"EVALUATION OF FUNCTIONAL OUTCOME OF INTRA-ARTICULAR PLATELET RICH PLASMA (PRP) INJECTION FOR EARLY OSTEOARTHRITIS OF KNEE: A COMPARISON BETWEEN SINGLE VERSUS DOUBLE SPINNING TECHNIQUE"

By

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DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTER, KOLAR, KARNATAKA

In partial fulfillment of the requirements for the degree of

MASTER OF SURGERY

IN

ORTHOPAEDICS

Under the Guidance of

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ABSTRACT

BACKGROUND:Osteoarthritis (OA) is the most common type of arthritis in both developed and developing countries.It is a chronic progressive musculoskeletal disorder characterized by gradual loss of cartilage in joints, with a prevalence of 22% to 39% in India.

OA is more common in women than men, 45% of women over the age of 65 years have symptoms while 70% of those over 65 years show radiological evidence of OA, by 2050 people aged over 60 will account for OA more than 20% of the world's population. Treatment of knee OA is difficult due to the avascular and aneural nature of adult knee cartilage, which results in a low regenerative capacity, and thus limited healing potential for the joints. The joint destruction arising from OA occurs as a result of an imbalance in the equilibrium between the breakdown and repair of the joint tissue while a combination of cellular changes and biomechanical stresses causes several secondary changes in the joint.

PRP is a simple and minimally invasive method to obtain a high concentrate of autologous GFs in physiological proportions, which can be easily and safely placed directly into the lesion site. Moreover, the risk of allergy or infection is negligible, due to the autologous nature of the platelet extract.

PRP concentrations have been reported to range widely, and the numerous preparation methods present many other different variables, such as the presence of other cells, activation and storage modalities, and many other aspects that are not of secondary importance for determining PRP properties and clinical efficacy. Platelet concentration is one of the most topical factors in PRP treatment.

Platelet concentration in the published literature on knee OA has been variable and not consistently reported. Some authors suggest that the PRP platelet concentration should be at least two times the whole blood platelet concentration; however, concentrations up to eight times that of blood levels have been reported with good results. Single-spinning centrifugation results in platelets up to three times that of baseline level whereas double-spinning centrifugation results in platelets up to eight times the baseline level with a high leucocyte content. However, there are very few studies which compare the use of the two techniques of preparation of PRP in early OA knee.

The aim of this study is to explore this novel biological treatment for degenerative lesions of articular cartilage and OA by comparing two products, already used in clinical practice, which are based on different preparation approaches: single- versus double-spinning procedures.

MATERIAL AND METHODS: It is a prospective, comparative, observational, time bound, hospital-based study conducted from November 2018 to May 2019, after obtaining institutional Ethical committee approval. 68 patients were included in the study following the inclusion and exclusion criteria. The patients were randomized into 2 groups using a standard randomization technique (odd and even number method). Group A (odd numbers) received single spin PRP injection and group B (even numbers) received double spin PRP injection. Out of the 68 patients, 4 patients were lost to follow-up, which gave a final sample size of 64 patients.Patients were selected from R L Jalappa Hospital and Research centre, Department of Orthopaedics, Kolar, on outpatient and in-patient basis who meets inclusion criteria.

After clinical examination & radiographs of the knee joint in standing position (antero-posterior views and lateral views) were taken, blood sample of the patient was collected and PRP prepared in Blood bank. Infiltration was done in the operation theatre under strict aseptic conditions.

Patients were assessed with "VAS" (visual analogue scale) for pain, "WOMAC" (Western Ontario McMaster Universities Arthritis Index) scoring, and Oxford knee scoring, before giving the PRP injection & after giving the injection at periods of 1 month, 3 & 6 months. The decrease in WOMAC score & VAS score and increase in Oxford knee scores was suggestive of improvement in patient's condition.

RESULTS: The mean age was 57.63 ± 7.4 in SS-PRP and it was 50.75 ± 7.33 in DS-PRP. The participants in both groups are above 40 years of age.

The mean pre-injection VAS score was 8.25 ± 0.62 in SS-PRP, it was 8 ± 0.51 in DS-PRP. The mean VAS score at 1 month was 6.28 ± 0.73 in SS-PRP, it was 5.88 ± 0.61 in DS-PRP. The mean VAS score at 3 months was 5.16 ± 0.57 in SS-PRP, it was 4.63 ± 0.61 in DS-PRP. The mean VAS score at 6 months was 4.28 ± 0.58 in SS-PRP, it was 3.34 ± 0.65 in DS-PRP. From above observations it can be deduced that PRP injections helps in reduction of pain in osteoarthritis over a period of 6 months which is evident by reduced VAS scores. Another observation that can be made from above results of present study is that the reduction in VAS scores is slightly higher for group injected with double spin PRP at 1,3 and 6 months follow up.

The mean pre-injection WOMAC score was 76.75 ± 3.62 in SS-PRP, it was 74.84 ± 3.02 in DS-PRP. The mean WOMAC score at1 month was 67.28 ± 4.46 in SS-PRP, it was 61.25 ± 2.75 in DS-PRP. The mean WOMAC score at 3 months was 60.66 ±

5.38 in SS-PRP, it was 52.72 ± 3.09 in DS-PRP. The mean WOMAC score at 6 months was 54.78 ± 5.95 in SS-PRP, it was 44.53 ± 3.03 in DS-PRP. The difference in WOMAC scores between single spin PRP and double spin PRP groups was again similar to VAS scores. There was slightly higher reduction in WOMAC scores in DS-PRP group than in SS-PRP group.

The median pre-injection Oxford knee score was 20 in SS-PRP, it was 19 in DS-PRP. The median Oxford knee score at 1 month was 24 in SS-PRP, it was 24 in DS-PRP. The median Oxford knee score at 3 months was 26.5 in SS-PRP, it was 27.5 in DS-PRP. The median Oxford knee score at 6 months was 29 in SS-PRP, it was 30 in DS-PRP. From above observations it can be deduced that improvement was noted in knee scores for both single spin and double spin PRP groups with slightly better outcomes in the double spin group.

ABBREVATIONS

OA	Osteoarthritis
PRP	Platelet rich plasma
AAOS	American Academy of Orthopaedic Surgeons
NSAID	Non-steroidal anti-inflammatory drugs
HA	Hyaluronic Acid
MSC	Mesenchymal stem cells
IA	Intra-articular
GF	Growth factor
IGF	Insulin like growth factor
PRF	Platelet rich fibrin
PPP	Platelet poor plasma
SS-PRP	Single spin platelet rich plasma
OKS	Oxford knee score
VAS	Visual Analogue Scale
WOMAC	Western Ontario And Mcmaster Universities Osteoarthritis Index
DS-PRP	Double spin platelet rich plasma
BMP	Bone morphogenic proteins
GFP	Green fluorescent protein
HSP-47	Heat shock protein 47
IKDC	International Knee Documentation Committee
SF-36	Short Form- 36
PRGF	Platelet rich in growth factors
HW-HA	High molecular weight hyaluronic acid
LW-HA	Low molecular weight hyaluronic acid
aPRP	Activated platelet rich plasma
aPPP	Activated platelet poor plasma

ELISA	Enzyme linked immunosorbent assay
MMP	Matrix metalloproteinases
PIP	Procollagen type 1 carboxyterminal peptide
ACL	Anterior cruciate ligament
IA-PRP	Intra-articular platelet rich plasma
KL	Kellgren-Lawrence radiographic grading
JKOM	Japanese knee osteoarthritis measure
KOOS	Knee injury and osteoarthritis outcome score
PRP-LP	Low white blood cell and platelet concentration
PRP-HP	High white blood cell and platelet concentration
IL	Interleukin
PCR	Polymerase chain reaction
ADAMTS-5	A disintegrin and metalloproteinase with thrombospodin motifs-5
TIMP-1	Tissue inhibitor of metalloproteinases-1
VEGF	Vascular endothelial growth factor
PDGF-A	Anti-platelet derived growth factors
OARSI	Osteoarthritis research society international score
ACP	Autologous conditioned plasma
KQoL	Knee quality of life
PA-PRP	Photo activated platelet rich plasma
ROM	Range of motion
COLL2-1	Collagen 2-1
ACR	American college of rheumatology
COMP	Cartilage oligometric matrix protein
RCT	Randomized control trial
MCII	Minimal clinically important improvement
BRG	Bad responders group

VGRG	Very good responders group
IM	Inflammatory mediators
TNF-α	Tumor necrosis factor alpha
H-PRP	Healthy male platelet rich plasma
OA-PRP	Osteoarthritis platelet rich plasma
OMERACT-OARSI	Outcome measures in rheumatology clinical trials and osteoarthritis research society international
QUOROM	Quality of reporting meta-analysis
LCL	Lateral collateral ligament
PGE2	Prostaglandin E2
PLA2	Phospholipase A2
COX-2	Cyclo-oxygenase-2
PTOA	Post traumatic osteoarthritis
BMD	Bone mineral density
JSN	Joint space narrowing
DMOAD	Disease modifying osteoarthritis drugs
ESCEO	European society for clinical and economic aspects of osteoporosis and osteoarthritis
FGF-18	Fibroblast growth factor 18
EGF	Epidermal growth factor
BP	Bisphosphonates
NGF	Nerve growth factor
APPA	Apocynin and Paenol
ACI	Autologous chondrocyte implantation
UKA	Unicompartmental knee arthroplasty
TKA	Total knee arthroplasty
DEPA	Dose, efficiency, purity, activation
HAQ	Health assessment questionnaire

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INTRODUCTION:

Arthritis is a significant contributor to the global disability burden, with Osteoarthritis (OA) being the most common in both developed and developing countries. Osteoarthritis is a multifaceted process in which the central role is played by mechanical factors and is has changes in structure and function of the whole joint. There is no cure and current therapeutic strategies are primarily aimed at reducing pain and improving joint function.[1]

The prevalence of OA varies according to the definition of OA, the specific joint under study, and the characteristics of the study population. The age-standardized prevalence of radiographic knee OA in adults age 45 years or older was 19.2% and 27.8% in two studies. Approximately 37% of participants older than 60 years had radiographic knee OA.[1]

It is a chronic progressive musculoskeletal disorder characterized by gradual loss of cartilage in joints, with a prevalence as high as 40% in India. OA has more preponderance in women than men, 44% of women over the age of 65 years have symptoms while 70% of those over 65 years show radiological evidence of OA, by 2050 people aged over 60 will account for OA more than 20% of the world's population.[2]

Treatment of knee OA is difficult due to the avascular and aneural nature of adult knee cartilage, which results in a low regenerative capacity, and thus limited healing potential for the joints. The joint degeneration arising from OA occurs as a result of an imbalance in the equilibrium between the breakdown and repair of the joint tissue while both cellular changes and biomechanical stresses causes several secondary changes in the joint.[3]

Weight loss, physiotherapy and diet control remains one of the first treatments recommended for the treatment of OA and has been shown to be a safe, effective method of reducing pain and improving function in OA patients. However, exercise has limitations as a treatment modality. One limitation being poor compliance,another limitation is that exercise can be painful for people with OA. It has been seen that intra-articular platelet rich plasma (PRP) injection in combination with exercise provided much better results than exercise alone.According to the 2013 AAOS evidence guidelines for knee osteoarthritis, oral NSAIDs were the only treatment modality that received a strong recommendation for treating osteoarthritis. But, it is known that continuous NSAID use can potentially lead to severe systemic side effects that limit their usefulness of as a long-term treatment method for OA, such as renal insufficiency, gastritis, peptic ulcer formation, and rare effects with the cardiovascular and cerebrovascular systems. PRP injections resulted in significantly better outcomes than treatment with NSAID, with the PRP group showing sustained improvement in knee function at 24 weeks post-injection. [4]

Hyaluronic acid (HA), an integral part of synovial fluid, assists the joint in different ways, providing lubrication, stress reduction, and substance transport across the synovium. Since HA concentrations have been found to be reduced in knees with OA, viscosupplementation with HA hopes to restore the elastic functions provided by HA back into the affected joint. But, it is important to note that unlike the autologous nature of PRP injections, HA injections are synthetically manufactured products. Several studies have shown that PRP injections gives better results when compared to HA injections. [4]

Stem cell treatment has received recent attention as a treatment method for KOA, with the majority of research focusing on mesenchymal stem cells (MSCs). Stem cell

harvest is also more invasive than the simple blood draw required for PRP, which could increase the likelihood of infection or complications in patients undergoing this therapy. Since there is a lack of strong clinical trial evidence supporting the use of MSCs for OA and a lack of studies directly comparing the effects of PRP and MSCs, it is not possible to recommend MSC therapy over PRP injections for OA, at this time, until further research is conducted.[4]

PRP is an autologous blood product that is created by first obtaining a small amount of blood through peripheral venesection, concentrating that blood sample through centrifugationand then administering the concentrated plasma back into the patient through an intra-articular (IA) injection. The concentrated plasma product contains a high concentration of platelets (at least two times greater than whole blood), which have critical roles in maintaining tissue homeostasis and regulating the inflammation and coagulation responses of the body such as chondrocyte apoptosis inhibition, bone and vessel remodeling, inflammation modulation and collagen synthesis.[4]

Recent research has identified a number of important biochemical pathways that could be treated therapeutically through biological intervention. Platelet-rich plasma (PRP) is one such intervention. PRP is an autologous plasma suspension of platelets, that has a platelet concentration higher than blood and is able to release growth factors (GFs) involved in and regenerative and reparative processes.[5]

PRP is a simple and minimally invasive method to obtain a high concentrate of autologous GFs in supraphysiological proportions, which can be easily and safely placed directly into the lesion site. Moreover, the risk of allergy or infection is negligible, due to the autologous nature of the platelet extract.[6]

Insulin-like growth factor (IGF) stimulates proteoglycan production and many other bioactive molecules are involved in cartilage regeneration and metabolism independently or with synergistic interaction.[6]

PRP concentrations have been known to range widely and the various preparation methods present many other different variables, such as the presence of other cells, storage and activation modalities and many other aspects that are not of secondary importance for determining PRP properties and clinical efficacy.[6]

Platelet concentration is one of the most crucial factors in PRP treatment. Platelet concentration in the published literature on knee OA has been variable and not consistently reported. Some authors suggest that the PRP platelet concentration should be at least two times the whole blood platelet concentration; however, concentrations up to eight times that of blood levels have been reported with good results.[7]

Single-spinning centrifugation results in platelets up to three times that of baseline level whereas double-spinning centrifugation results in platelets up to eight times the baseline level with a high leucocyte content. However, there are very few studies which compare the use of two techniques of preparation of PRP in early OA knee. [7]

Our aim is to explore this novel treatment for degenerative lesions of articular cartilage and OA by comparing two products, that are already used in clinical practice, based on different preparation approaches: single- versus double-spinning PRP techniques.

AIM AND OBJECTIVES

- To determine the effect of single spin platelet-rich plasma (SS-PRP) intra articular (IA) injections in early stages of osteoarthritis (OA) of the knee based on the functional outcome with the Western Ontario and McMaster Universities osteoarthritis index (WOMAC score), Oxford knee score (OKS) and pain by visual analogue pain scale (VAS) at end of 1st, 3rd and 6th month.

- To determine the effect of double spinplatelet-rich plasma (DS-PRP)intra articular (IA) injections in early stages of osteoarthritis (OA) of the knee based on the functional outcome with (WOMAC score), Oxford knee score (OKS) and pain with visual analogue pain(VAS) at end of 1st, 3rd and 6th month.

- To compare and determine the effectiveness of single spin versus double spin PRP injections with the above parameters.

REVIEW OF LITERATURE

The History of Platelet Rich Fibrin (PRF) started in 1970, when Matras described a fibrin glue, formed by polymerizing fibrinogen with thrombin and calcium, which was used to improve skin wound healing in a rat model in 1970. Because of the low concentration of fibrinogen in plasma, the stability and quality of fibrin glue were low. A few years later several research works proposed an upgraded concept for the use of blood extracts, termed "platelet-fibrinogen-thrombin mixtures" or "gelatin platelet- gel foam". In this new concept, the fibrin glues were presenting a significant concentration of platelets within the final preparation. The idea was first to reinforce naturally the fibrin gel, and also to combine the healing properties of the platelets with those of the fibrin. This improvement allowed to prepare more natural products, integrating more natural blood constituents as it should.[8]

In a study conducted in the year 1998 in Miami, the authors selected 88 elective cancellous cellular marrow graft reconstructions of mandibular continuity defects 5 cm or greater arising from benign and malignant tumor extirpations without radiotherapy and randomized them into 2 groups. 1 group received cancellous cellular marrow grafts without PRP, the 2nd group received grafts with PRP added during the bone-milling phase of graft preparation and applied topically after bone placement into the defect. The PRP was obtained in the operating room using a cell separator. It withdrew 400 to 450 ml of autologous whole blood through a central venous catheter placed during surgery. The blood was then centrifuged into its three components; red blood cells, PRP and PPP. The layers were separated, from the less to the more dense; therefore the first layer separated was PPP(200ml), second was PRP(70 ml), leaving the residual red blood cells (180 ml). Once the PPP was collected, the centrifuge speed was reduced to 2400 RPM to allow separation of the PRP from the red blood

cells. The bone grafts were allowed to mature and consolidate for 6 months.Radiographs were taken at the 2, 4, and 6-month intervals. The resultssuggested that PRP addition increased the rate and degree of bone formation in a bone graft through the first 6 months.[9]

A review article based in France in 2004 had used the deposition of a PRP-clot with or without autogenous bone in a series of 20 patients undergoing tooth extraction due to vertical fractures or severe periodontal disease. In patients with multiple extractions, PRP-clot was placed into the socket on one side of the mouth while the other served as the control. Biopsies of bone were taken at extraction sites between 10 and 16 weeks. In patients with PRP-clot, there was extensive bone regeneration and bone tissue was compact with well-organised trabeculae. In contrast, in the control group the cavity was filled with connective tissue mainly. They had also reviewed articles which stated that PRP was especially useful for the soft tissue and bony reconstruction encountered in facial plastic and reconstructive surgery and also better healing of diabetic ulcers. They concluded that platelets release multiple growth factors having a chemotactic and mitogenic effect on mesenchymal stem cells and osteoblasts and therefore accelerate bone healing.[10]

In 2005, a prospective study based in Italy hypothesized that bone marrow derived stromal stem cells associated with platelet-rich plasma are potent angiogenic inducers proven to release vascular endothelial growth factor. The goal was to test if stromal stem cells, when combined with platelet-rich plasma, was able to increase allograft integration in a large animal model at 4 months. A defect was made in the diaphysis of the metatarsal bone of 10 sheep; the study group received an allograft with stromal stem cells, platelet-rich plasma, and collagen (six animals) and the control group received the allograft only(four animals). Investigations done were,

radiographs, mechanical tests and histomorphometric analysis, including neo-vascularization. Results showed good new bone formation in the allograft of the study group. Bone formation was correlated with better vascular invasion and remodelling of the graft in the study group. The results confirmed the role played by stromal stem cells and platelet-rich plasma in bone repair. The authors also estimated the efficacy of PRP on proliferation of human stem cells and observed that there are markedly increased cell numbers with an increase in concentration of PRP from 1% to 10%.[11]

In a study that was held in Japan in 2007, the authors studied the effects of PRP on osteoblast differentiation and proliferation in vitro in the presence of bone morphogenetic proteins (BMPs). PRP and its soluble fraction produced differentiation of osteoblastic cells and myoblasts in the presence of BMP-2, BMP-4, BMP-6 or BMP-7. The soluble PRP fraction stimulated osteoblastic differentiation in 3 dimensional (3D) cultures using scaffolds of hydroxyapatite or collagen. These results suggested that platelets contain not only growth factors for proliferation but also novel potentiators for BMP-dependent osteoblastic differentiation. They also found dual effects of the soluble fraction prepared from PRP on proliferation and differentiation. At lower concentrations, the PRP fraction markedly stimulated the osteoblastic differentiation in the presence of BMPs, while at higher concentrations the same fraction stimulated proliferation and suppressed osteoblastic differentiation.[12]

In 2008, in a study in Japan, the authors hypothesized that circulation-derived cells play a crucial role in the healing processes of tissue. In the early phases of tendon healing, cells derived from circulation exist in the wounded area for a short period to initiate the healing process and decrease in number with time. They assumed that a delay of time-dependent reduction in circulation-derived cells could enhance the healing of tendons. In this study, the authors injected platelet-rich plasma (PRP)

with various growth factors into the wounded region of the patellar tendon and compared the effects on activation of circulation-derived cells and process of tendon healing with a control group (no PRP injection). To follow the circulation-derived cells, a green fluorescent protein (GFP) chimeric rat expressing GFP was used. In the PRP group, the numbers of GFP-positive cells and heat-shock protein (HSP47)-positive cells were higher than in the control group at 3 and 7 days after injury. At the same time, the immunoreactivity for types I and III collagen was more in the PRP group compared to the control group in the early phase of tendon healing. The findings suggest that PRP is useful in activating circulation-derived cells for improvement of the tendon healing process. It was also postulated that PRP can both inhibit excess inflammation and also augment stem cell proliferation and maturation, as demonstrated in, invitro studies.[13]

A prospective pilot study was done in Italy in 2010, one hundred consecutive patients, affected by chronic degenerative condition of the knee, were treated with PRP intra-articular injections. The procedure consisted of 150-ml of venous blood that was collected in a blood bag containing 21ml of sodium citrate. Then two centrifugations (the first at 1,800 rpm for 15 min to separate erythrocytes, and a second at 3,500 rpm for 10 min to concentrate platelets) produced a unit of 20 ml of PRP. 3 PRP units of 5 ml each were used. Patients were assessed before and at the end of the treatment, and at 6 and 12months follow-up. International Knee Documentation Committee (IKDC) score and VAS were used for clinical evaluation. A 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. The results remained stable from the end of the therapy to 6 months follow up, whereas they became significantly worse at 12 months follow up, even though it was still

significantly higher with respect to the basal level. The limitations of this study were the lack of a control group and the fact that the evaluation of the results only took place at a short term follow up.[14]

A non-randomized, prospective, longitudinal study was carried out in Spain in 2010, to evaluate the effectiveness of a cycle of 3 intra-articular injections of autologous PRGF, 1 every 2 weeks, in patients with OA knee and symptoms for at least 3 months. Participants were asked to fill out a questionnaire with following assessments: VAS, SF-36, WOMAC Index and Lequesne Index before the first infiltration of PRGF and 6 months after the last infiltration. A total of 20 ml of blood (4 samples of 5 ml) was collected in sodium citrate tubes. The tubes with were centrifuged at 1,800 rpm for 8 min to obtain a concentrate of platelets suspended in plasma, which was separated into three fractions. At 6 months post intra-articular infiltration of PRGF, improvements in function was documented. An improvement of the scores, point towards PRGF as a therapy for OA.[15]

In a prospective, comparative study held in Italy in 2010, 150 patients affected by cartilage degenerative lesions and early and severe OA were included. 50 symptomatic patients were treated with 3 autologous PRP intra-articular injections and were evaluated before the injection and at 2- and 6-month follow-up. The results were compared with 2 groups of patients treated with HA injections. High– molecular weight HA injections was used in one group; low–molecular weight (LW) HA was used in the other group. IKDC and VAS scores were used for clinical evaluation; adverse events and patient satisfaction were also recorded. 150-ml venous blood sample for every knee treated with PRP. Then, 2 centrifugations (the first at 1,480 rpm for 6 minutes to separate erythrocytes and the second at 3,400 rpm for 15 minutes to concentrate platelets) produced 20 mL of PRP. It was divided into 4 small units of

5 mL each. At 2 months' follow-up, similar improvements were seen in the PRP and LW HA groups, with higher results compared with the high– molecular weight HA group. At 6 months' follow-up, superior results were observed in the PRP group. The authors concluded that autologous PRP injections showed more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function, particularly in more active patients with a low degree of cartilage degeneration.[16]

In a prospective study in 2011 in Korea, the authors studied the effects of activated platelet-rich plasma (aPRP) and activated platelet-poor plasma (aPPP) on the remodelling of the extracellular matrix, a process that requires activation of dermal fibroblasts, which is essential for rejuvenation of aged skin. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared using a double-spin method and then activated with thrombin and calcium chloride. The proliferative effects of aPRP and aPPP were measured by thymidine incorporation assay, and their effects on matrix protein synthesis were assessed by quantifying levels of procollagen type I carboxy-terminal peptide by enzyme-linked immunosorbent assay (ELISA). The production of collagen and matrix metalloproteinases (MMP) was studied by Western blotting and reverse transcriptase-polymerase chain reaction. Levels ofprocollagen type I carboxy-terminal peptide PIP were highest in cells grown in the presence of 5% aPRP. Additionally, aPRP and aPPP increased the expression of type I collagen, MMP-1 protein, and mRNA in human dermal fibroblasts. They concluded that PRP promotes tissue remodelling in aged skin and may be used as adjuvant treatment to lasers for skin rejuvenation in cosmetic dermatology.[17]

In a comparative study conducted in Italy in 2011, the authors involved 144 symptomatic patients affected by cartilage degenerative lesions and OA. 72 patients were treated with 3 injections of platelet concentrate prepared with a single-spinning

procedure (platelet rich in growth factors-PRGF), the other 72 with 3 injections of PRP obtained with a double-spinning approach. The patients were evaluated prospectively at the enrolment and at 2, 6, and 12 months' follow-up with International Knee Documentation Committee (IKDC), Visual Analogue Scale (VAS) and Tegner scores; adverse events and patient satisfaction were also recorded. Single spin was prepared by obtaining a 36-ml venous blood sample for every knee treated. Four tubes of 9 ml of blood were centrifuged at 580 g for 8 min, obtaining a concentration suspended in plasma. Double spin was prepared by obtaining a 150-ml venous blood sample for every knee treated. Two centrifugations (the first at 1,800 rpm for 15 min to separate erythrocytes, and a second at 3,500 rpm for 10 min to concentrate platelets) produced 20 ml of PRP. The unit of PRP was divided into 4 small units of 5 ml each. The most important finding of this study was that both groups presented a similar statistically significant improvement in all the scores evaluated at all the follow-up times.

