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| Original Article |

Intra-articular Injections of Platelet-rich Plasma in Patients with Knee Pain of Articular Cartilage Origin (degenerative chondropathy and early OA).

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Abstract : Platelet-rich plasma (PRP) has been used as an alternative to non-operative treatments for increasing the rate of cure in bone and soft-tissue regeneration, although there are very few clinical studies regarding the treatment of articular cartilage damage. Therefore, our study proposes non-surgical intervention for patients with articular cartilage damage and who are experiencing knee pain caused by this damage. This study was conducted as a single medical center. It was an uncontrolled, prospective clinical trial, and the study subjects included 44 patients who were suffering from early osteoarthritis and degenerative chondropathy; they were between 18 and 65 years of age and were included in the study irregardless their sex. PRP was injected twice intraarticulary within an interval of four weeks. The pain scores and functional scores were compared two months, four months, and six months following the second injection was completed, using the VAS, the Lysholm knee scale, and the Cincinnati knee rating system. There were no complications related to the PRP injection. The pain experienced by the study patients two months after the PRP injection was reduced compared to the pain felt before the injection, and the reduction in pain after four and six months compared to the pain experienced two months after the PRP injection was statistically significant. From a functional viewpoint, there was a statistically significant improvement in their pain during the entire follow-up period. Our study results suggest that PRP injection is an effective and safe treatment for the management of early osteoarthritis and degenerative chondropathy, as seen in this clinical trial.

Key words: *platelet rich plasma, knee pain, early osteoarthritis, chondropathy*

1. Introduction

The number of people suffering from knee pain has increased in recent years due to the increasing number of people participating in sports and also to the increasingly aging population.[1] Articular cartilage defects rarely heal spontaneously to normal cartilage due to the avascularity and relative absence of cells capable of becoming mature cartilage cells.[2] Partial thickness cartilage injuries do not heal spontaneously and they remain injured or worsen without surgical intervention.[3]

To date, most mild or moderate articular cartilage injuries have been successfully managed by non-surgical interventions such as drugs, weight loss, and lifestyle changes. However, these management techniques are intended to control the symptoms rather than focusing on making changes in the biochemical environment of the joint or stopping progression of the disease. Articular cartilage injuries progress due to the imbalance between pro-inflammatory cytokines, such as IL-1α, IL-1β, and TNF-β, and anti-inflammatory cytokines such as IL-4 and IL-10. It is already known that this imbalance destroys cartilage by activating protease.[4] A number of studies are being conducted regarding the use of PRP as auto-PRP when it can then be successfully used to treat tendinopathies and chronic wounds. In fact, PRP provides growth factors for the affected areas and transfers anti-inflammatory signals.[5,6] Compared to the studies conducted with regard to chronic tendinopathy, there is essentially a total lack of research regarding the use of PRP as a

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successful treatment method for articular cartilage injuries.

The authors of our study hypothesized that intra-articular injection of PRP can prevent articular cartilage injuries from further progression and can even facilitate their recovery and subsequent symptom improvement. This study attempts to evaluate the effects of intra-articular injection of PRP for knee pain of articular cartilage origin.

2. Methods

Patients between 18 and 65 years of age with early osteoarthritis and degenerative chondropathy(Outerbridge grade I and II) who had previously taken medication for more than six months and without physical improvement, were assessed in an open clinical trial. The 44 patients who met the experiment criteria and who voluntarily gave written consent to PRP treatment, were finally selected. The knee joint had to be stable and without a severe deformity greater than 5 degrees in the valgus or varus.

The exclusion criteria included advanced osteoarthritis (Kellgren-Lawrence Grading Scale >2) and inflammatory arthritis with severe deformity exceeding the above range. Patellofemoral instability, a history of drug abuse, and/or psychological problems were also patient exclusion criteria.

