i> = 27)	4.93 ± 1.68 ^{bc}	3.67 ± 1.86	3.37 ± 2.32	< 0.001
	<i>p</i> **	0.059	< 0.001	< 0.001	
Movement	PRP (<i>n</i> = 30)	7.10 ± 2.52 ^{bc}	3.70 ± 2.20	2.80 ± 2.32	< 0.001
	Placebo (<i>n</i> = 27)	7.74 ± 1.85 ^{bc}	6.00 ± 2.86	5.15 ± 2.60	< 0.001
	<i>p</i> **	0.356	0.003	0.001	
Night	PRP (<i>n</i> = 30)	4.23 ± 2.75 ^{bc}	2.00 ± 2.01	1.10 ± 1.35	< 0.001
	Placebo (<i>n</i> = 27)	4.63 ± 2.32 ^{bc}	3.18 ± 2.27	3.19 ± 2.53	< 0.001
	<i>p</i> **	0.627	0.037	< 0.001	

n: Number of patients; \bar{X} : Mean; *S*: Standard deviation; *Friedman Test; **Mann-Whitney U Test;
b: In post-hoc paired comparison, statistically significant difference was detected compared to "1st month after the treatment"; c: In post-hoc paired comparison, statistically significant difference was detected compared to "6th month after the treatment".

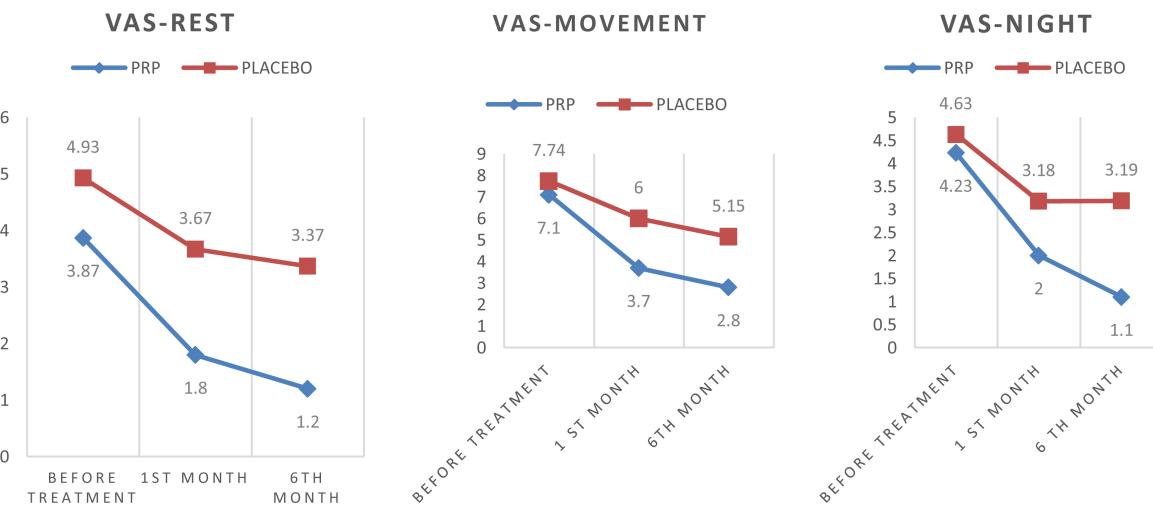


Fig. 5. Change in the VAS scores of the study groups over time.

3.2. Physical function

The physical functions of the patients were evaluated using WOMAC. The WOMAC subgroup and the total scores of both groups were improved statistically significantly in the first and sixth months after the treatment compared with before the treatment ($p < 0.05$). In the PRP group, this improvement was more pronounced in the pain subgroup in the first month and in all WOMAC subgroups and total scores in the sixth month ($p < 0.05$) (Table 3).

