Survey of Platelet-Riched Plasma Injection with and without Calcium Gluconate in Treatment of Patients with Knee Osteoarthritis

Abstract

Introduction: The most common cause of knee pain is knee osteoarthritis, many surgical and non-surgical treatments have been proposed but the preferred treatment remains unknown. One of the non-surgical treatments was PRP (plasma reach platate) injection in these patients. Various studies have shown the improvement of the effect of PRP injection with substances such as calcium gluconate, so the aim of this study was to evaluate the effect of PRP with and without calcium gluconate on the clinical results of intra-articular injection of this substance in patients with knee osteoarthritis.

Methods: This was a cohort study in which patients were divided into two groups: PRP injection and PRP injection with calcium gluconate. The results of the study were evaluated by KOOS questionnaire and pain by VAS. A significance level of 0.05 was considered.

Result: Generally, during the study, the pain level in patients after receiving both treatments significantly decreased during the six-month period, while the pain in the case group significantly decreased. (P Value <0.05).

Discussion: From the results of this study, it can be concluded that simultaneous injection of PRP and calcium gluconate can further improve the results of injection.

Key words: Platelet-Rich Plasma,Knee, Osteoarthritis of knee,Calcium Gluconate,Conservative Treatment

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Introduction

Knee osteoarthritis is one of the most frequently treated clinical musculoskeletal complaints by physicians [(1)]. The most common cause of knee pain is degenerative arthritis [(2)]. Osteoarthritis is a multifactorial degenerative disease that affects various joints, most notably those that bear weight. Conservative measures such as lifestyle changes, analgesic prescriptions, and intra-articular knee injections are used to treat knee osteoarthritis [(3, 4)].

Numerous intra-articular knee injection studies have demonstrated an increase in biological treatment options for knee disorders, including platelet-rich plasma (PRP), mesenchymal stem cells, and growth factors [(5)]. PRP is defined as an autologous preparation obtained from centrifuged peripheral blood of patients containing a concentration of four to seven times that of the basal level.

Several studies have demonstrated that this method has a more significant beneficial effect than hyaluronic acid or a placebo. It is widely regarded as one of the best treatments options for knee osteoarthritis [(6)]. Various studies have been conducted on the simultaneous injection of PRP and other substances, including sodium bicarbonate, erythropoietin, hyperosmolar dextrose, and calcium gluconate, with varying results [(7-9)]. Additionally, several studies have evaluated the intra-articular injection of sodium bicarbonate alone or in combination with calcium gluconate, with positive results following a six-month follow-up [(10, 11)].

According to relevant studies, calcium gluconate can form bonds between cartilage cells and bone proteins, restoring the hemostatic mechanism in cartilage and reducing apoptosis due to forming an alkaline environment. Moreover, it has anti-inflammatory properties by inhibiting COX-2 [(12)].

CaCl2 and thrombin are frequently used in PRP today to activate platelets [13]. Calcium is a major second messenger in platelet activation and determines platelet response characteristics, including deformation, granular secretion, and platelet aggregation [14]. Platelets can be activated in vitro by adding 10% calcium gluconate during the PRP preparation process [15].

Meanwhile, some believe that inactive platelets can become active when placed in an endogenous environment or when they come into contact with tissues [16]. Injections of activated and non-activated PRP have demonstrated varying outcomes in numerous studies [17, 18]. Considering the controversy surrounding PRP injection alone and in combination with other substances, the purpose of this study was to compare the effect of PRP with and without calcium gluconate on clinical outcomes following intra-articular injection of this substance in patients with knee osteoarthritis.

Methods

This study was a cohort study conducted at Kerman's Shahid Bahonar Hospital. All patients referred to the hospital with a diagnosis of knee osteoarthritis were included in the statistical population. The Kerman University of Medical Sciences ethics committee approved the research (IRKMU.REC.1397.068). The intervention and subsequent follow-ups were conducted on eligible patients with knee osteoarthritis.

Initially, the intervention methods were thoroughly explained to the patients, and written informed consent was obtained before conducting the research. Furthermore, during the history-taking process, data on the subjects' demographic characteristics (height, weight, age, occupation, history and amount of physical activity, history of diseases and medications, absence or presence of pain, abnormality, damage, or knee surgery) were collected.

