



Original Article

## Effect of platelet concentration on clinical improvement in treatment of early stage-knee osteoarthritis with platelet-rich plasma concentrations

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**Abstract.** [Purpose] To compare two platelet-rich plasma kits with different platelet concentrations for treatment of knee osteoarthritis. [Subjects and Methods] Male and female patients with knee osteoarthritis who had confirmed diagnosis with X-ray and magnetic resonance imaging were included in this retrospective study. Eligible patients were divided into two groups: Group I, which received platelet-rich plasma kit I, and Group II, which received platelet-rich plasma kit II. Platelet concentrations of both kits were measured by manual counting. For each group, platelet-rich plasma kit was injected twice with a one-month interval between injections. The Western Ontario and McMaster Universities Osteoarthritis Index and the Visual Analog Scale were applied for clinical evaluation before the first injection and one, three and six months after the second injection. [Results] Kits I and II contained 1,000,000 and 3,000,000 platelets/ $\mu$ l respectively. In both groups, initial Western Ontario and McMaster Universities Osteoarthritis Index and Visual Analog Scale scores were significantly higher compared to the latter evaluations. However, no significant difference was observed between groups in terms of clinical evaluations. [Conclusion] Similar clinical results were found in groups receiving different platelet concentrations, therefore, a concentration of 1,000,000 platelet/ $\mu$ l is considered sufficient for pain relief and functional recovery.

**Key words:** Knee, Osteoarthritis, Platelet-rich-plasma

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### INTRODUCTION

Osteoarthritis (OA) of the knee is a progressive disease involving the intra-articular (IA) tibiofemoral and patellofemoral cartilage<sup>1)</sup>. Conservative treatment modalities are the first choice in younger and middle-aged populations with cartilage damage and OA of the knee<sup>2)</sup>. Treatment of knee OA includes non-pharmacological methods such as exercise and lifestyle

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modification, as well as pharmacological therapies, such as analgesics, non-steroidal and steroidal anti-inflammatory drugs, and corticosteroid injections. The pharmacological therapies often have side effects and sometimes do not provide adequate benefits<sup>3,4</sup>.

Platelet-rich plasma (PRP) has been gaining popularity in treatment of knee OA due to its simplicity, safety and minimally invasive features<sup>5</sup>. PRP is an autologous blood product with an elevated platelet (PLT) concentration that contains many different granules<sup>6,7</sup>. PLT granules include a variety of growth factors (GFs), including PLT-derived GF, transforming GF-beta, insulin-like GF-1, and epidermal GF. The concentrations of these GFs may vary between patients and within the same patient at different times. These molecules are believed to be important in maintaining joint homeostasis, tissue healing and tissue regeneration<sup>8</sup>. PLT granules also store substances such as adenosine diphosphate, adenosine triphosphate, histamine, dopamine, serotonin, cathepsin D, cathepsin E, elastases and hydrolases, which are believed to play an important role in tissue regeneration<sup>8-10</sup>.

PLT concentration is mentioned in the literature as an important factor in PRP treatment. Scientific evidence is currently limited with regard to optimal PLT concentration for the treatment of knee OA and requires further investigation. Although *in vitro* studies reveal that PRPs with higher PLT concentrations release more GFs than PRPs with lower concentrations (4.69 × versus 1.99 × concentrated PLTs), it remains unclear whether more GFs yield better clinical results<sup>11</sup>.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire is one of the most widely used tools for evaluation of patient's functional status in rheumatic diseases, especially knee OA. Domains of stiffness, pain and functional limitation are measured in this questionnaire<sup>12</sup>. Similarly, the Visual Analogue Scale (VAS) is frequently used to evaluate clinical outcomes.

The aim of this study was to use WOMAC and VAS evaluations to compare the clinical outcomes of early stage-knee OA patients treated with two PRP kits with different PLT concentrations.

## SUBJECTS AND METHODS

Data from male and female patients with knee OA (Kellgren Lawrence stage II and III) who were admitted to our physical therapy and rehabilitation clinic and diagnosed by clinical examination and X-ray imaging between January 2013 and December 2014 were retrospectively included in this study. Patients who did not have routine clinical evaluations (pre- and post-treatment WOMAC and VAS) were excluded from the study. Magnetic resonance imaging (MRI) records were also used for excluding joint effusion, meniscal degeneration, anterior cruciate ligament oedema and other knee injuries which may result in knee pain.