When the patients in the PRP group are analyzed in terms of their K-L grades, the WOMAC subgroup and total scores in the sixth month after the treatment of patients with stages 1 and 2 OA were significantly lower than the patients with stage 3 OA ($p < 0.05$). No

statistically significant differences were detected in the WOMAC scores in the first month after the treatment ($p > 0.05$) (Fig. 6).

The rates of decrement in the VAS and WOMAC scores of both groups at the end of the sixth month are shown as percentages in Table 5.

We also compared the delta values (change of the scores compared to baseline) between the groups in Tables 6 and 7.

3.3. Quality of life

The patients' quality of life was evaluated using the SF-36. In the PRP group, the scores for the main factors of physical and mental health were statistically significant in the first and sixth months after the treatment compared with before the treatment ($p < 0.05$).

Table 3

Distribution of the WOMAC scores between the study groups and within each study group before and 1st and 6th months after the treatment

<i>WOMAC</i>		Before treatment $\bar{X} \pm S$	1st month $\bar{X} \pm S$	6th month $\bar{X} \pm S$	<i>p</i> *
Pain	PRP (<i>n</i> = 30)	11.13 ± 4.27 ^{b,c}	6.87 ± 3.76	4.73 ± 3.58	< 0.001
	Placebo (<i>n</i> = 27)	12.00 ± 2.91 ^{b,c}	9.22 ± 3.85	8.74 ± 4.14	< 0.001
	<i>p</i> **	0.490	0.024	< 0.001	
Stiffness	PRP (<i>n</i> = 30)	4.57 ± 1.89 ^{b,c}	2.87 ± 1.43	1.90 ± 1.79	< 0.001
	Placebo (<i>n</i> = 27)	4.59 ± 1.91 ^{b,c}	3.48 ± 1.74	3.52 ± 1.74	0.002
	<i>p</i> **	0.827	0.134	0.002	
Physical function	PRP (<i>n</i> = 30)	40.60 ± 13.51 ^{b,c}	26.03 ± 13.01 ^c	18.23 ± 13.83	< 0.001
	Placebo (<i>n</i> = 27)	39.44 ± 13.61 ^{b,c}	31.30 ± 13.33	30.22 ± 13.46	< 0.001
	<i>p</i> **	0.987	0.131	0.003	
Total	PRP (<i>n</i> = 30)	56.40 ± 18.71 ^{b,c}	35.77 ± 17.57 ^c	24.87 ± 18.79	< 0.001
	Placebo (<i>n</i> = 27)	57.04 ± 15.12 ^{b,c}	43.93 ± 17.99	42.37 ± 18.64	< 0.001
	<i>p</i> **	0.905	0.087	0.001	

n: Number of patients; \bar{X} : Mean; *S*: Standard deviation; *Friedman Test; **Mann-Whitney U Test; b: In post-hoc paired comparison, statistically significant difference was detected compared to “1st month after the treatment”; c: In post-hoc paired comparison, statistically significant difference was detected compared to “6th month after the treatment”.

