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Original Research Paper

Orthopaedics

ROLE OF OF LEUKOCYTE-RICH PLASMA IN SEVERE KNEE OSTEOARTHRITIS

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ABSTRACT

Aim : To determine whether LR-PRP is effective for severe degeneration, and if it provides a therapeutic effect. To compare dynamic time-series changes in the visual analog scale (VAS) score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and MOAKS for bone marrow lesions (BMLs). **Material And Methods:** 120 subjects were included in this study. One intra-articular LR-PRP injection was administered every 4 weeks, for 6 months, by a well-trained, senior orthopedic surgeon with 10 years of experience. Patients were clinically evaluated using subjective and objective assessments before injections and at 3, 6, 12, and 24 months following treatment to determine the primary clinical outcomes of LR-PRP therapy. **Results :** The average improvement in the VAS score was 58.12%. There were no correlations between age and VAS score improvement or between body mass index and VAS score improvement. Comparison of the KOOS-Q before and after treatment with each K-L grade indicated that the KOOS-Q was significantly lower before injections. **Conclusions:** Manually prepared Continuous LR-PRP injections reduced pain and improved quality of life in severe knee degeneration . Furthermore, the use of repeated doses of LR-PRP over time helped to determine new conservative treatment strategies for patients with advanced KOA and mild degeneration.

KEYWORDS :

INTRODUCTION :

Knee osteoarthritis (OA) is a painful and debilitating ailment. Despite its extensive incidence, which is quickly increasing due to a growing elderly population, there are no effective prophylactic treatments or acknowledged disease-modifying medications. [1] Usually total knee arthroplasty (TKA) is the last go for patients with knee osteoarthritis (KOA) who have not responded to conservative treatment, which includes weight loss, rehabilitation, hyaluronic acid (HA) injections, corticosteroid (CS) injections, and/or nonsteroidal anti-inflammatory drugs. As a result, significant pain relief is essential prior to TKA, with regenerative medicine emerging as a viable therapy option. [2]

Biologic therapies, including as platelet-rich plasma (PRP) injections, have recently gained popularity as a treatment for OA. Specifically, leukocyte-poor platelet-rich plasma (LP-PRP) concentrate contains anabolic cytokines that may enhance cartilage regeneration while eliminating proinflammatory catabolic cytokines. [1]

Platelet-rich plasma (PRP) therapy has been shown to reduce pain caused by KOA. In individuals with mild knee OA, PRP infiltration was found to be more effective than traditional pharmaceutical therapy in terms of reducing pain and increasing function. The rationale for employing PRP injections for KOA treatment is based on modulating the intra-articular (IA) environment by infusing autologous blood products into the joint, lowering inflammatory distress, and encouraging chondrogenesis. [3]

LP-PRP contains therapeutic quantities of IL-1ra and TGF-b, leading to an inflammatory environment and progression of OA. Intra-articular LP-PRP infiltrations can prevent cartilage degeneration and slow the evolution of OA in its early phases. [3] Consecutive leukocyte- rich PRP (LR-PRP) injections have been demonstrated to have therapeutic effects, independent of the degree of degeneration, indicating benefits for patients with severe knee degeneration.

The present study aimed to compare dynamic time-series changes in the visual analog scale (VAS) score, Knee Injury and Osteoarthritis Outcome Score (KOOS), and MOAKS for bone marrow lesions (BMLs). It also aimed to determine whether LR-PRP is effective for severe degeneration, and if it provides a therapeutic effect.

MATERIALS AND METHODS :

The 120 subjects in this study were selected according to the following inclusion criteria: difficulties in performing activities of daily living despite continuous conservative treatment, involving weight loss, rehabilitation, HA or CS injections, and/or nonsteroidal anti- inflammatory drugs to avoid surgery, and no treatment with HA or CS injections, or any other knee injections during the LR-PRP therapy period.

The exclusion criteria were as follows: history of a systemic disorder, such as rheumatoid arthritis, malignant cancer, hematological disease, infection, or immunodeficiency; recent intra-articular CS or HA injections during the past 4 or 2 weeks, respectively; recent administration of anticancer drugs or immunosuppressive drugs; and children and adolescents without closure of the epiphyseal plane.

