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Treatment With Platelet-Rich Plasma Is More Effective Than Placebo for Knee Osteoarthritis

A Prospective, Double-Blind, Randomized Trial

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Background: Specific growth factors have been proposed as therapeutic proteins for cartilage repair.

Hypothesis: Platelet-rich plasma (PRP) provides symptomatic relief in early osteoarthritis (OA) of the knee.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 78 patients (156 knees) with bilateral OA were divided randomly into 3 groups. Group A (52 knees) received a single injection of PRP, group B (50 knees) received 2 injections of PRP 3 weeks apart, and group C (46 knees) received a single injection of normal saline. White blood cell (WBC)–filtered PRP with a platelet count 3 times that of baseline (PRP type 4B) was administered in all. All the groups were homogeneous and comparable in baseline characteristics. Clinical outcome was evaluated using the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire before treatment and at 6 weeks, 3 months, and 6 months after treatment. They were also evaluated for pain by a visual analog scale, and overall satisfaction with the procedure and complications were noted.

Results: Statistically significant improvement in all WOMAC parameters was noted in groups A and B within 2 to 3 weeks and lasting until the final follow-up at 6 months, with slight worsening at the 6-month follow-up. The mean WOMAC scores (pain, stiffness, physical function, and total score) for group A at baseline were 10.18, 3.12, 36.56, and 49.86, respectively, and at final follow-up were 5.00, 2.10, 20.08, and 27.18, respectively, showing significant improvement. Similar improvement was noted in group B (mean WOMAC scores at baseline: 10.62, 3.50, 39.10, and 53.20, respectively; mean WOMAC scores at final follow-up: 6.18, 1.88, 22.40, and 30.48, respectively). In group C, the mean WOMAC scores deteriorated from baseline (9.04, 2.70, 33.80, and 45.54, respectively) to final follow-up (10.87, 2.76, 39.46, and 53.09, respectively). The 3 groups were compared with each other, and no improvement was noted in group C as compared with groups A and B (P < .001). There was no difference between groups A and B, and there was no influence of age, sex, weight, or body mass index on the outcome. Knees with Ahlbuck grade 1 fared better than those with grade 2. Mild complications such as nausea and dizziness, which were of short duration, were observed in 6 patients (22.2%) in group A and 11 patients (44%) in group B.

Conclusion: A single dose of WBC-filtered PRP in concentrations of 10 times the normal amount is as effective as 2 injections to alleviate symptoms in early knee OA. The results, however, deteriorate after 6 months. Both groups treated with PRP had better results than did the group injected with saline only.

Keywords: platelet-rich plasma; osteoarthritis
investigated in vitro and in vivo. Nevertheless, the complex OA process involves an interplay of several growth factors needed in joint homeostasis and cartilage metabolism. Autologous platelet-rich plasma (PRP), which contains a pool of growth factors, appears to offer an easy solution for delivering multiple growth factors needed for tissue repair.

Today, PRP is being portrayed as a “wonder drug,” without sufficient evidence to support its application in almost all the areas in which it is used; this may be similar to the way local steroid injections were used when they first came into practice. Despite the promising preclinical findings and the huge interest in its clinical application, most questions on PRP applicability and efficacy remain unanswered. Indeed, the literature contains more reviews than clinical studies, which consist mostly of a limited number of anecdotal reports or case series, and many of the recent publications are either poorly designed or are retrospective, have small sample sizes, or do not have a placebo control. Additional issues are lack of clarity about the platelet concentration for injection, the role of white blood cell (WBC) filtering during preparation, the site of injection into the knee, and most importantly, the number and frequency of injections for appropriate effectiveness. Keeping in view these gray areas in our knowledge, this prospective clinical trial was designed to evaluate the role of PRP in the early stages of knee OA.