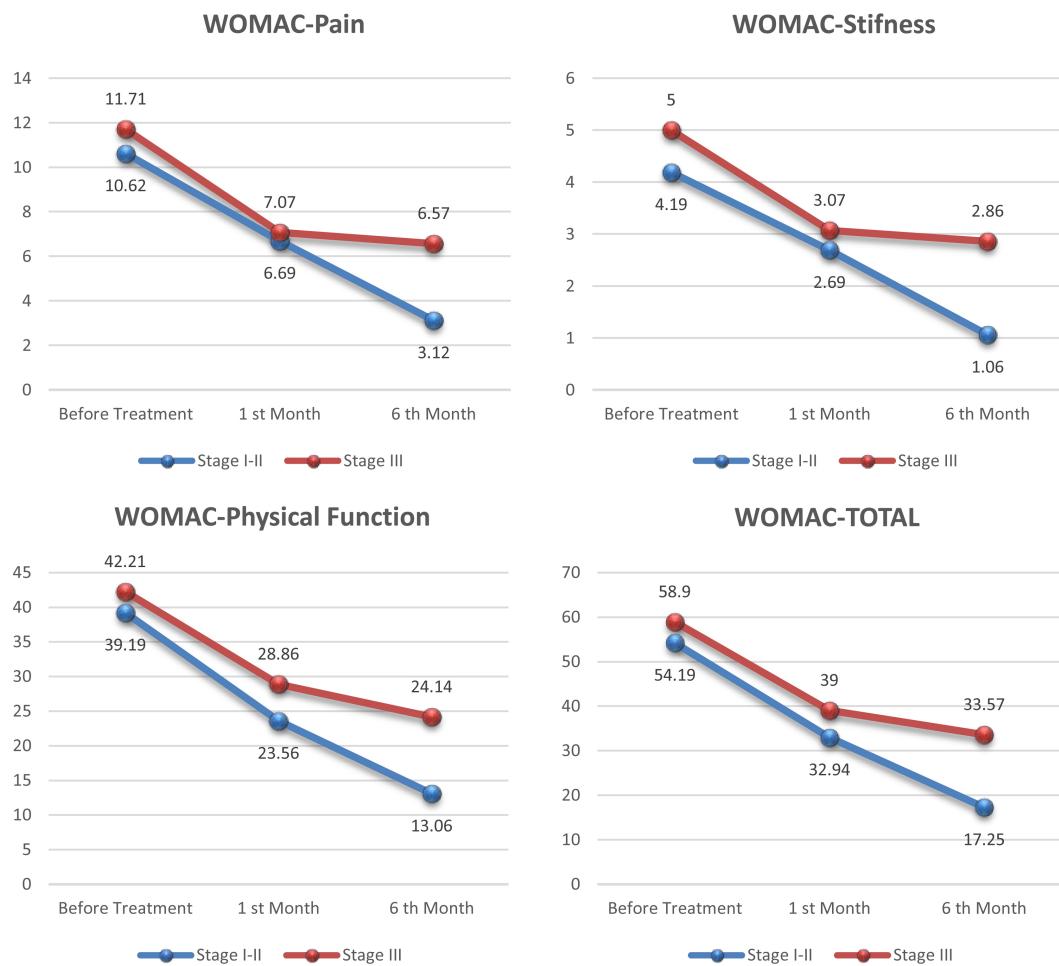


Fig. 6. Change in the WOMAC scores over time depending on the stage of osteoarthritis in the PRP group.

Table 4

Distribution of the SF-36 scores between the study groups and within each study group before and 1st and 6th months after the treatment

