



# The Clinical Effect of Platelet-Rich Plasma Prepared Through Different Activation Methods on Patients with Knee Osteoarthritis

## Farklı Aktivasyon Yöntemleriyle Hazırlanan Trombositten Zengin Plazma'nın Diz Osteoartritli Hastalardaki Klinik Etkisi

The Different Activation Methods of Platelet Rich Plasma

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### Özet

**Amaç:** Trombositten zengin plazma'nın (TZP) eklem içi uygulaması, diz osteoartriti tedavisinde alternatif bir yöntem olabilir. Bu çalışmanın amacı, eklem içine uygulanmadan önce trombositten zengin plazmanın aktivasyon yöntemlerinin karşılaştırılmasıdır. **Gereç ve Yöntem:** 51 hasta (76 diz) rastgele iki gruba ayrıldı. 1. grupta TZP aktivasyonu kalsiyum klorid (CaCl) eklenerek sağlandı. 2. grupta ise TZP aktivasyonu, hazırlandıktan sonra -70 derecede 24 saat bekletilip, tamamen çözülmesi için 37° suda 5 dakika bekletilerek eklem içine uygulandı. Hastalar VAS ve WOMAC ağrı skorları ile başvuru anında, ikinci, altıncı ve onikinci aylarda değerlendirildiler. **Bulgular:** VAS ve WOMAC skorları başvuru anında; 2., 6., ve 12. aya göre kıyaslandığında istatistiksel olarak anlamlı derecede yüksekti (p=0.06). 2., 6., 12. aylarda gruplar arasında istatistiksel anlamlı fark saptanmasa da 2., 6., ve 12. aylarda her iki skorda kademeli olarak düşme eğilimi olduğu görüldü. **Tartışma:** Hastalar her iki aktivasyon yönteminden de oldukça fazla klinik fayda gördüler. Klinik faydaları açısından TZP'nin CaCl veya -70 derecede aktivasyonu arasında anlamlı fark yoktur. Bu nedenle -70 derecede aktivasyon CaCl gibi ilave malzeme ihtiyacını ortadan kaldıracağından öncelikli tercih edilebilir.

### Anahtar Kelimeler

Trombositten Zengin Plazma; Osteoartrit; Diz

### Abstract

**Aim:** Intra-articular application of platelet rich plasma (PRP) can be an alternative treatment method for knee osteoarthritis. The objective of this study was to compare the activation methods of platelet rich plasma before intra-articular application. **Material and Method:** A total 51 patients (76 knees) was randomly selected into two groups. In group 1, activation of PRP was managed by adding calcium chloride (CaCl). In group 2, activation of PRP was managed by keeping the solution at -70° degrees for 24 hours after preparation and immersed in water at 37°C for a period of 5 minutes for complete dissolution. Then PRP was applied. The patients were assessed with VAS and WOMAC pain scores both baseline and after 2nd, 6th and 12th months of the treatment. **Results:** VAS and WOMAC pain scores were significantly higher at baseline compared to the results obtained at the 2nd, 6th and 12th months (p=0.06). Following 2nd, 6th and 12th months a gradual downward tendency was seen in both scores, even though no significant difference was found between the groups after 2nd, 6th and 12th months. **Discussion:** Patients received some clinical benefits from both activation methods. There is no significant difference between activating PRP by CaCl or -70°C which compared in terms of clinical benefits. Therefore, blood storage at -70°C may be preferred primary due to no need for additional material such as CaCl.

### Keywords

Platelet-Rich Plasma; Osteoarthritis; Knee

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## Introduction

Osteoarthritis (OA) is a degenerative chronic joint disease which is generally progressive. It is a musculoskeletal system problem seen remarkably widespread in people above the age of 50 years [1]. In primary OA, the knee joint is one of the most frequently involved joints and this leads to degenerative lesions in cartilage. Oxidative stress, lipid peroxidation and proteolytic enzymes may have a role in pathogenesis of knee osteoarthritis (gonarthrosis) [2].

However, there is limited self-healing of cartilage lesions and treatment presents a challenge for orthopedic surgeons [3]. As initial treatment, non-steroidal anti-inflammatory drugs (NSAIDs) are the first choice; however these drugs have toxic effects on the cardiovascular system and gastrointestinal system [4]. Topical agents have a short-term effect on cases with moderate pain. There have also been studies where intra-articular steroid injection has been applied, although only short-term benefits have been determined [5]. The effectiveness of glucosamine, chondroitin sulfate, vitamin E, hyaluronic acid and arthroscopic debridement on chronic severe chondropathy or osteoarthritis is still controversial [6].