Better results were achieved in younger patients with a lower degree of cartilage degeneration. The comparative analysis showed more swelling and pain reaction after double spinning injections but similar final improvement, thus suggesting the potential of both platelet concentrates in treating joint degeneration processes.[6]

In a study in the year 2012, in Spain, the authors reviewed several articles surrounding the therapeutic applications of PRP in Orthopaedics and sports medicine. The authors reviewed articles on repair of tendon injuries where PRP was injected into the tendon fibers after the tendon was sutured. After closing the paratenon and before closing the overlying skin, the affected area was covered with the fibrin scaffold. The results showed that those receiving the PRP-therapy experienced a

significant acceleration in functional recovery compared with a matched group that underwent conventional surgery.

A preliminary study where PRP injection was used in ACL repairs was reviewed in which the author described a procedure for treating bone tunnels and for conditioning the graft prior to implantation with PRP. They compared a group of 50 patients treated with surgery and pure PRP with another group of 50 patients who underwent surgery alone. The authors reported better integration of PRP-treated grafts within the tunnels, as assessed by X-rays, and a larger number of completely stable knees in the PRP group.[18]

In Japan, a prospective study was held in 2013 administered single spin IA-PRP injection in 10 patients with radiographically documented knee osteoarthritis of grades 1 to 3, according to the Kellgren-Lawrence (K-L) radiographic classification. Single spinning was done by drawing approximately 36 mL of venous blood from the antecubital vein in an effort to avoid irritation and trauma to the platelets. The blood was collected in four extraction tubes containing 3.8% sodium citrate as an anticoagulant. Subsequently, the tubes were centrifuged at 2100 rpm for 8 minutes at room temperature. 6-ml of PRP was injected in the knee joint three times at 1-week intervals. All patients were evaluated before injection and at 1, 3, and 6 months after the treatment. Complications, the VAS score, Japanese Knee Osteoarthritis Measure (JKOM) score and Japanese Orthopedic Association score were evaluated. The authors concluded that single spin IA-PRP is safe for use in patients with knee osteoarthritisand while local and minor adverse events related to PRP injection occurred, all symptoms disappeared within 48 hours. This therapy has the potential to induce pain relief that is maintained for up to 6 months.[19]

A study done in China, in 2013, stated that PRP is promising for treating injuries of cartilage. PRP has shown, anabolic effect on both chondrocytes and bone-marrow derived stem cells with resulting increases in proliferation of cells and production of matrix & anti-inflammatory effect via catabolic signalling pathways downregulation. Maybe it is a feasible, economic and secure way to induce mesenchymal stem cells (MSC) differentiation into chondrocytes integrally and expand cartilage cells in vitro.[20]

In Korea, a non-randomized, prospective study was done in 2013 where the authors used a double spinning method of preparation of PRP in 65 patients with early OA knee. The patients were evaluated at 1, 3, 6, 9, and 12 months after the procedure using VAS score and IKDC score. Both VAS and IKDC scores showed significant improvement post injection. The patients reported relapsed pain 9 months after the procedure. They also concluded that developing degeneration according to the Kellgren–Lawrence grade reduced the effects of PRP and also accelerated the time of relapsed pain. The limitations of their study was that it was not a randomized trial with a formal control group, nor a comparative study.The authors concluded that, even though intra-articular PRP injection can be used for the treatment of early OA, increasing age and developing degeneration hampers the potential for PRP injection therapy. [21]

A cohort study done in 2013 in Czech Republic used double spin PRP injection in 50 consecutive and strictly selected patients, affected by Grade II or III tibiofemoral chondromalacia. Outcomes were measured using the Lysholm, Tegner, International Knee Documentation Committee(IKDC), and Cincinnati scores. Magnetic resonance imaging was used to evaluate degree of degeneration and thickness of cartilage. The authors had seen a significant improvement in the outcome

measures at the end of 12 months. Cartilage assessment revealed no significant cartilage regeneration. They concluded that the technique they used is safe and provides pain reduction and improved function. They confirmed their hypothesis that PRP improves knee condition and clinical outcomes.[22]

A prospective case study done in Italy in the year 2013 used leukocyte-poor platelet-rich plasma (PRP) preparation for the treatment of knee osteoarthritis. 45 patients were included in the study and treated with a cycle of 3 weekly injections of autologous conditioned plasma. 6 patients were affected by bilateral OA, hence 51 knees were treated in total. The patients initially underwent a baseline evaluation and then after a follow-up of 14 months, using the following outcome measures: International Knee Documentation Committee (IKDC), Visual Analogue Scale(VAS), Tegner, and Knee Injury and Osteoarthritis Outcome (KOOS) scores. The authors concluded that in the "early/moderate OA" knee group, there was a significant improvement in the IKDC, VAS, Tegner and KOOS scores. Although an improvement was also recorded in the "severe OA" group, the clinical outcome of the patients in this group was significantly poorer and they reported less benefit. [23]

In a prospective, randomized study held in 2014 in Italy, the authors planned to assess the outcome of intra-articular platelet rich plasma (PRP) injections into the knee in patients with early stages of osteoarthritis (OA) and wanted to determine whether cyclical dosing would affect the end result. In this study, 93 patients (119 knees) were followed up for a minimum of 2 years. 50 knees were randomly selected before the 1st injection, to receive a 2nd cycle at the end of 1 year. A cycle comprised of three injections, each given at a monthly interval. The outcome was measured using Knee Injury and Osteoarthritis Outcome (KOOS), Visual Analogue Scale (VAS), Tegner and Marx scoring systems, measured before the first injection and then

at 12, 18 and 24 months. They found that, at 18 months, except for KOOS and Tegner score, all other parameters showed a difference between the two groups in favour of the patients who had received the second cycle. At 2 years, the scores reduced in both groups but remained above the pre-injection value with no significant difference between the groups despite the patients with two cycles showing higher mean values for all the scores. The authors concluded that intra-articular PRP injection for early stages of OA with symptoms, are a valid treatment option. Even though the beneficial effects are ill sustained after 2 years, the results are encouraging when compared to the pre-treatment function.[24]

A controlled lab study that was held in Connecticut, U.S.A in 2015 assessed 2 different PRP preparations and their anti-inflammatory effects over time on human OA cartilage and synovium. One preparation was a single spin yielding low white blood cell and platelet concentrations (PRP-LP) and the other preparation was a single spin yielded high white blood cell and platelet concentrations (PRP-HP). The PRP-LP was prepared by drawing 50 ml of peripheral venous blood and then centrifuged immediately at 1500 rpm for 5 minutes. The PRP-HP was prepared by centrifuging the collected blood at 3200rpm for 15 minutes. A coculture of osteoarthritic cartilage and synovium from 9 patients who underwent total knee arthroplasty was made. Interleukin-1b was added to each coculture to induce inflammation. Either PRP-LP, PRP-HP, or medium was added to the coculture wells. Control wells contained OA cartilage and synovium but neither IL-1b nor PRP. Normal cartilage was taken to establish baseline gene expression levels. PCR was used to evaluate changes in markers of inflammation in the tissues (a disintegrin and metalloproteinase with thrombospondin motifs–5 [ADAMTS-5], tissue inhibitor of metalloproteinases–1 [TIMP-1], vascular endothelial growth factor [VEGF], aggrecan and type I collagen)

at 0, 24, 48, and 72 hours. Treatment with PRPLP or PRPHP reduced expression of TIMP-1 and ADAMTS-5, increased aggrecan expression in cartilage, and reduced ADAMTS-5, VEGF, and TIMP-1 expression in synovium compared with control. They concluded that, both had a significant anti-inflammatory effect on gene expression; however, there was no difference in the anti-inflammatory effect between the 2 preparations. [25]

In Saudi Arabia in 2015, a study was carried out on a rat model of surgicallyinduced OA to assess the changes in the synovial membrane and to evaluate the effects of intra-articular injection of PRPin these cases. 45 male albino rats were put into 3 equal groups; control, surgicallyinduced OA and surgicallyinduced OA followed by intra-articular injection of PRP. Knee joints were evaluated for immunohistochemical and histological staining with anti-platelet derived growth factor (PDGF-A) and anti-vascular endothelial growth factor (VEGF) and the percentage of area of immunostaining was measured. The osteoarthritis research society international (OARSI) score was higher in OA than in control and in PRPtreated tissues. The immunostained area for PDGF-A was significantly higher in PRPtreated tissues than in OA and control tissues. They concluded from their study that intra articular PRP injection could produce optimizing effects in surgically induced OA in the form of; decreasing the OARSI score, improving the inflammatory events in synovium and modulating the PDGF-A and VEGF serum levels and synovial tissue immunoexpression. These effects could be reflected positively on the associated chondral defect.[26]

A review of overlapping meta-analyses in 2015 in U.S.A aimed at providing a framework for analysis and interpretation of the best available evidence toprovide recommendations for use of PRP in the setting of knee OA. Literatures on the use of

PRP versus corticosteroids, hyaluronic acid, oral nonsteroidal anti-inflammatory drugs or placebo was obtained. The clinical data and the quality of the meta-analysis was assessed. 3 meta-analyses met their criteria and ranged from Level II to Level IV evidence. They authors conferred that the use of PRP led to improvements in patient outcomes at 6 months post injection and these improvements were seen starting at 2 months and were at the same levels for up to 12 months. The limitation of the study was that the authors were unsure if the use of multiple PRP injections, the double-spinning technique or activating agents leads to better results. They concluded that intra articular-PRP injection is a viable treatment for osteoarthritis knee & this method has the potential to produce symptomatic relief. Increased risk of local adverse reactions after multiple PRP injections was observed. This method offers better symptomatic pain relief for the patients having early degenerative changes in knee and use of this method should be considered in patients with knee OA. [27]

A one-year, non-placebo-controlled, randomized clinical trial was done in 2015 in Iran where the authors involved 160 patients affected by knee OA, grade 1–4 of Kellgren–Lawrence scale. In the PRP group (87 patients), 2 intra-articular injections were administered at 4 week intervals, and in the HA group (73 patients), 3 doses of intra-articular injection at 1-week interval were applied. All patients were evaluated before and at 12 months after the treatment by WOMAC scores and SF-36 questionnaires. The PRP was prepared by centrifuging 35-40 ml of the patients' blood, first at 1600 rpm for 15 minutes and then the buffy coat layer and plasma layer was centrifuged for another 2800 rpm for 7 minutes. The resultant 4-6ml double spin PRP was injected. At the end of 12-months, WOMAC pain score and bodily pain improved in both groups; however, better results were found in the PRP group compared to the HA group. Other WOMAC and SF-36 parameters improved only in

the PRP group. In both the groups, better improvement was achieved in patients with grade 2 OA. The authors concluded that PRP injection is more efficacious than HA injection in improving quality of life & also reducing symptoms. It is a therapeutic option in knee osteoarthritic patients who have not responded to conventional treatment.[28]

A randomized, double-blind, placebo-controlled clinical trial was conducted in 2016 in Columbia where 30 patients with either Grade 2 or 3 OA knee according to Kellgren-Lawrence grading were included in the study. These patients were randomly assigned to receive either autologous conditioned plasma(ACP) (15 patients) or saline placebo (15 patients) for 3 weekly injections. WOMAC scores served as the primary efficacy outcome measure and the patients were followed up for 1 year. The ACP was prepared by drawing 15ml of blood from the patient and spinning it a single time at 1500rpm for 5 minutes and then by removing the yellow leukocyte-poor PRP layer. At the end of 12 months, there was an overall improvement in WOMAC scores by 78% from the baseline score in the ACP group, compared to 7% for the placebo group. Thus, the authors concluded that Intra-articular autologous conditioned plasma injections is safe and provides quantifiable benefits for pain relief and functional improvement with regard to knee OA.[5]

In Australia in the year 2016, a double-blind, randomized controlled pilot study was conducted where 37 people with OA knee were randomly allocated to receive 3 injections of either photo-activated PRP or HA. The outcomes were measured using VAS, KOOS, Knee Quality of Life (KQoL) scale, maximum hopping distance and number of knee bends in 30 seconds at 4 and 12 weeks. The PA-PRP was prepared by collecting 48.5ml of the patient's blood and then centrifuging it 2,000 rpm for 5 minutes. The plasma and buffy coat containing platelets was drawn

from the top of the sample and placed in a sterile tube and centrifuged again at 3,000 rpm for 3 minutes. This double-spin approach produced PRP that is higher in leukocytes, which was preferred for this study as photo-activation is thought to act at least in part by influencing the pro- and anti-inflammatory properties of leukocytes. The PA-PRP group showed improvements in the VAS, KOOS Pain, KQoL subscales at 4 and 12 weeks. The PA-PRP group also significantly improved hopping and knee bends at 4 and 12 weeks. At 12 weeks, the only improvement showed by The HA group was in the KOOS Function subscale. The authors concluded that intra-articular injection of photo activated platelet-rich plasma in patients with knee osteoarthritis showed improvement in self-reported pain, symptoms, lower extremity function. [29]

A randomized, double-blind, comparative study conducted in Iran in 2016 included 41 participants who suffered from Grade 2 or 3 OA knee according to Kellgren-Lawrence grading and randomly divided them into 2 groups: intra-articular injection of PRP and corticosteroid. KOOS, 20 meter walk test, active and passive ranges of motions (ROM), flexion contracture, and VAS scores were assessed before, 2-months, and 6-months after interventions. The authors observed that compared to the group treated with corticosteroid; pain relief, activities of daily living and quality of life were significantly better in the PRP group. PRP was also more helpful the 20-meter walk test than corticosteroid. [30]

A systematic review of level 1 studies was conducted in U.S.A in 2016 with the aim to determine differences in outcomes between PRP and corticosteroid injections or viscosupplementation or placebo injections at 6- and 12-months post-injection. Six articles that were analyzed showed significant improvements in statistical and clinical outcomes, including pain, physical function and stiffness with PRP. Mean post-treatment WOMAC scores for PRP were significantly better than for

HA at 3 to 6 months and at 6 to 12 months. None of the included studies used corticosteroids. They concluded that, in patients with symptomatic knee OA, PRP injection results in significant clinical improvements up to 12 months post-injection. Outcomes were significantly better after PRP versus HA at 3 to 12 months post-injection. The limitation of their study was a lack of evidence for comparing leukocyte-rich versus leukocyte-poor PRP or PRP versus steroids. [31]

A prospective, comparative study conducted in Turkey in 2017 aimed To compare two platelet-rich plasma kits with different platelet concentrations for treatment of knee osteoarthritis. Patients were divided into: Group I, who received platelet-rich plasma kit I, and Group II, who received platelet-rich plasma kit II. Platelet concentrations of both kits were measured. For each group, platelet-rich plasma kit was administered twice with a one-month interval between injections. The WOMAC and VAS were applied for clinical evaluation before the first injection and 1, 3 and 6 months after the second injection. Kits I and II contained 1,000,000 and 3,000,000 platelets/μl respectively. In both groups, initial WOMAC and VAS scores were significantly higher compared to the latter evaluations. However, no significant difference was observed between groups in terms of clinical evaluations. They suggested that the PRP platelet concentration should be at least two times the whole blood platelet concentration; however, concentrations up to eight times that of blood levels have been reported with good results.[7]

In the same study they concluded that the risk of adverse events in PRP-treated participants was not significantly increased in comparison with other knee osteoarthritis treatment options. The first major limitation of the study was the retrospective design of their study, which restricted the sample size. The second major limitation was that biochemical changes of each kit could not be evaluated.[7]

A prospective case study conducted in Italy in 2017 included Twenty-five patients affected by grade I and II knee primary OA according to the Kellgren–Lawrence scale and they received a single intra-articular PRP injection. Patients were prospectively evaluated for 6 months. VAS, WOMAC, and KOOS scoring scales were used to evaluate clinical outcomes. The PRP was prepared by taking 50ml of blood from the patient and centrifuging it at 3,200 rpm for 15 minutes. At the end of 6 months follow up, improvements of WOMAC, KOOS and VAS scores was seen. The authors concluded that treating knee OA with PRP injection is safe. A single dose of PRP seems to be effective in managing pain and improving quality of life in patients with low-grade knee OA. [32]

In a study conducted in 2017, the authors aimed to evaluate the effect of one dose of intra-articular injection of PRP in the knee joint on a specific biomarker of degeneration of cartilage in OA, Collagen 2-1 (Coll2-1), over a short period of 3 months. The aim was extended to clarify the effect of PRP on the functional status of the osteoarthritic knee joint. 60 patients with unilateral primary knee OA diagnosed according to the American College of Rheumatology (ACR) classification criteria of knee OA were included in the study. They were divided into two groups according to the Kellgren and Lawrence (KL) scale: Group I: including patients with < grade 3 knee OA and Group II: including patients with ≥ grade 3 knee OA. Patients were asked to complete the WOMAC questionnaire.

About 35mL of venous blood was collected from each study subject by sterile venipuncture, about 3 mL allowed to clot naturally for 30 minutes, then centrifuged at 1000g for 15 minutes, sera were separated and kept frozen at -80° C till used for ELISA. The remaining 30 ml of blood samples were collected in sodium citrate tubes.

The tubes were first centrifuged at 1800 rpm for 15 min to separate erythrocytes, then for 10 min at 3500 rpm to concentrate the platelets. By this method, 5 mL of PRP- were obtained and was injected immediately without storage. Serum Coll2-1 was measured using a commercially available ELISA kit.

Comparison between mean serum Coll2-1 level at base line and 3 months after PRP injection showed a statistically significant decrease. Comparison between WOMAC index variables at base line and follow up visits in patients with unilateral primary knee osteoarthritis showed a statistically highly significant difference too. In the study, authors observed synovitis after PRP injection in four patients and temporary pain following the injection in eight patients that resolved within three days.

The authors concluded by stating that reduction in S.Coll2-1 following intra-articular PRP injection showed that PRP could be a promising safe and tolerable effective therapeutic option which improves function from basal states in primary knee OA patients.[33]

A blinded controlled experimental study performed on male guinea pigs, proved to be a model in which knee OA occurs spontaneously and bilaterally. Two models of 12 animals each were made (group I and II). Out of each model (group I and II), 6 animals were euthanized at 3 months post intervention (subgroup IA and IIA – early outcome analysis) and the other 6 at 6 months (subgroup IB and IIB- late outcome analysis). Another group of 6 donor animals was taken for harvesting blood for allogenic PRP.

PRP was administered to the experimental knees three times in a week, with administration of saline to the contralateral control knees in group I. 15 ml of blood was taken from one donor animal under aseptic precautions via cardiac puncture

which was then sent for platelet count. Blood was centrifuged for 20 minutes at 800 rpm which separated the blood into a red cell layer and a buffy coat.

The buffy coat was pipetted out and subjected to hard spin for 15 minutes at 2200 rpm. This produced platelet poor plasma overlying a platelet pellet at the bottom of the tube. Two-third of the platelet poor plasma was removed and the rest was dissolved to produce a solution. This method gave 4 ml of allogenic PRP.Under aseptic precautions, activated PRP (1 part 0.025 M CaCl2 to 4 parts of PRP) was injected into the experimental knee joint through the patellar tendon and the same amount of saline was injected in the control knee of the same animal (200 microliter).

The knee was subsequently aspirated using a 26-gauge needle through the same approach. This fluid was then subjected to centrifugation at 2000 rpm for 10 minutes to remove cell debris and the supernatant was collected and stored at -80°C for analysis. After aspirations, the knee joints were surgically harvested. This tissue was fixed in 10% formaldehyde and sent for histological examination immediately.

Synovial fluid cartilage oligomeric matrix protein (COMP) levels were measured using enzyme-linked immunosorbent assay (ELISA) technique. The collected samples were processed according to the algorithm given by the Osteoarthritis Research Society International (OARSI) initiative. Synovial inflammation and cartilage degeneration were graded using the semi-quantitative scores given by OARSI. The synovial vascularity was also assessed and >3 blood vessels per high power field was considered increased synovial vascularity.

The statistics for all 4 subgroups were calculated for all parameters (i.e. weight, platelet counts in whole blood and PRP, synovitis scores, COMP concentration and articular cartilage scores). The results showed an improvement in synovitis and a reduction in synovial vascularity in PRP treated knees. The COMP levels of

synovium were also lower in knees treated with PRP, albeit, at short term analysis only. The effect on articular cartilage, however, remains equivocal since the beneficial effect of PRP was observed into some extent in group I but was not reproducible in group II.[34]

An updated systematic review was performed in 2017 to evaluate the temporal effect of PRP on knee pain and physical function. Human RCTs comparing the efficacy and/or safety of PRP infiltration with other intra-articular injections were reviewed. A summary and quality assessment was done for all the studies finally included for analysis. For studies reporting outcomes concerning WOMAC or adverse events, a random-effects model was used for data synthesis. 14 RCTs comprising 1423 participants were included. The control consisted of saline placebo, HA, ozone, and corticosteroids. The follow-up period was from 12 weeks to 12 months. On comparison with the control, PRP injections significantly reduced WOMAC pain subscores at 3, 6, and 12 months follow-up. The review concluded that intra-articular PRP injections probably are more efficacious in the treatment of knee OA in terms of pain relief and self-reported function improvement at 3, 6- and 12-months follow-up, compared with other injections, including saline placebo, HA, ozone, and corticosteroids. [35]

In 2017, a systematic review of meta-analyses was published, evaluating platelet-rich plasma injection in the treatment of knee OA. The objective of this study was to evaluate the similarities and differences between the variety of PRP formulations, preparation, and uses of these techniques and to try to determine characteristics of the PRP which tend to give the best result. A total of 19 articles were selected. The outcomes that were assessed and reported by each study, including WOMAC,VAS, IKDC and KOOS were listed. The minimal clinically important

improvement (MCII) was defined to help determining whether an observed difference is clinically important. The different studies in 2 groups were classified depending on the outcomes into bad responders group (BRG < MCII) and very good responders group (VGRG > 2xMCII).In 4 out 7 studies of the VGRG, 1 or 2 injections were given, against 3 in all the studies of the BRG. Volume injected varied from 2.5 mL to 8 mL in the VGRG. Centrifugation technique was variable. Single spinning technique was the one used most and appeared to give better results than double spinning technique. The study helped identify features of PRP recommended for knee OA treatment, such as the use of a single spinning technique, a platelet concentration lower than 5 times the baseline (from 3 to 4), and avoiding RBC and WBC and recommended leveraging this information about PRP for future studies.[36]

A meta-analysis held in 2017 in Finland reviewed articles comparing local injections of platelet-rich plasma versus control to evaluate the effectiveness of PRP local injections for androgenetic alopecia. The primary outcome was taken as the difference in number of hairs per square centimeter. Secondary outcomes were hair cross section increase, hair regrowth, and thickness percentage increase. They concluded that local injection of PRP for androgenic alopecia might be associated with an increased number of hairs and some hair thickness improvement.[37]

A prospective, comparative study was conducted in India in 2018, where the authors administered single spin PRP injection in 150 patients with early OA knee. Group A (75 patients) received a single injection of PRP, group B (75 patients) received 2 injections of PRP 3 weeks apart.Clinical outcome was evaluated using the WOMAC questionnaire before treatment and at 1 month, 3 month, and 6 months post injection. Although a significant reduction of WOMAC scores were seen in both groups, they were not statistically significant. Thus, they concluded that PRP injection

in Grade I & Grade II (Ahlback's radiological grading) does give pain relief and improves knee stiffness and functionality without any major adverse effects so this can be recommended as a viable modality of treatment. [38]

From the same series, authors also opined about the duration of the effect, which reduces early in patients who continue to pursue heavy works compared to those with sedentary lifestyle. But since there was no significant difference between single versus double injection protocol, they concluded that double dose doesn't offer any additional advantage. [38]

A study conducted in 2019, included individuals with chronic knee pain persisting for longer than three months between 40 and 80 years of age who were diagnosed with osteoarthritis based on the American College of Rheumatology (ACR) criteria and followed as outpatients. The study enrolled patients with grade 2-4 knee osteoarthritis based on the Kellgren-Lawrence (K-L) classification who provided voluntary informed consent.This study measured the short-term efficacy of PRP on chronic pain in patients with OA of the knee. Approximately 10 mL of blood was taken from patients for each knee and was revolved at 3000 rpm for 8 minutes. By doing so, approximately 4 mL of leukocyte-poor PRP was obtained. The PRP prepared was injected into the knee involved within 20 minutes. The study was launched with 88 knee joints of 69 patients. After the first PRP injectionand after the second PRP injection, 11 patients were lost to follow-up; the third PRP injection was delivered to 71 knee joints of 53 patients. The study was completed with 60 knee joints in 42 patients who had received three PRP injections that attended the last follow-up at week 12 and the statistical analysis was performed. It was observed that PRP injections provided a meaningful improvement in chronic knee pain in patients

with knee OA throughout a 12-week period. The pain reduction response to PRP was better in patients with early-stage knee OA.[39]

A 2019 review stated that with the average life expectancy and the rising prevalence of obesity, osteoarthritis (OA) is creating an increasingly large financial and physical burden on the U.S. population. As the body ages and experiences trauma, articular cartilage surfaces in joints are gradually worn away, leading to OA. Traditionally, treatment options have included lifestyle modifications, pain managementand corticosteroid injections, with joint replacement reserved for those who have exhausted nonsurgical measures. More recently, hyaluronic acid, micronized dehydrated human amniotic/chorionic membrane tissue and platelet-rich plasma (PRP) injections have started to gain traction. PRP has been shown to have both anti-inflammatory effects through growth factors such as transforming growth factor-β and insulin-like growth factor 1 and stimulatory effects on mesenchymal stem cells and fibroblasts.