The following inclusion criteria were used: diagnosis of knee osteoarthritis grade 1 or 2 using the American College of Rheumatology's (ACR) criteria [19], use of at least one osteoarthritis treatment, such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs), aids, lifestyle changes, and weight loss, and a minimum of two weeks since stopping NSAIDs or corticosteroids.

The following exclusion criteria were used: lactation or pregnancy, diagnosis of rheumatoid arthritis or severe cardiovascular disease, skin infection at the injection site, use of immunosuppressive drugs, use of anticoagulants (e.g., warfarin and heparin), a history of hemorrhagic diseases, coagulation disorders, or anemia, current low back pain spreading to the lower extremities (due to possible impacts on the following assessments), a history of knee surgery within the last three months, active infection or malignancy within the last six months, substance abuse, and intra-articular corticosteroid or hyaluronic acid injection within the last six months.

According to previous research, the sample size in this study was estimated to be 60 (30 per group). The patients were divided into two groups: case and control. Following that, the researcher divided the subjects into two groups: those receiving PRP treatment with calcium gluconate and those receiving PRP treatment without calcium gluconate. A total of 8ccs of venous blood were drawn from patients using an 18-gauge butterfly needle and poured into a special tube containing citrate and Bio ACT gel.

Blood samples from participants were centrifuged at 3000 rpm for 8 minutes. After the procedure, 4ccs of PRP were extracted from the tube. After administering 1cc of lidocaine aseptically, the substance was injected into the knee with a 22-gauge needle following the addition of calcium gluconate (0.5ccs per 4ccs of PRP) before gelation [20]. The control group's PRP preparation and injection were identical to those of the intervention group, except for the absence of an activator. Notably, all injections were performed by a single individual (a resident of the orthopedic ward).

Afterward, patients in both groups had their injured knees bent three times inactively, and participants were asked to rest in a supine

position for ten minutes. In the event of knee pain, patients were given acetaminophen and hydrocodone and advised to avoid using the injured knee for the following 24 hours. It is worth noting that no standard physiotherapy protocol was followed during or following the injection. Additionally, those who used alternative therapies such as physiotherapy and acupuncture or took NSAIDs in the following five days, despite the researcher's recommendations, were excluded from the study.

In this study, a visual analog scale (VAS) was used to assess patients' perceptions of pain during rest and while slow-walking before and after PRP injection plus two weeks, three, and six months later. Patients carried out the procedure under the supervision of an orthopedic ward resident. The VAS is a 100-mm horizontal scale with the words "no pain" at one end and "severe pain possible" at the other. The patient draws a line through the point that they believe best represents their current state. Pain is quantified by calculating the length of the line drawn from the continuum's start to the patient's mark. The scale mentioned above has been widely used in research on pain, and its validity has been established [21].

Furthermore, the Knee Injury and Osteoarthritis Outcome Score (KOOS) was used to assess and quantify the symptoms and pain associated with knee osteoarthritis before and following the injection and the level of difficulty associated with daily activities, leisure activities, sports activities, and life quality associated with knee performance. The participants completed the tool before PRP injection and after two weeks, three months, and six months, under the supervision of an orthopedic ward resident. The KOOS is a 42-item patient-centered questionnaire that assesses five concepts of pain, disease symptoms, daily life activities, sports and recreational activities, and life quality concerning knee problems [22].

After a six-month follow-up period, all outcomes and complications of PRP injection were recorded in both groups, and data analysis was performed in SPSS version 22 using an independent sample t-test (to compare the results of evaluations over time in the two groups). Notably, statistical significance was defined as a P-value less than 0.05.

Results

In total, 60 patients were randomly assigned to one of two groups: case (PRP injection with calcium gluconate) or control (without calcium gluconate). The participants were randomly divided into two 30-person groups, with an effort to create two groups that were identical concerning age and gender. Males made up 19 of the case group's participants and 18 of the control group's participants, while females made up the remainder. There was no significant difference in this regard between the two groups (P=0.791).

The mean age of all subjects was 59.35±4.74 years, whereas subjects in the case and control groups were 60.16±4.71 and 58.53±4.74 years, respectively. There was no significant difference in mean age between the groups (P=0.185). Table 1 summarizes the findings regarding patients' pain intensity. In both groups, pain decreased significantly over six months. However, the case group experienced a more significant reduction in pain than the control group (P<0.05).

