Original Article

Role of Autologous Platelet Rich Plasma in Treating Symptomatic Patients with Osteoarthritis Via Double Spinning Technique

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ABSTRACT

Background: Platelet rich plasma (PRP) is a hemoderivative containing a platelet concentration well above the normal baseline rich in platelet degranulation products such as cytokines/vasoactive amines and growth factors, which make it a good therapeutic tool for repair and healing in many speciality fields of orthopaedics, dentistry, plastic surgery (including cosmetic and burn therapy) and dermatology. The present study was conducted with aim of seeing the response of PRP on symptomatic alleviation and functional restoration in patients of osteoarthritis.

Methods: The present study was undertaken in the department of transfusion medicine in conjunction with department of orthopedics at a tertiary care teaching hospital on 50 cases of OA graded according to Kellgren-Lawrence grading system. In all the cases single intra-articular injection of PRP made by double spin technology was injected and response of the patient followed up at 3, 6 and 12 months with visual analogue score (VAS) and Western Ontario & Mc Master Universities Arthritis (WOMAC) index. Apart from simple statistics of mean and averages; the means of paired samples were compared for the VAS with Student's t parametric test. The WOMAC index was tested by the non-parametric Wilcoxon's rank sum test. In both the settings a value < 0.05 was deemed significant.

Result: Statistically significant differences ($p<0.001$) between pre-treatment and follow up values were found for pain, stiffness and functional restoration of the joint as measured by WOMAC index and VAS pain scores. There were no adverse events related to PRP infiltration.

Conclusion: The study hence concludes that intra-articular infiltration of autologous PRP prepared by double spinning approach in OA of the knee according to the established protocol is a safe, tolerable and effective tool which results in reduction of pain and is also associated with functional improvement of the joint as well.

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Introduction

Platelet rich plasma (PRP) is a hemoderivative containing a platelet concentration well above the normal baseline. [1,2] According to application of the technique utilized it is classified conventionally as pure platelet rich plasma (P-PRP), leucocyte and platelet rich plasma (L-PRP), pure platelet rich fibrin (P-PRF), leucocyte and platelet rich fibrin (L-PRF) based upon the technique used to prepare and the final content of the product.[3]

Different derivatives have different physiological responses for example PRF is reportedly has a better therapeutic response in comparison to PRP owing to better platelet degranulation [3,4] and leucocyte poor PRP are better in treatment response owing to pauci-immune response in such types of PRP. [5] Since late 90's its usage is increasing and getting more and more popular in field of orthopedics, dentistry, ophthalomology, wound healing, plastic surgery including burn management and maxillofacial surgeries which require active tissue regeneration and repair .[6]

The therapeutic effect of PRP is attributed to the rich plethora of cytokines/vasoactive amines and growth factors which are released into it via the alpha granules of the platelets, namely; platelet-derived growth factor (PDGF), transforming and fibroblastic growth factors-β, insulin-like growth factor-1/2 (IGF-1/2), vascular endothelial growth factor, epidermal growth factor amongst others with these growth factors acting as inexpensive, efficient, biologically safe (autologous hemoderivative) adjunct therapy to maintain physiological hemostasis (in case of orthopedic sciences as an aid to repair and remodel the skeletal and soft tissue compartment). Thus PRP itself is coming up in various fields as an alternative to the conventional pharmacological and instrument based therapies which have proven associated side effects or morbidity. [7-12]

The rationale behind the injection of autologous blood preparations lies in the exploitation of body's own natural response to injury and to release growth factors [13,14] or the production of anti-inflammatory cytokines by blood components as explained above [11,15]

Blood is an important and unique source of cellular and protein products that has been explored more intensively over the last three decades for the production of biomaterials for clinical use .[10]

In the field of transfusion medicine apart from supply of the "safe blood and its components" and apheresis an added responsibility of provision of PRP has been incorporated. As a recent addition the safety and standaridization protocols are still being evolved targeting the specific fields of medicine and surgery where they are being utilized.