SF36		Before treatment $\bar{X} \pm S$	1st month $\bar{X} \pm S$	6th month $\bar{X} \pm S$	p^*
Physical function	PRP ($n = 30$)	37.17 ± 18.04^{bc}	55.33 ± 19.99^c	68.17 ± 21.43	< 0.001
	Placebo ($n = 27$)	36.30 ± 17.35^c	40.00 ± 19.41	46.85 ± 18.61	0.008
	p^{**}	0.676	0.008		< 0.001
Difficulty in physical role	PRP ($n = 30$)	25.00 ± 34.74^{bc}	52.50 ± 40.13	76.67 ± 39.90	< 0.001
	Placebo ($n = 27$)	23.15 ± 35.31^c	37.04 ± 36.27	46.30 ± 40.85	0.015
	p^{**}	0.928	0.151		0.004
Pain	PRP ($n = 30$)	25.43 ± 20.59^{bc}	57.03 ± 24.20	70.77 ± 27.75	< 0.001
	Placebo ($n = 27$)	25.00 ± 17.35^{bc}	42.96 ± 23.75	46.56 ± 22.34	< 0.001
	p^{**}	0.735	0.028		0.001
Overall health	PRP ($n = 30$)	43.10 ± 19.53^c	48.70 ± 21.52	56.80 ± 22.80	< 0.001
	Placebo ($n = 27$)	42.78 ± 24.26	45.11 ± 21.49	47.48 ± 23.76	0.157
	p^{**}	0.981	0.378		0.128
Vitality	PRP ($n = 30$)	44.17 ± 10.43^c	50.67 ± 17.85	58.17 ± 16.48	0.007
	Placebo ($n = 27$)	48.33 ± 19.71	48.70 ± 21.11	42.59 ± 20.26	0.388
	p^{**}	0.391	0.515		0.004
Social isolation	PRP ($n = 30$)	50.67 ± 26.08^c	62.32 ± 23.04	75.45 ± 22.90	0.001
	Placebo ($n = 27$)	46.96 ± 27.36	56.74 ± 23.86	53.04 ± 26.15	0.283
	p^{**}	0.509	0.185		0.001
Difficulty in emotional role	PRP ($n = 30$)	43.23 ± 42.12^{bc}	49.77 ± 36.84	83.22 ± 28.82	< 0.001
	Placebo ($n = 27$)	34.30 ± 31.17	31.82 ± 26.75	38.15 ± 43.02	0.986
	p^{**}	0.479	0.044		< 0.001
Mental health	PRP ($n = 30$)	65.333 ± 16.52^{bc}	65.73 ± 19.50	75.07 ± 15.24	0.003
	Placebo ($n = 27$)	62.81 ± 18.16	63.56 ± 18.38	58.22 ± 16.05	0.515
	p^{**}	0.791	0.575		< 0.001
Physical health	PRP ($n = 30$)	27.65 ± 7.69^{bc}	38.67 ± 9.14	43.59 ± 11.33	< 0.001
	Placebo ($n = 27$)	27.78 ± 8.08^{bc}	32.96 ± 10.07	35.89 ± 10.44	< 0.001
	p^{**}	0.885	0.041		0.018
Mental health	PRP ($n = 30$)	45.09 ± 8.86	44.13 ± 8.66^c	50.61 ± 6.55	0.054
	Placebo ($n = 27$)	42.93 ± 9.60	42.70 ± 7.13	39.78 ± 11.28	0.162
	p^{**}	0.477	0.648		< 0.001

n: Number of patients; \bar{X} : Mean; S: Standard deviation; *Friedman Test; **Mann-Whitney U Test; b: In post-hoc paired comparison, statistically significant difference was detected compared to "1st month after the treatment"; c: In post-hoc paired comparison, statistically significant difference was detected compared to "6th month after the treatment".

Table 5

Unit percentage of the decrement in VAS and WOMAC scores of both groups at the end of the 6th month

	WOMAC			VAS		
	Pain	Stiffness	Physical function	Total	Rest	
PRP group	57.5%	58.4%	55.0%	55.9%	68.9%	60.5%
Placebo group	27.1%	23.3%	23.3%	25.7%	31.6%	33.4%

In contrast, in the placebo group, statistically significant results in the scores for the main factors in physical health were obtained only in the sixth month after the treatment. Regarding the SF-36 sub-parameters, the PRP group was much better than the placebo group. The detailed information is shown in Table 4.

3.4. Adverse effects

The patients in both groups were questioned about any adverse effects in their first-and sixth-month con-

trols. None of the patients had experienced severe adverse effects, such as septic infection, long-term pain, or bleeding. In the PRP group, five patients (16.6%) described mild pain that lasted 1–2 days and did not require treatment. Three patients (11.1%) in the placebo group described mild pain that lasted for 24 hours after the injection.