All patients provided written informed consent after being counselled about the potential benefits of LR-PRP, treatment procedures, and follow-up period. PRP was injected monthly for 6 months to determine the peak effect. This protocol was based on our previous finding of sufficient fibrocartilage coverage in the cartilage defect approximately 7 months after meniscal repair with PRP and platelet-rich fibrin.

Continuous administration of LR-PRP for ≥6 months centrifuged for 5 min at 1000 G (Desktop Centrifuge is done. The upper and middle buffy coats formed by the first centrifugation were collected in a single dry glass, and the tubes were subsequently centrifuged for 15 min at 1500 G. This second centrifugation separated the blood into three different layers. The upper layer was removed, and 3mL of LR-PRP was

obtained.

Injections: One intra-articular injection was administered every 4 weeks, for 6 months, by a well-trained, senior orthopedic surgeon with 10 years of experience. The PRP produced was injected and administered within approximately 7 min. The LR-PRP injection technique with free hand technique over standard Antero-lateral portal method was performed with the patient's knee slightly bent at an angle of approximately 90 .

The injection was administered under sterile conditions using a 21-gaugeneedle and a Anterolateral approach from the outside of the knee. and LR-PRP was injected following confirmation that there was no resistance. If fluid was present in the joint, PRP injection was implemented following aspiration of as much of the fluid as possible. Aspiration was performed before injections in patients with joint effusion. Following injection, patients were instructed to unrestrictedly perform their activities of daily living.

Clinical assessment

Patients were clinically evaluated using subjective and objective assessments before injections and at 3, 6, 12, and 24 months following treatment to determine the primary clinical outcomes of LR-PRP therapy. Radiographic and MRI examinations were also performed at 3, 6, 12, and 24 months.

Furthermore, we examined the average number of injections required to reduce pain by 50% or more. Pain was assessed using the VAS.19 Clinical assessments included the KOOS, KOOS-total, KOOS-- symptoms, KOOS-pain, KOOS-activity, KOOS-sports, and KOOS-quality of life (KOOS-Q)20

Therapeutic efficacy was determined by the Outcome Measures in Rheumatology-Osteoarthritis Research Society International (OMER- ACT-OARSI) responder criteria for osteoarthritis. Furthermore, the severity, During the six-injection protocol, PRP was discontinued upon nature, and duration of adverse events associated with the study pro-achievement of subjectively satisfactory outcomes as qualified by each patient. However, clinical and imaging follow-ups were continued despite discontinuation of treatment.

Self-funded PRP therapy for adults who did not consent to surgical treatment and showed no improvement despite conservative management as provided by insurance was performed. Hence, the patients' baseline data before treatment was used as control data. Their progress was closely monitored following the commencement of therapy.

Processing LR-PRP

Preparation: The duration of venous blood extraction for participants was within 1 min. Blood was immediately transported and subsequently centrifuged. Two-time centrifugation with different centrifugal forces was required to create the LR-PRP. First, 1 mL of anti- coagulant (sodium citrate solution) was added to a 20-mL blood sample

Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS version 24.0 for Windows

RESULTS:

According to the K-L classification, 20 patients had grade-I KOA ,27 had grade-II KOA, 53 had grade-III KOA, and 20 had grade-IV KOA. There were 120 responders at 24 months. (36.4% with K-L grade I; 14.9% with K-L grade II; 12.3% with K-L grade III; and 16.7% with K-L grade IV).

There was a significant difference between the average age of participants with each K-L classification ($P< 0.0002$). however, there was no significant difference between participants with K-L grades I and II (P 0.07) and those with K-L grades III and IV (P0.19).

An examination of the time and group interactions indicated a significant difference in the time series transitions between the K-L classifications, VAS scores, and KOOS-Q (VAS, P 0.015; KOOS-Q, $P < 0.001$). Comparisons of the VAS score with each K-L grade before and after treatment showed that the VAS score was significantly lower before the injection ($P < 0.001$).

The average improvement in the VAS score was 58.4 %. There were no correlations between age and VAS score improvement (r 0.05; $P < 0.01$) or between body mass index and VAS score improvement (r 0.04; $P < 0.01$). Similarly, there were no correlations between the white blood cell concentrations (r 0.12; $P< 0.01$) and platelet concentrations (r 0.17; $P < 0.01$) and the rate of improvement in the VAS score. There were no obvious changes observed on radiographs and no obvious adverse events.