In field of orthopaedics, Osteoarthritis (OA) is a chronic degenerative condition of hyaline cartilage accounting for profound morbidity, pain and health care expenses. The consequences to the individual and to the population as a whole are very significant, particularly with our aging population. There are few validated interventions that improve the clinical condition of a patient once the degenerative process becomes symptomatic. Given the lack of response of the body's healing mechanisms to degenerative conditions generally, injection of growth factors and cytokines is sensible. Lab and animal models exist for using PRP in OA with generally favorable results.[15] A recent article by Kon et al indicates improved functional outcomes. [16] It is still unknown whether PRP acts by local paracrine factors to alter pain, by new hyaline or fibrocartilage formation or a combination of both or neither.[17]

Materials and Methods

The study was conducted in Department of Transfusion Medicine in conjunction with department of orthopedics between November 2013- December 2014 at a tertiary care teaching hospital. The hospital ethical committee approved the study. Patients included in the study were between the age group 39-60 years who attended the orthopedics outpatient department with complains associated with joint pain and ultimately diagnosed as suffering from osteoarthritis (OA) according to the criteria's laid down by American College of Rheumatology (ACR). [18]

The aim of the present study was to judge the response of PRP injected intra-articularly in knee joint by double spin technique on pain relief and functional restoration of the joint by measureable clinical indicators.

Study: Patients included in the study were those having a body mass index (BMI) < 30, non responsive to anti-inflammatory drugs > 3 months without any previous therapeutic invasive procedure or history of fracture.

A pre-study questionnaire designed at the start of the study to record patient details such as age, BMI, current work status (active/passive/retired), dominant extremity and the two tests_VAS (Visual analogue score) and WOMAC (Western Ontario and Mc Master Universities Arthritis) index were duly filled up.

Exclusion Criteria: Patients with thrombocytopenia (count < 1,00,000), anemia (< 10 gm/dl hemoglobin), immunodeficiency states/ immunosuppressive therapy, positive for transfusion transmitted infections (TTI's), advanced tri-compartmental OA, possible infective arthritis of knee joint and patients with skeletal knee deformities as a squeal of advanced OA were excluded.

Also excluded were patients using consistent NSAID's or use of local corticosteroid injection and systemic usage of corticosteroids within 2 weeks.

Informed consent of all the patients was obtained. In total 50 patients were finally included in the study of OA knee having grade 1, grade 2 and grade 3 criteria of Kellgren-Lawrence grading system [19] done on anterio-posterior / lateral knee and skyline/ standing 45 degree flexion knee radiograms. [Table-1]

All the 50 patients were treated with one intra articular injection of autologous PRP produced by double spinning approach with pre-procedural complete blood count (CBC), coagulation profile and tests for transfusion transmissible infections (TTI's).

Technique for Preparation of PRP: To prepare 20 ml of PRP with platelet concentration of 4-6 times the average normal value; 100 ml of venous blood from antecubital vein under aseptic precaution was collected in an adult double bag (Polymed/J.Mitra) with 14ml of citrate phosphate dextrose and adenine as an anticoagulant. Blood with CPDA -1 was centrifuged twice- first at 1800 rpm for 15 min to separate RBC and a second at 3500 rpm for 10 min to concentrate platelets. The final PRP was extracted for intra-articular injection in two 10 ml syringes (approximately 8 ml per knee) and 0.5 ml PRP was sent to laboratory for platelet counting and validation of the procedure.

Procedure: Under aseptic conditions, PRP thus obtained were injected through a classic supra lateral approach using a 22 G needle in a supine position with knee in full extension. Immediately post procedure, the patients were told to actively flex and extend their knees a few times to allow the PRP to spread throughout the joint before it coagulates spontaneously (gelling). Patients were sent home after 15-20 min of rest with instruction to have rest, to limit weight bearing and to use cold packs 3-4 times a day for 10 min for the next 48 hours. Patients were prohibited from using NSAIDS, steroids or any medications, which might influence platelet count or function. Exercise or physical treatment were also restricted during the study period to eliminate any synergistic effects.

Post-procedural Follow Up: All the patients were followed up for a minimum period of one year. Follow up was done on a regular interval (3 months, 6 months and 12 months) with both clinical and functional evaluation for pain relief as per VAS (Visual analogue score) and WOMAC (Western Ontario and Mc Master Universities Arthritis) index.

