Use of intraarticular injections of platelet-rich plasma in the treatment of knee osteoarthritis: A review article

Naveed Baloch,[1] Obada Hasan,[1] Zain Baig,[2] Umme-e-Hani Abdullah,[1] Mohammad Atif,[3] Hiroshi Ohuchi[4]

[1]The Aga Khan University Hospital, Karachi, Pakistan; [2]Health Quest, Sharon, CT, USA; [3]Ghurki Trust Teaching Hospital, Lahore, Pakistan; [4]Kameda Medical Centre, Kamogawa, Japan

Abstract

Osteoarthritis is one of the most common degenerative conditions affecting knee joint. As our understanding in the disease pathogenesis is evolving, so do the treatment modalities. One of the postulated mechanisms suggests the production of inflammatory cytokines secondary to repeated micro trauma than in turn lead to cartilage damage overtime. Cartilage being avascular structure has limited potential for repair. Based on this, recent studies have been focusing on stimulating cartilage-healing process by growth factors. This is where platelet-rich plasma comes in to light. Literature is showing promising results of platelet-rich plasma in treating knee osteoarthritis. In this review we have discussed the preparation, composition, classification, uses of platelet-rich plasma as well as evidence for its efficacy and complications.

Introduction

Osteoarthritis is one of the most common diseases affecting the knee joint. It is a chronic degenerative disorder that due to its widespread prevalence is a major economic burden on the healthcare system of a country.[1] It is characterized by progressive degradation in the articular cartilage, osteophyte formation and subchondral sclerosis.[2] Osteoarthritis is known to be in the top ten causes of disability around the world. In the United States, it is the second most common cause of productive work time loss (after low back pain being the first).[3] And although it can involve any joint, for the purpose of this review we would be restricting ourselves to Osteoarthritis of the knee only.

According to Rayegani *et al.*,[4] approximately 11 percent of women and 7 percent of men suffer from some degree of knee osteoarthritis. It is hence a significant cause of pain, loss of functionality and deteriorating quality of life in a major proportion of the population. It is postulated that due to the increase in life expectancy and the increasing trend of obesity, we are likely to see an increase in the incidence and hence prevalence of Osteoarthritis in years to come.[1] It is therefore crucial for new treatment modalities to come forth in order to address this growing global burden.

One of the proposed mechanisms of development of knee osteoarthritis suggests that repetitive mechanical injury leads to a constant production of inflammatory cytokines such as IL-1 and TNF-alpha, which leads to significant cartilage damage overtime.[5] Cartilage being an avascular structure has low potential for regeneration and repair.[6]

Treatment of knee osteoarthritis is confined to symptomatic relief with the aim of reducing pain, joint stiffness and increasing joint mobility. Weight loss and exercise are recommended life style modifications.[7-9] The use of walking aids is also sugested.[5] Pharmacological therapy includes the use of acetaminophen and non-steroidal anti-inflammatory drugs.[10] Intra articular treatment with Hyaluronic acid, corticosteroids and platelet-rich plasma (PRP) has also come into limelight.

Recent studies have been focusing on stimulating the intraarticular cartilage healing process via growth factors.[2] This is where Platelet-rich plasma has sparked interest. It is believed that since PRP has growth factors and plasma proteins, it will be able to modulate anti-inflammatory signals and promote angiogenesis.[4] Literature review has shown promising results of PRP therapy in patients with knee osteoarthritis. It is being used as an alternative therapeutic agent, given intraarticularly in the affected knee. Studies show reduction in pain and overall improvement in joint motion. PRP has chondrogenic[11,12] and anabolic differentiating[13,14] properties found *in vitro* (at cell culture level) as well as *in vivo* in mice and rabbits[15] showing major role in regeneration of osteoarthritic cartilage.

What is platelet-rich plasma?

