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Single Blind Randomized Placebo Controlled Prospective Study to Assess the Efficacy of Autologus Platelet Rich Plasma Injectionin Bilateral Early Osteoarthritis of the Knees

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ABSTRACT:
Objective: Over the years, with increase in prevalance, osteoarthritis of the knee has become one of the major cause of disability in the world. Among number of methods used for treating knee OA,the use of growth factors present inautologus platelet rich plasma has shown a promising results. The science being called as Orthobiologics or Tissue engineering, where human body's own regenerative potentials are concentrated and they try to regenerate and rebuild the damaged tissues. The present study aims to assess the efficacy of Autologus Platelet Rich Plasma Injection in patients of Osteoarthritis Knees and to compare the PRP Injection in Osteoarthritis Knees with the Placebo effect of the Saline Injection.
Design: In this randomized, controlled, single blind, prospective study 30 patients of either sex with upto grade 2 osteoarthritis (K. L. grading), who met study criteria were randomly given autologus PRP injection in one knee and normal saline injection(placebo) in the other and followed up at 1 month, at 6 months and at 12 months period. Clinical outcomes were evaluated using VAS scale, modified Lequesne score and analgesic requirements during follow ups.
Results: The study showed that the group receivingPRP injection have significant reduction in VAS score at 1($p<0.05$), 6($p<0.0001$) and at 12($p<0.0001$) months. Similarly the severity of disability was reduced as illustrated by the modified Lequesnescore at 1(p),6(p) and 12(p) months. The test group also showed no or decrease in analgesic requirement at 1(p), 6(p) and 12(p) months.
Conclusion: The findings of the results support the use of autologus platelet rich plasma injection in the early osteoarthritis (grade 1 and 2) of the knees.The improved results in the controlled group were seen for shorter duration and it is attributed to the placebo effect.
Keywords: Osteoarthritis, Knee, Autologus platelet rich plasma, Normal saline.

I. INTRODUCTION:

Osteoarthritis (OA) represents a clinical classification of pathological conditions involving a progressive degeneration of articular cartilage, a remodelling of sub-chondral bone and a synovitis which is usually limited. The condition is variously described as a part of a process of age-related change or a disease. It is twice more prevalent in women than men and increases in incidence with age, there being a major rise after 60 years. It is believed that the changes that lead to the development of OA are slow (insidious). That in idiopathic OA clinical presentation may result from changes over 15-20 years. The disease may involve primarily one or two large joints or may be generalized. Following joint trauma there is an increased incidence of OA which probably results from accelerated degeneration over a period of about 10-15 years[1]. In contrast, familial OA presents very early, often following cessation of growth, as a consequence of alterations in cartilage matrix structure which leads to the manifestation of joint degeneration following the cessation of growth. An example is provided by patients with a mutation in the type II collagen gene[2].A wide variety of treatment options are available to the patient with knee OA, categorized as non-pharmacological, pharmacological, and surgical. Commonly utilized non-pharmacological treatments include weight loss, lateral wedge insoles, bracing, and physical therapy. Pharmacological treatments include analgesics, nonsteroidal anti-inflammatory drugs (NSAIDS), opioids, hyaluronic acid or corticosteroid injections, and various drugs purported as disease-modifying osteoarthritis drugs (DMOADs). Surgical options include arthroscopic debridement and lavage, high tibial osteotomy, and unicompartmental and total knee arthroplasty. Despite the fact that all of the 12 existing guidelines for knee OA management dictate that optimal management of OA requires a combination of non-pharmacological and pharmacological modalities.[3]

Whilst total knee replacement is well established in the elderly with predictable and reproducible results , many orthopaedic surgeons are reluctant to perform TKA in younger patients which may be a result of concerns that high levels of activity may lead to increasing wear and aseptic loosening.[4-6] This is also compounded with the

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increased technical challenges, and complications associated with multiple revisions are additional causes for concern in these patients[7]. Because of these limitations recent trend has been shifted towards the use of autologus platelet rich plasma injections in knee osteoarthritis.