3.5. Cartilage thickness

No statistically significant differences were detected

Table 6
Comparison of the delta value in WOMAC scores after the treatment

WOMAC		Δ	
		Before treatment – 1st month	Before treatment – 6th month
		$\bar{X} \pm S$	$\bar{X} \pm S$
Pain	PRP (n = 30)	4.27 ± 3.53	6.40 ± 4.09
	Placebo (n = 27)	2.78 ± 2.91	3.26 ± 3.98
	p*	0.062	0.011
Stiffness	PRP (n = 30)	1.70 ± 1.56	2.67 ± 1.71
	Placebo (n = 27)	1.11 ± 1.48	1.07 ± 1.80
	p*	0.125	< 0.001
Physical function	PRP (n = 30)	14.57 ± 10.35	22.37 ± 12.59
	Placebo (n = 27)	8.15 ± 12.67	9.22 ± 14.33
	p*	0.068	0.001
Total	PRP (n = 30)	20.63 ± 14.45	31.53 ± 17.58
	Placebo (n = 27)	13.11 ± 14.00	14.67 ± 18.08
	p*	0.045	0.001

n: Number of patients; \bar{X} : Mean; S: Standard deviation; *Mann-Whitney U Test.

Table 7
Comparison of the delta value in VAS scores after the treatment

VAS		Δ	
		Before treatment – 1st month	Before treatment – 6th month
		$\bar{X} \pm S$	$\bar{X} \pm S$
Rest	PRP (n = 30)	2.01 ± 1.48	2.67 ± 2.06
	Placebo (n = 27)	1.26 ± 1.63	1.56 ± 2.15
	p*	0.052	0.161
Movement	PRP (n = 30)	3.40 ± 1.83	4.30 ± 2.41
	Placebo (n = 27)	1.74 ± 2.25	2.59 ± 2.48
	p*	0.005	0.027
Night	PRP (n = 30)	2.23 ± 1.89	3.13 ± 2.53
	Placebo (n = 27)	1.44 ± 1.55	1.44 ± 2.42
	p*	0.110	0.044

n: Number of patients; \bar{X} : Mean; S: Standard deviation; *Mann-Whitney U Test.

between the medial, intercondylar, and lateral femur cartilage thicknesses before and six months after the treatment in both groups ($p > 0.05$).

4. Discussion

This study was conducted to determine the effects of PRP treatment on pain, functionality, quality of life, and cartilage thickness in patients with knee OA. The results showed that the PRP did not have any effect on cartilage thickness, whereas all other parameters were improved in the first and sixth months after the treatment. This improvement was more pronounced in patients with lower K-L grades than in patients with higher K-L grades in WOMAC scores.

The number of studies on the use of PRP in musculoskeletal system diseases has increased in recent years. Interest in this novel treatment method has increased because of the positive results obtained in *in vitro* and animal studies. Although numerous clinical studies have been conducted on humans, most have been reported as being methodologically weak [9]. In most studies in the literature, the PRP treatment in knee OA is compared to the hyaluronic acid (HA) treatment. Spakova et al. [10] and Sanchez et al. [11] found that PRP treatment was superior to HA treatment over a follow-up period of six months, whereas Vaquerizo et al. [12], Reissadat et al. [13], and Cole et al. [14] found that it was superior to HA treatment over a follow-up period of one year. In contrast, Cerza et al. [15] found that PRP treatment was superior to HA treatment in all grades (K-L grades 0–3) over a long-term follow-up period of two years. However, in a study by Filardo et al. [16], it was found that both treatment methods were effective in OA treatment, and neither was superior to the other. In all these previous studies, the greatest limitation was the lack of a placebo group.

There is a lack of prospective, double-blind, and randomized studies with a placebo control. So far, there are only three studies in the literature. In the first study by Patel et al. [17], Seventy eight patients with early-stage bilateral knee OA were divided into three groups: two doses of PRP; one dose of PRP; and one dose of a saline injection. In both PRP groups, the WOMAC and VAS scores were decreased significantly during the follow-ups. In the saline group, the scores for the same parameters were worsened compared to the beginning of the treatment. In the study by Görmeli et al. [7], One hundred and sixty-two patients with knee OA were divided into four groups: three doses of PRP; one of dose PRP and two doses of saline; three doses of HA and three doses of a saline injection. At the end of the sixth month, all groups showed statistically significant improvement compared with the placebo group. Three doses of PRP injections in early-stage OA showed more significant improvement compared to single-dose PRP and HA injections. In another study that included a saline control group, Smith [18] performed PRP injections in 15 patients and saline injections in 15 patients. Before the treatment and one year after the treatment, the patients were evaluated based on their WOMAC scores. In the PRP group, the total WOMAC score was decreased by 78%. In contrast, in the group that was administered saline, the decrease was 7%. In our study, the total WOMAC scores at the end of the sixth month were decreased by 55.9% in