Table 1. Mean pain score of patients in both case and control groups

		N	Mean	P-Value
Pain before the injection	Case	30	7.7667	1.000
	Control	30	7.7671	
Pain two weeks after the injection	Case	30	7.2667	0.322
	Control	30	7.4667	
Pain three months after the injection	Case	30	6.9333	0.374
	Control	30	7.0667	
Pain six months after the injection	Case	30	6.0667	0.000
	Control	30	6.8667	

In terms of pain, the KOOS questionnaire results indicated that patients in both groups experienced a significant reduction in pain intensity at the end of the six-month follow-up. On the other hand, a decrease in pain in

the case group was more significant than in the control group (P=0.000).

Table 2. Mean pain score based on the KOOS questionnaire in both case and control groups

		N	Mean	P-Value
Pain before the injection	Case	30	85.4333	1.000
	Control	30	84.6667	
Pain two weeks after the injection	Case	30	84.6000	0.322
	Control	30	83.7000	
Pain three months after the injection	Case	30	80.3000	0.374
	Control	30	82.6000	
Pain six months after the injection	Case	30	77.8667	0.000
	Control	30	80.6333	

In terms of symptoms, both groups showed a significant reduction in pain intensity at the end of the six-month follow-up period, according to the KOOS questionnaire results. Meanwhile, decreased symptoms in the case group were more significant than in the control group (P=0.034).

Table 3. Mean score of symptoms based on the KOOS questionnaire in both case and control groups

		N	Mean	P-Value
Score of symptoms before the injection	Case	30	83.7000	0.456
	Control	30	82.6333	
Score of symptoms two weeks after the injection	Case	30	79.8000	0.155
	Control	30	81.8000	
Score of symptoms three months after the injection	Case	30	78.5000	0.103
	Control	30	80.8667	
Score of symptoms six months after the injection	Case	30	76.7667	0.034
	Control	30	79.8333	

In terms of ADL, at the end of the six-month follow-up, the KOOS questionnaire results indicated a significant decrease in the ADL score of patients in both groups. However, the case group saw a more significant reduction in score than the control group (P=0.027).

Table 4. Mean ADL score based on KOOS questionnaire in case and control groups

		N	Mean	P Value
ADL score before the injection	Case	30	86.2000	0.696
	Control	30	85.7000	
ADL score two weeks after the injection	Case	30	84.5000	0.553
	Control	30	83.8000	
ADL score three months after the injection	Case	30	81.1333	0.191
	Control	30	82.6000	
ADL score six months after the injection	Case	30	79.0000	0.027
	Control	30	81.4333	

At the end of the six-month follow-up, the KOOS questionnaire results indicated a significant decrease in the sports score of patients in both groups. Meanwhile, the sports score significantly decreased in the case group than in the control group (P=0.027).

Table 5. Mean sports score based on the KOOS questionnaire in both case and control groups

		N	Mean	P-Value
Sports score before the injection	Case	30	83.5333	0.696
	Control	30	82.4333	
Sports score two weeks after the injection	Case	30	82.2667	0.553
	Control	30	81.5333	
Sports score three months after the injection	Case	30	80.2000	0.191
	Control	30	80.2667	
Sports score six months after the injection	Case	30	76.8000	0.027
	Control	30	78.8000	

Regarding knee-related QOL, the KOOS questionnaire results showed that patients in both groups had a significant decrease in their QOL score at the end of the six-month follow-up. However, the QOL score decreased more significantly in the case group than in the control group (P=0.027).

Diagram 6. Mean QOL scores based on the KOOS questionnaire in both case and control groups

		N	Mean	P-Value
QOL score before injection	Case	30	82.1333	0.696
	Control	30	82.0333	
QOL score two weeks after the injection	Case	30	80.5000	0.553
	Control	30	80.9000	
QOL score three months after the injection	Case	30	76.2000	0.191
	Control	30	78.4000	
QOL score six months after the injection	Case	30	75.5000	0.027
	Control	30	77.0000	

Discussion

This study was a six-month clinical trial involving 60 patients who were identical in age and gender and were randomly assigned to one of two case and control groups. According to the findings, while pain decreased significantly in both groups after a six-month follow-up, the case group's reduction was significantly more significant than the control group's. The five-section KOOS instrument revealed that both groups' pain, symptom, sport, ADL, and QOL scores significantly decreased at the end of the intervention. However, when compared to the control group, the case group's reduction was significantly higher.