Platelet-rich plasma (PRP) is defined as "a sample of autologous blood with concentrations of platelets above baseline values".[16] Normal platelet counts in blood ranges from 150,000 to 350,000 platelets per microliter. However, PRP has counts two or four times higher than this.[17]

Platelets contain a high number of cytokines and growth factors which are believed to be able to slow down the degeneration process and may even initiate chondrogenesis.[18]

Historically PRP was first used in dental surgery to accelerate the healing of implants and jaw reconstruction. Seeing as it is a cheap and minimally invasive modality its use has been explored in a wide variety of medical fields such as neurosurgery, ophthalmology, dermatology, otolaryngology and orthopedic surgery.[6]

In recent years, the popularity of PRP in sports medicine has grown exponentially. The reason for this rapid development is its removal from the list of banned performance enhancers in 2011 (It was previously classified as such due to its growth factor content) and its approval by the International Olympic committee.[19]

Classification of platelet-rich plasma

To increase standardization of PRP reporting and to facilitate the interpretation of various clinical studies, several classification systems for PRP have been suggested. One of these was the Sports medicine Platelet-rich Plasma Classification system suggested by Mishra *et al.*[20,21] and summa-

Correspondence: Obada Hasan, Section of Orthopedics, department of surgery, The Aga Khan University Hospital, Karachi, Pakistan.
Tel.: +92.333.3302009.
E-mail: obada.husseinali@aku.edu

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rized in Table 1. According to this nomenclature, PRP preparations were divided into 4 categories based on platelet concentrations, WBC content and activation status. Type 1 and Type 2 consist of preparations with WBCs increased over baseline. Type 1 does not include the addition of an exogenous activator such as calcium or thrombin whereas Type 2 does. Similarly Type 3 and Type 4 have minimal WBCs, with Type 3 having no exogenous activator and Type 4 having an exogenous activator. Each subtype is then further subdivided into 2 groups; A and B with group A having more than five times platelets over baseline and group B having less than five times platelets than baseline. Due to the development of a double spin system, which allowed greater concentration of platelets to be achieved with little or no WBCs or RBCs, this classification was deemed insufficient by Mautner *et al*. in their review published in 2015.[21] In 2009, another classification was published by Dohan *et al*.[22] This classification stratified PRP according to platelet concentrations, leukocyte concentrations and the presence of fibrin. Preparations were divided into four groups namely; P-PRP (pure PRP), L-PRP (leukocyte and PRP), P-PRF (pure platelet-rich fibrin) and, L-PRF (leukocyte and platelet-rich fibrin). However, this was again deemed insufficient by Mautner *et al.* due to its lack of information on RBCs and WBCs along with its limited application to nonoperative conditions. The next classification was suggested by DeLong *et al.*[23] in 2012. This was the "PAW" classification system that looked at platelet concentration (P), activation (A) and WBCs (W).

Accordingly, platelets were stratified into four groups with P1 having baseline platelet concentrations and p4 having greater than 1.2 million platelets per milliliter. Activation was either exogenous or not and WBCs were categorized as above or below baseline. Since there was no information on RBCs and the stratification of WBCs was just above or below baselines, Mautner *et al.*[21] argued that this was an understatement regarding their role in PRP action. Mautner and collegues[21] then proposed their own classification system namely the PLRA system. This has been summarized in Table 2. It takes in to account the concentration of platelets, WBCs, RBCs and the addition of an activating agent.

Preparation of platelet-rich plasma

It is important to appreciate the fact that different PRP formulations were used in different studies. To date there is no standardized method of PRP preparation. Studies vary in the initial amount of autologous blood used to prepare 4-5 mL of PRP as well as the type of anticoagulant used and the centrifugation rates in their PRP preparation. Such differences make it difficult to objectively compare different studies with one another.