Platelet rich plasma (PRP) is prepared from autologous blood by centrifugation to obtain a highly concentrated sample of platelets, which is four to five times higher than that of normal blood[8].Platelets actively participate in healing processes by delivering a broad spectrum of GFs (insulin-like growth factor, transforming growth factor b-I, platelet derived growth factor, and many others) and other active molecules (e.g., cytokines, chemokines, arachidonic acid metabolites, extracellular matrix proteins, nucleotides, ascorbic acid) to the injured site[9]. These factors altogether contribute to comprehensive roles of PRP, including chondrogenesis, bone remodeling, proliferation, angiogenesis, antiinflammation, coagulation and cell differentiation[10,11].In knee OA, PRP injections aim to promote cartilage repair and relieve osteoarthritic symptoms, potentially delaying the need for joint replacement surgery[12]. Some studies have reported a reduction in PRP efficacy in moderate and advanced (Kellgren Lawrence grade 3–4) knee osteoarthritis, as this group of patients have higher pain and functional impairment, which require more medical attention[13,14]. The various growth factors and their effects on chondrocytes/cartilage, synoviumand mesenchymal stem cells in vitro and in vivo is summarized as follows :

SUMMARY OF THE EFFECT OF GROWTH FACTORS ON CHONDROCYTES/ CARTILAGE, SYNOVIUM, AND MESENCHYMAL STEM CELLS IN VITRO AND IN VIVO [15]

Growth factor	Chondrocytes/cartilage	Synovium	Mesenchymal stem cell
TGF-b1	Stimulates synthesis of ECM Decreases catabolic activity of IL-1 and MMPs	Causes synovial proliferation and Fibrosis Induces chemotaxis of inflammatory leukocytes to synovium Induction of osteophyte formation	Increases proliferation and ECM production Down regulatZes collagen type 1 gene expression
BMP-2	Stimulates synthesis of ECM Partial reversal of dedifferentiated phenotype in OA Increased ECM turnover (increased aggrecan degradation)	Presumed role in maturation of osteophytes Multiple injections lead to synovial fibrosis Stimulates synovial thickening in experimental OA	Increases proliferation and ECM production Down regulates collagen type 1 gene expression
BMP-7	Stimulates ECM synthesis Decreases cartilage degradation through decreasing activity/ expression of numerous ILs and MMPs	Decreases expression of MMPs and aggrecanase Does not appear to cause osteophyte formation or synovial fibrosis	Inhibits cell proliferation Inconsistent ability to induce chondrogenesis Alone Potentiates chondrogenic differentiation with TGF-bs resulting in increased ECM synthesis and decreasing collagen type 1 compared with TGF-b alone
IGF-I	Stimulates ECM synthesis Decreases matrix catabolism except in aged and OA cartilage	Protective effect on synovium resulting in decreased thickening and decreased evidence of chronic inflammation	Stimulates cell proliferation Increases expression of ECM Additive effect when combined with TGF-b
FGF-2	Decreases aggrecanase activity Antagonizes PG synthesis Up regulates MMPs	Induces synovial proliferation Inflammatory and induces osteophyte formation when used alone	Increases PG synthesis Increases cell proliferation
FGF-18	Increases chondrocyte proliferation and stimulates ECM in vitro and in injured joints but not in normal joints	Induces synovial thickening Enlargement of chondrophytes in experimental OA	
PDGF	No adverse effect in normal joints	No adverse effect in normal joints	Induces proliferation

TGF-b1 = transforming growth factor-b1; BMP = bone morphogenetic protein; IGF-I = insulin growth factor I; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor; ECM =

extracellular matrix; IL = interleukin; MMP = matrix metalloproteinase; OA = osteoarthritis; PG = proteoglycan.

Kellgren-Lawrence Grading System for OA,1957[16]

(This is radiological assessment in which x-ray of both knees AP view in standing position are taken).
Grade 0: Normal, no features.
Grade 1: Questionable presence of osteophytes/questionable presence of joint space narrowing / both.
Grade 2: Definite presence of osteophytes with possible joint space narrowing or definite mild joint space narrowing
Grade 3: Definite moderate joint space narrowing (at least 50%) Osteophytes usually present, Cysts/sclerosis may be present
Grade 4: Severe joint space narrowing withsubchondral bone sclerosis and possible deformity of bone ends.

Material and methods:

The study was conducted on patients admitted for treatment of osteoarthritis knee.