Patel et al. looked at 78 patients with knee osteoarthritis divided into three groups, two of which were given PRP injections. After a six-week, three-month, and six-month follow-up, the results indicated a significant relationship between PRP injection and patient improvement in the case group when compared to the control group [23]. Gobbi et al. (2013) followed 80 patients with knee osteoarthritis grade 3 (as determined by the Kellgren and Lawrence system) for a year. Finally, PRP treatment reduced pain and improved patients' quality of life [24]. In this respect, our findings are consistent with the findings of the studies mentioned.

In another study by Kalbkhani et al., the results showed that PRP has a remarkable effect on repairing cracks in rabbit femur articular cartilage, with a significant difference between the treatment and control groups in this regard [25]. In a study, Kon et al. compared the effects of three injection treatments in patients with articular cartilage lesions, finding that the injection improved knee performance and quality of life in patients with low degrees of joint degeneration, particularly in young people [26].

While the studies' results were consistent with our findings, the current study did not focus on a specific age group and instead attempted to create homogeneous groups. As previously stated, there was no significant age difference between the groups.

Platelets contain cytokines that regulate the tissue repair process and are a rich source of growth factors. Platelet-secreted bioactive molecules impact cell trafficking and proliferation, as well as anti-inflammatory and complex catabolic/anabolic activities [27].

Despite the claims made regarding PRP, numerous investigations have found a range of contrasting views. However, the heterogeneity of the results could be explained by the difficulty of obtaining a single result due to the variety of platelet extraction, preparation, and treatment techniques used. Numerous studies indicate that platelet activation in the target environment is critical for achieving a positive outcome [28, 29].

The term "activation" refers to two aspects of PRP: 1- platelet degranulation to release growth factors from α-granules, and 2- fibrinogen cleavage to form an initial matrix and allow growth factor secretion and the repairing process to begin [30]. Platelet activation before PRP administration has been studied in vitro and before injection with thrombin or calcium chloride. On the other hand, some physicians prefer platelets to activate spontaneously after injection in the presence of native tissue collagen [31].

As a result, despite numerous studies, there is disagreement about the best injection and

platelet activation method, and no uniform protocol has been reported to improve the process' results. Exogenous collagen, for example, is a weak factor in platelet activation when compared to other substances. Growth factors are small, potential molecules that can affect the repair process and are influenced by several factors [32]. As a result, one of the principles of PRP treatment protocols appears to be selecting the best platelet-activating factor to achieve acceptable results.

The time of platelet activation is also a factor that affects them. The time of activation and secretion of growth factors is effective in treatment results because the half-life of growth factors and platelets is limited, which is another reason for the discrepancy between the findings of numerous studies. Cavallo et al. [15] demonstrated that 24 hours of calcium chloride ($CaCl_2$) administration resulted in growth factor release and platelet activation.

Meanwhile, Fufa et al. found that administering $CaCl_2$, thrombin, and collagen type 1 at the same time accelerated growth factor activation and secretion [33]. Caamano et al. examined the effect of intra-articular sodium bicarbonate and calcium gluconate injections in patients with bilateral knee osteoarthritis. According to their findings, compared to sodium bicarbonate injection alone, the combination of sodium bicarbonate and calcium gluconate had a more significant impact on improving patient performance. On chondrocytes, cartilage, and bone binding proteins, calcium gluconate has been shown to have a protective effect [10].

In comparison to calcium alone or no activation, Roh et al. discovered that a low dose of thrombin/calcium activation increased the overall cytokine release of PRP preparations over seven days. Researchers and physicians should evaluate the laboratory and clinical outcomes of using any activator type when applying PRP and choose the optimal method based on those outcomes [34]. Based on our findings, calcium gluconate may help patients experience less pain and symptoms and achieve better results. Silva et al. found no significant difference in the rate of growth factor increase between calcium gluconate and bovine thrombin when PRP was extracted from cat samples [35], which contradicts our findings.

A comparison of the current study's findings to those from other studies revealed that the administration of calcium gluconate or other substances combined with PRP remains a point of contention in the treatment protocol for patients with knee osteoarthritis. As a result, additional research in this area is necessary.

One of the study's major limitations was the absence of a control group to evaluate the effect of PRP alone and PRP in combination with calcium gluconate, which may not be negligible due to ethical concerns. Further research with larger sample sizes is recommended in order to confirm or refute these findings.

Conclusion

According to the present study's findings, simultaneous injection of PRP and calcium gluconate had a more significant effect on improving injection results while improving performance in patients with osteoarthritis grades 1 and 2.

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