Multiple studies have indicated that PRP is superior to hyaluronic acid and corticosteroids in terms of improving patient-reported pain and functionality scores. Unfortunately, there are many variations in PRP preparation and lack of standardization in factors, such as speed and duration of centrifugation, leads to wide ranges of platelet and leukocyte concentrations. The review concluded that there is clear evidence in the literature to support the use of LP-PRP in OA for improvement in patient-reported pain scores, joint stiffness and physical functioning.

Currently, PRP seems to be the most beneficial for early/low K-L grade OA compared with more advanced OA. Better outcomes are seen with younger individuals with cartilage defects or earlier OA and worse outcomes tend to be seen in patients over the age of 50 and those with further degenerated joints.[40]

A study based in California, USA, in 2019 conducted a prospective study where they hypothesized that i) "lower" levels of the inflammatory mediators (IMs), interleukin-1βand tumor necrosis factor α (TNF-α) and (ii) "higher" levels of the growth factors (GFs), insulin-like growth factor 1, and transforming growth factor β1 within leukocyte-poor PRP correlate with more favourable chondrocyte and macrophage responses in vitro.

Samples were collected from 10 healthy young male (23-33 years old) patients (H-PRP) and nine older (62-85 years old) male patients with severe knee OA (OA-PRP). The samples were separated into groups of "high" or "low" levels of IM and GF based on multiplex cytokine and enzyme-linked immunosorbent assay data.

Three-dimensional (3D) alginate bead chondrocyte cultures and monocyte-derived macrophage cultures were treated with 10% PRP from donors in different groups. Gene expression was analyzed by quantitative polymerase chain reaction. Contrary to their hypotheses, the effect of PRP on chondrocytes and macrophages was mainly influenced by the age and disease status of the PRP donor as opposed to the IM or GF groupings. Their data suggested that PRP from older individuals with OA contain factors that may suppress chondrocyte matrix synthesis and promote macrophage inflammation in vitro.[41]

Another study conducted in 2019 aimed at documenting the efficacy of a single PRP injection to treat knee OA and validate a routine care procedure. Out of the 61 screened patients, 60 were injected and 57 were finally analyzed. Patients were aged 63.3 ± 9.6 years old and presented grade 2 and grade 3 knee OA according to the Kellgren-Laurence scale. 42 and 6 patients had previously had IA injection of HA or corticosteroids, respectively. The final injected volume was 8.8 ± 1.1 ml. The mean

increases in platelets and leukocytes compared with blood were 1.4 ± 0.4 and 0.1 ± 0.1, respectively. The percentage of platelets was 96.2 ± 2.5% with low contamination from RBCs (3.7 ± 2.4%) and leukocytes (0.1 ± 0.1%). The main objective of the study was reached with 84.2% ofOutcome Measures in Rheumatology Clinical Trialsand Osteoarthritis Research Society International (OMERACT-OARSI) responders at three months.

This result was stable with 82.5% and 80.7% of responders one and six months after the injection, respectively. Single injection of PRP was effective in improving knee functional status with a significant increase in KOOS total score, six months after the procedure. This significant difference was observed already after one month after the treatment and was reflected on all KOOS subscores. Assessment of pain through a 50-foot walk test also resulted in significant decrease of pain from the baseline as of one month after the injection. Assessments of damages caused by the arthrosis were also reducedat six months with significant reduction at all follow-up. The authors concluded that a single injection of a large volume of very pure PRP is associated with a responders' rate of around 80%, up to six months after the injection, supporting the initiation of daily PRP injection in OA patients.[42]

A meta-analysis conducted in 2020 in China reviewed 10 studies that compared the effects of HA injection to PRP injection in OA knee cases on patient's pain levels and functionality improvements. Among these studies VAS, WOMAC and IKDC scores showed significant improvements in PRP injections whereas the KOOS score did not show any statistical difference. Thus, the authors stated that PRP appears to be better than HA at achieving pain relief and self-reported functional improvement.[43]

A comprehensive meta-analysis was conducted in China in 2020 comparing platelet-rich plasma (PRP) injection with hyaluronic acid (HA) and placebo injection for KOA patients. Meta-analysis quality was assessed using the Quality of Reporting of Meta-analyses (QUOROM) systems and the Oxman-Guyatt quality appraisal tool. 4 meta-analyses were included in their studyand all of these articles were Level I evidence. The QUOROM score of each included meta-analysis range from 14 to 17 points (mean score 15, maximum score 18), and the Oxman-Guyatt score range from 4 to 6 points (mean score 5, maximum score 7). 3 meta-analyses indicated PRP showed more benefit in pain relief and functional improvement than the control group and the other one suggested no difference between these groups. All included meta-analyses found no statistical difference in adverse events between these groups. In addition, a meta-analysis conducted by Shen et al. got the highest methodological quality score and suggested that PRP provided better pain relief and function improvement in the treatment of KOA. The analysis concluded that for short-term follow-up (≤1 year), intra-articular PRP injection is more effective in terms of pain relief and function improvement in the treatment of KOA patients than HA and placebo. There is no difference in the risk of an adverse event between PRP and HA or placebo.[44]

RELEVANT ANATOMY

The largest and the most complex joint in the body is the knee joint. The femur has a lateral and a medial condyle which corresponds to the two condyles of the tibia. The knee joint basically contains two different joints, one between femur and tibia and the other one between the patella and femur. The one between femur and tibia is a hinge variety of synovial joint which allows some degree of rotatory movement, while the one between femur and the patella is a plane gliding variety of synovial joint. [45]

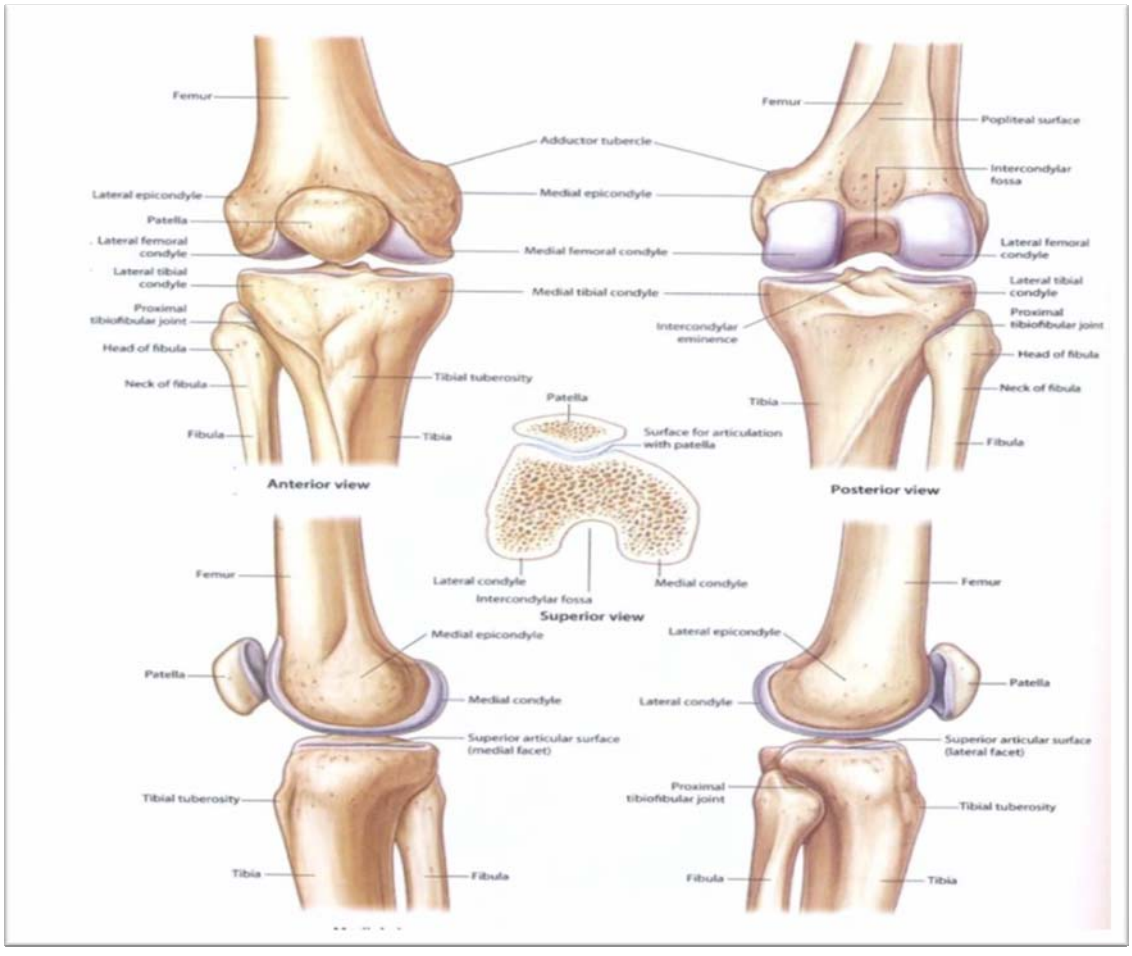


Figure 1: Bones forming knee joint.[45]

ARTICULATIONS: -

The knee joint bounded above by the rounded condyles of femur, below by the condyles of the tibia and their cartilaginous menisci, in front is the articulation between the lower end of the femur and patella.

The articular surfaces of the femur, tibia, and patella are covered with hyaline cartilage. The articular surfaces of the medial and lateral condyles of the tibia are often referred to clinically as the medial and lateral tibial plateaus.[45]

CAPSULE: -

The knee joint contains a capsule which is attached to the margins of the articular surfaces and which also surrounds the sides and the posterior aspect of the joint. The capsule is missing in front of the joint which permits the synovial membrane to form a pouch upwards below the quadriceps tendon and thus forming a suprapatellar bursa. The capsule of knee joint is further strengthened by expansions from the tendons of vastus lateralis and vastus medialis. An expansion from the semimembranosus muscle called oblique popliteal ligament strengths the capsule from behind. The popliteal ligament emerges through an opening in the capsule behind the lateral tibial condyle.[45]

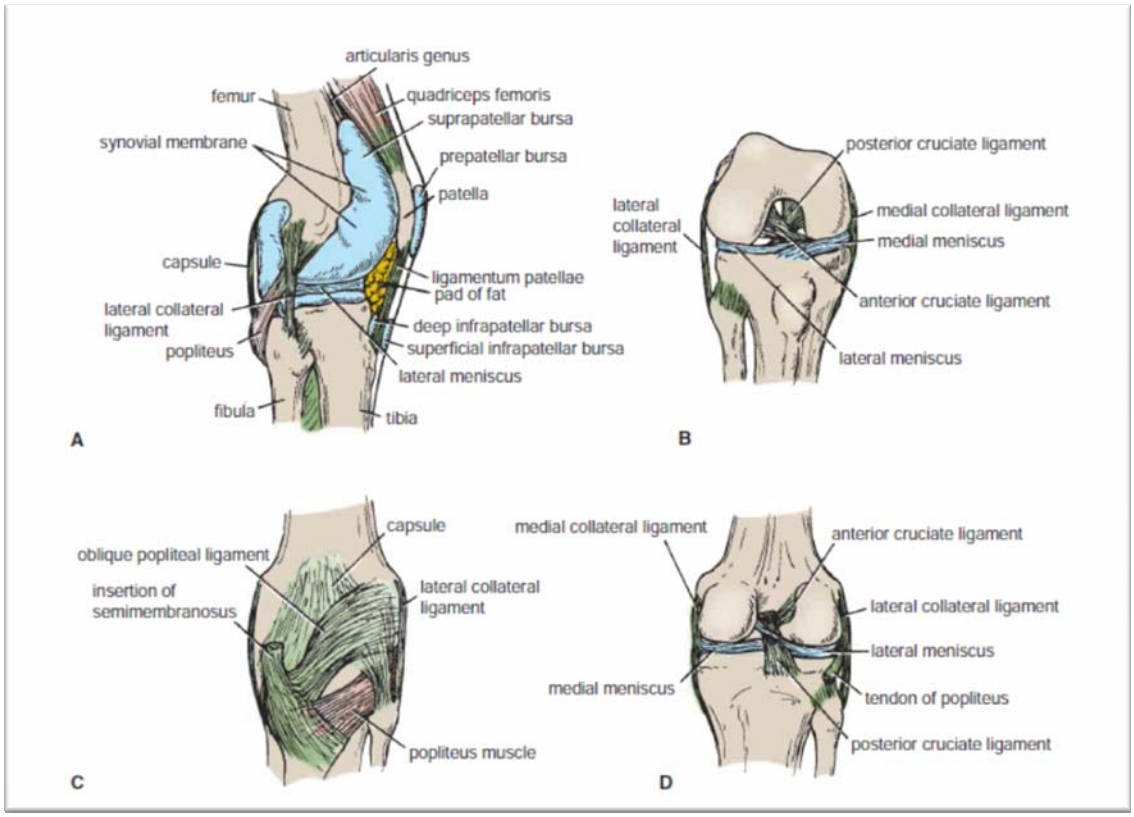


Figure 2: A. The right knee joint as seen from the lateral aspect. B. The anterior aspect, with the joint flexed.

C, D. The posterior aspect. [45]

THE VASCULAR SUPPLY:-

The major arteries which supply the knee joint are derived from five major arteries which form a rich anastomosis around the knee joint. These major arteries are superior medial and lateral, the middle and inferior medial and lateral genicular arteries.

The descending genicular arteries and the anterior tibial recurrent artery also form an anastomosis around the knee joint. The major vessels provide blood supply to specific areas of the knee joint, branches from these vessels form anastomoses around

the knee joint. The femoral and tibial condyle and the patella have rich intraosseous blood supply.

In addition to this, soft tissues including major ligaments and the peripheral parts of menisci also have rich blood supply. The major arteries which supply blood to the knee joint are derived mainly from the branches of femoral and popliteal arteries. These include the descending branches of the lateral circumflex artery, the descending genicular branch of the superior femoral artery, descending genicular branch of the superior femoral artery.

In addition to this the genicular arteries from the popliteal artery, recurrent branches from the tibial arteries and a small branch from circumflex fibular artery. [46] All these arteries form a rich network of anastomosis around the knee joint. On the posterior side of the knee the popliteal artery which is a continuation of superficial femoral artery passes through the adductor hiatus and then terminates into anterior and posterior tibial arteries.

The major structures of the knee joint are supplied by blood vessels present in popliteal fossa. The major arteries that pass through the popliteal fossa are:

Popliteal artery: This is a continuation of femoral artery at the adductor hiatus which runs down the popliteal fossa and divides into tibial arteries.

Genicular arteries: There are many branches of this artery which form a network of arteries around the knee. The genicular arteries which are involved in this anastomosis are the superior lateral, superior medial, middle, inferior lateral and inferior medial genicular arteries. These arteries form a network of arteries surrounding the knee.

Anterior tibial artery: The anterior compartment of the knee contains anterior tibial artery. This artery supplies blood to the anterior compartment of the leg and continues till the ankle.

Posterior tibial artery: This artery runs to the posterior compartment of the leg to provide blood to structures in the posterior and lateral compartments.

Fibular artery: The posterior tibial artery branches off to form fibular artery and then runs down the posterior part of the knee. It also provides blood to the lateral and posterior compartment of knee. [46]

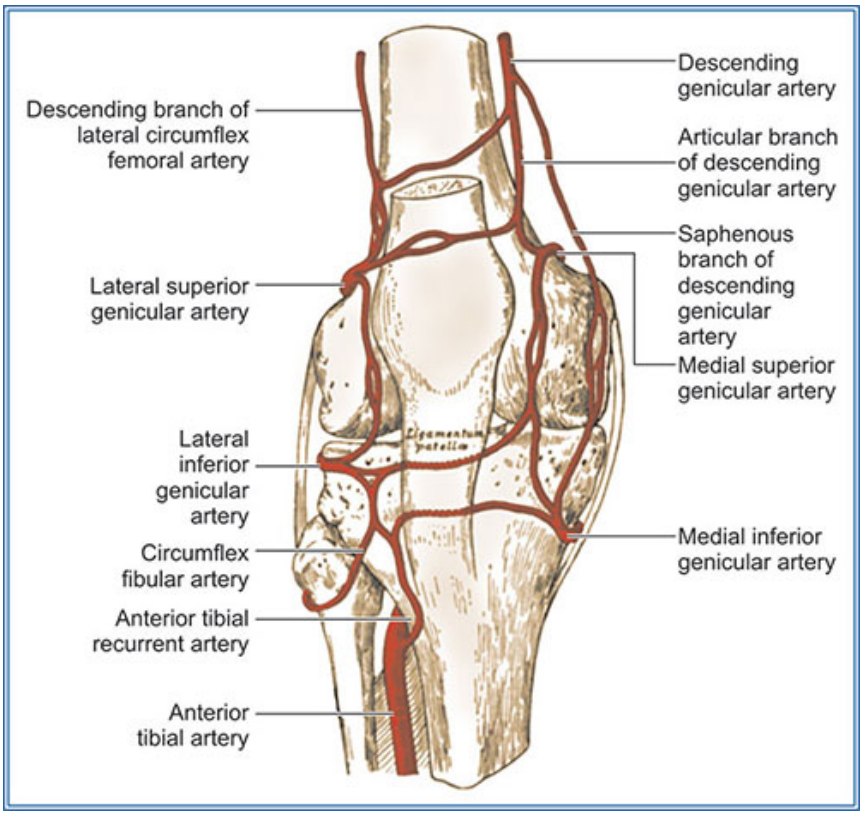


Figure 3: Arterial supply around knee joint.[46]

VENOUS SUPPLY:-

The major vein of the knee is located in the popliteal fossa. It originates from the posterior tibial vein and travels up through the popliteal fossa up in the thigh and continues as femoral vein. Following are other veins that serve the knee and leg:

Lateral superior genicular vein: Located superior to the lateral condyle of the femur; drains into the popliteal vein

Lateral inferior genicular vein: Below the lateral condyle of the tibia; also drains into the popliteal vein

Great saphenous vein: A superficial vein that travels from the foot, along the medial side of the knee, and all the way up to the hip

Small saphenous vein: A superficial vein that travels along the lateral side of the ankle to the posterior leg and empties into the popliteal vein. [47]

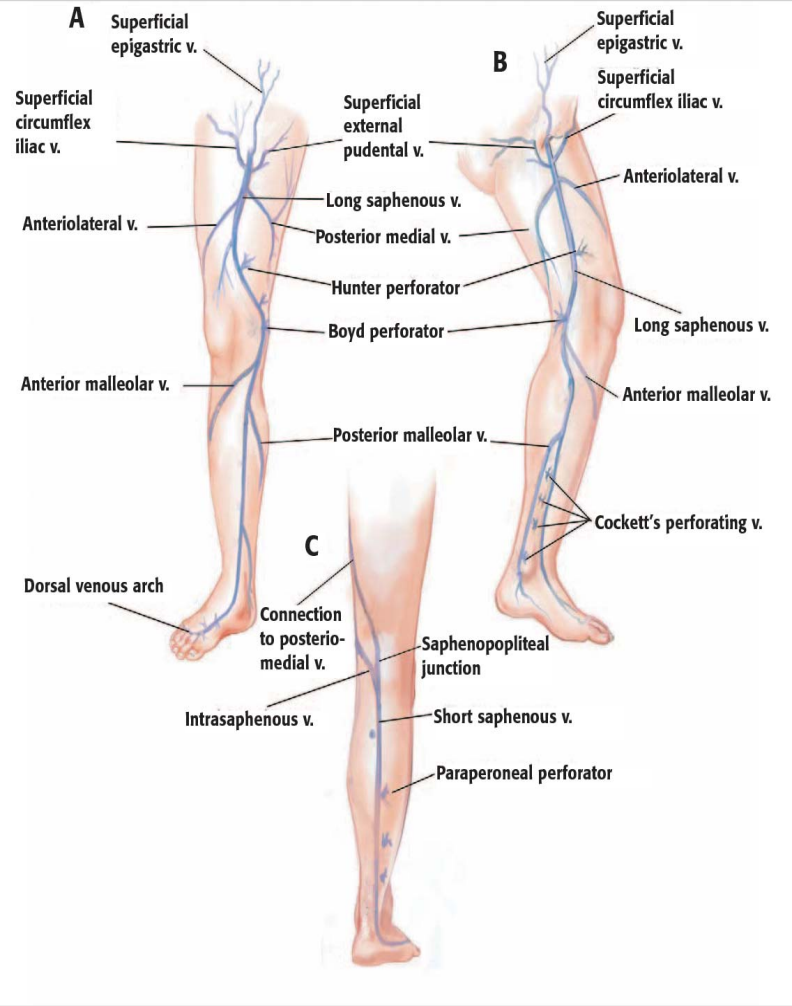


Figure 4: Venous supply around knee joint.[47]

THE LYMPH NODES:-

The lymph from the joint capsule, deep and superficial lymphatic vessels drain lymph into the popliteal lymph nodes situated in the popliteal fossa. The popliteal nodes also receive lymph from the lateral side of the leg through superficial lymphatic vessels. The popliteal nodes also receive lymph from the deep lymphatic vessels which accompany the deep veins of the leg. The deep lymphatic vessels run along sides the deep veins of the leg to the popliteal nodes. Ultimately the lymph gets drained in to the deep inguinal nodes of the thigh through deep lymphatic vessels.[48]

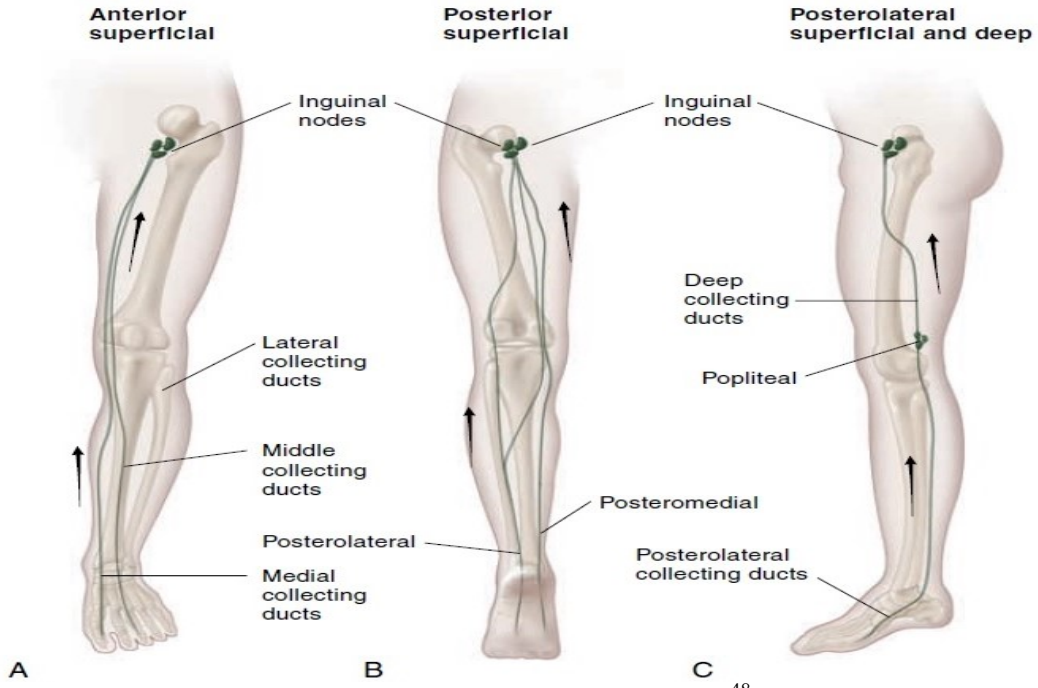


Figure 5: Lymphatic drainage of the lower limb.[48]

NERVE SUPPLY:-

The knee joint is innervated by the branches of important nerves like sciatic nerve, obturator nerve and femoral nerve. The branches from these nerves supply various muscles around the knee joint.