the PRP group and by 25.7% in the placebo group. In our study, at the end of the sixth month, in the placebo group, the benefit rate from the treatment was found to be higher than that found in other studies. We think that this outcome could be attributed to several factors. In addition to the three studies with the placebo group, we also assigned both groups an exercise program. Hence, regarding ethics, the group that was administered saline alone would not be left untreated. Because of the positive effects of the exercise program, which is a non-pharmacological treatment method that has proven efficacious in many treatment regimens, the patients might have recovered, or their pain might have decreased [3–5]. Moreover, we might have increased the placebo effect in our patients because we also collected blood from the placebo group, and we covered the fluid in the injector during the injection so that it would not be seen. Another factor that could have affected the outcome of our study is that invasive treatments increase the efficacy of treatment in patients. Finally, although it has not been proven, there are published scientific data on the effects of isotonic saline solution on decreasing nociceptive pain in knee OA patients [19].

Although many factors affect the pathophysiology of OA, cartilage loss has a major role in this process. In new treatment methods, the aim is to stop this loss and increase cartilage production [20]. However, a previous study showed that PRP did not induce chondral anabolism or reduce the catabolic process. In the same study, it was also reported that although PRP did not affect cartilage, it modulated joint homeostasis and cytokine levels and decreased synovial hyperplasia [21,22]. In *in vitro* animal experiments, it was shown that in particular, inflammatory mediator levels in the synovial fluid, IL-1b, and TNF-a were decreased by PRP treatment [23]. Cole et al. was the first clinical study on this subject. It included a synovial fluid analysis in addition to a clinical follow-up. In that study, which was prospective, randomized, double blinded, and controlled, PRP and HA were compared, and a significant decrease in the IL-1b and TNF-alpha levels was detected in the PRP group compared to the HA group [14].

In our study, we evaluated the effects of PRP on cartilage by measuring the distal femur cartilage thickness using ultrasonography. Although previous studies on the efficiency of PRP treatment measured pain, functionality, and quality of life, only a few studies have measured cartilage thickness. Sampson et al. [24] did not find a change in the cartilage thickness with ul-

trasonography in the sixth month after three doses of PRP. Halpern et al. [25] also did not detect any changes in the cartilage on MRI at the end of the first year after single-dose PRP. In contrast, Çalış et al. [26] performed three PRP injections at one-week intervals in 82 patients with knee OA. Their findings showed that their VAS and WOMAC scores were improved at the end of the sixth month, and there was a significant increase in knee cartilage thickness measured by ultrasonography. Although the number of patients was higher than in the first two studies, the fact that the person performing the ultrasound-guided assessment was not blinded to the treatment increased the probability of bias. In our study, ultrasonography at the end of the sixth month showed no statistically significant differences between the cartilage thickness in both groups or in the evaluations within the PRP group.

In studies on PRP treatment, because of the differences in the intervals between the applications and the number of repeats, it is difficult to compare the outcomes. Indeed, the main controversial issue regarding PRP concerns the length of the intervals between injections in applications that are performed at least twice as well as the number of repeats. Although in some studies, the applications were in one-week intervals [11,12,15,18], in other studies, one-month intervals were used [13,24]. Spakova et al. explained that they performed injections at one-week intervals because the thrombocyte half-life is 8–10 days [10]. The meta-analysis by Ornetti et al. [9], included only five studies, all of which were prospective, used HA as the comparison, and performed the injections at one-week intervals. In all these studies, PRP was found to be more effective than HA, particularly in younger patients and in those with early-stage OA. Only Cerza et al. [15] found that PRP was significantly more useful than HA in all stages. Furthermore, there is no clear consensus on how frequently PRP treatment should be repeated. In a meta-analysis by Chang et al. [27], it was reported that two or three doses of PRP were more effective than a single dose. In light of these previous results, in our study, we administered three doses of PRP treatment at one-week intervals.