The basic mechanism of creation of PRP includes withdrawal of 35-40 mL[4,24] of the patient's own peripheral blood. This may be done by using an 18G needle to collect blood from the patient's upper limb from the cubital vein. The next step involves the addition of an appropriate anticoagulant such as acid citrate dextrose[4,18,24] and then passing the blood through two cycles of centrifugation. The first centrifugation (done at 1600rpm for 15 minutes according to Rayegani *et al.*[4] and Raeissadat *et al.*[24]) stratifies blood into three strata. The basal layer called red layer, consists of erythrocytes, the middle (white) layer is filled with leukocytes; and the top or yellow layer contains plasma, platelets and growth factors. A second phase (done at 2800 rpm for 7 min)[4,24] is used to concentrate platelet-rich and platelet poor plasma components.[25,26] The use of a manual centrifuge, easily available in affordable prices in most medical centers and hospitals, can assure the availability of PRP injections to higher number of patients. Multiple cost-effective preparation methods mentioned in literature which can be effectively utilized in developing countries.[27,28] Obtaining PRP by automated methods is expensive and can be prohibitive in resource constrained countries.[29,30]

Composition of platelet-rich plasma

Platelets

Platelets are small anucleated cells formed from megakaryocytes.[31] The lifespan of platelets is 5-9 days, after which they are predominately cleared by Kupffer cells and hepatocytes.[32] Platelets can be thought of as a modified version of smooth endoplasmic retinaculum, containing lysosomes, mitochondria, ribosome,[33] and most importantly granules.

Three types of granules are found in platelets, namely alpha, dense and lysosomes. Alpha granules consist of more than 300 different proteins.[34] Dense granules are few in number and contain serotonin, histamine, calcium, pyrophosphate and different phosphate compounds like ADP, ATP, and GDP.[35] Lysosomes play a role in clot lysis.[36]

Studies show that different proteins from platelet secretion have major roles in chemotaxis, angiogenesis, cell proliferation

Table 1. Sports medicine platelet-rich plasma classification.

	White Blood Cells	Activation	Platelet concentration
Type 1	Increased	No activation	A=5X or > B= <5X
Type 2 B= <5X	Increased	Activated	A=5X or >
Type 3		No activation	A=5X or > B= <5X
Type 4		Activated	A=5X or > B= <5X

Table 2. PLRA classification system.

Classification	Criteria	
P: Platelets count	_P Volume Injected	_M Cells/microlitter
L: Leukocyte content[a]	>1% <1%	+ -
R: Red Blood content[b]	>1% <1%	+ -
A: Activation	Yes NO	+ -

[a]If white blood cells are present (+), the percentage of neutrophils should be reported. bThe method of exogenous activation should be reported.

and differentiation. Some proteins have bactericidal and fungicidal properties and other cause aggregation of macrophages, mesenchymal stem cells and osteoblast resulting in removal of dead tissues .This accelerates wound healing.[37]

Growth factors

As previously mentioned Platelet-rich plasma comprises of tissue growth factors and inflammatory mediators (IL-1 receptor antagonist, IL-6, TNF, alpha-2-macroglobulin) (Table 3).[19,38-43] These serve to increase angiogenesis, promote chondrogenesis and increase epithelial cell, osteoblast and fibroblast proliferation. They also stimulate the production of collagen and hyaluronic acid.[19]

Various techniques for preparation of PRP and isolation and differentiation of growth factors from PRP are being studied. The following growth factors have been isolated from PRP.[44]

Platelet alpha granule derived

They are: Platelet derived growth factor (PDGF); Vascular endothelial growth factor (VEGF); Transforming growth factor beta-1 (TGF-b1); Epidermal growth factor (EGF); Basic fibroblast growth factor (bFGF); Insulin-like growth factor-1 (IGF-1).

Plasma derived

They are: Hepatocyte growth factor (HGF); Insuline like growth factor-1 (IGF-1).

Leukocytes

Depending on the preparation technique PRP may be leukocyte rich or leukocyte poor. It is postulated that leukocytes produce metalloproteinases and free radicals which are detrimental for the joint and increase post injection pain.[4,19] However, a possible advantage may be restriction of microbial growth around the injection site.[19] Other researchers believe that the leukocyte release of enzymes is crucial for the repair process as well.[4]

Clinical use of platelet-rich plasma

Platelets were initially used in plastic and maxillofacial surgeries in 1990s with favorable results.[45] Later on, the technique gained recognition in its usage in different orthopedic surgeries like in bone grafts,[46] to increase spinal fusion and fracture healing.[47] PRP is recently widely used for treatment of osteoarthritis as one of biological therapies because of its simplicity in preparation and cheap availability with absence of risk of immunological reaction or disease transmission.

