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BIOLOGICAL TREATMENT OF PRIMARY OSTEOARTHRITIS OF KNEE: EARLY RESULTS

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ABSTRACT

PRP contains cocktail of growth factors helping in inhibiting activation of cartilage destroying proteolytic enzymes and preventing progress of osteoarthritis. 50 patients with 82 knees were injected with 3 injections of PRP. VAS, KOOS and WOMAC scores improved significantly from baseline to 6 months and after one year. Ultrasonic measurement did not show any change in cartilage thickness at one year. No major complication was noted. PRP injection is safe treatment modality for early osteoarthritis but it's role in reversing diseased process has not been proved ultrasonographically.

KEYWORDS : Osteoarthritis, Platelet Rich Plasma, Intraraticular, Injection, Ultrasound

Osteoarthritis (OA) is the leading cause of musculoskeletal disability in the age group 40-60 years. Although most of the research has been done with the destruction of cartilage in mind, arthritis is now more often considered as like an organ failure. It's a poorly understood disease. Pathogenesis involves all the major articular tissues including cartilage, synovial membrane, sub chondral bone and other connective tissues like ligaments and tendons (Haywood et al., 2003).

Treatment for OA knee aims to alleviate pain and improve functions so as to lessen the reduction in activity. Medical management of OA as per the guidelines of American College of Rheumatology 2012 includes both pharmacological and non-pharmacological therapies. Initial management involves education, exercise, appropriate rest, various appliances, braces and weight reduction as the non-pharmacological therapies. Pharmacological modalities recommended for initial OA includes oral and topical NSAIDS, tramadol, intraraticular injections of steroids and hyaluronate (Hochberg et al., 2012, Christensenet al., 2005). Opoids are recommended in patients who do not respond to initial therapies. Patients, who are not relieved adequately with medical therapies, may have to be considered for surgical intervention like high tibial osteotomy, unicompartment or total joint replacement (TKR). Though TKR is the commonest and ultimate treatment option for OA knee, but non-operative modalities should be exhausted before contemplating surgical procedures (Steadman et al., 2007, Manen et al., 2012).

Current treatment modalities available (exercise, medication and surgery) provide symptomatic relief but do not in general address the process itself. Many a times the adverse effects/ complications and the presence of co-morbidities in the patient even restrict the use of these interventions. Thus, there is a need to investigate a treatment modality which not only provides symptomatic management but also directly addresses the diseased process (Abramson and Yazici, 2006, Lauder et al., 2007). Lately therapies for OA include the use of biological markers which inhibits IL-1 thereby reversing the cartilage destruction. Also the potential use of growth factors(GFs) as therapeutic proteins for cartilage repair are being explored. Autologous PRP, when injected intraraticular, delivers large pool of GFs{insulin-like growth factor-1(IGF-1),platelet derived growth factor(PDGF) and transforming growth factor β(TGF β) }and proteins implicated in tissue repair mechanism. These factors favor cartilage stabilization by regulating metabolic activities of chondrocytes and subchondral bone. This "Preparation Rich in Growth Factors" (PRGF) also increases hyaluronic acid concentration, stabilizes angiogenesis, encourages chondrogenesis and enhances the limited capacity of cartilage to repair itself (Schmidt et al., 2006, Anitua et al., 2005).

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TGF β is essential for cartilage integrity and is a powerful agent to prevent or repair cartilage damage. A strong correlation exists between lack of TGF β and predisposition to cartilage damage. It was suggested that the solution to cartilage damage might not be restricted to blocking a single cytokine or stimulation of a single growth factor but selection of factors that together possess anti osteoarthritic properties which can be provided by TGF β (Davidson et al., 2007)

Anitua et al hypothesized that the PDGF restore hyaluronic acid concentration and also induce hepatocyte growth factor production by synovial fibroblasts. In their study they cultured synovial cells of osteoarthritic patients and observed that PRP significantly enhanced HA secretions from these cultured cells vis a vis platelet poor preparations. They concluded that intra articular PRP might be helpful in restoring HA concentrations and changing angiogenesis to a more balanced status (Anitua et al., 2006).

In view of the available literature, we aimed in our study to evaluate the role of PRP in symptomatic primary osteoarthritic patients and also to explore the effects of PRP in restoring the cartilage thickness by measuring with ultrasonography.

