



## Original article

## Comparative analysis of conventional and biological treatment in healing of bone disease

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## ABSTRACT

The healing of Bone tissue consists of a complex process. Hence, we designed our study to evaluate chondrial diseases, which are as they have a very low healing capacity. Seventy two elderly osteoarthritis (OA) and 54-paediatric juvenile idiopathic arthritis (JIA) patients were included. The group was divided as 24 OA patients and 18 JIA patients in each group. Group I received Hyualuronic acid and glucocorticoides. Group II received platelet rich plasma and fibrin glue. Group III received PRP, fibrin glue, and MSC. 40 control patients received only PRP treatment. Out of 72 OA patients 35 (48.6%) male and 37 (51.4%) female with mean age of  $48 \pm 6.5$  years. 64 (88.9%) Patients had pain and swelling. 52 (72.2%) lacked flexibility. 42 (58.3%) had hypertrophy. 28 (38.9%) had less cartilage thickness. 34 (47.2%) were in grade 3, grade 2 has 28 (38.9%) and grade 1 has 10 (13.9%) patients respectively. Among 54 JIA patients 28 (51.9%) male and 26 (48.1%) female patients with mean, age  $4.6 \pm 3.8$  years. 39 (72.2%) had pain and swelling. 32 (59.3%) lacked flexibility. 29 (53.7%) children's had functional disability. Group I patients showed 30% improvement with no statistical significance ( $P < 0.21$ ). Group II showed 45% improvement with statistical significance ( $P < 0.01$ ). In Group III 80%, improvement was observed with statistical significance ( $P < 0.001$ ). In 40 control patients, 60% improvement was observed. In conclusion, use of these MSC, PRP, and PPP are safe and less cost effective for treating OA and JIA.

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## 1. Introduction

Bones are biological tissue composed of active cells forming an integrated framework. The Bone tissue healing consists of an array of complex process involved (Mehra and McCarthy, 2006). Many recent studies have come up to understand the mechanism behind the healing of bone disease few of them are use of growth factors. To improve healing platelet gel or platelet rich plasma (PRP), Fibrin glue (Platelet poor plasma-PPP), Hyualuronic acid, Mesenchymal stem cell usage are the few substances which are used for clinical application (Khan et al., 2003).

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Platelets are the smallest cells of the blood circulation (Harrison, 2005). Platelets are packed with multiple growth promoting factors like platelet derived growth (PDGF) factors,  $\beta$ -transforming growth factor (TGF- $\beta$ ), Fibroblast growth factor (FGF), Epidermal growth factor (EGF), Connective tissue growth factor (Civinini et al., 2013). Platelets are also known to possess an anti-inflammatory property, which helps in faster healing by promoting the tissue/cell regeneration in the damaged site. In recent years the use of Mesenchymal stem cells (MSC) as a cellular therapy are gaining importance due to the ability of the MSC cells in faster healing with rapid regeneration of the cells. In recent times the use of MSC for healing of bone disease are widely used (German et al., 2012). MSC is multi-potent cells with the potential to differentiate itself into lineages of tissues such as cartilage, bones, marrow etc (Owen and Friedenstein, 1998).

Hyualuronic acid is an acidic mucopolysaccharide which was used as a conventional lubricant used for treatment of osteoarthritis (OA) or cartilage degeneration. Though Hyualuronic acid is known to decrease pain and reduce friction between joints in some

cases it leads to an allergic reaction and also the effect does not last for a long time.

Many osteoarticular disorders in paediatric age group have a severe consequence throughout their lifetime. MSC have found as a cure for these disorders not only in paediatric age group, but also for the elderly person, at least in reducing the pain and sequelae of the disorder (Wakitani et al., 2011).

Hence, our study was designed to evaluate chondrial diseases, which are more challenging as they have a very low healing capacity. The natural regeneration takes a longer time or sometimes even fails to regenerate in some cases. Hence, we aim to evaluate the effect of PRP, PPP, and MSC against the conventional anti-inflammatory drug Hyaluronic acid and glucocorticoides in elderly patients who have osteoarthritis and juvenile Idiopathic arthritis.

## 2. Materials and method

### 2.1. Study details

A total of 72 elderly patients with osteoarthritis and 54 paediatric patients with juvenile idiopathic arthritis were recruited from the outpatient ward of the Chinese hospital during the period of May 2014 – Nov 2015. The diagnosis was made based on the American college of Rheumatology (ACR) Classification criteria (Altman et al., 1986). The study was approved by the institutional ethical board. An informed consent was obtained either from the patient or from parents in case of paediatric population.