Studies to see effects of PRP therapy in osteoarthritis have shown that it improves anabolic capacity of chondrocytes. Huang *et al*.[48] and Kilian *et al*.[49] demonstrated *in vitro* that PRP is capable of induction of mesenchymal proliferation. Work of Mishra *et al.*[11] showed that PRP increases fibroblast proliferation *in vitro*. They also added that PRP increase gene expression for chondrogenic and osteogenic differentiation. *In vitro* study, Nakagawa *et al*.[50] demonstrated increased chondrocytes proliferation and synthesis of collagen.

Efficacy of platelet-rich plasma in the treatment of knee osteoarthritis according to previous research and trials

We did a literature search of clinical trials published in this arena. We included all studies that were available in English script and were accessible to us as full text articles (Supplementary Table S1).

Multiple clinical trials divided the study patients into two groups; one of them received intraarticular PRP injections and the other was a control group. The control group was given HA injections or acetaminophen. The dosage, number of injections and time interval varied in each study. Follow up was done till 6 months and in some trials till 12 months.[24,51]

Evaluations were done using Knee Injury and Osteoarthritis outcome (KOOS), Western Ontario and McMaster University's Arthritis Index (WOMAC), Visual Analogue Scale (VAS), International Knee Documentation Committee (IKDC) and SF-36 questionnaire and significant improvement in their mean scores was seen in the PRP group.[4,18,24,51,52] Physical and mental components in SF-36 questionnaire also showed significant improvement in the PRP group. In two trials, VAS score improved significantly in both the groups.[18,53] When analyzed for osteoarthritis grades, early osteoarthritis (grade II) groups showed more improvement with PRP treatment compared to the late osteoarthritis groups.[24,51,53] Pain improved significantly in the PRP group compared to the control group at follow up.[4,18,51-54]

Recently different systemic reviews and meta-analysis studies have been published to determine the efficacy of PRP therapy in comparison to other intra-articular therapies for treating osteoarthritis.

In 2017, Shen *et al*.[55] conducted a systemic review and meta-analysis of fourteen randomized control trials (RCTs) from 2011 to 2016. The control comprised of saline placebo, HA, ozone and corticosteroids. A total of 1423 patients were included by randomly. PRP therapy group included 12 to 96 patients control group include 11 to 96 patients. Average follow up was 12 weeks to one year. Follow up assessment after one year showed better outcome with PRP therapy. Patient assessment was done using WOMAC score. Limitations of this review were high heterogeneity among studies, blinding of participants not done reliably and substantial placebo effect.

Laudt *et al*.[56] published a study in 2015 for determination of efficacy of PRP intra articular injection therapy. They included ten trials. Outcomes were measured by visual analog scale and numeric rating scale. Scores were improved with PRP therapy as compared to placebo or HA at 6 months follow up. Limitations included small number of studies and domain-based evaluation done for assessing risk of bias rather than using a scale or checklist.

Wang-Saegusa *et al.*[57] studied 312 patients with knee osteoarthritis. Three injections of plasma-rich plasma at 2-week intervals were given. At 6 months, the patients reported a significant improvement

Table 3. Effect of PRP derived growth factors on Chondrocytes (Cartilage).

Growth Factor	Effect on Chondrocytes
Platelet derived growth (PDGF)	Enhances cellular proliferation and proteoglycan production[38]
Transforming growth factor-beta (TFG-β)	Stimulates extracellular matrix (type II collagen and proteoglycan),decreases catabolic activity of interlukin-1(IL-1) and matrix metalloproteinase(MMP)[39,40]
Fibroblast growth factor-2 (FGF-2)	Stimulates proliferation of chondrocytes[41]
Insulin like growth factor-1 (IGF-1)	Increases synthesis of type II collagen and proteoglycan while reducing amount of type I collagen[42,43]

in pain, stiffness and function.