Furthermore, the pretreatment scores were low for those with K-L grade I and high for those with K-L grade IV; however, a large decrease in time until improvement was observed for those with

- VAS score with grade I: 51.9 [95% confidence interval: 43.6–60.2] to 15.0 [95%CI: 2.727.4] at 2 years, difference = —36.9;
- VAS score with grade II: 62.7 [95% CI: 57.5–68.0] to 27.5 [95% CI: 19.0–35.9], difference 35.2).
- VAS score with grade III: 59.9 [95% CI: 55.8–64.1] to 43.7 [95% CI: 37.1–50.4], difference = —16.2;
- VAS score with grade IV: 69.8 [95% CI: 63.9–75.6] to 44.8 [95% CI: 34.7–54.8], difference25.0).

Therefore, it was found that the VAS score at 2 years was significantly lower for those with K-L grades I and II than for those with K-L grades III and IV.

Comparison of the KOOS-Q before and after treatment with each K-L grade indicated that the KOOS-Q was significantly lower before injections in all groups (all $P < 0.001$). A comparison of the K-L classifications revealed no statistically significant difference among the values before treatment.

The magnitude of the increase in the KOOS-Q score tended to decrease in those with K-L grades II to IV compared with those with K-L grade I

- KOOS-Q score with grade I: 32.0 [95% CI: 24.2–39.8] to 63.2 [95% CI: 52.2–74.2], difference = 31.2;
- KOOS-Q score with grade II: 34.5 [95% CI: 29.6–39.4] to 53.5 [95% CI: 46.0–60.9], difference = 19.0;
- KOOS-Q score with grade III: 30.6 [95% CI: 26.7–34.6] to 43.4 [95% CI: 37.5–49.4], difference = 12.8;
- KOOS-Q score with grade IV: 30.5 [95% CI: 25.1–36.0] to 39.7 [95% CI: 31.3–48.0], difference 9.1).

Therefore, it was determined that the KOOS-Q score at 2 years was significantly higher for those with K-L grades I and II than for those with K-L grades III and IV.

Regarding the KOOS-pain and KOOS-activity, a significant increase was observed with time for each K-L classification, although no significant difference was observed in the time-series transition between these classifications (KOOS-pain: P 0.230; KOOS-activity: P 0.280). However, we also observed higher scores for those with K-L grades I and II compared with those with K-L grades III and IV at 2 years.

Regarding the MOAKS for BMLs, no significant difference was found in the time-series transition between K-L classifications (P 0.542). A statistically significant decrease was observed at 2

years only for those with K-L grades III and IV; however, it was confirmed that the score at 2 years was not lower than the score before treatment for those with K-L grades I and II (grade III: 8.54 [95% CI: 7.72–9.37] to 6.68 [95% CI:5.28–8.09], P = 0.007; grade IV: 12.34 [95% CI: 11.22–13.47] to 8.94 [95% CI: 6.97–10.90], P < 0.001) (Table 3).

Comparisons of time to 50% improvement in the VAS score:
The period required for a minimum improvement of 50% in terms of the VAS score was recorded from the start of therapy. An improvement in the VAS score of 50% or more has a significant impact on the OMERACT-OARSI responder criteria. The average time to achieve this improvement was significantly longer for those with K-L grades III and IV than for those with K-L grade II, and it was also significantly longer for those with K-L grade IV than for those with K-L grade I (grade I: 7.57 [95% CI: 3.97–11.18] months; grade II: 4.03 [95% CI: 3.14–4.92] months; grade III: 10.68 [95% CI: 8.65–12.72] months; grade IV: 12.95 [95% CI: 10.04–15.86] months; grade III vs. grade II, P < 0.001; grade IV vs. grade II, P < 0.001; grade IV vs. grade I, P = 0.006).

Comparison of treatment periods until the peak of VAS improvement was achieved:
There were no statistically significant differences between K-L classifications during the treatment period, peak VAS score, and peak improvement within 12 months. An improvement of up to 75% within 12 months occurred in patients with K-L grade IV, and an improvement of up to 60% within 12 months occurred in patients with K-L grade I; however, no significant difference was detected (P = 0.283).