Recently, the efficacy and safety of contemporary treatment methods for the cartilage damage have been evaluated and compared in several studies. Autologous biological therapy for regenerative purposes, hyaluronic acid, matrix metalloproteinase inhibitors, gene therapy, cytokine inhibitors, and the growth factors have been assessed [7-8]. Among them, platelet-rich plasma (PRP) is a natural concentration which contains autologous growth factors in normal blood and contains higher concentrations of natural platelet-derived growth factor, transforming growth factor, insulin-like growth factor, vascular endothelial growth factor and epithelial cell growth factor [9]. These growth factors are secreted from the alpha-granules after platelet activation and facilitate treatment by reaching the damaged zone [10]. The benefits of intra-articular application of PRP in the treatment of degenerative arthritis of knee on the clinical findings have been shown previously [11-13].

Routine preparation of PRP is consisting of centrifugation of whole blood from patient. PRP was prepared by centrifugation varying the relative centrifugal force, temperature, and time. Another method consists of activation of PRP with thrombin and calcium chloride for increasing growth factor levels [14]. The influence of activation of PRP using thrombin and calcium chloride on the secretion of growth factors and on the amount of platelets concentrated in plasma was evaluated previously [14].

The purpose of this study was to identify if any differences exist clinically between the two activation and preparation methods for intra-articular injection of PRP.

## Material and Method

The study group consisting of a total of 51 patients (76 knees) who had received a diagnosis of osteoarthritis was divided into 2 groups, of 38 knees in each. Intra-articular PRP injection was applied to all knees in both groups. In Group 1, immediately after PRP was prepared, 0.22 mEqCaCl was added and applied as an intra-articular injection. In Group 2, the prepared PRP was stored at -70°C for 24 hours then reconstituted in a water tank

at 37°C for 5 minutes before being applied as an intra-articular injection.

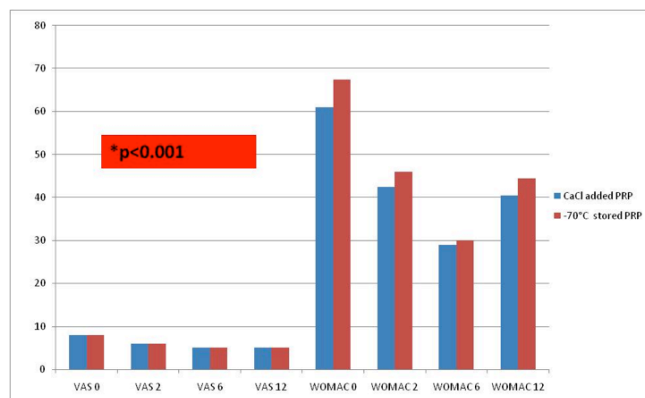
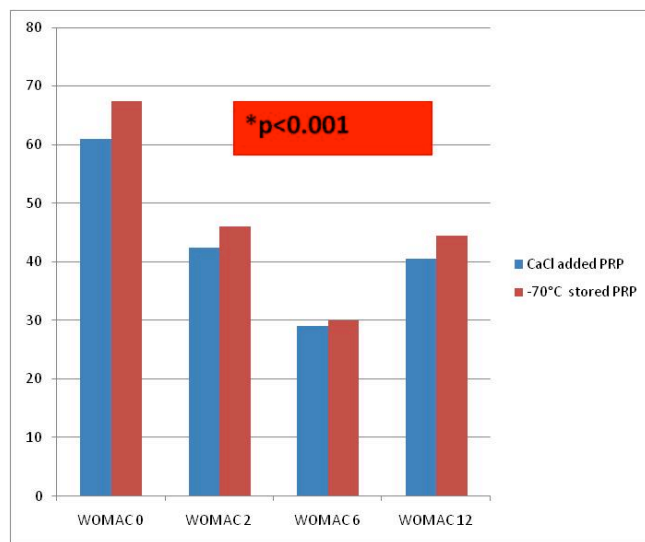
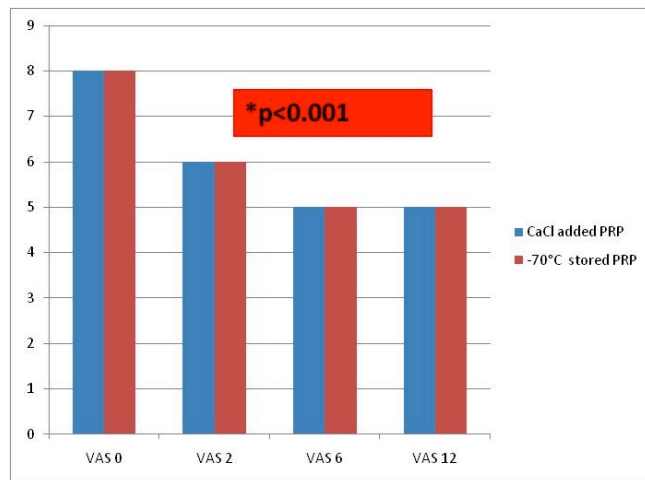
Criteria for admission of patients to the study were the presence of chronic knee pain for at least 4 months or complaints of swelling and degenerative changes determined according to the criteria defined by Kellgren and Lawrence [15]. Patients with systemic chronic disease (Diabetes Mellitus, Rheumatoid Arthritis), major deviation ( $\geq 5^\circ$  varus or valgus), hematological disorders (coagulopathy), severe cardiovascular disease, diagnosed infection, or diagnosed immunodeficiency, and those undergoing anticoagulant/anti-platelet therapy, using NSAID within the previous 5 days, with hemoglobin value under 11 mg/dl and platelet count less than 150000/ml were not included in the study.