Patients were divided into two groups. Group I patients who received the Easy PRP Kit (Neotec Biotechnology Ltd., Istanbul, Turkey) and Group II patients received the Ycellbio PRP Kit (Ycellbio MEDICAL Co. Ltd., Seoul, South Korea).

For preparation of the Ycellbio PRP kit<sup>®</sup>, 3–4 ml of PRP with a concentration of 9–13 times the average normal value, and 2 ml of anticoagulant were drawn into a 20-ml syringe. Then, 14 ml of blood was collected from the patient. In total, a blood sample of 16 ml was carefully injected at a 45° angle into a Ycellbio kit<sup>®</sup>. The sample was centrifuged at 3,700 rpm for seven minutes to concentrate the PLTs. Using the control lever in the bottom of the Ycellbio kit<sup>®</sup>, 3–4 ml of PRP containing leucocytes was raised to the mid-line and drawn into a 5 ml syringe. The injection site was prepared, and the PRP was injected under sterile conditions using a 22G needle in the classic approach for IA knee injections (Fig. 1). For preparation of the Easy PRP kit<sup>®</sup>, 1–3 ml of PRP with a concentration of 4–6 times the average normal value, and 8.5 ml of blood were collected. Then, 1.5 ml of ACD-A was added to the sample as an anticoagulant. The prepared Easy PRP Kit<sup>®</sup> was centrifuged for five minutes at 1,200 RCF. Afterward, the blood was separated into plasma and red blood cells (RBCs). Next, RBCs, which are collected in the RBC collector section, were discarded from the device. In order to concentrate PLTs, a second centrifuge process was performed for 10 minutes at 1,200 RCF. The final product was 1–3 ml of PRP containing leucocytes. An exogenous factor was not used for the process of activation, but allowing the PLTs to be in direct contact with the joint collagen helped the activation. The injection site was prepared, a local anesthetic injection was administered, and the PRP was injected under sterile conditions using a 22G needle in the classic approach for IA knee injections. For each patient, the anesthetic injection consisted of 2 cc of a mixture of 0.5 cc lidocaine and 5 cc of physiological serum.

Each patient was injected twice by the same physiatrist with a four week interval between injections. The PLT concentration of the two different PRP kits was evaluated by manual counting in the same microbiology laboratory.

The WOMAC and the VAS were applied for objective clinical evaluation before the first injection and one, three, and six months after the second injection. Patients were asked to complete the 24-question WOMAC osteoarthritis index questionnaire, which assesses pain, stiffness, and physical functions of OA. Higher WOMAC scores indicate impairment of the measured function. Level of pain was evaluated by the patients using a VAS with a 10-cm line with 'no pain' at one end and 'worst pain' at the other end.

This study was conducted in accordance with Declaration of Helsinki and approved by the ethics committee of Istanbul Kanuni Sultan Suleyman Training and Research Hospital with approval number KAEK/2014/2. A written informed consent was obtained from each subject.

The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. For the analysis of quantitative data, the Mann-Whitney U test was used. Qualitative data were analyzed using the  $\chi^2$  test. For descriptive statistics of the data, mean,



**Fig. 1.** Intraarticular injection technique

The knee is slightly flexed while the patient is in supine position. The patella is stabilized and the needle is inserted underneath the supralateral surface of the patella. The needle is aimed toward the center of the patella, and then directed into the knee joint.

**Table 1.** Demographic features

		Group I		Group II	
		Mean $\pm$ SD/n (%)	Med (Min–Max)	Mean $\pm$ SD/n (%)	Med (Min–Max)
Age		56.4 $\pm$ 6.8	58 45–69	60.4 $\pm$ 5.5	61 49–69
Gender	Female	19 95%		24 96%	
	Male	1 5%		1 4%	
Affected joint	Right	3 15%		5 20%	
	Left	14 70%		3 12%	
	Bilateral	3 15%		17 68%	

standard deviation, median, minimum, maximum, frequency and ratio values were used. SPSS 22.0 software (IBM, Armonk, NY, USA) was used for analysis.