While some of these nerves supply the capsule of the joint and the ligaments; others innervate the capsule to supply the synovial membrane. The nerve supply to knee involves both sensory and motor fibres.

Some gives both sensory and motor fibres to the arteries that accompany them while other are pure sensory fibres.

The fibrous capsule of the knee joint containing sensory fibres is of two types: a) Algesic type and b) Proprioceptive type. The former is responsible for the painful sensation especially when the capsule in overstretched or torn; the later transmit information to all parts of the central nervous system including the cerebellum and the cerebrum. This information includes the position of a resting joint and the rate and extent of motion at a moving joint.

The fibrous capsule of the knee joint is innervated by the sensory nerves at many points which reach the synovial membrane only after innervating the capsule. These sensory nerve fibres then form a wide meshed network in the sub-synovial layer of the joint capsule. These sensory nerves are basically algesic in nature and stimulation of these nerves causes diffuse pain unlike the nerve fibres to the fibrous capsules which causes localized pain. These nerves are found in those areas of the synovial membrane where the fatty pads are in abundance. These fibres are also present in peripheral parts of the articular cartilage especially in the non-articular parts, discs and the menisci. This is the reason why injury to these structures causes excruciating pain. The articulating of the articular cartilage has no nerve supply. [45]

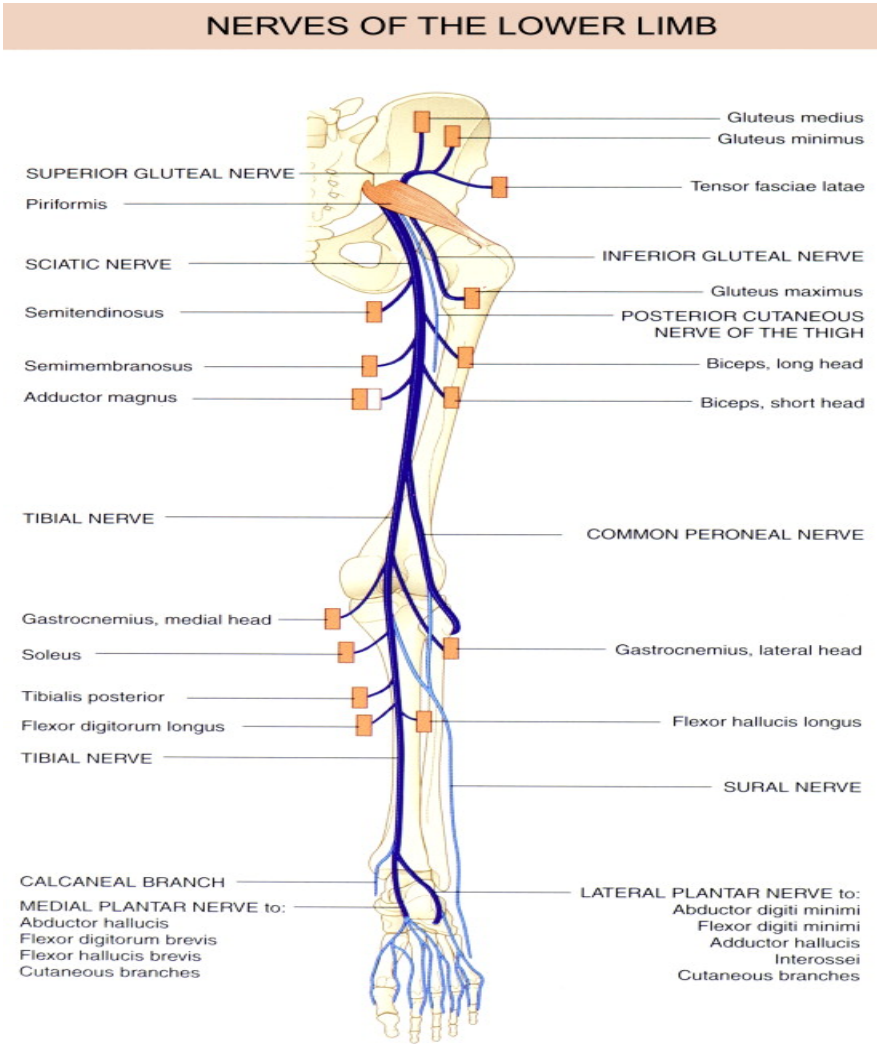


Figure 6: Nerve supply around knee joint and lower limb.[45]

LIGAMENTS: -

Knee joint consists of ligaments into two parts; those which are lying outside the capsule and those lie within the capsule.

Extracapsular Ligaments: -

Ligamentum patellae- It lies outside the capsule. It is the central portion of the common tendon of insertion of quadriceps femoris. The tendon is attached to the apex of the patella above and to the tuberosity of the tibia below.

Lateral collateral ligament- It is a cord like ligament that is superiorly attached to the lateral epicondyle of the femur just above the popliteal groove.

Inferiorly, it is joined by the tendon of the biceps femoris and is attached to the head of the fibula in front of its apex. It represents the femoral attachment of the peroneus longus. The LCL acts as a primary restraint to varus force.

Medial collateral ligament- It is a flat band that is superiorly attached to the medial epicondyle of femur just below the adductor tubercle. Inferiorly, it is divided into superficial and deep part. The superficial part is attached below to the medial border and posterior part of the medial surface of shaft of tibia. Posterior or deep part is short and blends with the capsule and medial meniscus. It is attached to the medial condyle of the tibia above the groove for the semimembranosus.

The posterior surface of the capsule is strengthened by a tendinous expansion derived from the semimembranosus muscle called oblique popliteal ligament.

Intracapsular Ligaments: -

The two strong intracapsular ligaments called cruciate ligaments cross each other within the joint cavity.

Attached to the anterior intercondylar area of the tibia is a ligament called ACL or anterior cruciate ligament, it passes upward, backward, and laterally, to be attached to the posterior part of medial surface of the lateral femoral condyle. A ligament called anterior cruciate ligament or ACL is attached to the tibia around its anterior inter condylar area and then passes upward, backward and laterally and attached to the medial surface of the posterior part of lateral femoral condyle.

The posterior intercondylar area of tibia attaches the posterior cruciate ligament (PCL) and passes upward and then forward and medially to be attached to the anterior part of the lateral surface of the medial femoral condyle. [45]

MENISCI: -

The menisci in the knee joint are basically C shaped fibrocartilages whose peripheral borders are thick and attached to the capsule. The inner border of these menisci is thin and concave in shape and form a free edge. The menisci are C-shaped sheets of fibrocartilage. The upper surfaces are in contact with the femoral condyles. Whereas the lower surfaces of the menisci are in contact with the tibial condyles. [45]

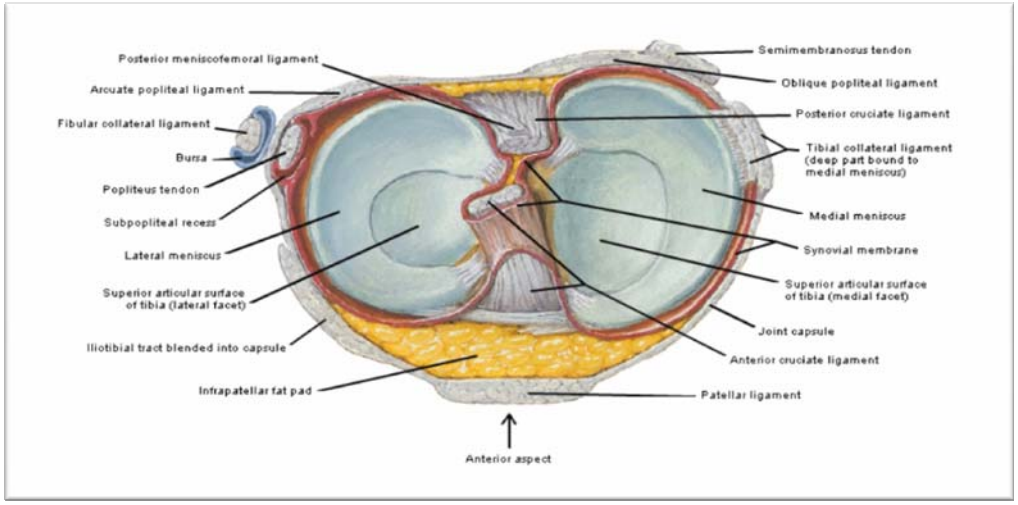


Figure 7: Superior view of knee joint.[45]

JOINT METABOLISM AND NUTRITION: -

The fibrous capsule of the knee has small blood supply and thus has very low rate of metabolism and thus heal slowly when injured. The cells or the chondrocytes of the articular cartilage is responsible for its metabolism.

They have similar rate of carbohydrate metabolism like other cells elsewhere in the body and is not affected by age. On the other hand, the consumption of oxygen

by these cells or chondrocytes reduces with age. This proves that the metabolism in these cells is primarily carbohydrates and proteins and not fats.

The formation of chondroitin sulphate and keratosulphate molecules, the main ingredients of the cartilaginous materials are formed by passage of sulphur from blood through synovial fluid to the chondroitin sulphate and then to the matrix.

Sulfonated hyaluronic acid is chondroitin sulphate which is the characteristic constituent of synovial fluid. The chondrocytes that are responsible for formation of cartilage is confirmed by its presence in the matrix and not in the synovial fluid.

As the age advances, the proportion of chondroitin sulphate reduces and that of keratosulphate increases in the cartilage showing reduction of metabolic activity of the cells. In the skeletal system of the body; only the cartilages, discs and menisci are nourished by the synovial fluid but the rest are supplied by blood vessels directly. [49]

MUSCLES AROUND KNEE JOINT: -

Quadriceps Femoris- A group of four muscles that are present on the front side of the thigh are called quadriceps and are responsible for extending of legs. These muscles then join to form a single tendon called quadriceps tendon which then gets attached to tibia via patella and patellar ligament. These include Vastus medialis, Vastus lateralis, Vastus intermedius and Rectus femoris.

Sartorius- A thin muscle that comes from anterior part of pelvis and attached to medial part of tibia.

Gracilis- This muscle gets attached to the medial part of tibia after coming down from pelvis. This is not a very strong muscle but helps with many movements of hip and knee.

Hamstrings- Three muscles Semimembranosus, Semitendinosus and Biceps femoris forms hamstring muscles and are situated on the posterior side of the thigh and helps in bending of knee.

Gastrocnemius- The medial and lateral gastrocnemius muscles are attached to the heel bone and helps in bending of knee. [45]

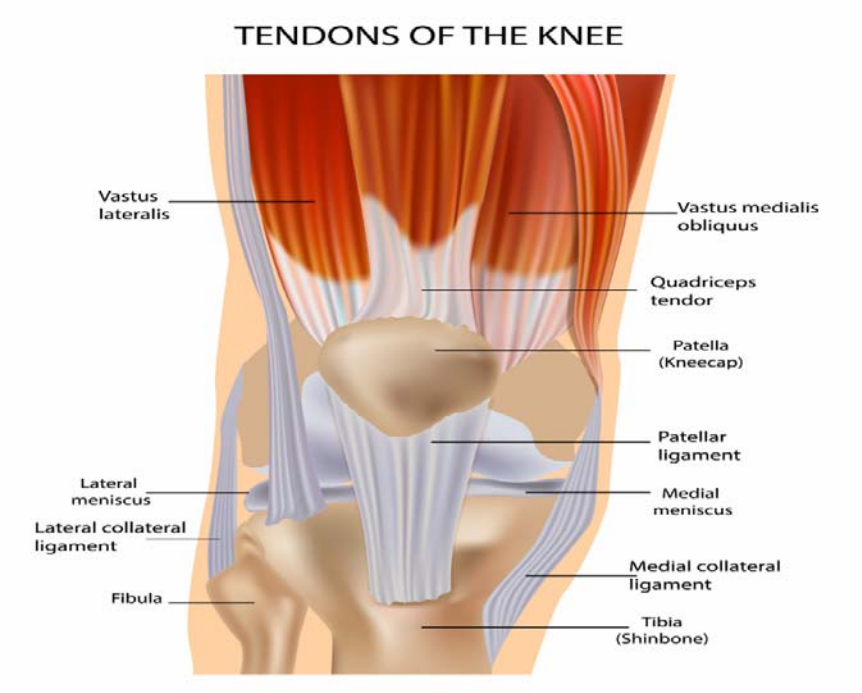


Figure 8: Muscles and tendons around the knee.[45]

SYNOVIAL MEMBRANE: -

The capsule of the knee joint is lined by synovial membrane and the same is attached at the margin of the articular surfaces. The synovial membrane forms a pouch in front and above the knee joint and it extends beneath the quadriceps femoris muscle above the patella for about three finger breadth and thus forming suprapatellar bursa.

The same synovial membrane is prolonged downwards at the back of the joint on the deeper surface of the popliteus tendon forming popliteal bursa.

From the posterior part of the capsule the synovial membrane is reflected forward around the front of the cruciate ligaments. The synovial fluid does not come in contact with the cruciate ligaments because they lie behind the synovial cavity. [45]

BURSAE RELATED TO THE KNEE JOINT

The main function of bursa is to reduce friction between adjacent moving structures.

1. Anterior Bursae:-

- The suprapatellar bursa-

  It is located between the femur and quadriceps muscle, it is attached to articularis genu muscle and communicates with synovial cavity.

- The prepatellar bursa -

  Lies between skin and patella.

- The superficial infrapatellar bursa-

  Lies between skin and tibial tuberosity.

- The deep infrapatellar bursa-

  Lies between patellar ligament and upper tibia.

2. Posterior Bursae: -

- The popliteal bursa
- The semimembranosus bursa-

  Lies between semimembranosus and the medial head of gastrocnemius; may communicate with the bursa under the medial head of the gastrocnemius and thereby the synovial cavity

3. The remaining four bursae are found related to

- The tendon of insertion of the biceps femoris;
- Related to the tendons of the sartorius, gracilis, and semitendinosus muscles;
- Beneath the lateral head of origin of the gastrocnemius muscle; and
- Beneath the medial head of origin of the gastrocnemius muscle. [45]

MOVEMENTS: -

The major function of knee joint includes extension, flexion and rotation. The knee joint can flex, extend, and rotate. In full extension of the knee joint, the femur undergoes medial rotation resulting in twisting and tightening of all major ligaments of the joint and in this position the knee becomes a mechanically rigid structure and the cartilaginous menisci are compressed like rubber cushions between femoral and tibial condyles. This position fully extended knee is called locked position of knee. [45]

MOVEMENT	**PRINCIPAL MUSCLES**
EXTENSION (from sitting on a chair to standing)	Quadriceps femoris (four heads)
LOCKING	Vastus Medialis
UNLOCKING	Popliteus
FLEXION	Biceps femoris Semitendinosus Semimembranosus
MEDIAL ROTATION OF FLEXED LEG	Popliteus Semimembranosus Semitendinosus
LATERAL ROTATION OF FLEXED LEG	Biceps femoris

Table 1: Muscles producing movements at the knee joint. [45]

ARTICULAR CARTILAGE

Hyaline (literally, 'glass-like') cartilage coats the articular surfaces of synovial joints. It is composed of individual chondrocytes bound together by an extracellular matrix.

It is avascular, aneural, alymphatic and almost nonimmunogenic. It is nourished entirely via diffusion from the synovial fluid.

The constituents of the extracellular matrix are

a. Water (75 per cent wet weight of articular cartilage),
b. Proteoglycans (10–15 per cent wet weight).
c. Type II Collagen fibres (almost exclusively type II) constitute around 10–20 per cent wet weight (40–70 per cent dry weight), forming a meshwork with high tensile strength.
d. Chondrocytes (5 per cent wet weight) manufacture and maintain the extracellular matrix. [50]

STRUCTURE OF ARTICULAR CARTILAGE

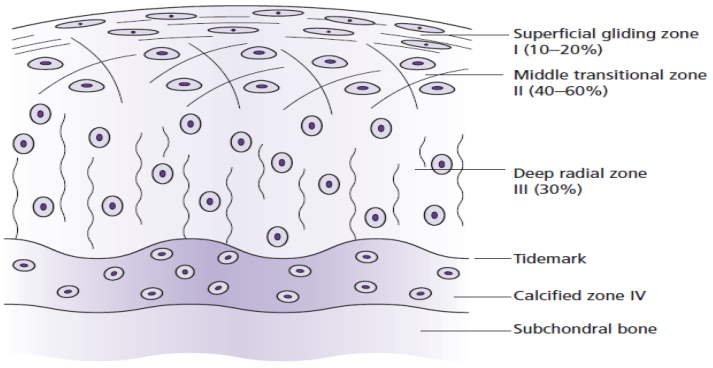


Figure 9: Layers of articular cartilage seen on histological section.[50]

FUNCTIONS OF ARTICULAR CARTILAGE

The major function of hyaline cartilage is to distribute weight-bearing forces and joint lubrication (allowing movement between opposing surfaces with the minimum of friction and wear) and shock absorption (distributing joint loads and therefore reducing the stresses experienced). [50]

CHANGES IN OSTEOARTHRITIC CARTILAGE

	AGEING	OSTEOARTHRITIS
Water content	Decreases	Increases (90% compared with normally 65-80%)
Synthetic activity	Decreases	Increases
Collagen	Unchanged	Breakdown of matrix framework leads to decrease in collagen, but relative concentration increases due to loss of PGs
PG content	Decreases (length of protein and GAG chains decreases	Decreases
PG synthesis	Decreases	Increases
PG degradation	Decreases	Increases very significantly
Chondroitin sulphate (4- and 6-)	Decreases	Increases
Keratan sulphate	Increases	Decreases
Chondrocyte size	Increases	
Chondrocyte number	Decreases	
Modulus of elasticity	Increases	Decreases due to increased water content; increased water content also causes increased permeability and decreased strength
Enzymes		Increased activity of MMPs
Matrix subunit molecules		Increased levels e.g. COMP, aggrecan (in synovial fluid and serum)

Table 2: Biochemical changes seen with ageing and osteoarthritis in cartilage.[50]

OSTEOARTHRITIS OF KNEE

Osteoarthritis (OA) is a chronic degenerative disease of the joint resulting from the degradation of articular cartilage, degradation and proliferative reformation of subchondral bone & a low degree of synovitis that leads to a reduced quality of life (QoL).[51]

Osteoarthritis is a chronic arthropathy, which is characterized by debilitating pain and consequent hampering of day-to-day activity, most commonly affecting the knee and the hip joints. Due to the high use and stress, knee joint is a frequent site for painful conditions including OA.[52]

Osteoarthritis is the most frequent joint disease with a prevalence range of about 22% to 39% in our country. OA is more commonly seen in women than in men, but the prevalence increases dramatically with the age. Nearly about 45% of women > 65 years age have symptoms, while radiological evidence can be found in 70% of those > 65 years.[53]

Osteoarthritis knee is one of the leading causes of mobility impairment, particularly among females & is estimated as the 10^{th} leading cause of nonfatal burden. Osteoarthritis knee is found to have high prevalence rate compared with other sites of OA. The incidence increases with age & with longer lifetime and higher average weight of the population, particularly in obese women.[54]

It is a chronic disorder which has disintegration and softening of articular cartilage with reactive phenomena like osteoblastic activity and vascular congestion in the subarticular bone, new growth of cartilage and bone at the joint margins and fibrosis of the capsule. OA is not accompanied by any systemic illness and although there are some signs of inflammation, it is not an inflammatory disorder.