### 2.2. Study design

#### 2.2.1. Inclusion criteria

- Patient with chronic pain in knee for more than six months
- Both sexes
- Swelling in the knee joint
- Radiographic proof showing osteophyte from moderate to severe OA based on the Kellegren-Lawrence scale of grading (Kellegren and Lawrence, 1957)
- Age 45–80 in case of elderly patients and 2–14 years in case of juvenile Idiopathic arthritis

#### 2.2.2. Exclusion criteria

- Patient with Rheumatoid arthritis
- High Obese patients
- Patients with uncontrolled diabetes mellitus
- Patients receiving immunosuppressive drugs
- Patients with known severe disease conditions.

All the patients' demographic details were taken from the hospital record. Clinical assessment was done for both elderly OA patients and Paediatric patients with juvenile Idiopathic arthritis. The arthritis was graded based on the International Knee documentation committee (IKDC), along with X-ray was also taken to find the OA (Kellegren and Lawrence, 1957). Doppler was also done with Ultrasonography to find out the hypertrophy of the synovial joint, thickness of the cartilage, vascularity and patient's flexibility of the knee was examined in supine position (Lee et al., 2008). From the Ultrasonography the cartilage degeneration was evaluated from grade 1 to grade 3. For juvenile paediatric arthritis the standard criteria of paediatric Rheumatology International trail organization, group (Len et al., 1998) were followed; functional disability; pain in knee; not able to move freely; ESR; negative for antinuclear antibodies.

### 2.2.3. Exclusion criteria

- Patients with juvenile diabetes
- Patients below one year
- Patients positive for autoimmune diseases were excluded

### 2.3. Preparation of PRP

Around 50 ml of blood from the vein were collected from the same patient who were about to undergo the PRP therapy. Blood was collected in tubes containing anticoagulants. After which the tube was centrifuged at 2500 rpm for 20 min to separate RBC's and at 4000 rpm for 15 min to separate the platelets. The PRP obtained were quantified by ELISA method. The freshly obtained platelets were used in the therapeutic applications.

### 2.4. Fibrin glue (PPP)

The preparation of fibrin glue is similar to that of PRP but platelets will be excluded in it. It is also available commercially. In our study, we prepared fresh fibrin glue and used it for therapy.

### 2.5. Mesenchymal stem cell preparation (MSC)

Mesenchymal stem cell was obtained from various sources. In our study, we obtained the MSC from human embryonic tissue of placenta and from the adipose tissue because of the ease of availability. In our study, we divided the patients into three groups viz; group I consists of 24 patients with 14 male and 10 female patients in the age 48–65 years diagnosed with OA. 18 paediatric patients with JIA in the age of 6–12 years were included in which 11 were male and 7 were female children's. They were treated only with Hyaluronic acid and glucocorticoides steroids. Group II consists of 24 patients in the age group of 38–60 years. Among the 24 patients, 12 were male and 12 were female patients. 18 patients with JIA in the age group of 4–11 years were included of which 9 were male and 9 were female patients. They received platelet rich plasma and fibrin glue for the therapy. Group III included 24 patients consisting of 15 females and 9 male patients in age 42–72 years, whereas in JIA population 18 patients were included out of which 8 were male and 10 were female patients in age 5–13 years. They receive PRP, Fibrin glue and MSC has the treatment.

All the patients were followed for a minimum of 1 year with every 3-month evaluation. 40 control group patients with 22 male and 18 female with complaints of knee pain served as controls. They received only PRP as treatment. All the patients were in the mean age group of  $34 \pm 10.1$  years.

### 2.6. Statistical analysis

SPSS software Ver 20.0 was used to analyze the data for statistics. The mean, standard deviations were calculated and t-test was done to differentiate between various therapies. Level of significance was calculated with  $p < 0.01$ .

## 3. Result

Our study was carried out with 72 OA patients and 54 JIA patients who were diagnosed clinically by symptoms like pain, swelling of the knee along with radiographic and ultrasonographic proof. Both the patient types divided into 3 groups. Out of 72 patients diagnosed with OA out of which 35 (48.6%) were male and 37 (51.4%) were female patients with a mean age of  $48 \pm 6.5$  years. The duration of the disease ranged from 8 to 36 months with the mean duration of  $19 \text{ months} \pm 2$ . Out of 72

patients 64 (88.9%) Patients had complaints of pain and swelling, 52 (72.2%) had complaints of lack of flexibility. 42 (58.3%) patients had hypertrophy. 28 (38.9%) patients cartilage thickness was very less which were found by ultrasonographic method. 18 (25%) patients had increased BMI of  $31.5 \pm 3.8$ . Of 72 patients 34 (47.2%) were in grade 3 (severe) OA, 28 (38.9%) were in grade 2 (moderate) OA and only 10 (13.9%) were in grade 1 (mild) OA classified based on Kellegren Lawrence scale. 12 (16.7%) patients had type II diabetes with blood glucose > 140 mg/dl (Table 1).