## RESULTS

A total of 20 patients (19 females) were treated in Group I, and 25 patients (24 females) were treated in Group II. Mean ( $\pm$  SD) age of the patients was 56 ( $\pm$  6.8) years in Group I, and 50 ( $\pm$  5.5) years in Group II. There were no significant differences between the two groups in terms of gender and age ( $p > 0.05$ ; Table 1).

In both groups, the initial WOMAC total and subgroup scores for pain, stiffness, and function were higher at pre-injection evaluation when compared to the evaluations performed one, three, and six months after the second injection. WOMAC scores were reduced by more than 50% for each group, and these reductions were significant in each group ( $p < 0.05$ ). However, no significant difference was observed between Group I and Group II in terms of WOMAC total and subgroup scores ( $p > 0.05$ ; Table 2).

Like the WOMAC scores, VAS scores of the patients reduced significantly ( $p < 0.05$ ) after the treatment in both groups when compared to pre-injection evaluations. No significant difference ( $p > 0.05$ ) was detected between the two treatment groups in terms of VAS evaluations performed at pre-injection, or one, three, and six months after the second injection (Table 3).

## DISCUSSION

There is no consensus regarding a standard regimen of PRP treatment in musculoskeletal disorders. When using PRP as

**Table 2.** The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) evaluations of Group I and Group II

	Group I			Group II		
	Mean ± SD	Med (Min–Max)		Mean ± SD	Med (Min–Max)	
<b>WOMAC pain</b>						
Pre-injection	15.3 ± 3.0	15	10–20	16.1 ± 2.6	15	12–25
1 month	7.5 ± 2.1	7*	5–15	6.9 ± 1.9	7*	0–10
3 months	7.3 ± 0.9	7*	5–9	7.4 ± 2.2	7*	0–13
6 months	7.0 ± 0.0	7*	7–7	7.0 ± 0.0	7*	7–7
<b>WOMAC stiffness</b>						
Pre-injection	6.0 ± 1.7	6	2–8	6.2 ± 1.2	6	3–10
1 month	3.2 ± 0.8	3*	2–6	2.9 ± 0.9	3*	0–4
3 months	3.2 ± 0.4	3*	3–4	3.1 ± 1.1	3*	0–6
6 months	3.2 ± 0.4	3*	3–4	3.4 ± 0.5	3*	3–4
<b>WOMAC function</b>						
Pre-injection	52.7 ± 12.1	51	34–68	54.0 ± 9.8	51	34–85
1 month	21.0 ± 6.2	19*	17–44	20.0 ± 7.2	19*	0–34
3 months	22.5 ± 3.6	21*	19–33	22.7 ± 6.0	19*	16–35
6 months	19.0 ± 0.0	19*	19–19	19.0 ± 0.0	19*	19–19
<b>WOMAC total</b>						
Pre-injection	74.0 ± 16.2	72	48–96	76.3 ± 13.5	72	49–120
1 month	31.6 ± 9.0	29*	24–65	29.8 ± 9.7	29*	24–48
3 months	32.9 ± 4.0	29*	29–45	33.2 ± 8.3	29*	16–54
6 months	29.2 ± 0.4	29*	29–30	29.4 ± 0.5	29*	29–30

Mann-Whitney U test/Wilcoxon test

\*Significant difference ( $p < 0.05$ ) when compared to the pre-injection evaluation of the same group

**Table 3.** Visual Analogue Scale (VAS) evaluations of Group I and Group II

	Group I			Group II		
	Mean ± SD	Med (Min–Max)		Mean ± SD	Med (Min–Max)	
<b>VAS</b>						
Pre-injection	7.2 ± 1.2	7	5–9	7.4 ± 1.1	7	4–9
1 month	3.6 ± 0.5	4*	3–4	3.7 ± 0.8	4*	1–5
3 months	3.5 ± 0.5	3*	3–4	3.0 ± 0.7	3*	1–4
6 months	2.7 ± 0.5	3*	2–3	2.6 ± 0.5	3*	2–3

Mann-Whitney U test/Wilcoxon test

\*Significant difference ( $p < 0.05$ ) when compared to the pre-injection evaluation of the same group

a treatment for OA, there are many variables to consider. These include preparation method, needle gauge for injection, PLT concentration, PLT granule secretion variability, leucocyte concentration, PLT storage, anticoagulant use, PLT pre-activation, local anesthesia use, image guidance use, injection volume, injection frequency, pre-injection and post-injection protocol, severity of OA being treated, and other patient factors<sup>13</sup>).