OA is best viewed as a dynamic repair process of synovial joint. OA is caused by disintegration of the cartilage that covers the ends of bones. As the cartilage wears away, the roughened surface of the bone is exposed which leads to pain and stiffness. With erosion of the surface, there is a tendency of fissuring of the cartilage. There is poor apposition of opposite joint surfaces due to the cartilage wearing out. The joint margins develop enlarging protuberances-called osteophytes. They are permanent and progressive and cause apparent swelling of the joints. Although, it is less crippling than in rheumatoid arthritis.[55]

ETIOLOGY:

The causes of OA are many that include physical trauma, mechanical forces, inflammation, many biochemical reactions, and metabolic derangements.[56] Role of inflammation in OA is not clear, it is still not known if inflammation stimulates OA or is secondary to OA.[57, 58] Earlier studies showed that only the cartilaginous tissue is responsible for the disease but now it is clear that only cartilaginous tissue cannot be responsible alone because it lacks both vascular and nerve supplies and thus cannot cause pain and inflammation. Therefore, it was realized that the pain in OA mainly comes from structures like capsule, ligaments, periarticular muscles and subchondral bones.[56, 57] As a result of involvement in the disease process, these structures get damaged that includes remodelling, formation of osteophytes weakening of muscles and ligaments and joint effusion.[58]

Inflammation of synovium is commonly seen in OA and can be observed even in the early stage of the disease and as the disease progresses the severity of inflammation increases.[59] The analysis of the synovial fluid in an advance case of OA shows many inflammatory mediators that also includes plasma proteins like C-reactive protein

(CRP), prostaglandin E2 (PGE_2), tumor necrosis factor (TNF), Interleukin (IL1β, IL6, IL15, IL17, IL18, IL21), transforming growth factor beta (TGFβ), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), nitric oxide, and complement components.[60] The major problem with increased levels of these components is that they stimulate many hydrolytic enzymes locally causing severe damage to the cartilage and collagen. However, body also has a protective mechanism to counter this unregulated damage by releasing macrophages and mast cells and also insulin like and platelet derived fibroblast 18 and transforming growth factor B. Unfortunately, in severe OA these protective factors get changed and become injurious to the joint.[61] Recent evidence suggests that OA is a process that is characterized an adaptive response of the synovial joint to genetic, biochemical and other environmental stress.[62, 63]

It has been postulated that movement of the joint is essential for its health because the main nutrition for the chondrocytes come from the synovium by the process of diffusion and this can happen only when the synovial fluid circulates due to movement of the joint. Now the moment the joint stop moving due to immobilization for various reasons, the chondrocytes cannot get the nutrition from the synovium and stops repairing the damaged cartilage.[64]

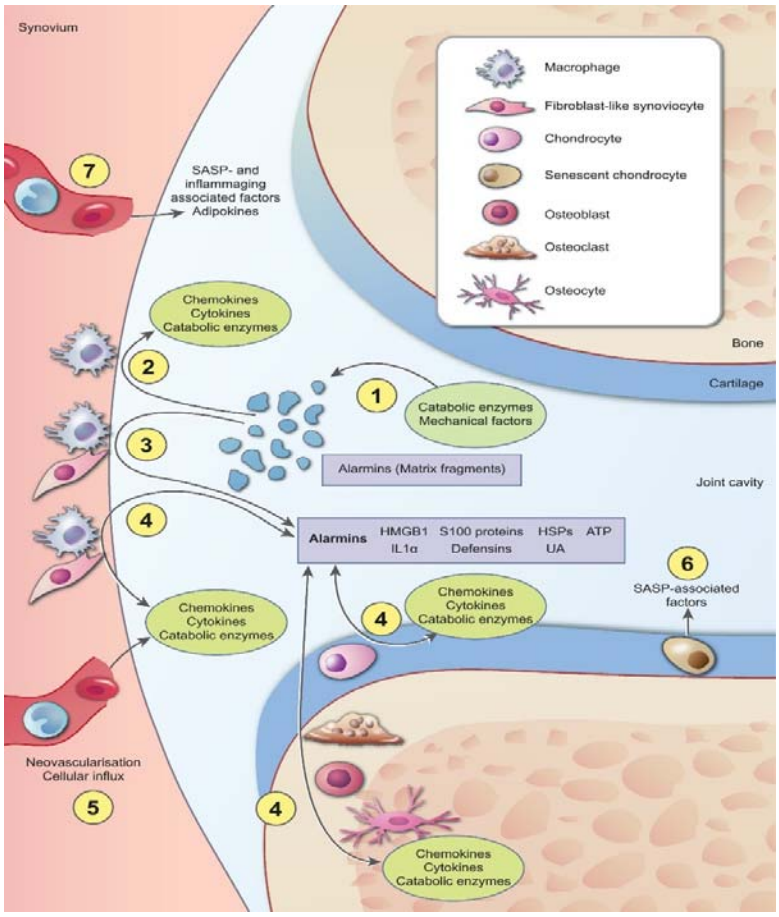


Figure 10: Schematic representation of a normal and OA affected joint.[60]

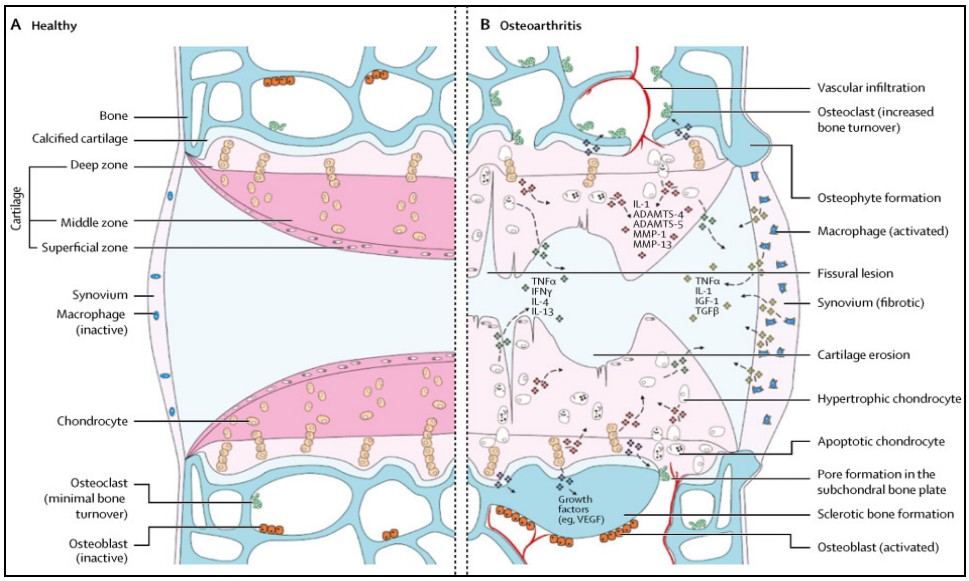


Figure 11: Comparison between normal and OA joint.[61]

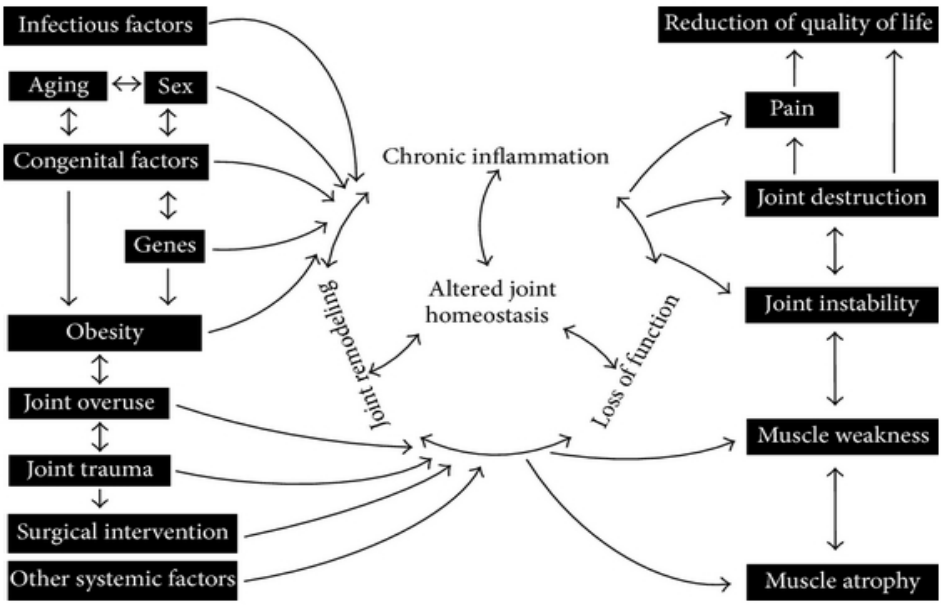


Figure 12: Schematic showing a closed disease circle comprising the disease progression of Osteoarthritis taking into accounts its cause and consequences.[64]

ROLE OF CYTOKINES IN THE ETIOPATHOGENESIS OF OSTEOARTHRITIS:

As discussed above since we cannot prevent some of the causes of OA like trauma, age, sex and genetics, however, we can at least control the progression of the disease by better understanding it at the cellular level through controlling the activities of cytokines. By better understanding of the activities of these cells, suitable antibodies and other biological factors can be developed to stop the progress of this debilitating disease. It seems that both inflammatory and anti-inflammatory cytokines are involved in the pathogenesis of this disease. In OA these cytokines act depending on the severity and duration of the disease.[61]

The total process of a balance in anabolism and catabolism in the tissues are important especially in maintaining the health of a joint and cytokines play a major role in

disturbing this process.[62] Once the balance gets disturbed there is rapid degeneration of the cartilage of the joint and as a result the disease process rapidly progresses with inflammation, degradation and destruction and as a result there is loss of joint function, pain and inflammation which together lead to a gradual loss of joint function and pain. We come across two types of cytokines in the body one is inflammatory and the other anti-inflammatory.[63] In OA the inflammatory cytokines play a dominant role and acting synergistically along with other cellular mediators initiates major catabolic activity in the joint. However, a minor role of anti-inflammatory cytokines cannot be ignored. [64]

INFLAMMATORY CYTOKINES

These cytokines play an important role in the pathogenesis of this disease. The catabolic and degenerative activity of these cytokines disturbs the homeostasis in the tissues like cartilage and causes rapid destruction. The major cytokines in this group are IL-1, TNF, IL-6, IL-7 and IL-18.[65]

I) Interleukin-1 Beta (IL-1β)

This is a major cytokine responsible for destruction of articular cartilage and other structures of the joint and induce inflammation. The synthesis of this interleukin is governed by the mononuclear cells present in the joint or entered the joint cells during inflammation.[65, 66] The proof of its role is confirmed by the elevated levels of this cytokine in the synovial fluid, cartilage, synovial membrane and subchondral bone of patients suffering from OA.[67, 68]

This cytokine also blocks chondrocytes from synthesizing of type II collagen and aggrecan and several other metalloproteinases. [69, 70]

In addition to the above, this cytokine stimulates the production reactive oxygen species thereby generating many peroxides hydroxyl radicals which cause major damage to the articular cartilage also this is further aggravated by the inhibition of protective oxidative enzymes.

II) Tumour Necrosis Factor Alpha (TNFα)

This is another key cytokine responsible for inflammatory responses in OA. Cells in the joint that secrete IL-1β also secretes this cytokine. The levels of this cytokine is also increased along with IL-1β.TNFα is secreted by the same cells in the joint cavity that synthesize IL-1β. The TNFα cytokine can attach to two receptors in the joint cavity, which are TNF-R1 and TNF-R2 but major destructive effect is observed through TNF-R1.[60, 61]

III) Interleukin-6 (IL-6)

This is considered a type of cytokine that stimulates immune system in the body and increases inflammatory response. In the OA affected joint chondrocytes, osteoblast, macrophages and adipocytes stimulates the release of IL-6 mediated by IL-1β and TNFα. Increased levels of IL-6 in the synovium and the synovial fluid correlates with the intensity of damage seen in radiographs. The basic effect of IL-6 on the cartilage is similar to other cytokines decrease in production of type II collagen and increased production of enzymes from matrix metalloproteinases (MMPs) group. Any injury to the joint enhances the damaging effect of IL-6. It causes changes in subchondral level bone layer and stimulates the release of osteoclast and thus enhances bone resorption. Its secretion by osteoblasts and chondrocytes is also affected by PGE2. The increased concentration of IL-6 is present in both the synovial fluid and serum and is positively correlated with the intensity of lesions in X-ray imaging.[71, 72]

IV) Interleukin-15 (IL-15)

Interleukin-15 (IL-15) helps in differentiation and proliferation of T cells and NK cells. It is implicated in the pathogenesis of Rheumatoid arthritis. About its role in OA, this cytokine has been found to be in high concentration in the synovial fluid in early cases.[73,74]

IL-15 has been found to be responsible for increased pain sensation and high degree of damage in OA.[74]

V) Interleukin-17 (IL-17)

IL-17 is considered a group of cytokines responsible for severe inflammation in OA. Basically IL-17 is a stimulated $CD4^+$ and mast cells that enter the synovial membrane and the joint through blood vessels and their levels were also found to be elevated in the serum and synovial fluid.[75, 76] IL-17 mainly affects the chondrocytes and fibroblast-like synoviocytes (FLS). Moreover, during the course of the disease IL-17 stimulates T cells against the membrane antigens of chondrocytes and fibroblasts. IL-17 also stimulates secretion of vascular endothelial growth factor (VEGF) by chondrocytes and favours excessive development of blood vessels in synovial membrane causing its hypertrophy. [77]

VI) Interleukin-18 (IL-18)

Interleukin-18 (IL-18) level has been found to be increased in synovial fluid, cartilage and blood serum and correlates well in terms of severity of the disease.[77, 78]

ANTI-INFLAMMATORY CYTOKINES

IL-4, IL-10 and IL-13 are the cytokines known to be anti-inflammatory in nature and are involved in pathogenesis of OA, are chondroprotective in nature.

I) Interleukin-4 (IL-4)

The activity of Interleukin-4 (IL-4) is mediated through some receptor system which is same for both IL-4 and IL-13. Production of IL-4 is mediated by T-cell via Th2 mechanism and it infiltrates the synovium of the joints after passing through blood vessels and their level has been found to be elevated in synovial fluid and cells. In patients suffering from OA, the levels of gene IL-4RA has been found to be elevated compared to normal individuals. This cytokine has chondroprotective effect but in advance cases the chondrocytes are found to be less susceptible to the protective effect of IL-4 which causes rapid degeneration of the articular cartilage. It also causes reduction in the secretion of PGE2, COX-2 and PLA2 and other inflammatory mediators under the influence of IL-4.[79, 80]

II) Interleukin-10 (IL-10)

This chondroprotective cytokine is related to interferons at least structurally. It also stimulates synthesis of type II collagen and aggrecan. It has been reported to increase the levels of proteoglycan synthesis and also its level in extra cellular matrix. It has been shown to stimulate chondrocyte proliferation and inhibits apoptosis. IL-10 is an important indicator for the OA. It has been reported that those patients whose blood samples does not show any increase in the level of IL-10 compared to base line values are running a 3-fold risk of developing OA compared to those who have high level of this cytokine. The anti-inflammatory and chondroprotective nature of IL-10 is composed of results of interesting studies of the influence of exercise on the secretion of cytokines in joint fluid and periarticular tissues in patients with OA of the knee joints.[81, 82]

III) Interleukin-13 (IL-13)

This cytokine is similar to IL-4 in effect and like IL-4 its activity is also mediated through the same receptor system. Like IL-4 this also has chondroprotective and anti-inflammatory actions. It acts on the cells of immune system synovium and the articular cartilage. It exhibits its anti-inflammatory actions by inhibiting the secretion of macrophages, monocytes, natural killer (NK) cells, B-cells and endothelial cells. IL-13 showed inhibitory effects on the synthesis of IL-1β, TNFα, and MMP-3 the pro-inflammatory cytokines and the same time increased the levels of interleukin-1 receptor antagonist (IL-1Ra).[83]

In comparison to IL-4, IL-13 does not affect phospholipase A2 (PLA2) production. IL-13 also selectively inhibit cyclooxygenase-2 (COX-2). The analysis of these results indicates the potential utility of IL-13 in the treatment of OA, as a compound that inhibits the inflammatory processes, protects chondrocytes, reduces the secretion of inflammatory cytokines and metalloproteinases, while stimulating the synthesis of IL-1Ra.[83]

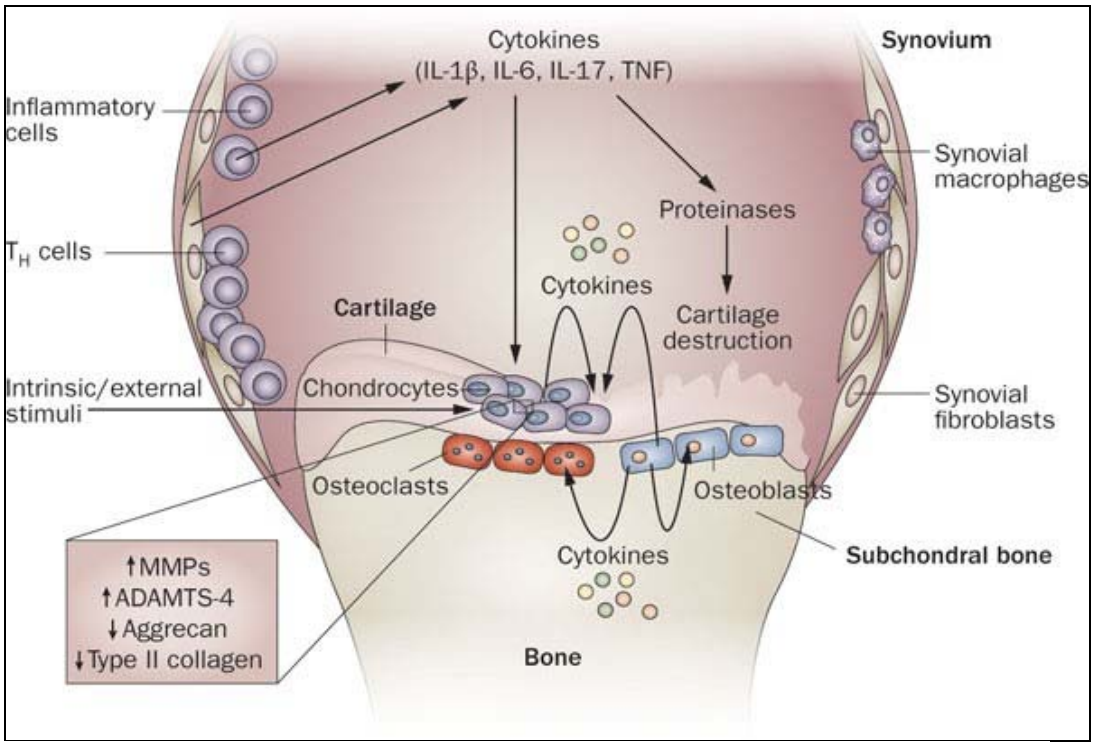


Figure 13: Role of proinflammatory cytokines in the pathophysiology of osteoarthritis.[83]

PRIMARY OA KNEE:-

Primary OA occurs more often than secondary. It is a slow, progressive condition that affects mainly the knees and hips which are weight-bearing joints of the body; but it can also affect the lower back, neck, and fingers. The main contributing factors are obesity and family history. There are many theories for the cause of primary OA, including the following:

1. Possible changes in the cartilage matrix: Chondrocytes (responsible for maintaining a normal blend of collagen), proteoglycans, and water, sometimes mix the balanced formula. The body compensates by producing more chondrocytes which, in turn, causes greater amounts of collagen and proteoglycans to be manufactured. There is

also an excess build-up of fluid which removes these newly synthesized molecules leaving fewer than before.

2. Out of control enzymes: While chondrocytes make collagen and proteoglycans, they are also responsible for producing enzymes that break down these ageing molecules. Consequently, more enzymes are produced when more chondrocytes are produced to try and rectify a possible change in the matrix. More cartilage is destroyed than formed which is the opposite of the intended purpose. When a joint is filled with these enzymes, the collagen fibers in the cartilage become smaller and the netting that they provide, starts to relax. Therefore, the proteoglycans, that are normally held in place by the collagen, now begin to move away and disappear. Without enough proteoglycans to attract and retain water, the cartilage begins to dry out, becoming more susceptible to cracking, fissuring, and wear and tear.

3. Trauma to the subchondral bone: This portion of the bone, located directly under the cartilage, may be damaged by an injury or by repeated stress to the joint, which leads to bony overgrowth and joint damage.

4. Bone disease: A problem with the blood supply can weaken the bone, which ultimately leads to tiny fractures and osteonecrosis (bone death). Excessive intake of alcohol, infection, and trauma are among the possible primary causes.

5. Abnormal liver function: The liver is a source of many hormones, growth factors and substances that aid cartilage and bone formation. Bony overgrowths and cartilage destruction occur if there is any chronic abnormality in its function.[84]

SECONDARY OA KNEE:

Secondary OA is quite different from the primary form. It often appears before the age of 40 and has a clearly defined cause. All of the following risk factors alter cartilage in some way and accelerate the rate of cartilage loss:

1. Trauma, as sprains, strains, joint dislocations and fractures.

2. Long-term mechanical stress associated with athletics, ballet dancing, or such repetitive physical tasks as tennis, gardening, typing, or even knitting.

3. The presence of inflammation in joint structures where inflamed cells release enzymes capable of digesting cartilage cells.

4. Joint instability caused by damage to supporting structures, usually of the joint capsule, ligaments or tendons.

5. Neurologic disorders, as diabetic neruopathy or Charcot's disease, where pain and reflexes are diminished or lost, increase the tendency for abnormal movement, positioning or weight-bearing.

6. Congenital or acquired skeletal deformities.

7. Blood or endocrine disorders, as hemophilia, which causes chronic bleeding into the joints or hyperparathyroidism which causes bone to lose calcium.

8. Drugs, as colchicine, indomethacin, and steroids which stimulate the activity of collagen-digesting enzymes in the synovial membrane.[84]

RISK FACTORS:

1. Age

Age is the major cause of development of OA because with increasing age with lack of exercise the muscles around the joint become weak, there is increased cartilage calcification, poor chondrocyte function and less joint proprioception. It has been

proven that at least 27% of those between 63-70 years of age have some radiological proof of OA, this percentage increase to 44% in persons who are more than 80 years of age.[85]

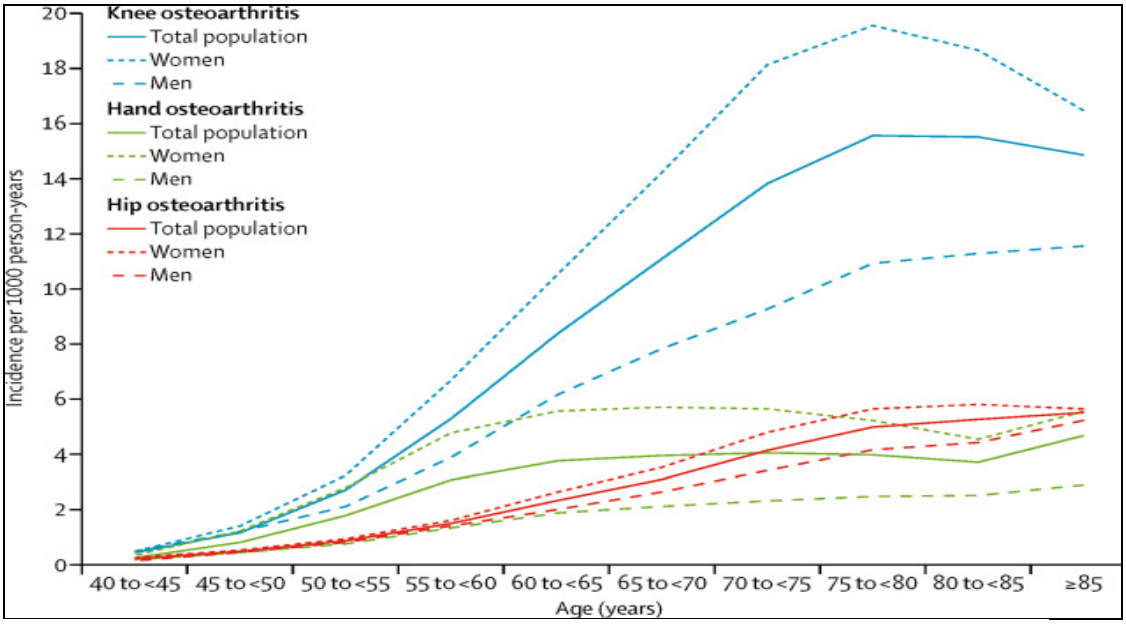


Figure 14: Age and sex –specific incidence rates (/1000 person-year) of knee, hand and hip osteoarthritis.[85]

2. Trauma

Any fracture or knee injury in the past increases chances of OA several folds. Any tear to meniscus, collateral ligament or cruciate ligament in the past leads to OA. For example, meniscectomy in a young adult post knee injury can lead to OA of knee. Many studies conducted so far show that joint injuries increase chances of OA many folds and the development of OA depends on age of the patients at the time of injury. Some studies have even reported an incidence of OA ranging from 20 to 50% in patients post injury. Most of the post-traumatic OA (PTOA) are seen in younger patients as compared to older patients.