In the sample obtained after centrifugation, there was a high number of leukocytes in addition to thrombocytes (platelets). Although some authors have reported that the presence of leukocytes was undesirable [11,17], many authors have argued that the enzymes and cytokines in leukocytes are protective against infections that can be caused by bacteria, such as *Staphylococcus aureus* and *E. coli* [16,28]. In a

study by Filardo et al., 72 patients were injected with a platelet-rich, leukocyte-poor growth factor (LP-PRP) obtained by single centrifugation method, and 72 patients were injected with leukocyte-rich PRP (LR-PRP) obtained by double-centrifugation method. In both groups, there were clinically significant changes, but one was not superior to the other, whereas symptoms such as pain and swelling were observed more frequently in patients in the LR-PRP group. The authors attributed this result to the leukocyte content [29]. In a meta-analysis by Riboh et al., 1,055 patients in six randomized controlled studies and three prospective comparative studies were included, and leukocyte-poor PRP (LP-PRP) was compared to LR-PRP. No statistical significant differences were detected between the two groups in efficiency and adverse effects [30]. However, there is also no consensus on this subject.

In a randomized study on 63 patients by Mariani et al. [31], HA was included as the control. LR-PRP obtained by the double-centrifugation technique was applied in three doses at one-week intervals. A synovial fluid analysis was conducted and pro- and anti-inflammatory cytokines were measured in blood drawn from both groups. The results showed no differences between the two groups in local and systemic pro- and anti-inflammatory cytokines. Contrary to the findings of *in vitro* studies, LR-PRP did not cause any significant changes in the levels of pro-inflammatory cytokines [31]. Patel et al. reported that among patients treated with PRP containing zero leukocytes obtained by leukocyte filter, the rate of adverse effects was 22% in those who received one dose of PRP, and it was 44% in those who received two doses of PRP. Pain was caused by the calcium chloride (CaCl_2) used for activation [17].

Activation has been performed using various substances before the PRP injection. In a study by Cavallo et al., it was found that activation was an important step in ensuring tissue health and in taking advantage of bioactive molecules. The results showed that when activation was performed with type-1 collagen, there was significantly less release of the growth factor (GF) than in other groups. The growth factors were released 15 minutes after activation was performed using 10% CaCl_2 ; this effect lasted up to 24 hours [32]. Some studies have shown that the activation procedure might have adverse effects and therefore should not be recommended [10,13]. However, the activation procedure has been recommended in many randomized prospective controlled studies [11,12,16,17]. In our study, we obtained LR-PRP using a double-centrifugation

method. Before applying it, we performed activation using 10% CaCl_2 . Although there were mild and transient symptoms in five patients (16.6%) in the PRP treatment, we did not encounter any severe side effects.

The limitations of our study are the small number of patients; the relatively short period of time; and the absence of a third group that was treated only with exercise. Nevertheless, we think that our results will guide future studies in this area.

5. Conclusion

Our study is the first to evaluate the effects of PRP on knee OA in terms of both clinical parameters and cartilage thickness using a double-blind, prospective, randomized, placebo-controlled design. While there were improvements in both the PRP and placebo groups in terms of the evaluated clinical parameters, they were more pronounced in the PRP group, particularly in patients with early-stage OA. At the sixth-month follow-up, the results also showed that the PRP treatment applied three times in one-week intervals did not increase cartilage thickness.

Conflict of interest

None of the authors declare any financial support or conflict of interest.

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