In addition, patients with positive HIV, HBV, HCV, HTLV, CMV, EBV, Syphilis test, severe anemia, severe heart disease or with the history of cardiac surgeries, active tuberculosis, pneumonia, pregnant women or women who were planning pregnancy within six months, women who were breast-feeding, patients with a history of drug abuse within the past six months, patients with uncontrolled infections, those who had taken anti-inflammatory drugs within the past five days, and patients who were judged to be inappropriate to participate in a clinical trial by the doctors of the experiment due to their psychiatric problems, were excluded from this study.

A total of 44 patients received the treatment, and there were 19 male patients (43%) and 25 female patients (57%). The mean age of the study subjects was 43 years (range: 19-63 years old), and their mean BMI was 24.9 (20.1-31.2). Fifteen of them had left knee pain, five of them had right knee pain, and 14 of them had pain in both knees.

This clinical study was in accord with the 'ethical principles of medical research with human beings' based on the declaration of Helsinki and with Korean good clinical practice standards (GCP, The Ministry of Food and Drug Administration 2009-211) and was conducted after being reviewed by Institutional review board (Catholic University of Korea).

2.1 Blood sample Collection and PRP Production

From all the patients who participated in the clinical trial, 27 ml of blood sample was collected with a 20-G needle from an antecubital vein so that the ratio of the blood and the anti-coagulant became 10:1. The collected blood samples were transferred to a prepared separation kit (Prosys, Tricell, Revmed, Seoul, Korea) and underwent centrifugation at the speed of 3,000 RPM for three minutes. The buffy coat layer and the plasma of the upper portion of the layer were obtained and were transferred to a concentration kit (Prosys, Tricell, Revmed, Seoul, Korea) using a 10-ml syringe. They again underwent centrifugation at the speed of 3,300 RPM for three minutes in order to obtain concentrated PRP.

2.2 PRP Injection

The injection area was sterilized aseptically and 3-4 ml of PRP were percutaneously injected into the knee joints. The patients were asked to keep bending and stretching their injected knees several times so that the PRP could be evenly spread. If there was any intra-articular effusion identified, it was removed before the PRP injection. The patients were then advised to rest for 24 hours and not to move the affected knee in an aggressive manner.

2.3 Follow-up After PRP Injection

The study subjects followed the instructions given by the doctors during the experiment and the second PRP injection was administered four weeks after the first injection. The pain score and the functional score were measured two months, four months, and six months after the second injection using the Visual Analogue Scale (VAS), the Cincinnati knee rating system (CKRS), and the Lysholm knee scale (LKS). The clinical score was calculated after determining the times of evaluation as level 1 (before the injection), level 2 (the second injection), level 3 (two months after the second injection), level 4 (in four months after the second injection), and level 5 (in six months after the second injection).

2.4 Evaluation Methods and Statistics

The measurements of each scoring systems were described in the average standard deviation in order to examine the effect of PRP for the pain caused by intra-articular cartilage injuries, and SPSS (version 12.0) was used for the statistical analysis. The Visual Analogue Scale (VAS), Lysholm Knee Scale, and the Cincinnati Knee Rating System were tested using Repeated Measure ANOVA and it was considered as significant when the p values were less than 0.05.

The effect scale (ES), the probability of type 1 error (α) and

Table 1. Average VAS, LKS, and CKRS scores of our study patients. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection. N=44

Level		Average	Standard deviation
1	VAS	6.25	1.433
	LKS	53.57	20.229
	CKRS	56.60	17.371
2	VAS	2.84	1.584
	LKS	88.84	8.787
	CKRS	81.82	13.569
3	VAS	2.05	1.524
	LKS	88.82	7.940
	CKRS	86.09	13.161
4	VAS	1.09	1.273
	LKS	91.82	9.284
	CKRS	85.82	13.968
5	VAS	.48	.876
	LKS	91.89	8.306
	CKRS	90.70	11.531

the statistical power analysis were determined at ES= .50, α= .05, and (1-β) = .90 for the selection of the study subjects, and G-Power 3.0.10 was used.