MATERIALS AND METHODS

The present prospective clinical study was conducted at Department of Orthopedics, Pt. B D Sharma PGIMS, Rohtak from June 2013- December 2015 after approval from Institutional Ethical Committee. Fifty five patients of either sex who fulfilled the criteria of Grade 1-3 OA with clinical symptoms of more than 3 months and Hb > 10gm/dl, were enrolled in the study after obtaining informed consent. The patients with grade 4 OA, secondary OA, pseudo-gout, gout, history of steroid treatment intake in last 3 months, rheumatoid arthritis, local or systemic infection, blood dyscrasias, malignancy, Hb<10 or immuno-depressive disease were excluded from the study. One patient did not complete full course of injection and four patients did not turn up for follow up, hence a total of 50 patients with 82 knees completed the study. There were 17 males and 33 females having mean age of 53.72 ±7.07 with a range from 39-66 years.

Preparation of Prp

PRP was prepared in the Dept of Blood Transfusion, PGIMS, Rohtak using standard preparation techniques on the day of application from patient's own blood. A total of 43 ml of blood was drawn from ante-cubital vein. Blood was anti-coagulated with citrate phosphate dextrose adenine with a ratio of 1:6 to blood. After 10 minutes of centrifugation at 2000 rpm blood was layered in 3 basic components; RBCs, platelets and platelet poor plasma (PPP). Due to different sediment coefficients the RBCs are the lowest level, platelets in the middle and the plasma is at the top. Supernatant including the platelets and plasma was drawn from the tube leaving the RBCS at the bottom. This supernatant containing platelets and plasma was then agitated and centrifuged again at 2600rpm for 10 minutes. After centrifugation two layers were formed in which the supernatant was PPP and the lower layer was concentrated platelets. About 3/4 of the supernatant was discarded and the residual PRP (Approx. 6 ml) was drawn into a syringe. Another syringe with 1ml of 10% calcium chloride was integrated with PRP syringe to activate the PRP (Figure 1).

Injection Procedure

Affected knee was exposed, cleaned and draped properly, the joint was approached through a supero-lateral site using 20 G disposable needle. The knee was aspirated and effusion if any was aspirated and discarded. PRP was injected into the joint with the help of 10 cc syringe. Adhesive dressing was applied at the needle site. Patient was instructed to bend and extend the knee several to allow equal distribution of PRP. Patient was discharged from OPD with advice of cold compression of knee for 24 hours, full weight bearing, oral antibiotics (tablet Levofloxacin 750 OD) and tablet Ibuprofen 400 mg SOS for 3 days. Patient was advised to come after one month for the next injection. A total of 3 injections at 1-month interval were given. Patients were followed at 3,6 and 12 months interval. Patients were also advised not to take any analgesic during the period of follow up.

Evaluation of Patients

All the patients were evaluated using Visual Analogue Scale (VAS), Knee injury and Osteoarthritis Outcome Score (KOOS) and Western Ontario and