In a prospective study of 100 consecutive patients, affected by knee osteoarthritis, were treated with PRP intra-articular injections (115 knees treated). Patients evaluated before and at the end of the treatment, and at 6 and 12 months follow-up. IKDC, objective and subjective, and EQ VAS were used for clinical evaluation. A statistically significant improvement of all clinical scores was obtained from the basal evaluation to the end of the therapy and at 6-12 months follow-up.[58]

More studies from china concluded that PRP therapy can be among successful options.

Complications of platelet-rich plasma injection

Post injection pain at the injection site is the most common complain reported in literature. These can be prevented by maintaining an aseptic environment during preparation and during the time of injection.

Rayegani *et al.*[4] also reports transient knee stiffness and local pelvic pain and sensation of swelling. These complaints subside after acetaminophen use and are not an indication to discontinue treatment.

Dhollander *et al.*[59] described a case of hypertrophy of regenerated cartilage tissue, treated with arthroscopic debridement.

Combination therapy with of platelet-rich plasma and hyaluronic acid

Abate *et al*.[60] was the first to publish a study in 2015 regarding combination therapy of knee osteoarthritis with both intraarticular injections of hyaluronic acid and PRP. In their study, they enrolled patients with mild to moderate knee osteoarthritis and stratified them into two groups. The first group received weekly injections of 2 mL hyaluronic acid added with 2 mL PRP for three weeks. While the second group received 4-5 mL of PRP only. Baseline evaluation was done using VAS, KOOS and weekly NSAID consumption. Standard Ultrasound of the knee was also done to look for effusion and synovitis. This was repeated at 1, 3 and 6 months. Intra group comparisons showed statistically significant improvement in both groups. However infra group comparisons failed to demonstrate any statistically significant differences. Hence it was concluded in this study that the combination regimen of Hyaluronic acid and platelet-rich plasma (PRP) gave no added advantage than PRP administered alone but in higher volumes. It was postulated in this study that hyaluronic acid has a synergistic effect with PRP by improving the activity of many molecules in PRP.

JFSD *et al.*[54] conducted a randomized control trial that compared hyaluronic acid, platelet-rich plasma and the combination of both in the treatment of mild and moderate osteoarthritis of the knee in 105 patients. The study had three groups of PRP, HA and combined PRP and HA. The study found that combining HA and PRP resulted in significant decreases in pain (P=0.0001) and functional limitation (P=0.0001) when compared to the HA group at 1 year follow up. Furthermore, physical function was significantly increased at 1 and 3 months when compared to the PRP group.

Conclusions

Our review of Literature shows promising effects of PRP therapy for tissue healing based on clinically and scientifically described studies. Its effectiveness as a therapeutic agent is still not proven however. Recently RCTs have elaborated its application in osteoarthritis patients but these studies often have a small sample size with short term outcome. In general, clinical trials have proved better outcomes as compared to other modalities of intra articular injections in patients with knee osteoarthritis. This is especially true for younger patients with low degree of injury.

Scientific evidence from animal model studies and *in vitro* studies is also well described in literature. It shows that PRP therapy increases anabolic capacity of injured cartilage hence repairing the damage.

PRP therapy can be considered as a therapeutic agent to delay the onset of irreversible damage to the tissue and hence surgical interventions in the form of prosthesis placement. Recent literature supports the use of PRP – with results substantiating as the most effective conservative treatment measure for Osteoarthritis. Further studies with large sample size, are still needed to determine the best formulation and processing technique of PRP therapy and application.

In our part of the world, where most health expenses are met out of pocket by the patient, PRP (by being endogenously produced) may help in reducing the total cost of treatment. It may also achieve this by decreasing the need for analgesics intake, hospital visits and delaying surgical intervention. We started this treatment in select patients at our institute and are planning to conduct further studies to form the basis for further usage.

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