3. Results

It was determined that level 1 was before the PRP injection, level 2 was the first follow-up, i.e. the second injection, level 3 was the second follow-up (within two months after the second injection), level 4 was the third follow-up (within four months after the second injection), and level 5 was the fourth follow-up (within six months after the second injection). Table 1 shows the average scores of VAS, LKS, and CKRS observed throughout the total five follow-up examinations, and repeated measure ANOVA was used to analyze these results.

The average VAS scores at each level were compared to the previous average VAS scores at previous levels using within-subject contrasts because there was a significant difference in within-subject effects (p=.000) which satisfied a sphericity test (p= .243) (Fig. 1). All of the VAS scores differed significantly from the previous VAS scores, and the patients' pain was significantly improved as the level progressed, as is shown in the graph.

Within-subject effects that satisfied Greenhouse-Geisser test (p=.470, p=.792) were referred because the mean LKS score and the mean CKRS score failed to satisfy a sphericity test (p<0.001, p= .020). The results showed that there were

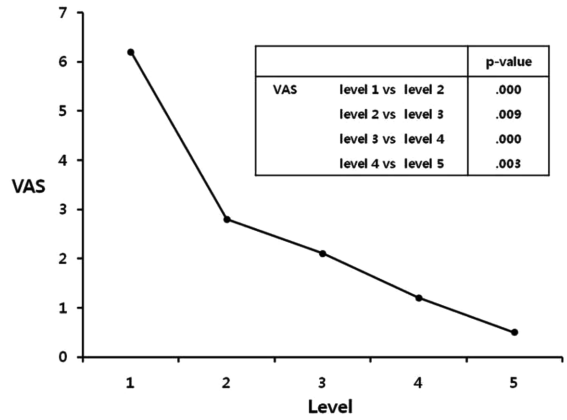


Figure 1. VAS change at each level. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

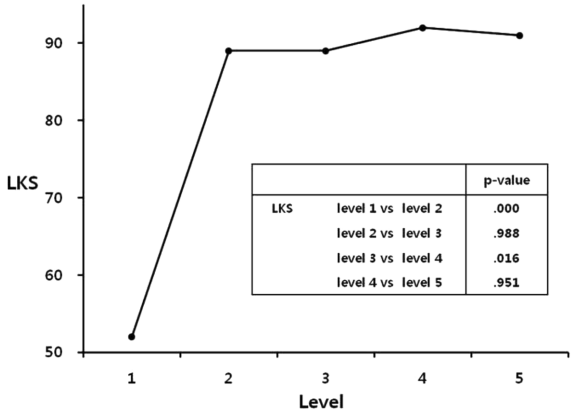


Figure 2. LKS change at each level. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

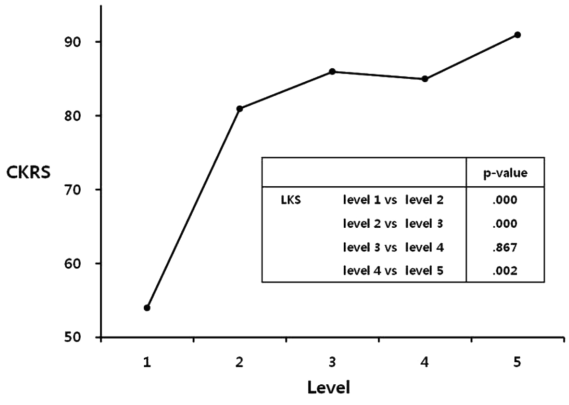


Figure 3. CKRS change at each level. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

significant differences ($p<0.001$, $p<0.001$), and each score was compared to the previous score by within-subject contrasts (Figs. 2 and 3). The LKS scores were improved at the first and the third follow–up examinations after the PRP injection, and the CKRS scores were improved at the first, second, and fourth follow–ups examinations after the PRP injection.