MATERIALS AND METHODS

Research Design

This was a double-blinded, randomized, placebo-controlled trial with 3 groups receiving 3 different lines of treatment (1 group serving as placebo controls). After institutional ethics board clearance, volunteer participants were blinded and subjected to a standardized injection protocol and were assessed on a number of variables (Western Ontario and McMaster Universities Arthritis Index [WOMAC] scoring, visual analog scale [VAS] for pain, satisfaction, and adverse effects) before the treatment and at 3 times after treatment (at 1.5 months, 3 months, and 6 months) by a blinded observer.

Sample and Sampling

The study was conducted on patients attending the orthopaedics outpatient department of the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. Patients who had early bilateral knee OA as diagnosed by American College of Rheumatology criteria and staged as per Ahlback radiological grading were included on a voluntary basis. Seventy-eight patients with bilateral early OA of the knee were selected on the basis of predefined inclusion criteria, that is, Ahlback grade 1 or 2 knees without significant deformity in patients who volunteered and signed a detailed informed consent form. Exclusion criteria were OA secondary to joint inflammatory diseases; patients with generalized OA, metabolic diseases of the bone, coexisting backache, and advanced stages of OA; patients who had received intra-articular injections within 3 months or arthroscopic lavage in the previous 1 year or who were receiving anticoagulant therapy; and patients with a hemoglobin level less than 10 gm% or associated comorbidities, infection, tumor, crystal arthropathies, or tense joint effusion.

Our sample size was based upon an assumed study power of 80% (β = .2), a false-positive rate of 5% (α = .05), and a predicted difference of 1.5 points on our VAS (standard deviation, ±1.5). Using these parameters, and adjusting our α for multiple comparisons, we required approximately 21 patients per treatment arm.

The participants were randomly divided by computer-generated random charts into 3 groups: 27 participants in group A (54 knees) were given a single injection of PRP, 25 participants in group B (50 knees) received 2 injections of PRP at an interval of 3 weeks, and 26 participants in group C (52 knees) received a single injection of normal saline (physiological control/placebo). Of the 27 patients in group A, 1 patient was excluded as he underwent total knee replacement elsewhere; thus, only 52 knees in group A were available for analysis. Of the 26 patients (52 knees) initially in group C, 3 patients later refused treatment when they reported for injection, and hence, 23 patients (46 knees) were available at follow-up (Figure 1). Randomization ensured that the baseline characteristics of the 3 groups were comparable with respect to age, sex, weight, height, body mass index (BMI), and preinjection WOMAC scores (Table 1).

PRP Preparation

The PRP required for injection was prepared and provided by the Department of Transfusion Medicine, PGIMER, Chandigarh, India. About 100 mL of venous blood was drawn under aseptic precautions from the antecubital vein atraumatically in an effort to avoid irritation and trauma to the platelets. The blood was collected in a 100-mL bag with CPD-A1 (citrate phosphate dextrose and adenine) as an anticoagulant. The whole blood was transferred from the blood bag into two 50-mL sterile tubes using a blood transfusion set inside a biosafety cabinet, class IIA (BIOAIR Safe flow, Euroclone, Siziano, Italy). The tubes were then centrifuged for 15 minutes at 1500 rpm on a table-top centrifuge, and the blood was separated into PRP and residual red blood cells with the buffy coat.
Hereafter, the procedure was completely performed inside the biosafety cabinet. The PRP was then extracted through a pipette and transferred to a test tube, and a leucocyte filter (Imugard III-PL, Terumo Penpol Ltd, Thiruvananthapuram, India) was then used to filter off the leucocytes. The final PRP was assessed for platelet count and was supplied for injection in a 10-mL syringe (approximately 8 mL per knee). Total leucocyte count and platelet count were measured from the patient’s peripheral blood as well as in the final PRP. Total leucocyte count was zero in our method. The mean platelet count achieved by our method was 310.14 × 10^9/L, and the mean quantity of platelets injected per knee was 238.56 × 10^6. None of the groups knew how much blood was extracted, as they were instructed to look the other way during extraction; only the investigator knew how much blood was extracted, as they were injected per knee was 238.56.