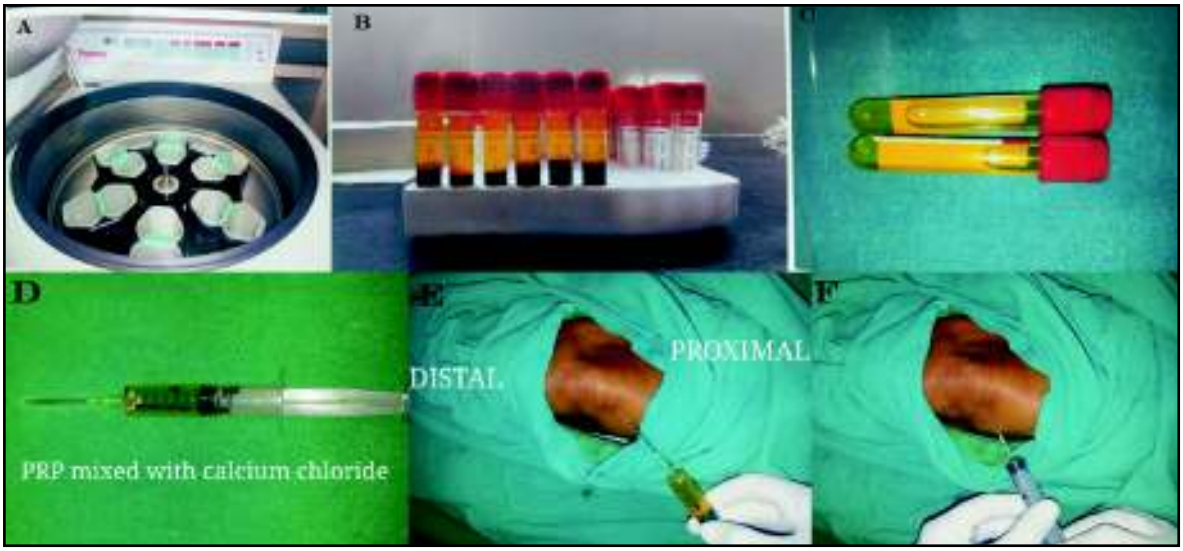

Figure 1: PRP Mixed with Calcium Chloride

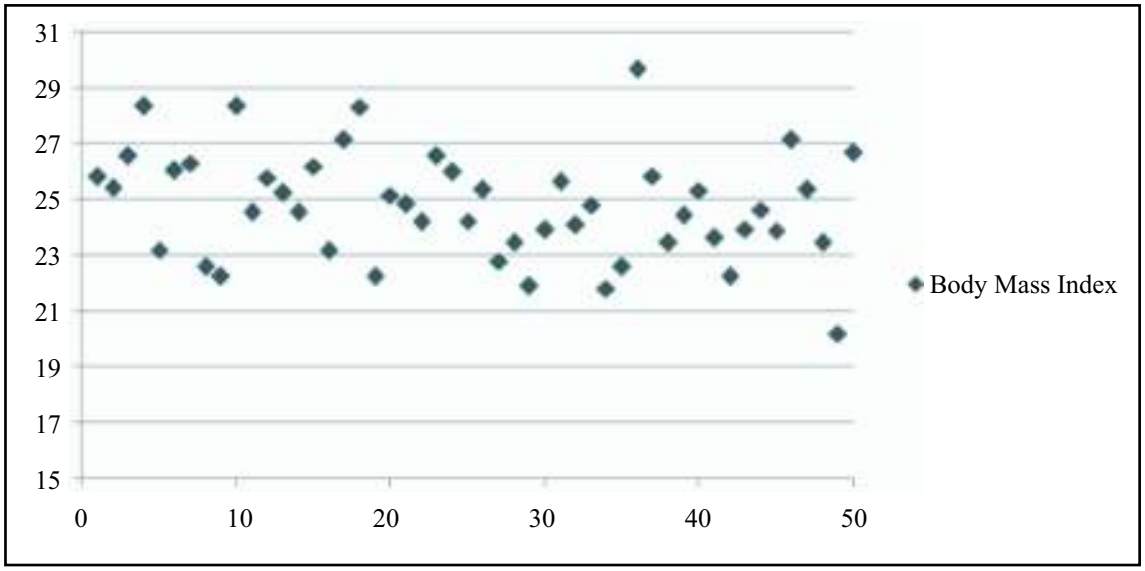

Figure 2: Scatter Diagram Depicting Body Mass Index (BMI) of the Study Subjects

McMaster university osteoarthritis index (WOMAC) scales prior to first injection, then subsequently at 3, 6 and 12 months. Thickness of knee articular cartilage was also measured at pre-injection time with knee in full flexion using high resolution linear ultrasound probe of 5-13 MHz frequency and then on every follow up.

The collected data was entered in a MS Excel spreadsheet, coded appropriately and later cleaned for any possible error in SPSS (Statistical package for social studies) for Windows version 20.0. Repeated measures ANOVA, Freidman's ANOVA and Mann Whitney Test were used to test for significance between baseline values and various follow up measurements.

RESULTS

82 knees of 50 patients with primary OA were enrolled in the study. 32 patients had bilateral involvement while 18 had involvement of single knee. Maximum number of patients n=23 had BMI of 25 and above with a mean of 24.77±1.92 ranging from 20.20 to 29.67 (Figure 2).

Table1: Depicting the Improvement in KOOS Parameters

Time	Normalised pain Mean±SD	Normalised symptom Mean±SD	Normalised ADL Mean±SD	Normalised Sports & Recreation Mean±SD	Normalised Quality of life Mean±SD
Pre - injection	34.52±15.04	63.11±12.37	46.70±13.04	35.30±14.52	24.67±11.08
3 month post - injection	55.66±12.14	74.00±10.44	65.64± 11.18	60.24±13.28	43.44±9.15
6 month post - injection	66.06±13.67	79.70±9.10	73.82±12.52	69.27±13.22	51.68±12.58
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
1 year post-injection	69.58±15.89	81.32±10.58	75.90±14.08	69.33±13.67	52.36±13.56
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2: Depicting WOMAC Parameters