30 patients of either sex with bilateral osteoarthritis knee (Grade 1 & Grade 2 as per Kellgren-Lawrence grading system of osteoarthritis knee) were selected for this study in the age group of 40 years and above. Radiological assessment was done as per Kellgren-Lawrence Grading System for OA. In all the 30 patients with the help of computer derived randomized charts (Pseudorandomised technique), one knee (could be right or left) received injection PRP while the other knee serving as control (could be right or left) was injected with normal saline. Patient were unaware of the fact that which knee had been given the placebo or the PRP. Following discharge from the hospital, the participants were followed up on a regular basis with clinical examinations and functional evaluations for pain relief as per VAS scale. Patients were followed up at 1months, 6 months and 12 months starting from the day of procedure. All the patients were evaluated as per the proforma attached and were followed up at 1 months, 6months and 12 months for pain relief as per VAS scale. Quantitative variables were described using mean ± standard deviation (SD) and categorical data by frequency and percentage. Quantitative variables were described using mean ± standard deviation (SD) and categorical data by frequency and percentage.Student's t-test and chi-square test was used to compare quantitative variables between groups of patients. In all tests, p value <0.05 (confidence 95%) was considered to be statistically significant.

Exclusion criteria were as follows:

1. Patients with advanced stages of primary OA i. e. Grade 3 & Grade 4
2. Patients having Secondary OA
3. Patients with generalized OA
4. Metabolic diseases of bone
5. Rheumatoid arthritis

And also patients with uncontrolled diabetes mellitus& having active infection in the body or local skin infection.

Preparation of autologus platelet rich plasma:

On the day of the surgery, the patients were taken to the Blood Bank, GND Hospital, Amritsar. A triple blood bag was taken, and 48 ml of CPD (citrate phosphate dextrose) was removed and discarded, leaving just 14 ml of CPD in the bag. 100 ml of patient's whole blood was drawn by a clean, single venepuncture, into the 1st blood bag. The bag was kept at room temperature (20-22 degrees C) before preparing platelet concentrate for not more than 6 hours. The bag was kept in the bucket of refrigerated centrifuge (HeraeusCryofuge 6000i) and balanced accurately, and centrifuged at 2000 rpm at 22 degrees C for 5 minutes. This separated the whole blood into red blood cell concentrate at the bottom and plasma above. 4/5th of the plasma was separated into the 2nd satellite bag, double sealing the tubing between the primary bag and the satellite bag. The primary bag with RBC concentrate was separated and kept aside. The remaining 2 satellite bags were again centrifuged at 4000 rpm at 22 degrees C for 10 minutes after balancing accurately. The plasma got separated into an upper layer of platelet poor plasma (PPP) and platelet concentrate (PRP) below. The PPP layer was expressed into the 2nd satellite bag, double sealed, separated and kept aside.The PRP (platelet concentrate) extract in the 1st satellite bag was approximately 12- 15 ml. This bag was sent to the OT immediately, where it was kept at room temperature before use.

Injection procedure for autologus platelet rich plasma and normal saline:

PRP was removed from the blood bag using aseptic technique, and will be put in a sterile container. Both knee were exposed and after proper cleaning & draping as per randomized chart, one knee was injected with PRP solution using anterolateral or superolateral portal using 18 G disposable steel spinal needle. Needle was removed & aseptic dressing done. Patient was instructed to

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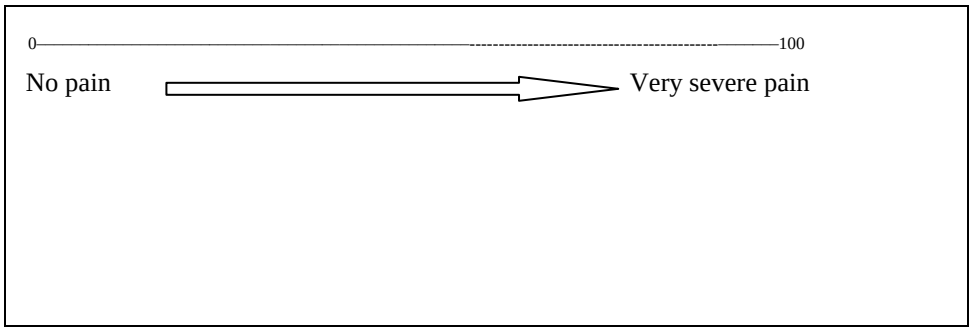
bend and extend the knee to allow equal distribution of PRP throughout the joint. The other knee was injected with normal saline. Similarly, patient was instructed to bend and extend the knee to allow equal distribution of normal saline throughout the joint. Patient was sent home immediately with no restriction of any day to day activities. One tablet of sustained release diclofenac sodium 100 mg was used for 3 days.