### Intervventional Procedure

The patient was placed in a supine position with the knee in full extension. Under aseptic conditions, 8 mL of either normal saline or platelet concentrate was injected into a suprapatellar pouch through a supralateral approach with an 18-gauge needle without local anesthetic. In the PRP group, 1 mL of CaCl₂ (M/40) was injected in a ratio of 1:4 for every 4 mL of PRP. The knees were then immobilized for 10 minutes after injection. The patients were discharged after 30 minutes of observation. Some patients who reported dizziness or sweating were observed for 2 to 3 hours and discharged when fully recovered. During the follow-up period, nonsteroidal anti-inflammatory drugs were not allowed, and paracetamol (dosage, 500 mg tds) was prescribed in case of discomfort; all patients were asked to stop medications 48 hours before follow-up assessment.

### Outcome Measures

The primary efficacy criterion was change from baseline in joint pain, measured using the WOMAC subscale. Secondary efficacy variables included change in joint stiffness, physical function, and global WOMAC. The WOMAC parameters were measured before injection and at 6 weeks, 3 months, and 6 months after injection. Patients were also assessed for pain by VAS and for satisfaction (satisfied, partly satisfied, not satisfied) at the end of 6 months. Adverse effects related to treatment were also recorded with respect to their nature, time of onset, duration, and severity.

### Statistical Analysis

Analysis of the data was conducted with the help of SPSS v.15 (SPSS Inc, Chicago, Illinois). Measurable data were tested for normality using the Kolmogorov-Smirnov test. The descriptive statistics (eg, mean, standard deviation) for normally distributed parameters were calculated for all 3 groups. The normally distributed parameters were compared for their means using the analysis of variance (ANOVA) followed by post hoc tests such as the Student-Newman-Keuls and Dunnett procedures. Nonnormal data were expressed as median and interquartile range, and their distribution for all 3 groups was compared using Kruskal-Wallis ANOVA followed by the Mann-Whitney U test. The association of various categorical/classified data, including complications, within the 3 groups was assessed using the Mann-Whitney U test. The association of various categorical/classified data, including complications, within the 3 groups was assessed using the Mann-Whitney U test. The association of various categorical/classified data, including complications, within the 3 groups was assessed using the Mann-Whitney U test. The association of various categorical/classified data, including complications, within the 3 groups was assessed using the Mann-Whitney U test.
follow-ups were analyzed using repeated-measures ANOVA followed by post hoc tests. A $P$ value of $<.05$ was considered to be significant in all tests.

RESULTS

Six patients (22.2%) in group A and 11 patients (44%) in group B had adverse effects at the time of injection. This was significant in comparison with group C, which had no adverse effects. In group B, 5 patients (20%) had adverse effects during the second injection. The various adverse effects were syncope, dizziness, headache, nausea, gastritis, sweating, and tachycardia. Four patients in group A and 3 patients in group B had pain and stiffness after injection for 2 days. It was noted that the adverse effect group had a significantly higher ($P = .02$) quantity of platelets injected (2.53 billion) compared with the group with no adverse effects (1.96 billion) (Table 2).

At the end of 6 months, 67.3% were satisfied, 7.7% were partially satisfied, and 25% were not satisfied in group A. In group B, 64% were satisfied, 4% were partially satisfied, and 25% were not satisfied. In group C, only 4.3% were satisfied and 6.5% partially satisfied, but 89.1% were not
satisfied with the procedure. This implies that patients in the PRP groups were satisfied (groups A and B) in comparison with normal saline (group C). Further, there was no statistically significant difference between group A and group B ($P = .585$), implying equal benefit of treatment in both groups.

**Primary Outcome: Pain Parameter**

In groups A and B, the mean pain score decreased from baseline at 6-week and 3-month follow-up, followed by a slight increase in pain at the 6-month follow-up, which was significant. However, the mean pain at 6 months was still less than that at baseline (Figure 2). The improvement was maintained from the end of the therapy to 6 months' follow-up, with only slight worsening at 6 months. In group C, however, the trend was of increasing mean pain scores at all follow-ups compared with baseline. The mean pain scores and percentage decrease in pain at each follow-up for all the groups are given in Table 3 and Figure 2.