Dividing the patients into two groups according to the mean CKRS score of 57 points before the PRP injection (CKRS1, CKRS ≤ 57; CKRS2, CKRS > 57), there were interactions noted in the changes of the CKRS scores ($p<0.001$) and the LKS scores of each group during the follow–up period. Therefore, each score was compared to the previous scores (Fig. 4). The results showed that there was a significant interaction of the CKRS and the LKS scores seen at the first follow-up examination following the PRP injection. According to the graph, the group with the lower CKRS score showed a significant improvement in both the CKRS score and the LKS score at the first follow-up compared to the other group.

Likewise, the study subjects were divided into two groups according to the mean LKS score of 54 points before the PRP injection (LKS1, LKS ≤ 54; LKS2, LKS > 54), and there were

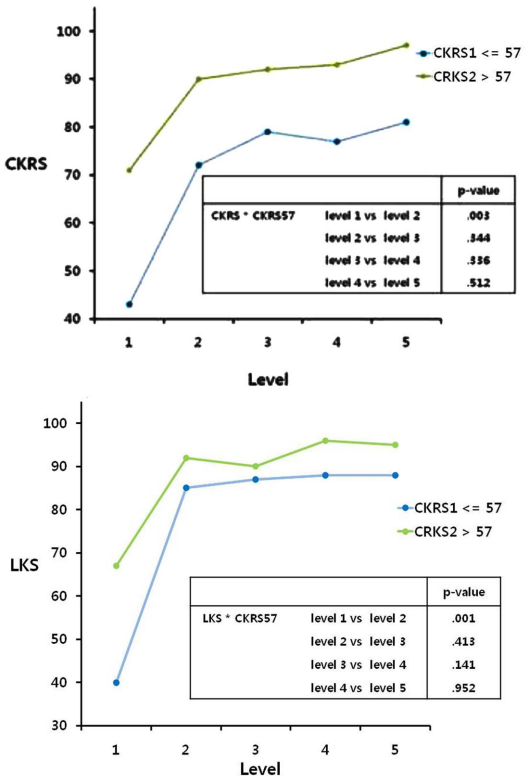


Figure 4. Comparison of CKRS and LKS with below and over average CKRS. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

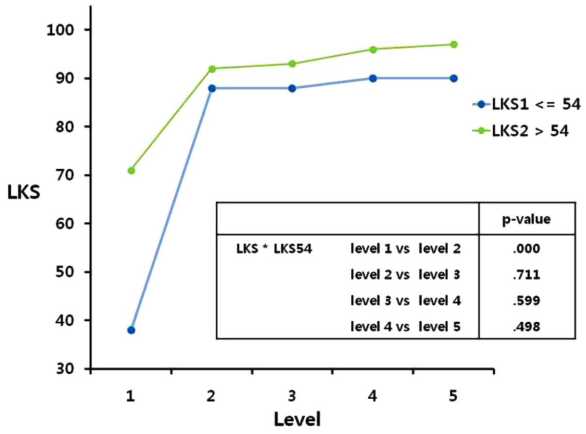


Figure 5. Comparison of CKRS and LKS with below and over average LKS. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

interactions in the changes of the LKS scores ($p<0.001$) of each group during the follow–up period. Therefore, each score was compared to the previous scores (Fig. 5) and the results showed that there was a significant interaction in the LKS scores seen at the first follow-up after the PRP injection and that the group with the lower LKS score showed a significant improvement in the LKS score at the first follow-up examination compared to the other group.

Summarizing both results, we concluded that the relatively high improvements in the articular function were shown immediately after the PRP injection when the knee function was relatively poor prior to the injection.