The post-traumatic OA mainly causes meniscal tear, injury to ligaments and chondral injury as well as intra-articular fractures. In most of the cases, PTOA cannot be diagnosed for months after the injury unless the patient develops symptoms. This may occur very early or may be delayed for months after sustaining the injury sometimes even after 10-20 years of sustaining the injury. Nevertheless, the pathological process in PTOA involves apoptosis of the articular chondrocytes, cellular infiltration and releasing of pro-inflammatory cytokines in the synovial fluid.[85]

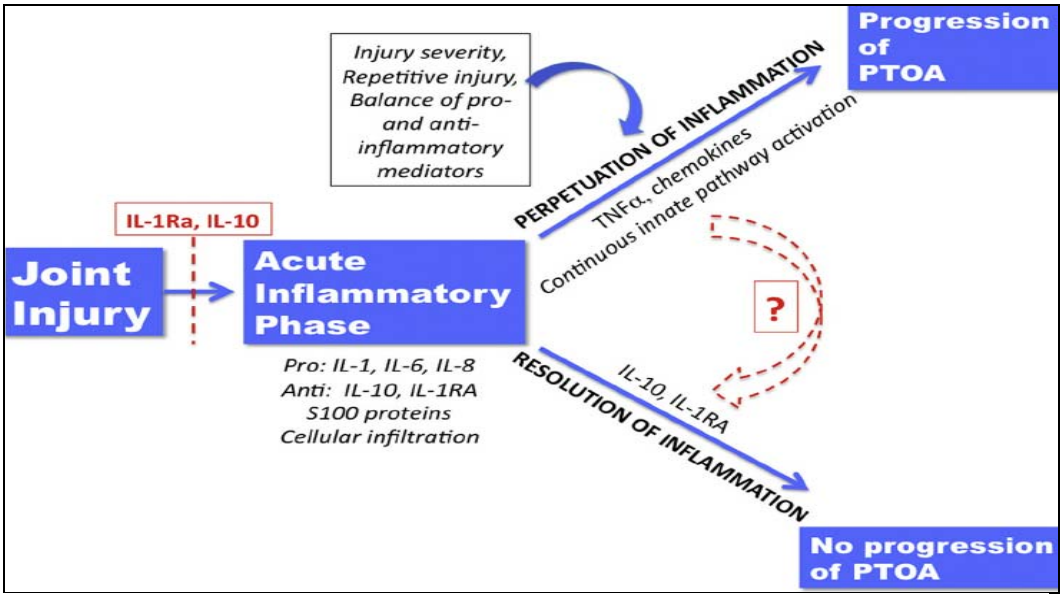


Figure 15: Perpetuation of inflammation as a mechanism leading to PTOA.[86]

3. Occupation

Excessive strenuous work, lifting heavy weight, frequent knee bending, squatting, kneeling leads to the development of OA.One of the most common occupational risk factors for knee OA is heavy physical work load.[56] Many other activities which can exaggerate the OA are like lifting heavy weight, frequent kneeling, bending, squatting and standing for more than 2 hours, walking for more than 3 km/day, jumping and vibration in knee.[87] Another study reported a five-fold increase in chances of OA among workers who are lifting heavy, squatting and climbing stairs and are more than

55 years of age. One study has shown that chances of OA is high among women who stand for a long time on a rigid surface and men who climb and jolt on the stair case regularly.[88]

4. Exercise

Recent studies have also shown that regular normal exercise for long period of time also does not affect the joint but subsequent studies have shown extensive and high intensity exercise in professionals at the highest level of participation causing likelihood of joint injury may develop OA over a period of time. Athletes who are in high impact sports are likely to develop OA because the repeated impacts the joint becomes weak and loses its stability resulting in weak quadriceps that reduce shock absorbing capacity and accelerate OA.[89]

5. Gender and ethnicity

Postmenopausal women have higher risk of developing OA and this may be because of lack of oestrogen which triggers the process; however, the scenario is just reverse under 50 years of age where men have higher risk of OA than women. At the moment role of oestrogen replacement therapy in the prevention of OA in post-menopausal women is still debated.[90]

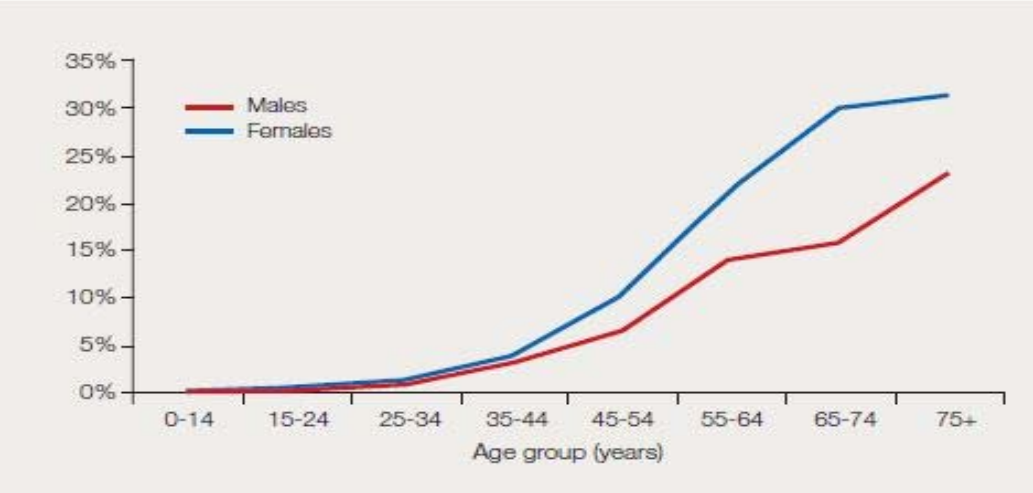


Figure 16: Incidence of OA in relation to Age and Sex.[91]

6. Genetics

Loughlin J. et al, in their study in 2000 aimed to test chromosome 2q for linkage to idiopathic osteoarthritis. The authors used a cohort of 481 OA families that contained at least one affected sibling pair with severe end-stage disease, following which they conducted a linkage analysis of chromosome 2q using 16 polymorphic microsatellite markers. They concluded that chromosome 2q is likely to contain at least one susceptibility locus for OA.[92] Many genes have been linked to osteoarthritis. OA risk variants are enriched near genes involved in skeletal development and morphology, and show genetic overlap with height, hip shape, bone area and developmental dysplasia of the hip. A study conducted in the US has shown a genetic component for OA which gets transmitted by a non-Mendelian way and is typical for a multifactorial disease. It has also been reported that depending on gender, joint affected and the severity of the disease the range of heredity is from 40-65%. The higher incidence of hereditary is for the hand and hip and less for the knee, but overall appear stronger for hand and hip OA than for knee OA. Another study in the UK clearly shows that OA is not a simple wear and tear disease and with the identification of many genetic loci this is now established that OA is a genetic linked disease. There are now studies to show

various phenotypes of OA cases the external driver being structures like synovium, articular cartilage and bone and internal drivers like injury, inflammation, immunity metabolic factors but at the end the synovial tissue and the joint playing a dominating role.[93]

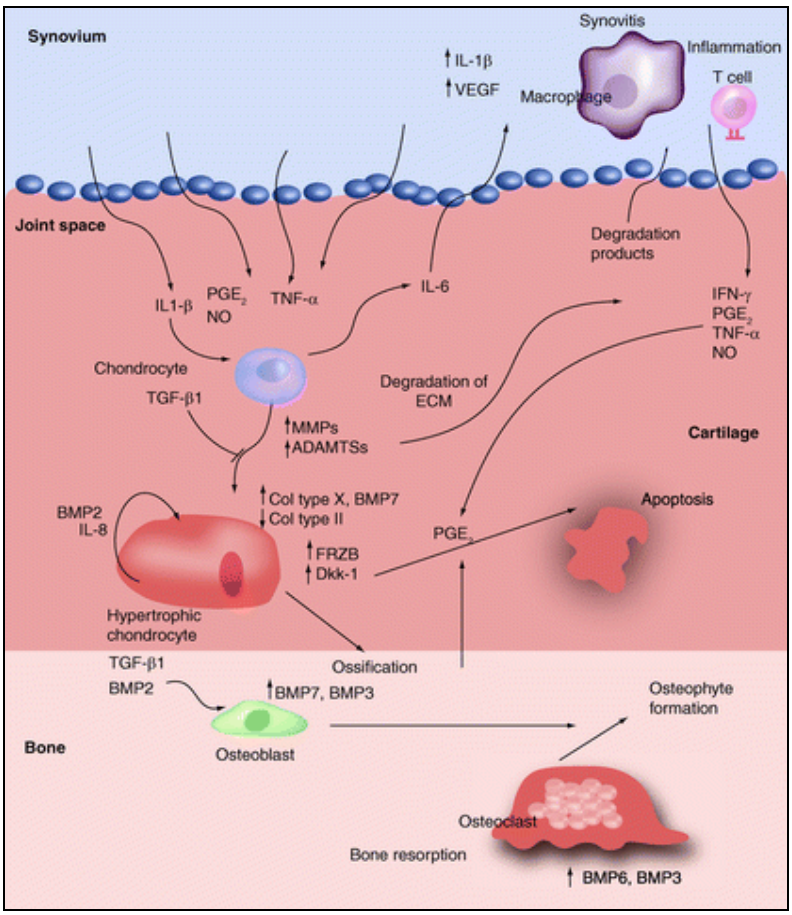


Figure 17: Molecular pathogenesis and genetics of osteoarthritis.[94]

7. Obesity

The major triggering factor for the developing OA is obesity. During walking 3-6 times of one's body weight is transferred to the knee while walking. Therefore, any increase in body weight if multiplied by this factor will be transferred to the knee joint thereby accelerating the OA. It has been observed that every two unit increase in body mass the chances of OA increases by 1.36.[95]

Increase or decrease in body weight in young age becomes a major risk factor to develop OA in old age. For example, if a person is obese at the age of 35-36, his or her risk of developing OA is extremely high in later age. However, weight reduction in a woman who is suffering from OA, the risk symptomatic OA reduces more than 50%.[96-99]

One study showed involvement of aberrant adipokine leading to destruction of joint tissue. These adipokines affect cartilage, synovium and bone and other joint tissue.[100] Adipokines like leptin and adiponectin exert effects on the joint tissue, including cartilage, synovium and bone. The receptors of these adipokines are located on the surface of chondrocytes, synoviocytes and subchondral osteoblasts. These adipokines acts by increasing the levels of destructive enzymes like nitic oxide and MMPs and then produces cytokines which are pro-inflammatory and thus causing wide spread destruction.Although adipokines are present in normal as well as overweight individuals but the way they respond in obese individual are totally different. The levels of these agents are also increased significantly in obese individuals with OA compared to controls. It was reported in a recent study that the level of these pro-inflammatory agents started reducing the moment the obese individual with OA started significant loss of weight.[101]

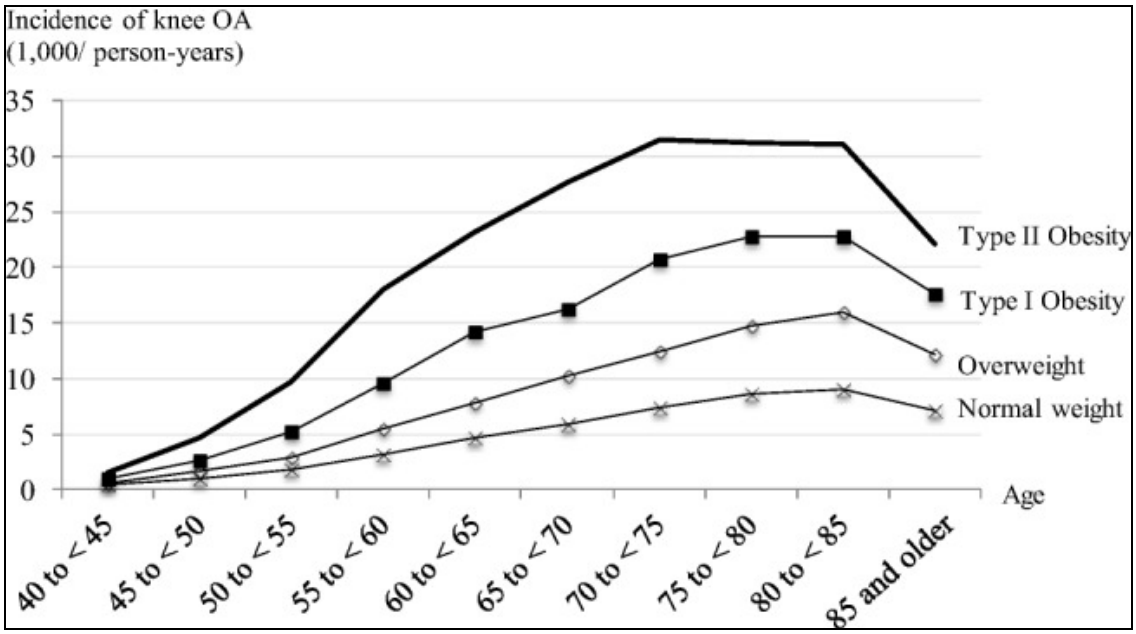


Figure 18: Incidence of knee osteoarthritis (OA) per age group according to each range of body mass index (BMI) per 1,000/person-years at risk.[102]

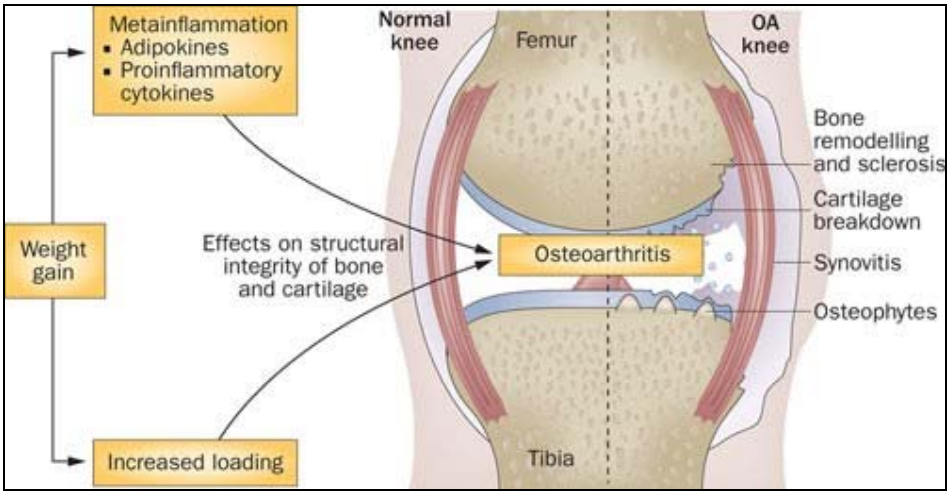


Figure 19: Effect of obesity in knee osteoarthritis.[103]

8. Diet

Regular intake of diet rich in vitamin C, antioxidants and vitamin D reduces the risk of OA. People with low vitamin C and vitamin D in blood may have higher risk of developing OA although role of vitamin D in prevention of OA is not proven.[104, 105]

9. Bone density

Recently, several longitudinal studies have shown higher bone mineral density (BMD) to be associated with a greater risk of developing subsequent radiographic knee OA, although findings have not been conclusive. Although the populations studied have been predominantly female, there is also evidence of a positive association between BMD and incident knee OA in male populations. A similar association between higher BMD and incident radiographic hip OA has been reported in postmenopausal women. Despite the weight of evidence that systemic BMD and radiographic OA are positively associated, some inconsistencies and areas of controversy remain. Studies examining the relationship between OA and rates of bone loss, also inversely related to BMD, have reached similarly opposing conclusions, with bone loss in individuals with OA found to be reduced, increased or variable depending upon the site of assessment of both OA and BMD. Findings from prospective studies that greater bone loss and turnover may be associated with more rapid OA progression would seem at odds with those discussed earlier, suggesting that higher, rather than lower, BMD is a risk factor for OA. One potential explanation is that OA develops over time in a phasic manner, with periods of active bone turnover corresponding with radiographic progression interspersed with quiescent phases in which bone turnover may be normal or reduced. [106-109]

PATHOLOGY

The pathology of OA provides evidence of the involvement of many joint structures in disease. Cartilage initially shows surface fibrillation and irregularity. As disease progresses, focal erosions develop there and these eventually extend down to the subjacent bone. With further progression, cartilage erosion down to bone expands

to involve a larger proportion of the joint surface, even though OA remains a focal disease with non-uniform loss of cartilage.[109]

After an injury to cartilage, chondrocytes undergo mitosis and clustering. While the metabolic activity of these chondrocyte clusters is high, the net effect of this activity is to promote proteoglycan depletion in the matrix surrounding the chondrocytes.[110] This is because the catabolic is greater than the synthetic activity. As disease develops, collagen matrix becomes damaged, the negative charges of proteoglycans get exposed and cartilage swells from ionic attraction to water molecules. [110]

Because in damaged cartilage proteoglycans are no longer forced into close proximity, cartilage does not bounce back after loading as it did when healthy, and cartilage becomes vulnerable to further injury. Chondrocytes at the basal level of cartilage undergo apoptosis. With loss of cartilage come alterations in subchondral bone. Stimulated by growth factors and cytokines, osteoclasts and osteoblasts in the subchondral bony plate, just underneath cartilage, become activated.[110]

Bone formation produces a thickening and stiffness of the subchondral plate that occurs even before cartilage ulcerates. Trauma to bone during joint loading may be the primary factor driving this bone response with healing from injury (including microcracks) producing stiffness.[111]

Small areas of osteonecrosis usually exist in joints with advanced disease. Bone death may also be caused by bone trauma with shearing of microvasculature, leading to a cut off of vascular supply to some bone areas. At the margin of the joint, near areas of cartilage loss, osteophytes form. These starts as outgrowths of new cartilage and with neurovascular invasion from the bone, this cartilage ossifies. Osteophytes are an important radiographic hallmark of OA. In malaligned joints, osteophytes grow larger on the side of the joint subject to most loading stress (e.g., in varus knees, osteophytes grow larger on the medial side). The synovium produces lubricating fluids that minimize shear stress during motion.[111]

Additional pathologic changes occur in the capsule, which stretches, becomes oedematous and can become fibrotic.In healthy joints, the synovium consists of a single discontinuous layer filled with fat and containing two types of cells, macrophages and fibroblasts, but in OA, it can sometimes become oedematous and inflamed. There is a migration of macrophages from the periphery into the tissue and cells lining the synovium proliferate. Enzymes secreted by the synovium digest cartilage matrix that has been sheared from the surface of the cartilage. [111]

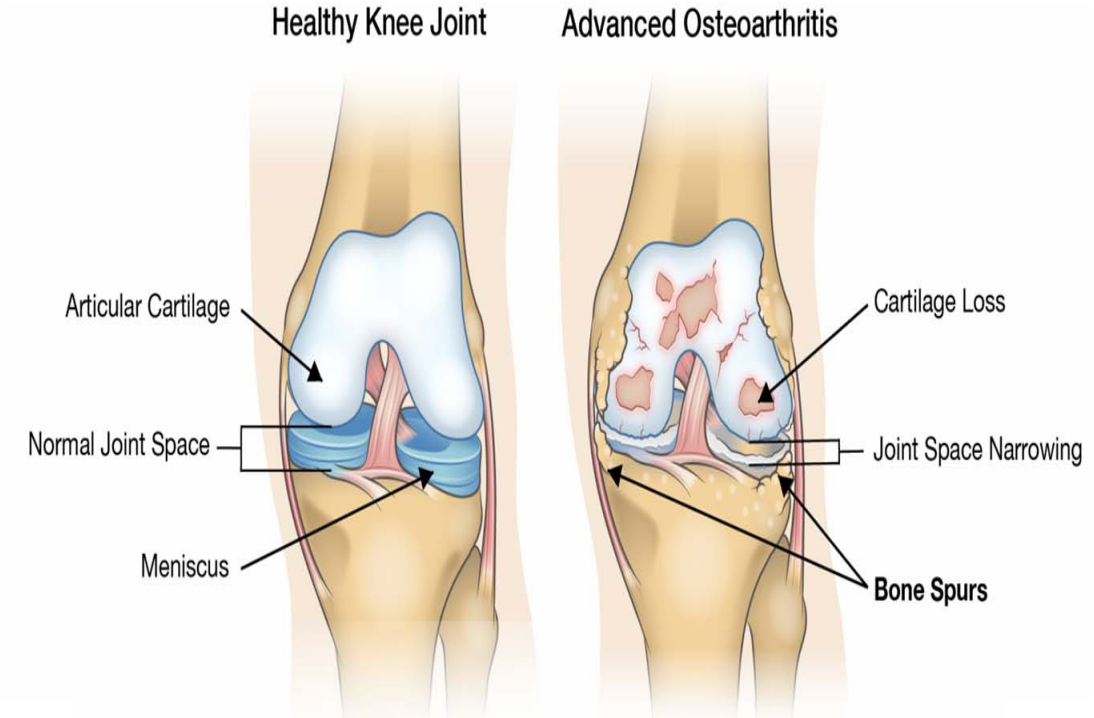


Figure 20: Osteoarthritis of knee. [112]

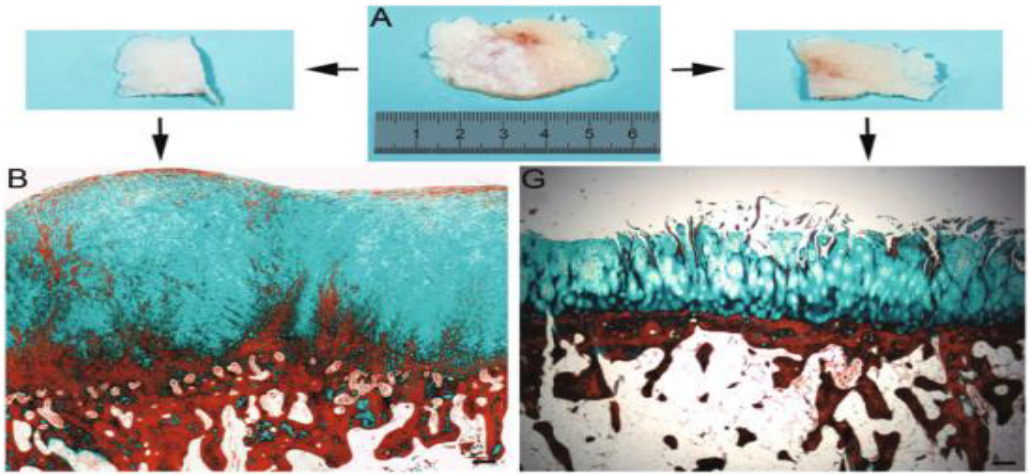


Figure 21: A) Macroscopic morphology of osteoarthritic cartilage,

B) and G) show panoramic images of the sample (Masson's trichrome staining). [113]

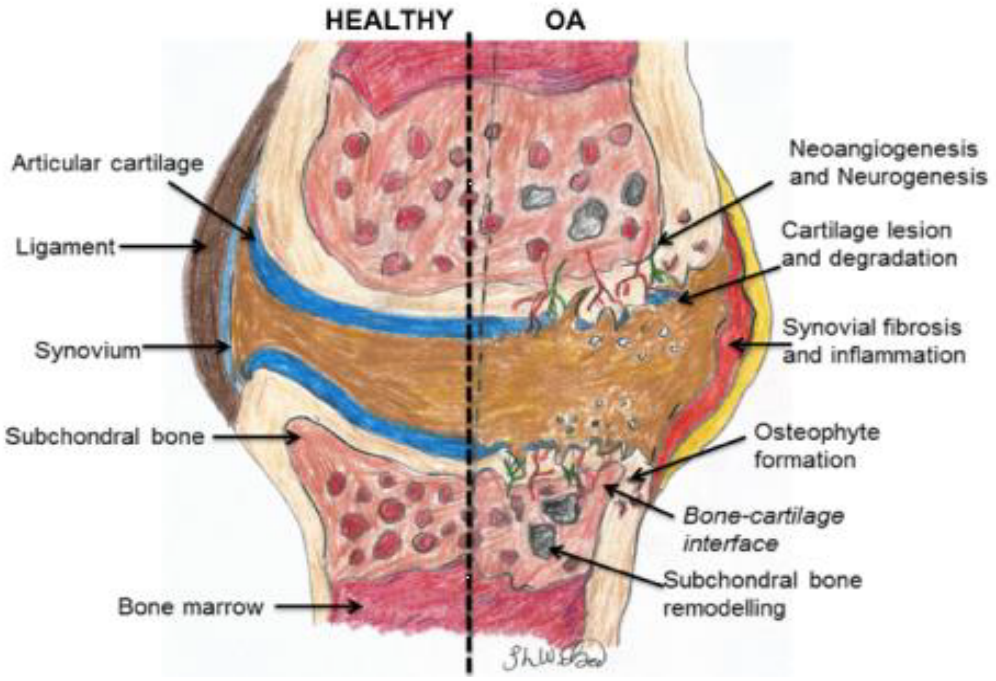


Figure 22: Comparison of a healthy (left) and OA knee joint (right).[114]

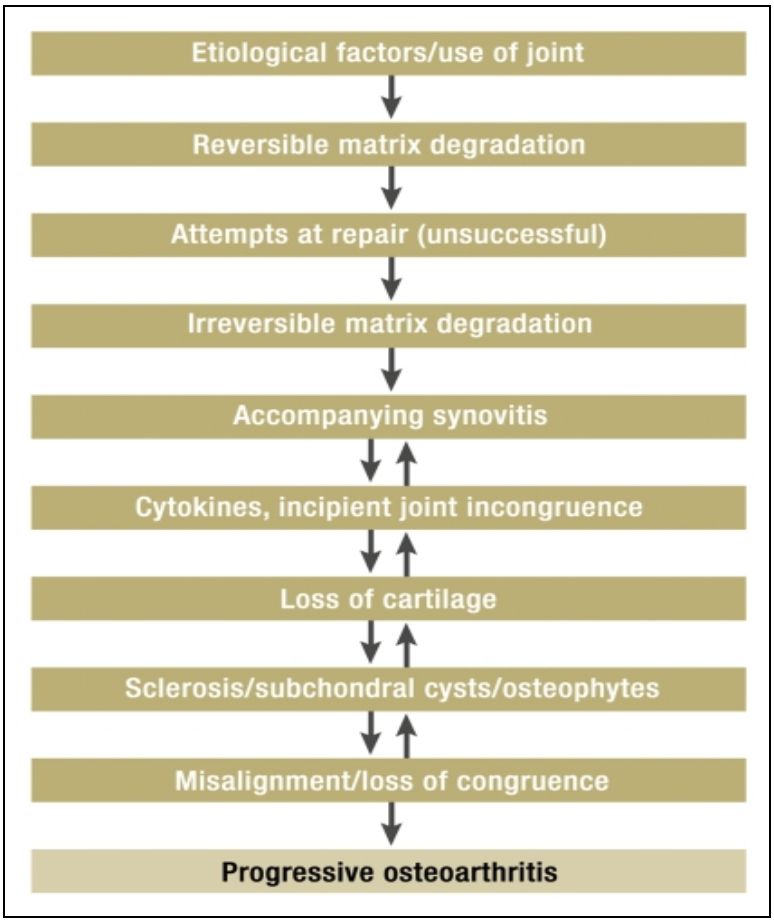


Figure 23: The Pathogenesis of Osteoarthritis [115]