Among the 54 JIA patients 28 (51.9%) were male and 26 (48.1%) were female patients with mean age  $4.6 \pm 3.8$  years. The duration of disease ranged from 11 months to 28 months with a mean range of  $14 \pm 6$  months. 39 (72.2%) patients had complaints of pain and swelling. 32 (59.3%) had lack flexibility of the knee. 4 (7.4%) had increased BMI of  $26.2 \pm 2.5$ . 29 (53.7%) children's had functional disability (Table 1).

The patients divided into 3 groups were assessed after 3 months of the start of the therapy. The data's were analyzed before starting the therapy and at every 3 months, the prognosis of the patients was recorded based on their treatment regime. Group I patients with Hyaluronic acid and glucocorticoides were used. In 24 OA patients, the mean age was  $42 \pm 12.5$  years, whereas in 18 JIA patients the mean age is  $7 \pm 2$  years. They were given Hyaluronic acid and Glucocorticoides for 8 months. After 3 months, they were assessed for the decrease in pain, stiffness with an increase in flexibility. No statistical significance of  $P < 0.21$  was observed in group I patients. Only 30% improvement was observed (Table 2).

Group II patients with 24 OA and 18 JIA patients among 24 OA patients the mean age was  $44 \pm 6.1$  years, whereas in JIA patients the mean age was  $5 \pm 2$  years. They receive PRP and fibrin glue as treatment regime. 45% of OA patients showed decrease in pain with increased flexibility compared to group 1 patients. Statistical significance was also observed in group 2 patients with  $P < 0.01$  (Table 3).

In group III patients 24 OA patients with mean age of  $46 \pm 10$  years. 18 JIA patients, with a mean age of  $7.1 \pm 4$  years were seen. They receive PRP, PPP and MSC injection as treatment. They showed 80% of improvement in reduction of pain with 70% increased flexibility at the end of the treatment. The group III patient had statistical significance of  $P < 0.001$  (Table 4).

In our study, none of the patients developed allergic reactions or other side effects due to the use of PRP, PPP, and MSC. However, in patients who received Hyaluronic acid and glucocorticoides 4 patients developed redness with bruising and skin rashes on the knee due to the use of HA and glucocorticoides. No statistical correlation was observed when BMI, Diabetes and OA of the patients were compared with  $P < 0.01$  whereas no correlation was observed

when BMI and JIA was compared. In patient receiving PRP, PPP and MSC there was decreased degeneration of cartilage and significant thickness was increased after 6 months of therapy. Among 40 control patients with knee pain after 6 of PRP treatment they showed 60% of improvements in flexibility and decrease in pain and swelling (Table 5).

#### 4. Discussion

There is a great challenge existing in the healing of various bone defects faced by clinicians worldwide. With many advancements in treatment with the use of growth factors in healing still there is a set back prevailing due to higher cost and their application complication prevailing (Becker et al., 2004). As a new advancement the use of PRP, fibrin glue (PPP) and MSC has gained importance due to less cost effective and simple procedure involved in the usage of those growth factors. PRP was first used by Marx et al. (1998) since then it has gained importance in various medical applications and are widely accepted due to very minimal or no side effect caused by these products. PRP are a natural concentrate with many growth factors that is cost effective and helps in rapid healing of the wounds, bone defects etc. The role of PRP in Osteoarthritis is a new therapeutic option with more effectiveness (De La Mata, 2013). The use of fibrin glue (PPP) also helps in quicker healing process because of its simpler clinical application (Tayapongsak et al., 1994).

In our study, we used PRP, PPP, and MSC, which showed progressive improvements both in OA and JIA patients. Fibrin glue is widely used as a sealant or adhesive and a carrier of growth factors with high fibrinogen level, which helps in healing the bone disease (Yavuzer et al., 2005). There are many controversial literature stating the action of PRP and fibrin glue, but in our study, we found a promising effect of PRP and fibrin glue in OA and JIA patients with significant improvement (Abiraman et al., 2002).

In our study, we studied on osteoarthritis (OA) and Juvenile Idiopathic Arthritis (JIA). OA is one of the major health problems worldwide (Monteforte et al., 2008). OA is a disorder involving muscular skeletal imbalance leading to damage of cartilage, which leads to pain and disability in affected individuals (Abramson and Attur, 2009). In our study, a significant improvement in patients who received PRP, PPP, and MSC was observed than in patients who received HA and glucocorticoides when various aspects such as reduced pain, swelling, increase of cartilage thickness and reduction in damage of cartilage analyzed. Statistically significance was also similar to our study and another study conducted by Hassan et al. (2015) who also showed statistical significance to PRP usage in OA patients.