The trend of other secondary WOMAC parameters, stiffness, physical function, and total WOMAC, was noted to be similar to pain. Details are given in Table 3 and Figures 3, 4, and 5.

**VAS Pain Scores**

The VAS scores decreased from 4.54 at baseline assessment to 2.16 at 6-month follow-up for group A. For group B, they decreased from 4.64 to 2.54. In group C, however, the VAS scores increased from 4.57 to 4.61. The benefit in pain reduction as measured by VAS was significant in groups A and B ($P = .001$) but not in group C ($P = .598$). There was no significant difference between groups A and B ($P = .410$) (Table 4).

The percentage change in scores from baseline for all WOMAC parameters at each follow-up was lower for knees with Ahlback grade 1 compared with those with grade 2. In group A, the difference was significant for pain ($P = .006$), stiffness ($P = .001$), physical function ($P = .001$), and total WOMAC ($P = .001$). In group B, even though the scores were low for Ahlback grade 1 knees, it was not significant statistically.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Analysis of Platelet-Rich Plasma (PRP)–Related Variables in Comparison With Occurrence of Complications$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP-Related Variable</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>PRP volume, mL</td>
</tr>
<tr>
<td>Platelet count, $\times 10^3/\mu$L</td>
</tr>
<tr>
<td>Quantity injected, $\times 10^7$ platelets</td>
</tr>
</tbody>
</table>

$^a$Values are expressed as mean ± standard deviation.

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There was no correlation of mean scores of all WOMAC parameters with age, sex, or BMI in either group A or B, which means that all patients irrespective of age, sex, weight, height, and BMI had equal benefit from the procedure.

The mean duration of benefit was 17.63 days in group A and 16.54 days in group B. Male patients had an earlier response compared with female patients in both groups A (19.1 days for female and 15.6 days for male patients; $P = .252$) and B (19 days for female and 13.5 days for male patients; $P = .103$).

Some of the knees with Ahlback grade 2 changes at baseline were determined to have grade 3 at follow-up by the senior investigator (M.S.D.), as they were considered borderline. This is reflected in Table 1.

**DISCUSSION**

A PubMed review performed in March 2012 revealed less than 38 hits with the keywords “osteoarthritis” and...
TABLE 3
Mean Scores and Percentage Change in Each Parameter of the WOMAC Score
Compared With Baseline at Each Follow-up for All 3 Groups

<table>
<thead>
<tr>
<th>WOMAC Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td><strong>Pain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.18</td>
<td>4.26</td>
<td>3.74</td>
</tr>
<tr>
<td>% changea</td>
<td>−61</td>
<td>−70</td>
<td>−56</td>
</tr>
<tr>
<td><strong>Stiffness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.12</td>
<td>2.12</td>
<td>1.76</td>
</tr>
<tr>
<td>% changea</td>
<td>−29</td>
<td>−48</td>
<td>−31</td>
</tr>
<tr>
<td><strong>Physical function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>36.56</td>
<td>18.98</td>
<td>16.98</td>
</tr>
<tr>
<td>% changea</td>
<td>−48</td>
<td>−58</td>
<td>−50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>49.86</td>
<td>25.36</td>
<td>22.48</td>
</tr>
<tr>
<td>% changea</td>
<td>−50</td>
<td>−60</td>
<td>−51</td>
</tr>
<tr>
<td><strong>P</strong> value</td>
<td>At each follow-up, the percentage benefit from baseline was greater in groups A and B than in group C (P &lt; .001); no difference between groups A and B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*0 = baseline value (preinjection); 1st = 6-week follow-up; 2nd = 3-month follow-up; 3rd = 6-month follow-up.

*P < .05.

aThe percentage change in the Western Ontario and McMaster Universities Arthritis Index (WOMAC) parameter compared with baseline. Negative % indicates improvement from baseline; positive % indicates worsening of the parameter.