EVALUATION OF RESULTS:

Results were evaluated according to

1. Visual Analogue Scale (VAS).
2. Modified Lequesne score
3. Analgesic requirements during follow up

1. VISUAL ANALOG SCALE



Before the surgical procedure the pain was considered at 100 mm in all patients and at every follow up; patient was asked to mark a point on the line to explain how much of pain relief he/she was having and then the distance from the 0 point was measured.

Results evaluation as per VAS Scale improvement

Excellent	> 70%	Improvement
Good	>50-70%	Improvement
Fair	>30-50%	Improvement
Poor	≤30%	Improvement

MODIFIED LEQUESNE SCORE FOR OA KNEES:

We modified the original Lequesne functional index system keeping in mind our study where we were comparing results of both the knees in the same patient and as such the criteria of distance walked could not be gauged properly. So, while modifying we excluded the locomotion criteria of 'Maximum distance walked'.

To gauge the severity of handicap after treatment with PRP injection modified Lequesne index score was used where all patients had extremely severe crippling of the bilateral knees to start with and during their follow up at 1 month, 6 months and 12 months, improvements in their disability were noted down.

The comparative evaluation of the results of clinical outcome as regards the pain relief was done for both the knees where one knee was treated with autologus platelet rich plasma (PRP) where as the other knee of the same patient was treated with normal saline injection.

3.ANALGESIC REQUIREMENTS DURING FOLLOW UP

We also evaluated the need for analgesics after the procedures at all the follow ups in both the groups and compared it with each other and with the analgesic requirements before the procedure. All the patients were taking Tab. Paracetamol 650mg thrice daily dose before the start of the therapy owing to pain in bilateral knees.

Observations and Results:

In this study 6(20%) patients were in the age group of 41-50, 14(46.67%) patients were in the age group of 51-60 and 6(20%) patients were in the age group of 61-70 and 4(13.33%) patients were in the age group of 71-80. None of the patients was >80 year old. Since the patients were same in both the groups with only difference of the knees, the age incidence has to be same in both the groups. Mean age group was 58.63 years. In our study 11 (36.66%) patients were males and 19 (63.33%) patients were females.Among group A, 12(40%) patients belonged to kellgren-Lawrence grading 1, 18 (60%) patients belonged to kellgren-lawrence grade 2 and none belonged to kellgren-lawrence grade 3 or 4. Among group B 15 (50%) patients belonged to kellgren-lawrence grade 1, 15 (50%) patients belonged to kellgren-lawrence grade 2 and none belonged to grade 3 or 4.

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VAS score at 1 month

Results were compared between both groups as per vas score comparison. In group A i.e. platelet rich plasma injection was given it was found that, out of 30 cases 18 (60%) patient had excellent results while 12 (40%) patients had good results while none of the patients had fair or poor result. Similarly in group B i.e. the group in which only normal saline was injected in the opposite knee of the same patient, it was found that 16 (53.33%) patients had excellent results while 14 (46.66%) patients had good result. The mean VAS score in group A was 25.16±5.49 mm while in group B it was 30.66±10.88mm and the difference between 2 groups was found to be highly statistically significant (p= 0.016).

VAS score at 6 months

In group A, it was found that, out of 30 knees 17 (53.33%) patient had excellent results while 13 (46.66%) patients had good results while none of the patients had fair or poor result. Similarly in group B, it was found that 13 (43.33%) patients had excellent results, 17 (56.67%) patients had good results and none of the patient had fair or poor results. The mean vas score in group A was 29.5±5.67mm while in group B it was 48.16±8.31mm and the difference between 2 groups was found to be statistically significant (p<0.05).