Dividing the patients into two groups according to the mean VAS score of 6 points before the PRP injection (VAS1, VAS ≤ 6; VAS2, VAS > 6), there were interactions in the changes of VAS ($p<0.001$), LKS ($p=.026$) and CKRS ($p=.033$). Therefore, each score was compared to the previous scores (Fig. 6), and the results showed that there was a significant interaction in the VAS, CKRS, and LKS scores at the first follow-up following the PRP injection and that the group with the higher VAS score showed a significant improvement in the VAS, CKRS, and LKS scores at the first follow-up compared to the other group. This result suggests that there was significant improvement in the articular function and decrease in pain immediately following the PRP injection even when the knee pain was relatively severe prior to the injection.

The improvement of knee function was compared with each level after PRP injection using repeated measure ANOVA according to the general characteristics of the study subjects, and the results showed that there was a significant improvement

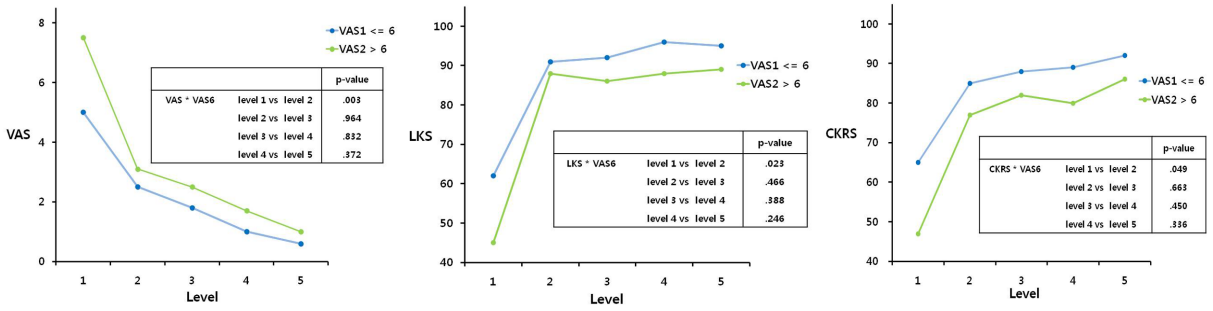
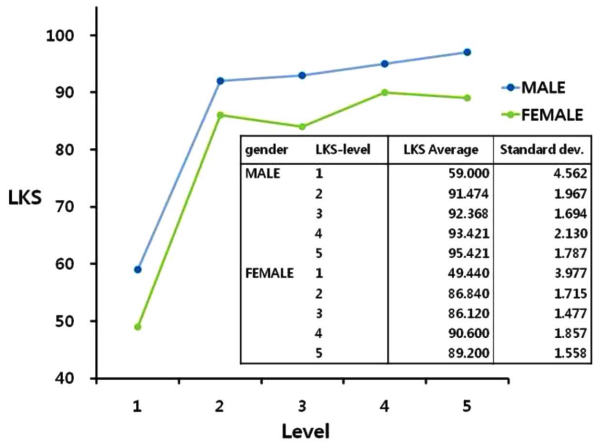


Figure 6. Comparison of VAS, CKRS, and LKS with below and over average VAS. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.



gender	LKS-level	LKS Average	Standard dev.
MALE	1	59.000	4.562
	2	91.474	1.967
	3	92.368	1.694
	4	93.421	2.130
	5	95.421	1.787
FEMALE	1	49.440	3.977
	2	86.840	1.715
	3	86.120	1.477
	4	90.600	1.857
	5	89.200	1.558

Figure 7. Comparison of LKS changes according to patient gender. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

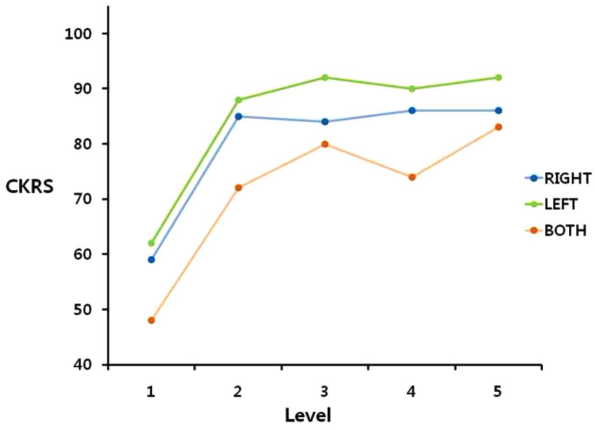


Figure 8. Comparison of CKRS changes according to right, left or both knees. level 1: pre-injection, level 2: 2nd injection, level 3: 2months after the 2nd injection, level 4: 4 months after the 2nd injection, level 5: 6 months after the 2nd injection.