“platelet rich plasma.” Only 7 previous studies had used PRP in knee OA; these studies however are of a diverse nature (see the Appendix, available in the online version of this article at http://ajs.sagepub.com/supplemental/). Some are small pilot studies, some have no controls, and some have used multiple injections without any specific rationale. The current literature is complicated by a lack of standardization of study protocols, platelet separation techniques, and outcome measures. As a result, there is uncertainty about the evidence to support the role of PRP in many situations. However, in all of the studies published to date in which PRP was used in knee OA, some improvement was noted; this however has only been vaguely quantified.8,10,12,15–17

There was no correlation of mean pain scores and other WOMAC scores with respect to age, sex, or BMI in our study, and WOMAC scores decreased equally with respect to all parameters. On the other hand, Kon et al10 noticed good response in young male and low BMI patients; their study had more male patients in contrast to our study and that of Sanchez et al,17 which had more female patients. The mean BMI of our study group and that of Kon et al10 were similar (25 ± 3), and both studies had a fewer number of overweight participants. Thus, no major comment can be made about the effect of BMI or sex on the outcomes.

In our experience, there was a definite correlation with Ahlback grading in both groups A and B, with grade 1 knees having lower mean pain and other WOMAC scores than grade 2 knees (ρ = 0.338 and P = .116 for group A; ρ = 0.338 and P = .005 for group B). Similar findings were noticed in a study by Kon et al10 in which patients with degenerative chondropathy achieved better results compared with patients affected by early OA, who had a higher improvement than patients with advanced OA. The strong points of our study are the comparison of a single-injection group with placebo controls as well as...
comparison with a double-injection group at an interval of 3 weeks. Our PRP preparation technique was standardized by our transfusion medicine department, and no commercial filters were used. We were able to get a standardized leucocyte-free concentration of platelets for all cases, and per the Mishra classification, this was type 4B. The number of platelets injected in our series was an average of 2.5 billion compared with 6.5 million used by Kon et al (almost 400 times higher).

Looking at pain as the primary outcome measure, we noted that mean pain scores decreased initially in both groups A and B, which received PRP; however, both groups showed a small increase in mean pain scores at the final 6-month follow-up. The final score was still far less than the baseline pain scores. The trend described by Kon et al was also similar to our findings, and they noted a slight tendency of worsening in International Knee Documentation Committee (IKDC) objective and subjective scores from 2 months to 6 months, which was not significant, and significantly decreased further at 12 months. They subsequently published their 24-month follow-up, wherein the objective and subjective scores further decreased. This opens other avenues of thought; because 1 injection is seemingly as effective as 2, and the improvement deteriorates over time, is there an option of giving serial single injections at 6-month or 1-year intervals, which may further relieve symptoms for longer periods and delay OA progression? This would be a good focus for future research.

The group that received PRP in the study by Sanchez et al showed a significant improvement in 33.3% of patients at 5 weeks, while Kon et al reported 80% satisfied patients. We had 67.3% satisfied patients in group A and 64% satisfied patients in group B at the 6-month follow-up, in comparison with the control group in which only 4.3% were satisfied. Further analysis of pain scores...
in these satisfied patients revealed a decrease in pain by 82% in group A and 74% in group B at the 6-month follow-up as compared with 53% for the entire group A and 43% for group B.

One feature unique to our study was the documentation of some systemic adverse effects; most of the adverse effects noted by us were immediate and systemic rather than local and were of short duration lasting 30 minutes. None of the adverse effects in either group was of severity or concern, and all subsided within half an hour when the patients were under observation. Most authors also report some injection pain, local inflammation of short duration, and reaccumulation of effusion,10,17 but the exact numbers are not mentioned. One finding of some note was that our patients with these adverse effects had a somewhat higher quantity of platelets injected (2.53 billion) compared with the group with no such effect (1.96 billion), and this may be postulated as a contributing factor. The possibility of CaCl₂, which was used as an activating agent, as a contributing factor to the adverse effects may be considered.