Time	Normalised pain Score±SD	Normalised stiffness score±SD	Normalised Global Function Score±SD	Normalised Total WOMAC Score±SD
Pre- injection	28.90±12.02	38.87±15.96	46.70±13.04	114.47±35.29
3 month post- injection	57.10±12.22	65.55±19.40	65.64±11.18	187.28±36.60
6 month post- injection	69.88±16.16	76.98±19.69	73.82±12.52	220.68±45.25
p-value	<0.001	<0.001	<0.001	<0.001
1 year post- injection	70.91±17.97	79.12±20.04	75.90±14.08	225.92±48.87
p-value	0.047	0.048	0.047	0.047

Table 3: Showing Mean VAS Score at Different Time Periods

Time	VAS Score Mean±SD
Pre- injection	5.70±0.60
3 month post- injection	4.01±0.88
6 month post- injection	3.33±1.28
p-value	<0.001
1 year post- injection	3.24±1.32
p-value	<0.001

36 knees (44%) fell in the grade 3 of Kellgren Lawrence grading system. Table 1 shows KOOS parameters at pre injection 3,6, and 12 months follow up. There was statistically significant improvement (p<0/001) in all the parameters of KOOS score. Similar improvement in each of subscale and in total score was observed in WOMAC score, which was statistically significant after 6 months and 1 year. Table 2 shows improvement in WOMAC score with time. The VAS score also showed statistically significant improvement with time. Table 3 shows VAS score at different time periods.

No significant increase in cartilage thickness over the time period was observed. Table 4 shows cartilage thickness at pre injection, 6 and 12 months follow up. Figure 3 shows cartilage thickness on ultrasound. No serious complication was observed in any patient. Only 1 patient had swelling of knee joint with moderate pain, which subsided without any further intervention. Mild pain at

Table 4: Showing Cartilage Thickness

Time	Cartilage Thickness Mean ±SD		
	Medial	Central	Lateral
Pre- injection	1.36±0.39	2.63±0.39	1.66±0.43
6 month post- injection	1.24±0.38	2.54±0.38	1.58±0.38
1 year post- injection	1.24±0.37	2.56±0.44	1.55±0.39

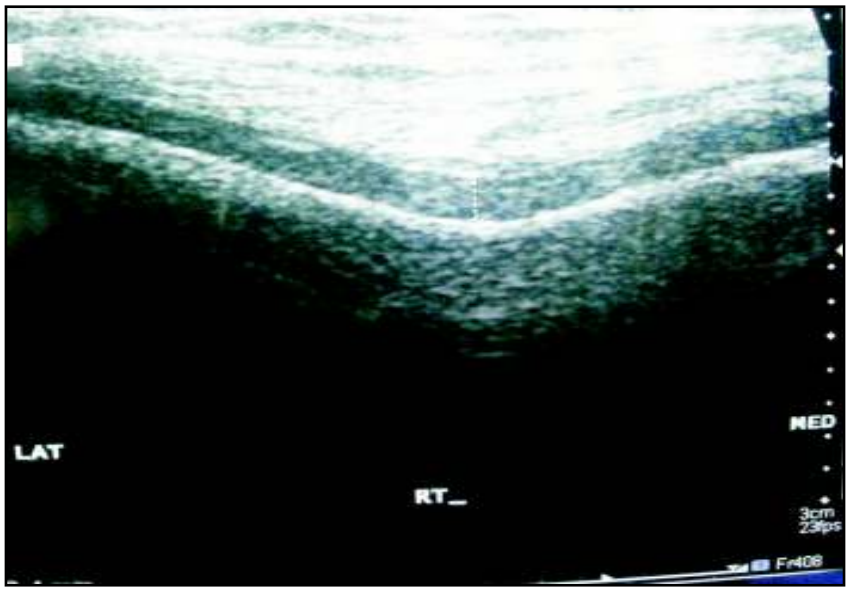

Figure 3: Cartilage Thickness on Ultrasound

injection site was complained by 20 patients, which relieved in 2-3 days spontaneously.