(p=.013) in the LKS scores in relationship to the patient's sex (Fig. 7). There was consistent improvement in the male patients compared to that seen in the female patients, although the improvements had decreased regardless the patient's sex at the time of the second follow-up compared to the first follow-up examination following the PRP injection.

There was also a significant change (p=.016) in the CKRS scores after the PRP injection, although depending on the affected areas, i.e. bilateral or unilateral (left and right) (Fig. 8). The least improvement was seen in those patients who were bilaterally affected at the beginning of the study, although the increase in the improvement in this group was greater toward the end of the study. Those patients who were unilaterally affected experienced a consistent improvement throughout the follow–up period. According to a post-hoc test, those patients with left knee problems experienced the greatest improvement in their articular function, as evaluated by CKRS scores, among all of the study patients.

There were no complications in relation to the PRP injection.

4. Discussion

Osteoarthritis or articular cartilage injuries of the knee occurs frequently. However, the initial management option is usually conservative management such as anti-inflammatory medicine or physiotherapy used to control the symptoms. These management options cannot be considered as a fundamental treatment as they do not delay or stop the disease progress, although they can alleviate subsequent symptoms. Also, conservative management includes passive management methods which wait for the joints to become severely damaged before surgery is recommended. For this reason, there is growing interest in disease-modifying OA drugs (DMOADs) such as glucosamine and chondroitin sulfate.[7, 8] Ochi et al. suggested that future cartilage regeneration methods would be based on minimally invasive tissue engineering such as injecting

cytokines or growth factors.[9] Among these methods, the PRP method uses a number of growth factors in platelets and is being widely researched in various clinical fields. There are storage spaces for growth factors in α-granules in platelets storing PDGF, TGF β, IGF-1, FGF, etc [10] Therefore, a higher than normal concentration of platelets can be obtained from the centrifugation of autologous blood and there will also be higher concentration of growth factors.[10, 11]

The effects of PRP on cartilage injuries have been reported in previous studies. Sanchez et al. conducted a retrospective study using a control group of patients which were injected with hyaluronic acid, and they reported that PRGF (autologous preparation rich in growth factors) was beneficial for the treatment of osteoarthritis of the knee.[12] PRGF was added to calcium chloride in order to activate it before it was injected into knee joints and 6-8 ml of PRGF was used in Sanchez' study. However, PRP was not activated before it was injected in this study, the rationale for which was that it was expected to be activated gradually when injected PRP reached the tissue. The injection dose was determined to be optimal at 3-4 ml as this dose created the highest compliance to patient and satisfaction level for the patients and 6-8 ml generated discomfort among the patients who participated in the clinical trials conducted before this clinical experiment was begun.

E. Kon et al. injected PRP in 100 patients with osteoarthritis of the knee three times with an interval of three weeks between injections, and these patients were observed for six months. They reported that there was a significantly positive effect.[13] It was suggested that storing PRP for a long period of time in the freezer with a temperature of -30°C for the second and the third PRP injections can cause changes in the form and function of the platelets, and the importance of leuko-reduction was also highlighted.[14] S. Sampson et al. injected PRP three times within an interval of four weeks into 14 patients with osteoarthritis; these patients were then observed for 12 months. They reported that there was a significant positive effect. They also attempted to observe using ultrasound whether there was an increase in the cartilage thickness, although they were unable to obtain any significant results due to their small number of study patients.[15]