Some previous authors have injections of PRP at 3-week intervals by drawing 150 mL of blood at one stage, preserving this at −30°C,176 and subsequently thawing before subsequent injections. Methods of PRP collection are often open systems; we have previously pointed out that it may not be justifiable to store these platelets,7 as storing platelets in freezing conditions may alter the shape and decrease the functional properties, including degranulation of alpha-granules.5 This may also become a variable in assessing and comparing the results of PRP in OA. In our study in which group B received 2 injections, we prepared fresh PRP both times by drawing blood, avoiding cold storing the PRP obtained at first injection as done by Kon et al,10 as ours was an open system, and we had doubts about platelet function being altered because of cold storage.

We also noted the time taken by the patients to start experiencing benefits after their first injection. The mean duration to the start of benefits was 17.63 days (95% confidence interval [CI], 14.59-20.68) in group A and 16.45 days (95% CI, 12.97-20.10) in group B. Therapeutic benefit has been postulated to be chondrogenesis by some authors,10,17 but this process would probably have taken more time for the patient to perceive benefits. Moreover, a slight worsening of WOMAC parameters was seen at the third follow-up compared with our second follow-up, indicating no sustained long-term effects and the beginning of waning of therapeutic benefits. If chondral remodeling was the cause for the improvement of symptoms, the benefit would have started later and lasted for a longer duration.

At the present moment, we presume that the improvement in our patients could be explained by the fact that injected platelets may act at different levels and are not stimulating the chondral anabolism or slowing the catabolic processes. Platelet-rich plasma may influence the overall joint homeostasis, reducing synovial membrane hyperplasia and modulating the cytokine level, thus leading to an improvement in the clinical outcome, even if only temporarily and without affecting the cartilage tissue structure and joint degenerative progression.4,14 Further studies are needed to confirm the results obtained and their longevity to understand the mechanism of PRP action and to evaluate if there is only a temporary symptom

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**Figure 5.** (A) Comparison of the 3 groups for percentage change in total Western Ontario and McMaster Universities Arthritis Index (WOMAC) score from baseline to 6 weeks (first follow-up), 3 months (second follow-up), and 6 months (third follow-up). Negative percentage change indicates a benefit compared with preinjection. (B) Trends in mean total WOMAC scores of all groups at baseline and subsequent follow-ups.

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**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 52)</th>
<th>Group B (n = 50)</th>
<th>Group C (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VAS score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At preinjection</td>
<td>4.54 ± 0.613</td>
<td>4.64 ± 0.563</td>
<td>4.57 ± 0.620</td>
</tr>
<tr>
<td>At 6-mo follow-up</td>
<td>2.16 ± 1.543</td>
<td>2.54 ± 1.717</td>
<td>4.61 ± 0.745</td>
</tr>
<tr>
<td>Difference (preinjection vs 6-mo follow-up)</td>
<td>2.380 ± 1.589</td>
<td>2.10 ± 1.669</td>
<td>−0.043 ± 0.556</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>.001</td>
<td>.001</td>
<td>.598</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± standard deviation.
improvement or if PRP plays a more important role through disease-modifying properties.

Study Limitations

Our study comprised predominantly bilateral knee OA, and randomization of patients was conducted rather than randomization of knees, and the patient received similar treatment in both knees. It would have been better if knees were randomized and the same patient would have received different treatments in his or her 2 knees, but this would have made the procedure cumbersome, and there were patient-blinding issues.

The primary imperatives of a new therapy remain the control of symptoms; because pain is the most pressing problem in OA, we evaluated only clinical parameters by using the WOMAC and VAS scoring systems. Radiographic follow-up investigation methods such as magnetic resonance imaging may be considered for evaluating cartilage regeneration (if any) in subsequent research efforts; we could not do this because of the cost and ethical issues.

CONCLUSION

The results of our study support the short-term effectiveness of PRP injection over a placebo for relieving pain and stiffness and improving knee functions in early knee OA. There are more benefits in early OA, and in our experience, a single dose of PRP is as effective as a double dose. The effect tends to taper off over time, leaving open the option of staged injections over many months as a potential future therapeutic regimen.

REFERENCES