DISCUSSION:

According to the leukocyte and fibrin content of PRP products, Dohan Ehrenfest et al. [4] initially suggested categorizing them into the following four groups: Leukocyte-poor or pure platelet-rich plasma (LP-PRP): this type of plasma has high platelet concentrations but little to no leukocytes; leucocyte and platelet-rich plasma (LR-PRP): this type of plasma has both a high platelet concentration and a large leukocyte count; (3) leucocyte-poor or pure platelet-rich fibrin (PPRF): this type of plasma has rich circulating fibrin but little to no leukocytes; and (4) leucocyte- and platelet-rich fibrin (L-PRF), this type of plasma has rich circulating fibrin and a significant number of leukocytes. LP-PRP and LR-PRP, two low-density fibrin formulations, are frequently utilized in injectable therapy for osteoarthritis.

Studies suggesting that PRP is only useful for treating moderate degeneration 2-7 employed a small number of cases, a short treatment duration, and a low amount of administration. In contrast, our technique effectively cured severe degeneration, with improvements in both the VAS score and the KOOS-Q. Although there were no statistically significant differences in the extent of improvement in pain or daily activities, the therapeutic impact was deemed adequate. Furthermore, in accordance to the K-L classification, the more severe the degeneration, the longer it took for the therapeutic impact to manifest.

The VAS score and KOOS-Q reduced with higher K-L classifications, but increased with higher starting K-L classifications before treatment; however, it did not fall below pretreatment levels for individuals with K-L grades I and II, despite noted improvements. Patients with K-L grades III and IV cannot expect to see a therapeutic response until they obtain the same VAS score and KOOS-Q as patients with K-L grade I before treatment. Thus, this treatment does not guarantee cartilage regeneration.

Although not statistically significant, patients with K-L grade IV tended to reach peak values one year after treatment, and these values then tapered compared to those of patients with K-L grade I, implying that more severe degeneration necessitates longer-term treatment and higher doses. Further research is required to determine whether there were histological changes on the cartilage surface and whether the connections with increased quality of life were coincidental findings.

Currently, LP-PRP is recommended for PRP treatment because it has fewer catabolic effects; however, PRP injections in the knee may benefit from less inflammatory cytokines. Therefore, it is vital to explore how continuous administration of LR-PRP impacts the regeneration of injured cartilages. Furthermore, histological investigations of the cartilage surface after LR-PRP administration are required.

Similar to our study , study by Peng Y-N [5] et al discovered that LR-PRP significantly improved WOMAC total scores, WOMAC pain scores, WOMAC physical function scores, and IKDC scores after 6 months as compared to the HA group. At 12 months after IA injection, the LR-PRP group had significantly improved WOMAC stiffness and physical function ratings than the HA group. There was no significant difference in adverse events between the LR-PRP and HA groups.

In par with our study, Dai et al. [6] conducted a study including 10 RCTs with 1069 patients and showed that IA PRP injection may have more benefits in WOMAC total, WOMAC pain, and WOMAC physical functions scores only at 1 year after injection as compared with HA and saline in treating knee OA patients

In accordance with present study, Belk et al.[7] examined 18 RCTs involving 811 participants and discovered that IA PRP injection can be predicted to improve clinical results compared to HA in treating knee OA patients. The trials that compared the LR-PRP to the LP-PRP reported no significant changes in the efficacy of WOMAC or VAS scores. However, the investigation revealed that LP-PRP may outperform LR-PRP in IKDC scores.

Limitations: limitation of this study was that there was no separate control group. Furthermore, just one set of injections was given. Because just LR-PRP was investigated, there was probably a strong placebo effect. Furthermore, individuals who received injections were required to pay for them. Furthermore, some patients were unable to visit the hospital due to the coronavirus disease 2019 (COVID-19) pandemic, making it difficult to gather and maintain case data, and reliable data may not have been obtained due to reduced activities and daily living constraints. Finally, there were various demographic characteristics, including age, BMI, and gender.

Our protocol was developed before the necessary information for studies evaluating biologics in orthopedics was set for PRP24, therefore individuals with comorbidities such as diabetes and smokers were included. As a result, this study demonstrates that the intervention's effect is genuine and independent of subject group variability.

CONCLUSIONS:

Manually made LR-PRP may alleviate pain and enhance quality of life, even in cases of severe degeneration. Continuous LR-PRP injections may help to improve pain and quality of life even in cases of severe knee deterioration. Furthermore, repeated dosages of LR-PRP throughout time aided in the development of new conservative treatment options for patients with severe KOA and mild degeneration. We verified LR-PRP, and by administering it many times every four weeks, we were able to demonstrate the treatment's efficacy regardless of the degree of deformation.

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Declarations of interest

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