The use of MSC seems to have the potential to heal various disorders such as JIA. The capacity to differentiate into number of growth factors has led to usage of MSC in immuno-suppressed patients and in those patients with less healing capacities. The use of MSC in paediatric disorders has many promising effect and safer in its clinical application with very less side effects observed. In our study JIA, patients showed 80% improvement with PRP and MSC therapy.

When other factors like improvement in hypertrophy using Doppler and ultrasound were used, we found more significant improvement, which is correlating with other studies that had showed synovial hypertrophy improvement in patients with OA and JIA. Most of the patients in our study with grade 3 OA moved to grade 1 after PRP, PPP and MSC treatment which is similar to other studies (Kon et al., 2011; Chang et al., 2014) where as in patients who received Hyaluronic acid as treatment showed less improvement either they remained in same grade or the conditions worsened (Pourcho et al., 2014).

**Table 1**  
Demographic details of OA and JIA patients.

Variables	OA (N = 72)	JIA (N = 54)
Sex		
Male	35	28
Female	37	26
Mean Age	$48 \pm 6.5$	$4.6 \pm 3.8$
Mean duration of disease	$19 \pm 2$ months	$14 \pm 6$ months
Presence of pain and swelling	64 (88.9%)	39 (72.2%)
Hypertrophy	42 (58.3%)	–
Thickness of cartilage	28 (38.9%)	–
Lack of Flexibility	52 (72.2%)	32 (59.3%)
Presence of Diabetes (>140 mg/dl)	12 (16.7%)	–
BMI	$31.5 \pm 3.8$	$26.2 \pm 2.5$
Functional disability	Not applicable	29 (53.7%)
Grading by Kellegren Lawrence scale		
Grade 1	10 (13.9%)	–
Grade 2	28 (38.9%)	–
Grade 3	34 (47.2%)	–

**Table 2**

Prognosis of the patients in Group I.

Parameters	OA (N = 28)				JIA (N = 18)			
	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)
Decrease in pain	0	3	7	11	0	3	5	7
Increased Flexibility	0	2	2	4	0	1	3	3
Response to treatment	0	12%	18%	30%	0	9%	15%	22%
Increase in cartilage Thickness	0	0	0	2	0	0	1	3

**Table 3**

Prognosis of the patients in Group II.

Parameters	OA (N = 28)				JIA (N = 18)			
	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)
Decrease in pain	0	18	19	24	0	9	12	15
Increased Flexibility	0	17	21	23	0	4	7	12
Response to treatment	0	22%	36%	45%	0	26%	39%	42%
Increase in cartilage Thickness	0	8	11	18	0	3	8	14

**Table 4**

Prognosis of the patients in Group III.

Parameters	OA (N = 28)				JIA (N = 18)			
	At start of therapy	After 3 months	After 6 months	At 1 year (End of treatment)	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)
Decrease in pain	0	15	19	23	0	5	9	15
Increased Flexibility	0	14	18	21	0	3	7	16
Response to treatment	0	54%	63%	80%	0	26%	39%	42%
Increase in cartilage Thickness	0	11	14	22	0	2	6	14

**Table 5**

Prognosis of the patients in Control group.

Parameters	Control group (N = 40)			
	At start of Therapy	After 3 months	After 6 months	At 1 year (End of treatment)
Decrease in pain	0	9	17	24
Increased Flexibility	0	10	16	22
Response to treatment	0	24%	43%	60%
Increase in cartilage Thickness	0	11	14	21

A recent literature supports our study which states that use of PRP, PPP, MSC increases the proliferation of cells and produces matrix which helps in anti-inflammatory effects which helps in faster healing of bone defects (Zhu et al., 2013). From our study we found that use of PRP, PPP, MSC were safe in our patients with no side effects reported. However, a maximum improvement was observed both in OA and JIA patients. Only limitation of the study is small patient population and only two bone disorders were taken. Further studies are required to find the clinical application of these PRP, PPP, and MSC in various bone disorders with huge population studies. Our is the first study to use PRP,PPP and MSC on OA and JIA patients with clinical application of PRP,PPP and MSC or any one growth factors were only used in their studies but ours we have used all the three growth factors.

## 5. Conclusion

In conclusion, from our study we found PRP, PPP, and MSC to be effective in restoring the cartilage thickness with increasing

flexibility reducing the inflammation. Hence, use of these MSC, PRP, and PPP are safe and less cost enables for treating OA and JIA.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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