VAS and WOMAC tests were used to evaluate 76 knees of 51 patients pre and post first injection then at the 2nd, 6th and 12th month. The patients were 6 males and 45 females. Group 1 consisted of 38 knees of 23 patients and Group 2 of 38 knees of 28 patients. CaCl was added to PRP and administered as an intra-articular injection to all the knees in Group 1. In Group 2, the prepared PRP was stored at -70°C for 24 hours then reconstituted in a water tank at 37°C for 5 minutes, before being applied as an intra-articular injection to all the knees in Group 2. PRP preparation for all patients used standard, specially produced, sterilized, disposable blood tubes and injector sets and special centrifuge apparatus compatible with these tubes. 16 ml blood was taken from each knee and 2 ml of sodium citrate was added. First, the blood taken was centrifuged at 1800 rpm for 15 minutes and was separated from erythrocytes. Then, it was centrifuged at 3500 rpm for 10 min and 3 ml PRP was obtained from the base of the solution. For Group 1, 0.22 mEqCaCl was added to the PRP and the injection was applied into the knee. The PRP prepared from Group 2 was stored at -70°C for 24 hours and then reconstituted completely in a water tank at a temperature of 37°C and applied into the knee. A total of 3 intra-articular injections were administered within 21 days. The injection was applied from the lateral of the knee. Following the application, a 24-hour rest and cold application were recommended. The use of NSAIDs was prohibited until the completion of the intra-articular injections. Follow-up examinations using WOMAC and VAS scoring were made in the 2nd, 6th and 12th months. Since continuous data obtained for Groups 1 and 2 did not conform with normal distribution, non-parametric tests were used. The differences in age, osteoarthritis score, VAS and WOMAC scores between both groups were analyzed with the Mann-Whitney U test, and the differences between the genders were analyzed using the Chi-square test. To determine differences between the VAS and WOMAC scores for both patient groups during the ensuing months of the follow-up period, Friedman Variance Analysis and Bonferroni corrected Wilcoxon-signed ranking test were used. Friedman ANOVA analysis was used in the comparison. Values of  $p < 0.05$  for Friedman Variance Analysis, chi-square test and the Mann-Whitney U test and  $p < 0.01$  cut-off value for Bonferroni corrected Wilcoxon-signed ranking test were accepted as significant.

## Results

The study comprised 76 knees of 51 patients; 6 males and 45

Figure 1. The differences in VAS and WOMAC scores in Calcium Chloride added PRP (Group 1) and PRP stored in -70°C (Group 2) between 0, 2, 6, and 12 Months. (Friedmann Variance Analysis  $p < 0.05$ ).



females with a mean age of 54.82 years. No difference was determined between the groups in terms of age, gender and osteoarthritis level. No significant difference was determined from the perspective of VAS and WOMAC scores between the two patient groups (Table 1). In Group 1, where CaCl was added to PRP, a significant difference ( $p < 0.01$ ) was found by Friedman Variance Analysis between VAS and WOMAC scores obtained at 0, 2nd, 6th, and 12th months. Similarly, in the group activated by PRP after having been stored at -70°C, a significant difference ( $p < 0.01$ ) was found by Friedman Variance Analysis between VAS and WOMAC scores obtained at 0, 2nd, 6th, and

Table 1. Statistical significance in VAS and WOMAC scores in Calcium Chloride added PRP (Group 1) and PRP stored in -70°C (Group 2). (Mann Whitney U Test  $p < 0.05$ ).

	CaCl added PRP	-70°C stored PRP	P-value*
<b>VAS SCORES</b>			
VAS 0	8	8	0,441
VAS 2	6	6	0,117
VAS 6	5	5	0,992
VAS 12	5	5	0,718
<b>WOMAC SCORES</b>			
WOMAC 0	61	67,5	0,144
WOMAC 2	42,5	46	0,196
WOMAC 6	29	30	0,529
WOMAC 12	40,5	44,5	0,747

\* No significant difference  $p > 0.05$ .

12th months. According to the Bonferroni corrected Wilcoxon-signed ranking test results, the values of the scores at 0 month were determined to be considerably greater than the values of the scores measured in the subsequent months.