VAS score at 12 months

In group A, it was found that, out of 30 knees 8(26.66%) patients had excellent results while 12 (40%) patients had good results and 10 (6.66%) had fair results and no patient had poor results. In group B, it was found that, out of 30 knees 4 (13.33%) patients had good results while 10 (33.33%) patients had fair results and 16 (26.66%) had poor results. The mean vas score in group A was 40.83±13.1mm while in group B it was 65.66±12.76mm and the difference between 2 groups was found to be statistically significant (p<0.05).

Modified Lequesne score at 1 month

At 1 month, it was found that severity of handicap in Group A was none in 4, mild in 23 and moderate in 3 knees whereas in Group B it was none in 2 , mild in 20 and moderate in 8 knees. Neither of the group had severe or extremely severe crippling. The mean Lequesne index score in group A was 2.38±1.55 compared to 3.41±1.81 in group B and the result was statistically significant. (P= 0.021)

Modified Lequesne score at 6 months

At 6 months, it was found that severity of handicap in Group A was none in 2, mild in 20 and moderate in 8 knees whereas in Group B it was mild in 14 and moderate in 12 knees and severe in 4 knees. The mean Lequesne index score in group A was 3.88±1.88 compared to 5.33±1.86in group B and the result was statistically significant. (p<0.05)

Modified Lequesne score at 12 months

At 12 months, it was found that severity of handicap in Group A was mild in 16 and moderate in 14 knees whereas in Group B it was mild in 4, moderate in 8 and severe in 12 and extremely severe in 6 knees. The mean Lequesne index score in group A was 4.95±1.37compared to 8.15±2.49in group B and the result was statistically significant. (p<0.05).

Analgesic requirement at 1 month

Before applying chi- square test, a null hypothesis was made that there is no significant difference in analgesic requirements between the two groups. At 1 month, 6 patients required analgesics in the form of Tab. Paracetamol 650 mg twice daily for 5 days for unilateral knee pain and it was found that all knees belonged to placebo group. Not a single knee from test group i. e. Group A required analgesics of any form. The value of chi-square 6.67 is more than 3.48 (confidence 95%) which means that there is difference and the null hypothesis is rejected.

Analgesic requirement at 6 months

It was observed that at 6 months, the need of analgesics increased proportionately where 20 patients required it because of unilateral knee pain and one patient due to bilateral knee pain. On analysis, it was seen 18 knees belonged to Group B and only 3 knees were from group A.

Analgesic requirement at 12 months

At the end of a year, all patients needed analgesics of which 9 patients required it for bilateral knee pain. It was discovered that 9 knees from the bilateral cases belonged to test group and all remaining 30 knees to the control group..

Complications

Among group B, one (3.33%) case had a superficial infection at the point of entry which was cured with short course of appropriate antibiotics for 5 days. Another 1(3.33%) case in group B had leakage of synovial fluid for about 5 days after the opening of the bandage. The problem was resolved with compression bandage for further 1 week. No

complication of any form was noted in group A except aggravation of pain for about 24 hours in 2(6.66%) cases.

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II. DISCUSSION

Osteoarthritis (OA) is the most common chronic joint disorder, and it causes detrimental effects on the quality of life and functional status. These are characterized by progressively occurring cartilage destruction, osteophyte formation, and subchondral sclerosis[20,21]. In older patients, who are refractory to conservative management, knee replacement surgery is the primary intended treatment for severe knee OA to relieve pain and improve function. Owing to the limited lifespan of joint replacements with implant wear and the associated risk for joint revision, conservative treatment modalities are the central focus in the younger and middle-aged population with cartilage damage and OA of the knee.Pharmacological therapies including analgesics, non-steroid and steroid anti-inflammatory drugs and corticosteroid injections provide only temporary benefit and often have side effects.There is an increasing clinical interest in autologous growth factor treatment such as the use of platelet-rich plasma (PRP) injections in OA of the knee.

Platelets contain significant amounts of cytokines and growth factors which are capable of stimulating cellular growth, vascularization, proliferation, tissue regeneration, and collagen synthesis. Delivery of high concentrations of cytokines and GFs to damaged tissues by PRP is considered to have a beneficial effect on tendon and cartilage tissue regeneration[22,23]. In some in vitro and in vivo studies, anti-inflammatory and reparative effects of PRP on cartilage, tendon, and ligament recovery have been shown[24-26].