VAS assessment was done with numbers from "0" to"10", equidistantly marked on a 10 cm line. The patients were explained that "0" meant they were experiencing no pain, "5" moderate pain and "10" unbearable pain, and they were asked to mark the appropriate score on the line describing their own pain during rest and physical activity. [20]

The WOMAC osteoarthritis index is a disease-specific questionnaire for the disease, which assesses pain, stiffness and physical functions of OA patients. It consists of 24 questions in total: 5 on pain, 2 on stiffness and 17 on physical functions. Individual subgroups score or the total score can be calculated. A Likert scale (1: none, 2: low, 3: medium, 4: high, 5: very high) is used to assess all parameters on the WOMAC OA index. High WOMAC scores are indicative of intense pain and stiffness and impairment of the physical function. In our study, the patients' WOMAC sub-scores (pain, stiffness, function) and total WOMAC score were calculated.[21]

Both the scores were done pre-procedure and after procedure on follow up visits and backed up with the standard blood investigation such as repeat CBC, ESR and CRP levels.

Statistical Analysis: Apart from simple statistics of mean and averages; the means of paired samples were compared for the VAS with Student's t parametric test. The WOMAC index was tested by the non-parametric Wilcoxon's rank sum test. In both the settings a value < 0.05 was deemed significant.

Result

In the study conducted on 50 patients there was a male preponderance (29 cases) with ages ranging from 39 years to 60 years with mean age of 49.8 years.PRP was injected mostly in right knees (32 cases; 62%).

The chondropathic grade distribution in the study according to the Kellgren-Lawrence grading system was mostly skewed towards grade 3 (40 %) cases followed by grade 2 (36 % cases) and grade 1 (24 % cases) respectively. No grade 4 lesions were included in the study as per pre-determined exclusion criteria. [Table -2]

It was noted in the study that the VAS score for pain which was 9.21 at pretreatment declined subsequently to 3.32 at 3 months and again showed a small fluctuation of 3.88 at 6 months and slight increase of 5.51 at 12 month follow-up, with statistically significant differences at each time point ($P<0.0001$). An improvement was documented in large group of cohorts (87.8% of patients). [Table-3]

The scores for WOMAC Index in the present study for pain, stiffness and functional capacity, respectively, were18.4, 7.44 and 54.4 at pretreatment**,** 3.21, 2.13 and 15.31 at

3-month, 4.55, 2.84, 18.5 at 6- month, and 8.81, 4.11, 28.8 at 12-month follow up with significant differences for all three periods (P<0.0001). The patients follow-up improvement was 84.4%, 67.7% and 81.1% for pain, stiffness and functional capacity respectively thus showing affable patient satisfaction overall. [Table-3]

In the study conducted no major signs or symptoms (adverse reactions) were recorded related to the PRP injections (both intra or post procedure); although in some cases slight pain was present during first 2 or 3 days but it regressed spontaneously without any administration of analgesics.

Table 1: Kellgren-Lawrence Classification.

Grade	Description
Grade 0	Normal
Grade 1	Doubtful narrowing of joint space and possible osteophytic lipping
Grade 2	Definite osteophytes and possible narrowing of joint space
Grade 3	Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, and possible deformity of bone ends
Grade 4	Large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone ends

Table 2: Patient demographic data and Kellgren- Lawrence grades.

No. of Patients	Male	Female	Right	Left	Grade 1	Grade 2	Grade 3
50	29	21	31	19	12	18	20

Table 3: Global results: pre/post treatment average values, percentage of improvement, statistical significance rates.

	VAS	WOMAC Pain	WOMAC Stiffness	WOMAC Functional capacity
Pretreatment	9.21	18.4	7.44	54.4
3 month	3.32	3.21	2.13	15.31
6month	3.88	4.55	2.84	18.52
12 month	5.51	8.81	4.11	28.80
%age of improvement	87.8%	84.4%	67.7%	81.1%
P value	<0.0001	<0.0001	<0.0001	<0.0001

Discussion

The present study was done to determine the effect of intra-articular injection of platelet rich Plasma (PRP) in symptomatic patients with knee osteoarthritis and to educate patients regarding importance of early intervention. Platelet-rich plasma (PRP) is a simple, efficient, and minimally invasive method of obtaining a natural concentration of autologous growth factors. Generation of PRP involves centrifugation of autologous blood to separate and extract the plasma and buffy coat portion of the blood, which contain high concentrations of platelets. The application of PRP has been documented in many fields. First promoted by Ferrari in 1987 as an autologous transfusion component after an open-heart operation to avoid homologous blood product transfusion, now its application is in many fields.[22]