Our study benefitted from the shortcomings of the previous studies. Fresh PRP was injected into 44 patients two times within an interval of four weeks and the results were evaluated using VAS, the Lysholm Knee Scale, and the Cincinnati Knee Rating System. Knee pain was measured by a visual analogue scale (VAS). This scale is a psychometric response scale which can be used in questionnaires and respondents specify their level of agreement to a statement by indicating a position along a continuous line between two end-points. Usually, the VAS score is determined by measuring in millimetres from the left hand end of the line to the point that the patient marks. Knee function was assessed with the use of the Cincinnati Knee Rating System and the Lysholm knee score. The Cincinnati Knee Rating System includes a functional assessment based on 6 abilities important for participation in sports. This can help evaluate change following surgery or other intervention. The Lysholm knee score has eight factors are rated to produce an overall score on a point scale of 0 to 100. The factors of limp, support, and locking are worth a potential of 23 points; pain and instability, 25 points each; swelling and stair-climbing, 10 points each; and squatting, 5 points.

Nevertheless, our study was also limited as we did not have a control group as there also was not in the previous studies and the actual cartilage restoration was not confirmed as it was difficult to compose a control group because most of our study patients were aware of the potential of PRP treatment and so preferred this treatment to the other options. In addition, evaluating the effects of PRP treatment based on the thickness of the articular cartilage can be unreliable because there are patients who complain of knee pain although their articular cartilage is thick enough whereas thin articular cartilage is not problematic in some patients.[16]

In this study, there was a noticeable improvement in pain within a short period of time after the PRP injection and there was also a statistically significant increase in the function scores, although the increase was slightly delayed compared to the pain scores (Figs.2 and 3). This was because it was time-consuming to build muscle strength as well as tissue around the cartilage for ambulation after the pain was alleviated. On the other hand, our patients experienced relatively higher functional improvement and reduction in pain immediately after the PRP injection if their knee function before the PRP injection was relatively poor or if their knee pain before the PRP injection was severe (Fig. 4, 5, 6). For this reason, the patients expressed their desire to undergo further treatment one year later. Comparing the effect of the injection according to patient sex, male patients showed higher functional improvement (Fig. 7). We believe that this resulted from the different attitudes of the male and female patients towards activities as the female patients were very cautious about activities despite their reduced pain and improved knee function, whereas male patients tended to test the level of improvement through more strenuous activities.

There were patients who only experienced pain after walking a long distance or doing protracted exercise, although there was not any abnormality seen in their physical examinations which would have suggested their inability to exercise or the possibility

of their experiencing severe knee pain. These patients carried out normal activities in their daily living without any problems, although their exercise activities were compromised. We designated this as 'relative chondromalacia' in this study.

As patients with knee problems can experience worsening of symptoms even while they are being treated with medications, their medications can be changed to more effective and stronger medications. However, these medications can cause gastrointestinal problems and as well as other complications which may eventually require them to be discontinued. On the other hand, one or two doses of PRP injection improved our patients' pain for six months and without major complications, thus allowing our study subjects to feel as though their articular cartilage had recovered. Although this clinical experiment evaluated only the short-term effect of PRP injection during a six–month period, we believe that PRP injection will become a method used to prevent osteoarthritis as long as the knee joint recovery can be maintained by regular PRP injections.

There is a still lack of research examining the effects of PRP on articular cartilage knee injuries, although research regarding PRP is currently being actively conducted. The significance of our study is that it suggests a successful alternative to medication treatment, physiotherapy or surgical treatment for managing articular cartilage injuries in order to overcome the shortcomings of those treatments. However, it is a preliminary study which will serve as the basis for further blinded, randomized controlled, clinical trials in the future. Please let me know if you think that any of the text should be modified. In conclusion, we believe the results of our clinical trial demonstrate that PRP injection is an effective and safe treatment for managing early osteoarthritis and degenerative chondropathy.

Competing interests

The authors declare that they have no competing interests.

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