CLINICAL MANIFESTATIONS OF OSTEOARTHRITIS:

Osteoarthritis is a debilitating illness affecting the knee joint most commonly. It is a chronic degenerative joint disease, progressing in severity with years. The symptoms of osteoarthritis include pain, stiffness, joint swelling and deformity occurs in rare cases. Pain is often due to the stimulation of capsular pain fibres, mechanoreceptors, periosteal nerve fibres and by perception of subchondral microfractures. Stiffness is a sequel of pain, due to lack of activity, especially initiating movement. The signs include coarse crepitus, bony enlargement due to remodelling and osteophytes, deformity, instability, restricted ability and stress pain.[112] The debilitating factors in osteoarthritis knee are pain and disability. In 2009, it was the 4th most common

reason for hospitalization in the United States of America.[117] A study done by MS Radha in Mysore showed that 63.3% of patients with OA suffered with pain, while 51.3% with stiffness and 67.3% with disability in performing physical functions based on WOMAC scores.[118] These manifestations have a direct impact on the Quality Of Life in terms of social interactions, mental functioning and sleep quality. For this reason, it is essential to assess Health Related Quality of Life (HRQoL). A study reported by Desmeules et al reported a HRQoL score below the 25th percentile among patients with OA knee awaiting Total Knee Arthroplasty.[119]

SOURCE	**MECHANISM**
SYNOVIUM	INFLAMMATION
SUBCHONDRAL BONE	MEDULLARY HYPERTENSION MICROFRACTURES
OSTEOPHYTES	STRETCHING OF PERIOSTEAL NERVE ENDINGS
LIGAMENTS	STRETCH
CAPSULE	INFLAMMATION, DISTENSION
MUSCLE	SPASM

Table 3: Mechanism of OA knee according to anatomical source.[119]

Physical examination:

I) Localized tenderness, bony or soft tissue swelling

II) Bony crepitus

III) Joint effusion

IV) Restriction of mobility. [119]

THE CARDINAL SIGNS OF OSTEOARTHRITIS:

- Joint space narrowing.
- Osteophytes.
- Bony cysts.
- Subchondral sclerosis.[120]

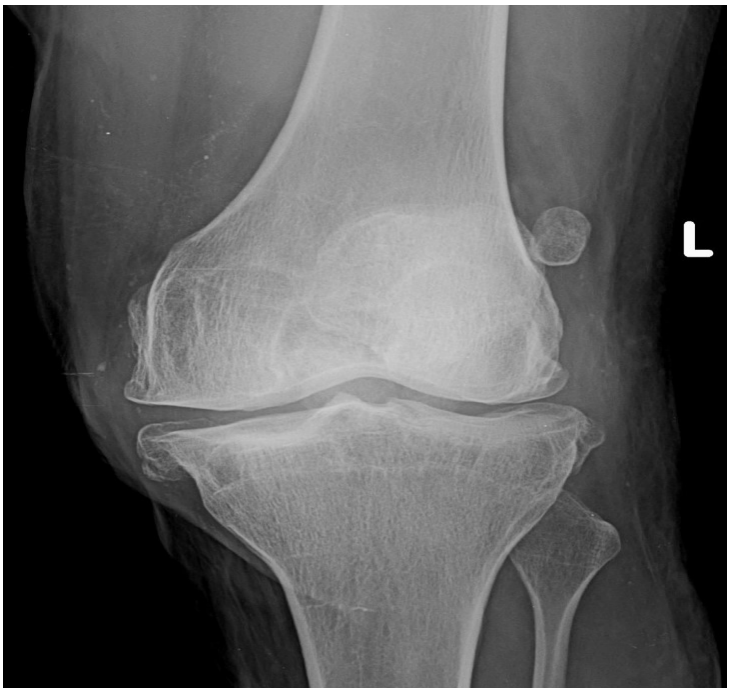


Figure 24: X-ray of knee joint showing OA changes

ASSESSMENT OF OSTEOARTHRITIS: -

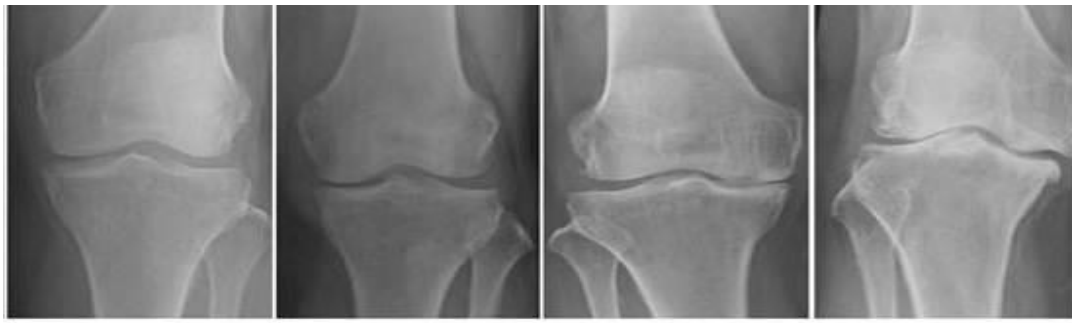
The assessment of OA knee is based on clinical and radiological methods. The clinical diagnosis uses various criteria and scores, of which American College of Rheumatology (ACR) criteria is quite popular. The ACR criteria evaluates based on pain, age, morning stiffness, crepitus on active motion, bony enlargement/ tenderness and palpable warmth as criteria for assessment clinically. The Kellgren& Lawrence (K&L) criteria is used for radiographic diagnosis which is graded in the following manner –

Grade 1 – Doubtful joint space narrowing (JSN) and possible osteophytic lipping

Grade 2 – Definite osteophytes and positive JSN on antero-posterior weight bearing radiograph

Grade 3 – Multiple osteophytes, definite JSN, sclerosis, possible bony deformity

Grade 4 – Large osteophytes, marked JSN, severe sclerosis, definite bony deformity.[121]



GRADE: 1 2 3 4

Figure 25: Kellgren-Lawrence criteria.[121]

MANAGEMENT OF OSTEOARTHRITIS IN CLINICAL PRACTICE: -

The following are the various methods used to treat a patient suffering from OA knee.

1. **Lifestyle-modifications**

i) Losing weight – The most important step is to control weight. By losing extra weight one can reduce load on the joints and thus slowing down the damage and loss of cartilage. Pain is the main annoying sign of OA, by reducing excess weight the pressure on the joint and at the same time pain can also be reduced. In OA shedding extra weight can slow down the complication and also damage to the cartilage

ii) Exercise – Thinking of exercise when in pain is unthinkable but light and regular exercise can not only alleviate pain but also strengthen muscle which can support the

joint better. Exercise can not only improve the muscles strength but also help support the joints, reduce pain, improve motion, reduces disability endurance and posture. Normal walking is an excellent exercise but sometimes pain can prevent one from walking and in that condition aqua exercise in in warm water is equally effective. Water supports the joints and warm water is always soothing for joints. Sometimes a gentle range of motion of various joints can improve mobility and reduce pain. The basic aim of exercise should be to train and strengthen those muscles required by the patient for his or her daily activity. The most effective way is to do some light aerobic exercise and resistance training. At the same time effort should be made to avoid any exercise that may increase pain in the joint. Thus, the main aim to help a patient to perfume these exercises would be to customize and individualize them to suit each one of them. The most effective exercise for OA knee were found to be both isotonic and isokinetic ones that strengthen both flexor and extensors when knee is flexed and extended against resistance.

iii) Nutrition and Dietary Supplements – Eating proper and balance diet is critical for good health and healthy joints. Healthy food reduces inflammation and also help reducing body weight.

iv) Acupuncture – Several controlled clinical trials suggest that the ancient Chinese practice of acupuncture is a very effective treatment for relieving osteoarthritic pain. It may also help improve joint function. A few clinical studies have found that people with OA experience better pain relief and improvement in function from acupuncture than from non-steroidal anti-inflammatory.

v) Balneotherapy (Hydrotherapy or spa therapy) – Balneotherapy is one of the oldest forms of therapy for pain relief for people with arthritis. The term "balneo" comes from the Latin word for bath (balneum) and refers to bathing in thermal or mineral

waters. Sulphur-containing mud baths, for example, have been shown to relieve symptoms of arthritis. However, hydrotherapy, which can be performed under the guidance of certain physical therapists, is occasionally used interchangeably with the word balneotherapy. The goals of balneotherapy for arthritis include:

- Improving range of joint motion
- Increasing muscle strength
- Eliminating muscle spasm
- Enhancing functional mobility
- Easing pain

vi) Yoga – This ancient Indian practice is well known for its physical, psychological, emotional, and spiritual benefits. In the West, it is often recommended to relieve musculoskeletal symptoms and some studies have found it can help relieve OA pain.

vii) Preventive Care – The risk of developing OA may reduce by:

- Protecting an injured joint from further damage
- Exercising
- Losing weight and maintaining a proper weight
- Avoiding repetitive motions.[122]

2. **Mechanical aids**

The osteoarthritis of knee causes severe pain and walking becomes a difficult task, wearing shock absorbing shoes reduces the impact of the load on the knees. Heal wedging is also a method to reduce pain. Sticks and other walking aids help in reducing pain and discomfort.[123]

3. **Pharmacotherapy**

i) Non-steroidal anti-inflammatory drugs (NSAID):

These drugs are helpful in reducing pain and inflammation and it has been reported that they reduce pain by about 30% and improvement in walking distance by 15% but they are never fund to be highly effective in relieving pain significantly in osteoarthritis patients.[124] However, they should be used very carefully in elderly patients for the fear of gastric ulceration and renal and hepatic complications. If required gastroprotective drugs should also be combined with these drugs. The newer COX-2 inhibitors are well tolerated and used regularly. But one should be very careful in prescribing these agents to elderly patients for the fear of cardiovascular and hematological complications.[125]

ii) Intra-articular corticosteroids:

Short term intraarticular injections of triamcinolone or methylprednisone can benefit for a short time but as expected they are associated with many side effects like skin atrophy, infection and chances of severe cartilage destruction if used excessively. Therefore, intra-articular injections are advised only if there is joint effusion indicating active inflammatory condition otherwise other therapeutic measures should be taken.[126]

iii) Hyaluronic acid derivatives:

In osteoarthritis the amount of hyaluronic acid, which is a high molecular weight polysaccharide, comes down which is major component of cartilage and synovial fluid. It was therefore suggested that intraarticular injection of this compound may increase synovial fluid viscosity. Hyaluronic acid is a high molecular weight polysaccharide, and is a major component of synovial fluid and cartilage. Many

studies have found the usefulness of this over intraarticular corticosteroids whose effect can last for 12 months.[127]

iv) Topical treatments:

Capsaicin an active substance found in red chilli is found to be effective in reducing pain in these patients. There is little evidence of efficacy of topical NSAIDs.[124]

v) Glucosamine sulphate and chondroitin sulphate:

These two nutraceutical supplements and are used extensively world over for osteoarthritis. They are supposed to be the derivative of glycosaminoglycans which is found in the articular cartilage, although their mechanism of action is not clear but they have been found to reduce symptoms by 20-25% and they have also been found to reduce medial compartment changes. They have also been found to reduce pain and the disability scores.[128]

vi) Other possible disease modifying osteoarthritis drugs (DMOAD):

a) Diacercin

A new drug that helps reducing the production of metalloproteins and interleukins called Diacerein have been found to be effective in osteoarthritis. Diacerein is a symptomatic slow-acting drug in osteoarthritis (SYSADOA) with anti-inflammatory, anti-catabolic and pro-anabolic properties on cartilage and synovial membrane. It has also recently been shown to have protective effects against subchondral bone remodelling. Based on a literature review of clinical trials and meta-analyses, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) confirms that the efficacy of diacerein is similar to that of non-steroidal anti-inflammatory drugs (NSAIDs) after the first month of treatment and superior to that of paracetamol. Additionally, diacerein has shown a prolonged effect on symptoms of several months once treatment was stopped. The use of

diacerein is associated with common gastrointestinal disorders such as soft stools and diarrhoea, common mild skin reactions and, uncommonly, hepatobiliary disorders. However, NSAIDs and paracetamol are known to cause potentially severe hepatic, gastrointestinal, renal, cutaneous and cardiovascular reactions. Therefore, the ESCEO concludes that the benefit-risk balance of diacerein remains positive in the symptomatic treatment of hip and knee osteoarthritis.

Because of high costs of knee OA management, therapeutic options that are effective on tissue healing have been taken into consideration in recent years in order to prevent the progression of OA.[129]

b) Sprifermin

Sprifermin is a very promising DMOAD and is an anabolic agent. This is a truncated version human fibroblast growth factor 18 (FGF 18) which is known to stimulate chondrocyte proliferation and production of cartilage matrix. A phase1b trial showed a significant reduction in femorotibial cartilage thickness in patients with symptomatic OA.[130]

c) BMP-7

This is another pro-anabolic drug and helps in articular cartilage repair. BMPs play an important role in protection against cartilage damage caused by inflammation or trauma, by binding to different receptor combinations and, consequently, activating different intracellular signalling pathways. [131]

d) Matrix metalloproteinases (MMP) inhibitors

MMPs and aggrecanases are responsible for cartilage destruction in OA. Inhibition of these proteases can prevent destruction of these cartilages. However, these agents are associated with severe side effects like arthralgia, edema, palmar fibrosis. As a result, these drugs are not developed further. These data demonstrate that compounds such as

sprifermin (FGF18) and BMP-7 have promising pro-anabolic effects on cartilage tissue, whereas the inhibition of catabolic factors such as proteases has not shown beneficial effects in cartilage so far owing to adverse effects.[132]

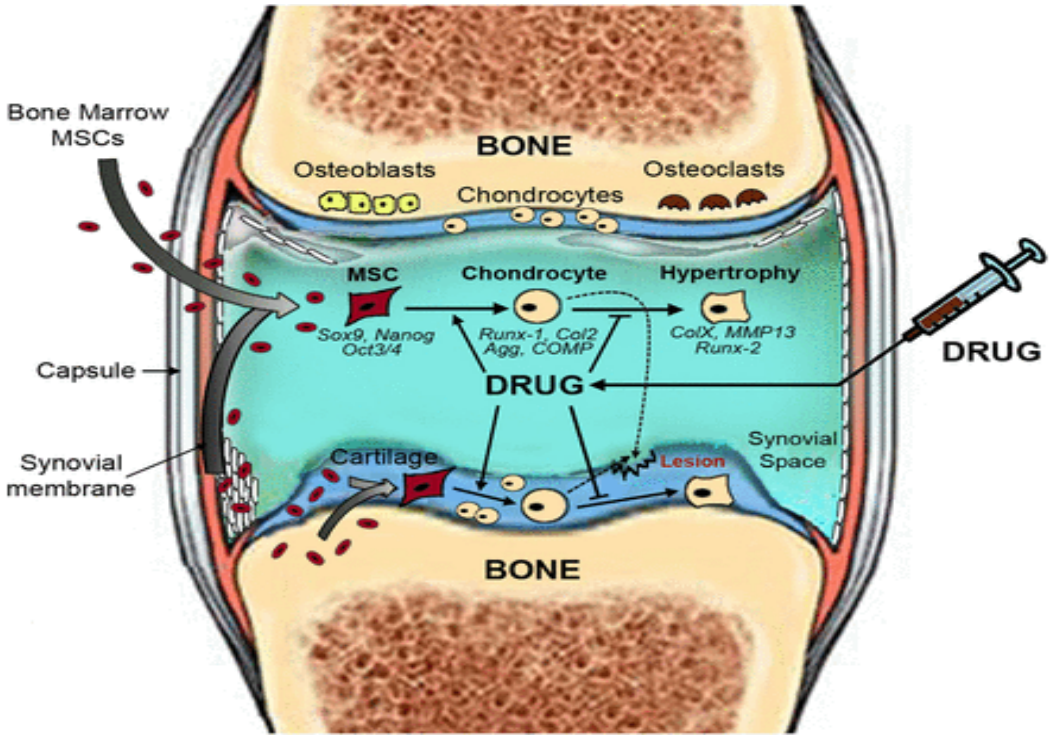


Figure 26: Localisation of mesenchymal cells, the chondrogenesis process in the joint and targets for a new DMOAD. [133]

vii)Regenerative therapies with stem cells

Stem cells therapy has shown great promise in regenerative medicine and can be a promising therapy for OA in the future. Now a day's mesenchymal stem cells are used as an injection into the joint has been found to be safe and along with Adipose derived stem cells are used in the treatment of OA. Mesenchymal stem cells (MSCs) are able to form cells of the mesodermal lineage, being able to differentiate towards osteoblasts, chondrocytes and adipocytes. Their presence throughout the body suggests an intrinsic role in tissue repair and regeneration. Several in vitro techniques

have been explored to assist MSCs to differentiate along a path of chondrogenesis. Both Transforming Growth Factor Beta 1 (TGFβ1) and Insulin-Like Growth Factor 1 (IGF-1) act synergistically to stimulate chondrogenesis. Along with their immunomodulatory and differentiation potential, MSCs have been shown to express essential cytokines including Transforming Growth Factor beta (TGFβ), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF) and an array of bioactive molecules that stimulate local tissue repair. Autologous MSCs can differentiate into cartilage and bone supporting their potential in the treatment in OA. Intra-articular injections of MSCs have resulted in pain and functional improvement in a number of preclinical and clinical trials. Importantly, recent limited case series evidence has shown regrowth of cartilage volume and disease modification following MSC injections. Despite initial concerns regarding MSC therapies, systematic review of clinical trials has indicated a relative safety in both intravascular and intra-articular injections. However, further randomized controlled trials are needed to evaluate the most effective application of MSCs in osteoarthritis management.[134]

viii) Therapies with bisphosphonates

Bisphosphonates (BPs) inhibits osteoclast activity in osteoporosis but their use in osteoarthritis is not proven. However, BPs might be especially beneficial in patients with high turnover of bones in an early state of OA. BPs directly affect osteoclast activity but they inhibit pain in OA. A research has shown that osteoclasts induce pain in OA by stimulating sensory nerve endings.[135]

ix) Drugs targeting bone cells

New molecule called cathespsin K neutralizer neutralizes cathespsin K which is the major proteolytic enzyme secreted by osteoclasts.[136]

x) Treatments targeting inflammatory mediators and pathways

It has now been proven that OA has an inflammatory component that may be dominant in some patients and joint tissues. The release of various pro-inflammatory mediators like prostaglandins, cytokines, and chemokines has been reported in patients. Synovitis has been seen in most of the OA patients and a number of factors like age, mechanical forces in extracellular matrix (ECM) are found to cause inflammation by triggering release of these inflammatory mediators.[137]

a) Anti-cytokine therapy

Otilimab an anti-granulocyte macrophage colony-stimulating factor (GM-CSF) antibody was found to be an effective drug in OA. However, since a single driver of inflammation in OA is not possible and thus targeting and blocking pro-inflammatory pathways should be an ideal way for OA.[137]

xi) Anti-nerve growth factor antibody treatment

This factor called nerve growth factor (NGF) gets released in response to stimulation from various inflammatory mediators like osteoclasts, chondrocytes, osteocytes and macrophages in human OA. Although initial trials using anti-NGF antibodies looked promising, further studies are needed to warrant treatment safety.[138]

xii) Therapies targeting senescence and aging

Age related changes in OA is well established. However, no human trials have proven the role of age and OA but some pre-clinical studies targeting age related factors may lead to the development of new drugs. Some Senolytes are known which can qualify as a potent therapeutic agent for OA.[139]

xiii) Serotonin–norepinephrine reuptake inhibitors

Recent evidence has implicated central sensitization as an important factor in mediating pain in osteoarthritis. The findings of Arendt-Nielsen et al lend support to

this theory, where the authors observed abnormal windup in their cohort of patients with knee osteoarthritis. This finding may explain the limited efficacy demonstrated by analgesics such as paracetamol and NSAIDs that target peripheral sensitization. Both noradrenergic and serotonergic neurons modulate nociceptive processing in the spinal cord and periaqueductal gray area and are potential targets in improving pain in osteoarthritis.[140]

xiv) Strontium ranelate

The rationale for testing strontium ranelate for the treatment of osteoarthritis was first proposed following post hoc analysis of spine radiographs from osteoporosis studies. A smaller proportion of patients treated with strontium ranelate experienced an increase in overall arthritis score and joint space narrowing compared with patients treated with a placebo. Strontium ranelate inhibits subchondral bone resorption by regulating the activity of osteoprotegerin, receptor activator of nuclear factor kappa-B (RANK) ligand, and matrix metalloproteinases (MMPs) produced by osteoblasts.[140]

xv) Apocynin and paeonol (APPA) are plant-derived compounds with anti-inflammatory and chondroprotective properties. Apocynin inhibits the neutrophil oxidative burst, while paeonol suppresses the expression of inducible nitric oxide synthase and cyclo-oxygenase-2. Hence, the combination of these compounds (APPA) may prove beneficial in improving pain and function as well as limiting disease progression in osteoarthritis.[141]

4.Surgical techniques

Surgical indication and choice of treatment is based on symptoms (e.g. pain and knee function), OA stage, and patient-related factors such as age, level of physical activity and patient's comorbidities. Radiological evidence of OA alone (joint space

narrowing, osteophytes, etc.) does not justify surgical intervention, which is indicated only in combination with relevant symptoms. Finally, it is the patient's degree of suffering, in correlation to radiological evidence of OA, which determines the time point of surgery. It is important that indication with OA surgery is always a relative indication. Only in case of progressive knee instability associated to OA surgical treatment (total knee arthroplasty) should not be unnecessary delayed.

i) Arthroscopic Lavage and Debridement

Arthroscopic techniques include lavage and debridement of the knee (e.g., shaving of rough cartilage or smoothening of the degenerated meniscus). In theory, arthroscopy for OA should relieve symptoms by removing the debris and inflammatory cytokines that cause synovitis. Debridement can remove torn meniscal fragments and loose cartilage flaps. However, the role of arthroscopy in treating knee OA is controversial. Although widely used, there is a lack of evidence showing it to have a significant benefit.

Patients who are of a younger age with mild to moderate OA knee or with lesions of meniscus, cartilage flaps benefit from this technique.[142]

ii) Autologous Chondrocyte Implantation (ACI)

In 1994, Brittberg presented the ACI technique whereby cultivated and proliferated autologous chondrocytes are re-implanted underneath a periosteal flap. Chondrocytes are harvested in a first procedure in which a small cartilage probe is taken arthroscopically. The cartilage is then digested and the harvested cells expanded during 3-4 weeks in monolayer culture before implantation.

Main indications for cartilage repair techniques are limited size cartilage lesions especially in younger patients. If cartilage damage tends towards an osteoarthritic lesion, cartilage repair procedures are not indicated. Exclusive cartilage repair will not

be successful if axial malalignment, ligamentous instability, or patella maltracking is the underlying cause or is associated with the cartilage lesion. The disadvantages of this technique are the two-stage procedure and the costs of the cell culture. [142]

iii) Osteotomies around knee

Osteotomies around the knee are an accepted method for the treatment of unicompartmental OA with associated varus or valgus deformity. Osteotomies around the knee alter the weightbearing axis of the lower extremity. The aim is to unload the damaged compartment and to transfer the weight load from the affected areas by slightly overcorrecting into a valgus or varus axis to reduce pain, slow the degenerative process and delay joint replacement. The classic inclusion criterion is OA of one compartment in combination with varus or valgus alignment. The femoropatellar compartment should not be affected by OA. Good mobility of the knee is a prerequisite, as well as ligament stability.

Instability is not an absolute contraindication because cruciate ligaments can be reconstructed together with correction of the axis. Age is a significant factor to consider. Age >60–65 years is a relative contraindication, whereas biologic age and activity must also be considered. Obesity and chondrocalcinosis are not strict contraindications, but the success rate and prognosis are compromised.