Many factors are contributive to the outcome in the patient, which are documented in literature such as increased concentration of platelet population from a baseline (lower type: 2.5-3.0 times or higher: 5.0-9.0 times), proportion of leucocytes within PRP (leukocyte rich or poor) and type of anti-coagulant.[23] It would seem intuitive that a higher platelet count would yield more growth factors and better clinical results, however, this has not yet been determined. Graziani et al suggested that the optimal concentration of PRP was 2.5x baseline and above this there may be an inhibitory effect. [23] OA is the leading cause of disability in the world and more than 10 % of the elderly population having symptomatic disease.[24] By mediating chondroprotective action PRP therapies can delay joint deterioration by interfering with the early catabolic and

inflammatory events and by promoting anabolic responses subsequently reducing OA pain. The central role of metalloproteinases is now well documented as the source of chondro-degeneration which is additionally aggravated by other physical and intrinsic elements notably amongst them an increase in BMI > 25.[25] In the present study maximum patients were of grade 3 (40 %) with very few patients of grade 1 is explained by the fact that in grade 1 the symptoms are very much mild and patient hence alleviates his/her pain with over the counter medications and doesn't present to the medical specialist.

PRP Technique and its correlation with Pain Improvement: Although there are 3 protocols for PRP production: selective blood filtration, single-spinning and double spinning procedures.[26] The latter two are associated with better results and patients compliance and is also cost effective. Double spinning is thought to increase the baseline platelet levels by 4- to 8- fold in comparison to 1 to 3 times in single spin technique. The method used in the present study was double spinning and there was significant improvement in all 50 patients at 3 and 6 months follow up ($p < 0.0001$), although from 6 months to 12 months patients with grade 2 and 3 started experiencing slight pain and stiffness but still the results were statistically significant ($p<0.001$) from baseline evaluation. This is in concordance with work done by researchers notably amongst them Kon et al who have employed intra-articular injections of PRP obtained after double spin technique in a cohort of 100 patients of OA with a two year evaluation period [16,27] .In a clinical trial conducted it was noted that technique of intra-articular autologous double-centrifuged PRP had better therapeutic efficacy than the hyaluronic acid (HA) injections in alleviating patient symptoms and also resulted in recovery of joint movements even in those patients afflicted by severe chondropathies. [28]

***VAS score*:** In our study baseline VAS score of patient was taken at 100 at the time of presentation to outpatient department. The reduction of mean VAS score at 3 month, 6 month and 12 month with increased proportion of percentage of improvement was found to be statistically significant with p value <0.0001. The percentage improvement of 87.8 % in our study was better than those seen in Kon et al , Wang et al and Sanchez et al who have reported them to be 80 % , 73.4 % and 33.3 % respectively.[16,29,30]

WOMAC score: The scores for WOMAC Index in the present study for pain, stiffness and functional capacity, respectively, were lesser in the 3 month follow up period than at the 6 and 12 month follow up period but still were lower than the pre-treatment levels. The finding was statistically significant in all three follow up periods ($p<0.001$). The patient's follow-up improvement was higher than the work conducted by Spakova et al, Patel et al and Creza et al.[31,32,33]

Conclusion

The study hence concludes that intra-articular infiltration of autologous PRP prepared by double spinning approach in OA of the knee according to the established protocol is a safe, tolerable and effective tool which results in reduction of pain in the long run. The study also proves emphatically that reduction of pain is also associated with functional improvement of the joint as well (measured in the study by WOMAC score). Apart from the improved clinical findings an additional benefit of PRP is absence of systemic toxic / adverse drug reactions/ idiosyncratic reactions associated with the conventional pharmaco-therapeutic agents.

Although blood banks in past apart from providing transfusion services have been entrusted additional services of therapeutic procedures such as aphaeresis with PRP being the new entrant. In the coming years blood banks are going to be an intrinsic and integral part of therapeutic interventions in many specialties. Hence work has to be done in framing of standardization for making PRP as a therapeutic tool in various specialties.

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Competing Interests

None Declared

Reference

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