In both patient groups, the VAS scores obtained in the 2nd and 6th months showed a tendency to decrease gradually. Nevertheless, no significant difference was determined between the 6th month scores and the 12th month scores (figure 1).

### Discussion

Recently, the efficacy and safety of contemporary treatment methods for the cartilage damage have been evaluated and compared in several studies. In an interesting study which criteria for admission of patients to the study and the patients assessment scores were similar to our study, Ediz et al., [16] emphasized that the addition of colchicin to acetaminophen produced significantly greater symptomatic benefit than acetaminophen alone with primary knee osteoarthritis.

In cartilage lesions of various etiologies, the benefit of PRP treatment has been proven by many studies [11, 12, 17]. This effect of PRP depends on the growth factors in the alpha granules [18]. Different methods are used for the release of these growth factors prior to PRP application [19]. Zimmermann [20] conducted a study to determine by which activation method the growth factor concentrate could be obtained at a higher rate prior to application of the prepared PRP substance. As a result of this in vitro study, the growth factors in CaCl-added material were compared with the growth factors in PRP stored at a temperature of -70°C and then reconstituted and it was observed that the growth factors in the CaCl-added substance was at a higher rate. The most significant finding of the current study was that PRP prepared with two different activation methods was no different in terms of clinical benefit. Therefore, rather than preparing more difficult CaCl-added material, the activation method of storing at a temperature of -70°C might be preferable as it can be utilized in all health centers with a blood center.

The application of autogenous PRP has started to be used in many fields together with orthopedics and sports medicine and different results have been reported in various diseases [21]. PRP can be obtained easily and does not cause disease transmission and it is an easy though invasive way of applying many

growth factors. In addition, there is very little concern on safety issues such as immunological reactions or carcinogenesis [22]. In a study by Sampson et al., [23] patients with primary and secondary knee osteoarthritis reported no adverse effects after the injection of PRP. During the current study, an evaluation was also made of potential side effects arising from the treatment. No complications such as infection, muscle atrophy, fever, hemorrhage, tissue hypertrophy, or cohesiveness occurred. Only mild side effects were observed such as slight pain reaction and effusion following the injection, which lasted at most for 2-3 days. Torrero et al., [24] reported significant improvements in clinical symptoms in knee chondropathies during a 6-month follow-up period following a single dose of intra-articular PRP and it could be considered an alternative treatment. The fact that the follow-up periods were longer in the current study and the preparation and application of multiple injections with comparison of different PRP preparation methods are superior aspects of this study. A statistically significant improvement was achieved in both groups in all the parameters evaluated in the current study. No statistically significant difference was seen between the two groups at the conclusion of the follow-up. In addition, clinical improvements were achieved in the majority of patients for daily activities.

In the current study, the VAS and WOMAC scores of both groups in the 2nd and 6th months showed a tendency to decrease gradually and an increase was observed in the WOMAC scores at 12 months. However, when this increase seen in the 12th month was compared with the scores of the 6th month, no significant difference was determined. In a study by Jang et al., [25] of 65 patients suffering from knee pain due to osteoarthritis, it was determined that although the clinical improvement within the first 6 months of the 1 year postPRP injection follow-up period was promising, it was emphasized that throughout such a 1-year follow-up, clinical improvement decreased and that the treatment was effective on early osteoarthritis but increasing age and degeneration decreased the effectiveness of PRP. Lee et al. [14] was evaluated the quantitative measurement of platelet counts and growth factor levels in PRP which was activated by thrombin and calcium chloride. They stated that there was no influence of activation of PRP using thrombin and calcium chloride on the secretion of growth factors and on the amount of platelets concentrated in plasma. In addition to this thrombin and calcium chloride was not considered to be necessary for the effective preparation of PRP or the release of growth factors [13]. Our clinical results were in accordance with this study. Addition of calcium chloride to PRP did not make any difference in the VAS and WOMAC scores compared to the method which the blood was stored in -70°C until the previous day of intra-articular injection procedure.

The methods of the current study could be open to discussion due to the absence of the control group. Another limitation of our study was the criteria for admission of patients, which was defined by Kellgren and Lawrence. The criteria had to be checked also by MR.

In conclusion, when PRP treatment activated by two different methods was applied to patients with osteoarthritis and those two methods were compared in terms of clinical efficacy, a high rate of benefit was observed without any difference between

the two groups. Therefore, the PRP activation method used from storage at -70°C may be preferred by health institutions which have a blood center as there is no need for additional material for PRP preparation. Also PRP can be applied with confidence due to a lower possibility of side effects. Further randomized, controlled studies are required with a larger number of patients and longer follow-up periods to establish a protocol for the intra-articular application of PRP.

### Competing interests

The authors declare that they have no competing interests.

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