DISCUSSION

Articular degeneration is difficult to treat and is a challenge for orthopedic surgeons due to the distinctive structure and function of the cartilage and its inherent low healing potential. Recently the trend has been on increased prevalence of use of biological products for treatment of OA that may provide both cellular and humoral mediators for tissue repair. PRP is one of such biological product obtained from patient's own blood, which contains number of growth factors (GFs). Many of these GFs have been identified to regulate articular cartilage particularly TGFβ, which is the leading factor involved in process of cartilage regeneration, inducing chondrogenic differentiation of mesenchymal stem cells, as well as matrix deposition and antagonizes suppressive effects of inflammatory mediator IL-1 on cartilage specific macromolecule synthesis. PDGF increases chondrocyte proliferation, proteoglycans synthesis and is a potent chemotactic factor for chondrocytes. Therefore, a characterized platelet derivative called PRGF is being encouraged to treat degenerative joint conditions, which enhances the limited capacity of cartilage to repair itself. PRGF has positive effects in variety of clinical situations involving connective tissue and also in OA synovial cell cultures. The availability of these natural biologically active molecules by PRGF within the joint is a safe strategy to induce positive results in improving conditions of articular damage. PRP achieved after centrifugation of patient's blood contains platelet concentration 4 to 5 times higher than that of normal blood. It is activated by addition of calcium chloride resulting in the formation of a sort of platelet gel and the release of cascade of growth factors (Abramson and Yazici, 2006, Buckwalter, 2004, Ochi et al., 2002).

The amount of about 6 ml of PRP was used in the present study as more amount of PRP would have required drawing larger amount of blood from the patient besides resulting in a bigger amount of intra articular injection leading to effusion in the joint, which may further hamper nutrition of the cartilage. A lesser amount would have been probably less effective due to reduced amount of GFs released by reduced number of platelets. Statistically significant improvement in VAS score was observed in the present study in baseline to 6 months and 1 year follow up, although there was little change from 6 months to 1 year. Kon et al in their study reported statistically significant improvement in from baseline to 6 months and baseline to 1 year in early OA with p value of 0.005 for both. However, there was worsening of score from 6 months to 1 year. Although the final score at 1 year was statistically significant above baseline score. In the present study, VAS score improved from mean of 5.70±0.60 at baseline to 3.33±1.28 after 6 months, though thereafter there was only meagre improvement to 3.24 ±1.32 at 1 year. Still it was statistically significant improvement at 1 year from baseline with p value of < 0.001 (Kon et al., 2010).

Statistically significant improvement in pain, stiffness and global function was observed in WOMAC score assessment. There was 58.64% improvement in pain from baseline to 6 months and 59.24% improvement after 1 year. Similarly, stiffness and global function subscales showed 49.50 and 36.74 % respectively after 6 months. As observed with pain there was very little improvement from 6 months to 1 year although the final outcome was statistically significant.

Normalized KOOS score outcome also showed significant improvement. Pain subscale showed 50.39% improvement from baseline to 1-year follow up with p value of 0.001 as compared to 26.61 % improvement (p0.0295) observed by Sampson et al. This might be possible because of the fact that their sample size was small and they included patients with both low and high grade OA. Statistically significant in symptom subscale was also observed along with improvement in ADL, sports and recreation and quality of life subscales with a p value of < 0.001 for all of them. Sampson et al also observed improvements in these scales but that was not statistically significant (Sampson et al., 2010).

Articular cartilage thickness was measured at pre injection; 6 months and 1 year follow up. No increase in cartilage thickness was observed at 1 year rather the thickness of cartilage decreased after 1 year corroborating the findings of Sampson et al. These findings do not agree with reports in literature about the positive role of PRP in cartilage regeneration. These findings do indicate that though PRP injections may provide symptomatic relief and reduce the requirement of analgesics in short term but the progression of disease in the form of reduction is articular thickness cannot be reversed, thereby indicating its limited usefulness on long term basis. However the ultrasonic measurement of cartilage thickness may not be absolutely accurate more so in case the change is very subtle. Ultrasonic measurements have an element of subjectivity, which may also result in, inter personal variations in cartilage thickness measurements. Possible a long term follow up with more definitive method of measurement of articular cartilage thickness like MRI or an arthroscopic evaluation of joint surface may throw some more light on PRP in reversing cartilage degeneration. As observed with all clinical score assessments PRP has early onset of action, which might be because of anti-inflammatory properties of PRP, which is further corroborated by the observation that there was little clinical improvement after 6 months (Sampson et al., 2010).

CONCLUSION

We conclude that intraraticular PRP injections are safe and a beneficial treatment strategy, which improves pain, stiffness, daily activity functions and overall quality of life. However, maximum effects were observed for 6 months only. The non-randomization, lack of comparative control limits the power and significance of the study, as we are not able to see the comparative effects of PRP. Also PRP did not result in improvement in cartilage thickness indicating not much significant role of PRP in reversal of disease process on long-term basis.

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