Our study being a single blind randomized one, eliminated the performance bias which occurs when subjects in particular group is given more attention than the other group making it difficult or impossible to conclude the results of the intervention. Randomly allocating the participants in different groups also eliminated selection bias. There was also no attrition bias in our study. In the present study maximum patients were in the age group of 5th and 6th decade. The youngest patient in the study had an age of 44 years and the oldest had an age of 78 years with mean age of 58.63 years. Similar trend was seen in a series of Lawrence RC, Helmick CG, Arnett FC, et al[27] while estimating the prevalence of arthritis and selected musculoskeletal disorders. It was observed that the incidence increases with age.Proprioception in general may decline with age, and there appears to be a specific correlation between increased age and decreased knee position sense in asymptomatic subjects.In our study of 30 cases, there were 19 females and 11 male patients. Female predominance is attributed to the hormonal changes due to menopause resulting in early onset of degeneration and the lesser bone.Hormonal differences between men and women may play a role in the development of osteoarthritis. Postmenopausal women, in particular, have an increased risk of developing arthritis and this has been linked to the decrease in estrogen during this time. The predominance of grade 2 is attributed to the fact that in grade 1 the symptoms are very mild and the patient is not bothered much to consult a specialist and usually household remedies are tried by these people, once these remedies fail or they progress to grade 2 and pain is not relieved, then they consult the specialist for treatment.

According to Kon E et al in their prospective study on one hundred consecutive patients, affected by chronic degenerative condition of the knee, treated with PRP intra-articular injections were clinically evaluated before and at 6 and 12 months follow-up[28]. 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 6 and12 months follow-up (P= 0.005). The results remained stable upto 6 months follow up. Similar sustained pain relief considering the VAS score was observed in PRP injection group in our study, indicating that the treatment with PRP injections is safe and has the potential to reduce pain and improve knee function and quality of life.

Lequesne score serves as an instrument to measure severity of disability (handicap) it was found that the group A which received platelet rich plasma fared well as compared to the Group B which received intraarticular normal saline injection and to support it a metaanalysis of 10 randomized controlled trials by Dai WL, Zhou AG, Zhang H, Zhang J where Lequesne score was used as functional score with a total of 1069 patients with OA knee also showed that at 12 months postinjection, PRP was associated with significantly better pain relief and improvement in functional score (Lequesne score, standardized mean difference 1.05, 95% CI 0.21-1.89, P = .01) than HA. Compared with saline, PRP was more effective for pain relief and functional improvement at 6 months and 12 months postinjection. It was also found that PRP did not increase the risk of adverse events compared with HA and saline[29].

As far as the complications are concerned No major adverse outcomes were recorded such as deep infection, muscle atrophy, deep vein thrombosis, fever, hematoma, tissue hypertrophy, adhesion formation, or other major adverse events occurred among study subjects in either group. In our study, 2 cases in group A showed temporary worsening of pain for about 24 hours which resolved spontaneously. This is attributed to the sudden increase in the volume of intracapsular contents leading to capsular stretch and hence pain. Once some absorption occurs the stretch pain settles down. Spakova T et al[30] in their study documented temporary mild worsening of pain in the knee joint after application of PRP in six cases out of 60, which was spontaneously resolved after 2 days. Another factor for such pain can be attributed to the quality of the PRP injected also. If the number of leucocytes and few RBC are more in the injected material, then the inflammatory cascade initiated will be more and as such can initiate the pain.

In our study one case group B had a superficial infection at the point of entry which was cured with short course of appropriate antibiotics for 5 days. Leakage of synovial fluid was seen in 1 case in group B which resolved with compression bandage for further 1 week. The leakage is due to slow healing of the entry track.

III. CONCLUSION

Autologus PRP injections results in the release of the growth factors and attract the Mescenchymal Stem cells so as to start the chondroprotective and chondroregenerative potential in knee joint cartilage and hence result in pain relief in the longer run. The improved effect in other group lasted only for shorter duration and it is attributed to the so called placebo effect for IA-NS injections. However the results of intraarticular PRP injections were far better than that of intraarticular normal saline injection. Considering the following results we highly recommend the use of Platele Rich Plasma injection in cases of early osteoarthritis knees to achieve pain relief and lower disability for longer duration.