In different studies, the average series of injections ranged from two to three at intervals of two to six weeks<sup>14–16</sup>). In this study, two injections separated by a four week interval were used, to allow sufficient time to alleviate the patients' symptoms. This protocol was chosen because inflammatory processes and patient symptoms are usually improved in two weeks<sup>15</sup>).

Two different PRP kits were used in this study, so two different centrifuge devices with different volumes of blood were used. Needle gauge may affect the activation of PLTs, and blood collection with small-gauge needles may lead to premature activation of PLTs<sup>11</sup>). Needle gauge is considered to be important for timing of GF release, since most GFs are released 10 minutes after activation<sup>6</sup>). To prevent this early activation, larger needles (at least 21G) may be used and in combination with slow aspiration for blood harvest<sup>17</sup>). To our knowledge, there are no solid data on the effect of needle gauge while administering PRPs, and further research is necessary to determine needle gauge effect on PLT activation<sup>17</sup>). However, to eliminate any

possible bias, the needle size (22G) was used for both blood harvest and PLT injection in both groups.

PLT concentration is one of the most topical factors in PRP treatment. PLT concentration in the published literature on knee OA has been variable and not consistently reported. Some authors suggest that the PRP PLT concentration should be at least two times the whole blood PLT concentration; however, concentrations up to eight times that of blood levels have been reported with good results<sup>18, 19</sup>. There is in vitro evidence that PRP with higher PLT concentration (4.69x) releases more GFs than PRP with lower PLT concentration (1.99x)<sup>11</sup>. In practice, there is evidence that positive clinical outcomes in knee OA can be obtained with relatively low PLT concentrations<sup>20</sup>. In the present study, although three times more PLTs were found in the Ycellbio PRP<sup>®</sup> kit (Group II), similar clinical results were obtained with the Easy PRP<sup>®</sup> kit (Group I). Consequently, PRP has multifactorial efficacy, including PLT granule secretion and leucocyte (and subtype) concentration.

In a meta-analysis, it was suggested that PRP injections significantly improve functional status in patients with degenerative knee pathology, and the beneficial effect was maintained for one year after treatment. It was added that the effectiveness of PRP was derived from a biological benefit which could not simply be explained by the placebo effect<sup>21</sup>. Scientific evidence is currently insufficient to suggest optimal PLT concentration for the treatment of knee OA and requires further investigation. Patel et al. compared the effects of one or two injections of PRP with a normal saline injection (as a control) in knee OA. According to their results, a single injection of PRP was shown to be as effective as two injections, and both were more effective than a normal saline injection<sup>22</sup>. Another study, conducted by Raeissadat et al. examined the effects of two injections of leucocyte-rich PRP (LR-PRP) separated by a four-week interval. Results indicated improvement in pain, stiffness, and functional capacity of patients with knee OA after six months. Improvements in quality of life after injections were significant. These changes were more significant in physical domains, including role limitation due to physical health, pain and physical functioning<sup>14</sup>. To date, there are no clear data about intervals between injections of PRP or frequency of injection. In this study, two injections of two different PRP-kits, with a four-week interval in between, revealed no difference in terms of WOMAC and VAS scores.

The use of local anesthesia for PRP injections is controversial. In vitro, local anesthetics have been shown to decrease the positive effects of PRP<sup>23</sup>. Furthermore, the chondrotoxicity of local anesthetics has been shown in multiple studies<sup>24, 25</sup>. In order to avoid negative impacts, no local anesthetic was administered intra-articularly; instead, a local anesthetic was applied at the injection site for patient comfort.

We recognize two major limitations of our study. The first major limitation was the retrospective design of our study, which restricted our sample size. The second major limitation was that biochemical changes of each PRP kit could not be evaluated.

As a result, similar clinical outcomes may be obtained with PRP kits containing different numbers of PLTs; we found receiving 1,000,000 PLTs/ $\mu$ l to be sufficient for pain relief and functional recovery in treatment of early stage knee osteoarthritis.

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