There are 4 basic types of proximal tibial osteotomy

a) Medial opening wedge

b) Lateral closing wedge

c) Dome osteotomy

d) Medial opening hemicallotasis.[142]

iv) Unicompartmental Knee Arthroplasty (UKA)

UKA is indicated in cases where OA involves only one of the three compartments of the knee: the medial tibiofemoral, lateral tibiofemoral or patellofemoral compartment. The commonest UKA replaces the contact surfaces of the medial tibiofemoral compartment with two metallic prosthetic devices and inserts a polyethylene inlay between them. For successful medial UKA, the initial conditions must provide a well-preserved lateral compartment with respect to meniscus and cartilage. The implant is unrestrained in the sagittal plane, so the stability of the prosthesis depends on intact cruciate ligaments. Considerable malalignment of the limb is a contraindication.[142]

iv) Total knee arthroplasty (TKA)

In advanced knee OA, with more than one compartment involved and failure of conservative treatments, TKA has been shown to be a highly effective treatment that results in substantial improvement in patient functioning and health-related quality of life. Until now it has been the first-line procedure for end-stage knee OA. The long-term results of TKA have been well documented with survival rates of up to 98% at 15 years. Results in younger patients are mostly reported to be inferior with 76% survival rates at 10 years.

The main complications are femoropatellar problems, loosening of components, infections and stiffness of the knee. Complications involving the extensor mechanism and the femoropatellar joint remain the primary non-infectious indications for revision TKA. [142]

Thus, PRP can be preferred in knee osteoarthritis patients to achieve symptomatic recovery and elevate improve quality of life in cases that do not respond to

conservative therapies or refuse arthroplasty. Delaying arthroplasty may be a more viable option in the future as results are obtained from studies investigating intraosseous or intraarticular plus intraosseous PRP application, PRP with hyaluronic acid, stem cell treatments and their various combinations. As studies continue, PRP may become an option in severe knee osteoarthritis providing improvements in pain, functional levels, and quality of life.

This will help decrease costs due to disabilities associated with knee osteoarthritis and surgery rates can be reduced in these patients.

PLATELET RICH PLASMA

Platelets are small anucleate cell fragments that have a characteristic discoid shape and range from 1 to 3 μm in diameter. Historically, platelets were referred to as cellular dust. Platelets are not only responsible for haemostasis, wound healing, inflammation and innate immunity but also angiogenesis.Platelets are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells, which reside in the bone marrow.[143]

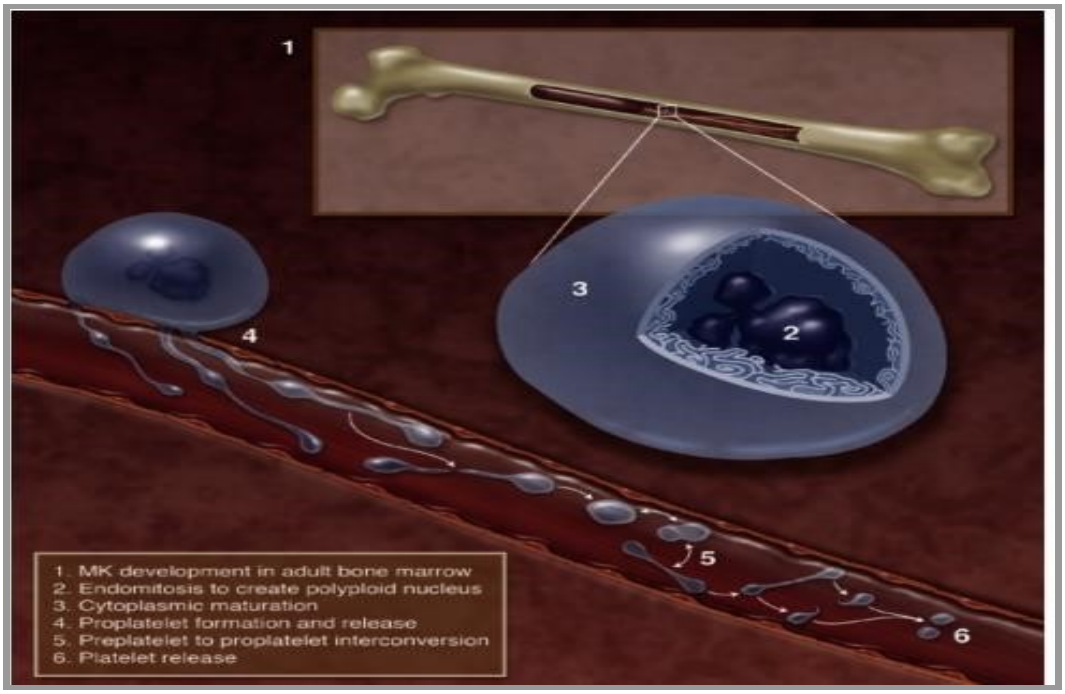


Figure 27: Formation of platelet from bone marrow. [143]

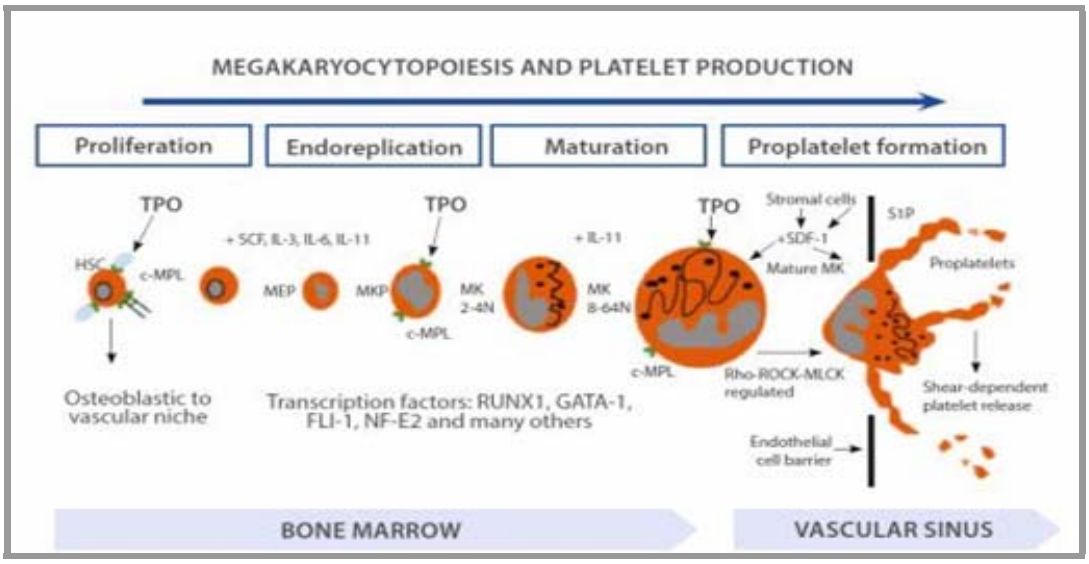


Figure 28: Schematic representation megakaryocytopoiesis and platelet production. [144]

After they are shed from the cytoplasm of megakaryocytes, platelets circulate in the bloodstream for 9 to 11 days.[145]

The two functional roles of platelets are haemostasis and the initiation of wound healing. The platelet cell membrane is trilaminar with a glycoprotein receptor surface overlying and partially interspersed with and penetrating a bilayer of phospholipids and cholesterol. They lack nuclei but contain organelles and structures such as mitochondria, microtubules, and granules (alpha, delta, and lambda).[146]

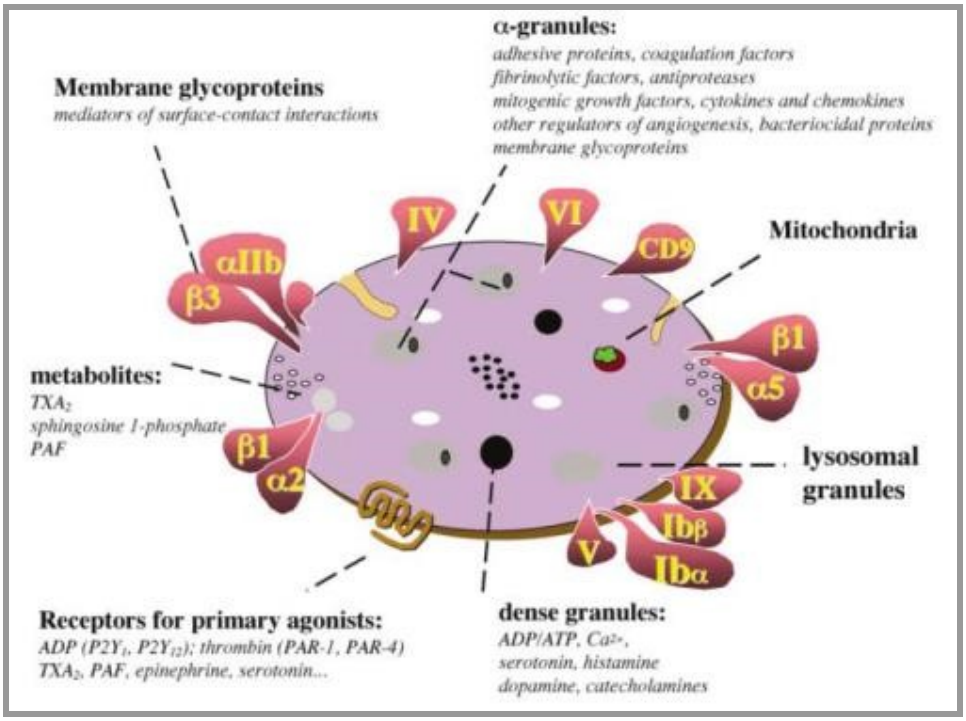


Figure 29: Structure of a Platelet. [144]

The alpha granules, bound by a unit membrane, are formed during megakaryocyte maturation, are about 200 to 500 nm in diameter, and number approximately 50 to 80 per formed platelet. They contain more than 30 bioactive proteins, many of which have a fundamental role in haemostasis or tissue healing.[147]

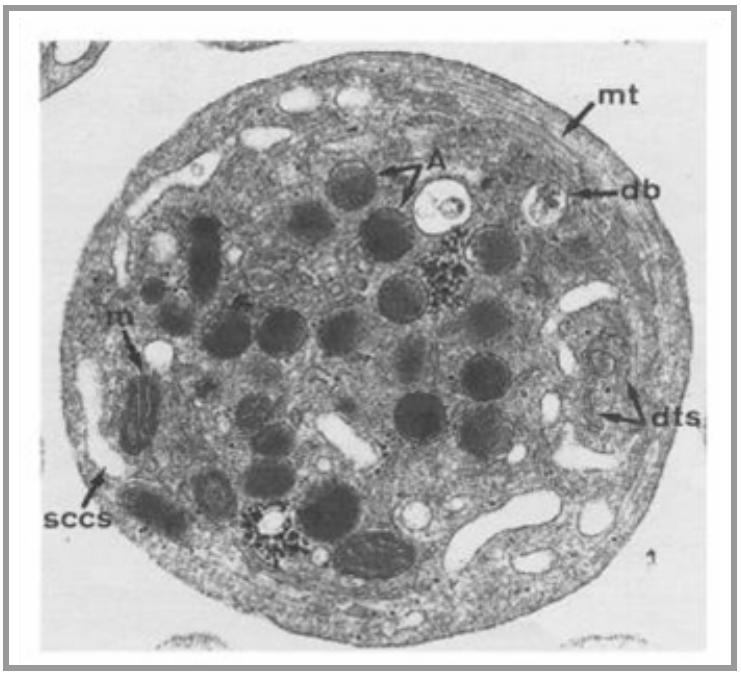


Figure 30: Platelet and its Granules.[147]

Platelets participate in tissue healing by secreting a variety of growth factors, cytokines, chemokines and other factors. For example, vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF a, b and c), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF) & insulin-like growth factor (IGF).[144]

NAME	ACRONYM	FUNCTION
Platelet-derived growth factor	PDGF	Stimulates fibroblast production, chemotaxis, stimulates transforming growth factor- ß1, collagen production, upregulation of proteoglycan synthesis
Transforming growth factor- ß1	TGF- ß1	Modulates proliferation of fibroblasts, formation of extracellular matrix, cell viability; increases production of collagen from fibroblasts, suppression interleukin
Basic fibroblastic growth factor	Bfgf	Produces collagen; stimulates angiogenesis, proliferation of myoblasts
Vascular endothelial growth factor	VEGF	Promotes angiogenesis
Epidermal growth factor	EGF	Promotes cell differentiation, angiogenesis, proliferation of mesenchymal and epithelial cells

Table 4: Growth factors present in platelet-rich plasma.[148]

PRP is concentration of human platelets (autologous) in small volume of plasma, where the concentration of platelet is higher (typically up to five times higher) than the normal platelet concentration in a healthy person's blood. Evidence suggests that PRP has potential to have a regenerative effect on certain body tissues, added to the main role platelets play in haemostasis.[149]

PRP is also known as platelet-rich growth factors (GFs), platelet-rich fibrin (PRF) matrix, PRF and platelet concentrate.

The concept and description of PRP started in the field of hematology. Hematologists created the term PRP in the 1970s in order to describe the plasma with a platelet count

above that of peripheral blood, which was initially used as a transfusion product to treat patients with thrombocytopenia.[150]

10 years later, PRP started to be used in maxillofacial surgery as platelet rich fibrin (PRF). Fibrin had the potential for adherence and homeostatic properties, and PRP with its anti-inflammatory characteristics stimulated cell proliferation.[151]

Subsequently, PRP has been used predominantly in the musculoskeletal field in sports injuries. With its use in professional sportspersons, it has attracted widespread attention in the media and has been extensively used in this field. Other medical fields that also use PRP are cardiac surgery, pediatric surgery, gynecology, urology, plastic surgery, and ophthalmology. [152]

Currently, there is a great discussion and no consensus regarding PRP preparation. PRP is prepared through a process known as differential centrifugation, in which acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity. [153]

Regarding the preparation of PRP, there are 2 techniques:

1. Open technique: The product is exposed to the environment of the working area and comes in contact with different materials that should be used for their production, such as pipettes or product-collection tubes. In the blood processing to obtain PRP with the open technique, it should be guaranteed that the product is not contaminated during microbiological handling.

2. Closed technique: It involves the use of commercial devices with marking (including centrifuge equipment and application) in which the product is not exposed to the environment. [153]

Briefly, the procedure requires the use of relatively small volumes of blood. The PRP is obtained from the blood of patients before centrifugation. The whole blood is

obtained by venipuncture in anticoagulated tubes (usually with acid citrate dextrose or sodium citrate solution). The blood is then centrifuged with single- or a double-spin centrifugation.

After centrifugation, the tube shows 3 basic layers: at the bottom of the tube, there are red blood cells with leukocytes deposited immediately above; the middle layer corresponds to the PRP, and at the top, there is the platelet poor plasma (PPP). The PPP is removed, and PRP is obtained. Platelets can be activated before application of the PRP, although there is no consensus on whether or not platelets must be previously activated before their application and with which agonist. [154]

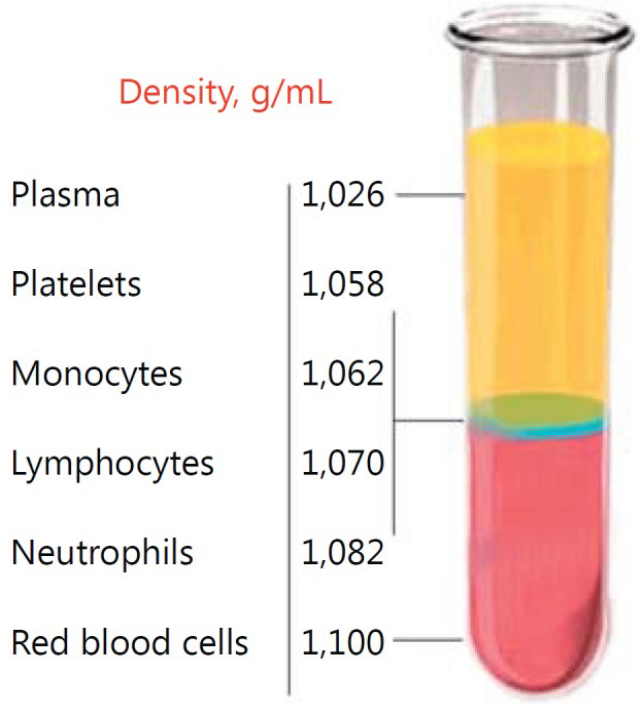


Figure 31: After centrifugation, the blood components (red blood cells, leukocytes, and platelets) are separated from the plasma due to their different densities. The platelets have the lowest density.[155]

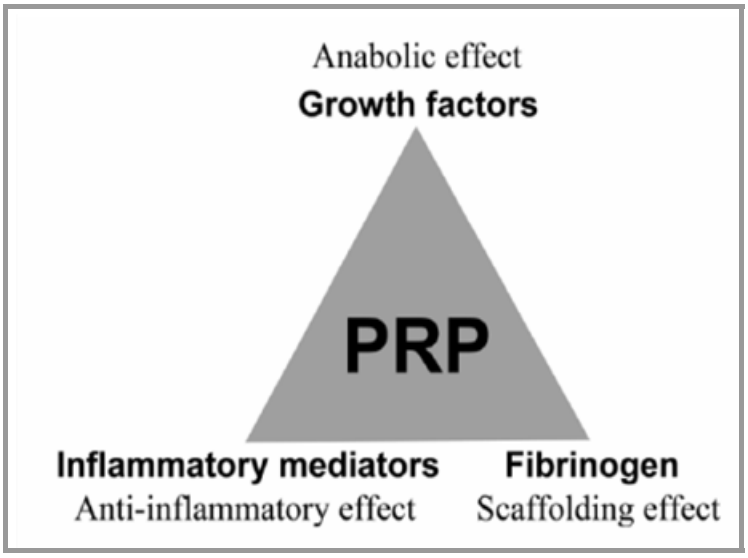


Figure 32:Principal components and potential effects and actions.[156]

In 2016, Magalon et al. proposed the DEPA classification (Dose, Efficiency, Purity, Activation) that focuses on the quantity of platelets obtained by the PRP kits as well as on product purity and on platelet activation prior to injection.

The DEPA classification is based on 4 different components:

1. Dose of injected platelets: calculated by multiplying the platelet concentration in PRP by the obtained volume of PRP. According to the injected dose (measured in billions or millions of platelets), it should be categorized into (a) very high dose of injected platelets of >5 billion; (b) high dose of injected platelets, from 3 to 5 billion; (c) medium dose of injected platelets, from 1 to 3 billion and (d) low dose of injected platelets, <1 billion.

2. Efficiency of the production: Corresponds to the percentage of platelets recovered in the PRP from the blood. It is categorized as follows: (a) high device efficiency, if the recovery rate in platelets is >90%; (b) medium device efficiency, if the recovery rate in platelets is between 70 and 90%; (c) low device efficiency, if the recovery rate

is between 30 and 70% and (d) poor device efficiency, if the recovery rate is <30% and corresponds to the relative composition of platelets, leucocytes, and RBCs in the obtained PRP.

3. Purity of the PRP obtained: Correlates to the relative composition of platelets, leucocytes, and RBCs in the obtained PRP. It is described as (a) very pure PRP, if the percentage of platelets in the PRP, compared with RBCs and leucocytes, is >90%; (b) pure PRP, between 70 and 90% of the platelets; (c) heterogeneous PRP, if the percentage of platelets is between 30 and 70%, and (d) whole-blood PRP, if the percentage of platelets in the PRP is <30% compared with RBCs and leucocytes.

4. Activation process: if an exogenous clotting factor was used to activate platelets, such as autologous thrombin or calcium chloride.[157]

Platelet-rich plasma has gained increasing attention as a promising procedure to stimulate repair of the cartilage, because of growth factors (GFs) stored in platelet. '**α** granules' which are found to play a role in regulation of articular cartilage.[158]

Extracting PRP is easy and convenient and processing is relatively simple and short, easy handling. It also offers multiple GFs at relatively inexpensive cost. Above all, its use is safe. It is a very minimally invasive method, to obtain a high concentrate of autologous GFs, which could be easily placed directly into the lesion site.[159]

PRP is safe from immune reaction and blood diseases because it is obtained from autologous blood and also PRP can be administered in the outpatient clinic easily. PRP therapy seems to delay operative approaches in early degenerative disease. In cases of advanced degenerative joint disease, operative approaches such as arthroscopy, osteotomy and arthroplasty can be better treatments.[160]

MATERIALS & METHODS

SOURCE OF DATA: -

It is a prospective, comparative, observational, time bound, hospital-based study conducted from November 2018 to May 2019, after obtaining institutional Ethical committee approval. 68patients were included in the study following the inclusion and exclusion criteria. The patients were randomized into 2 groups using a standard randomization technique (odd and even number method). Group A (odd numbers) received single spin PRP injection and group B (even numbers) received double spin PRP injection. Out of the 68 patients, 4 patients were lost to follow-up, which gave a final sample size of 64 patients. Patients were selected from R L Jalappa Hospital and Research centre, Department of Orthopaedics, Kolar, on outpatient and in-patient basis. After clinical examination & radiographs of the knee joint in standing position (antero-posterior views and lateral views) were taken, Blood sample of the patient was collected and PRP prepared in Blood bank. Infiltration was done in Operation theatre under strict aseptic conditions. Patients were assessed with"VAS" (visual analogue scale) for pain,"WOMAC" (Western Ontario McMaster Universities Arthritis Index) scoring,and Oxford knee scoring, before giving the PRP injection & after giving the injection at periods of 1 month, 3 & 6 months. The decrease in WOMAC score & VAS score and increase in Oxford knee scores was suggestive of improvement in patient's condition.

INCLUSION CRITERIA

- Patients aged between 40 and 70 years
- Patients clinically and radiographically diagnosed as Kellgren-Lawrence grade I-II primary OA knee, with no improvement in conservative management (activity modification and weight loss, physical therapy or NSAID).

EXCLUSION CRITERIA

- Previous lower extremity surgery.
- Poor diabetic control with HBA1c more than7%.
- Rheumatoid arthritis and Gout.
- History of infection or current infection at the affected joint.
- Hematological diseases with bleeding diathesis, hemophilia and documented anemia

SAMPLE SIZE ESTIMATION

Sample size is estimated based on the reduction of WOMAC score at 6 month evaluations in a study done by Patel S[13] reported an average variance estimate $(17.6)^2$.The sample size for the study is an estimation based on with 80% power and alpha error of 5% to detect the difference of 30% (effect) in mean WOMAC score. Estimated sample size per group of 32 patients. Hence the final sample size per group is considered to be estimated as 32 calculated using the formula:

$$n = \frac{2s_p^2\left[z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right]^2}{\mu_d^2}$$

$$s_p^2 = \frac{s_1^2 + s_2^2}{2}$$

Where,

- S_1^2 - Standard deviation in the first group
- S_2^2- Standard deviation in the second group
- M^2_d – Mean difference between the samples
- α- Significance level of 1%
- 1-β- Power of 80%

PATIENT SELECTION

All patients with primary knee osteoarthritis were evaluated clinically & radiographically. All the patients with grade I and II Kellgren and Lawrence radiological grading were included in the study, irrespective of age, sex & socioeconomic status.

Informed & written consent was obtained from patients participated in this study. Selected patient's blood sample was sent for CBC, random blood sugar and serology. Blood sample was evaluated to assess the WBC & platelet count prior to the infiltration.

Patients having platelet counts < 100000/cubic mm, HbA1c >7% and any documented anaemia, bleeding disorders or joint infections were excluded from the study. Patients were also asked about intake of any oral medications like NSAIDS, if anyone is on any analgesics, they were instructed to stop one week before administration of PRP. For the selected patients 'WOMAC' score, 'VAS' score and Oxford knee score were recorded in a chart for each patient & follow up scorings were also noted down similarly in the same chart of that patient.

PREPARATION OF PLATELET RICH PLASMA (PRP)

Single spin technique: 36-ml venous blood sample was collected in 4 tubes (9ml in each). Four tubes were centrifuged at 1500rpm for 15 min, obtaining a concentration suspended in plasma that will be extracted by pipetting carefully to avoid leukocyte aspiration. The platelet count was assessed in final PRP extract & was used for injection with a 10-mL syringe. The mean platelet count achieved in this method by us was around 4 times more than the normal platelet count of that patient.

Double spin technique: 150ml of whole blood was collected in a double bag having 63 ml of Citrate phosphate dextrose adenine (CPDA). Two centrifugations (the first at 1,800 rpm for 15 min to separate erythrocytes and a second at 3,500 rpm for 10 min to concentrate platelets). Next, blood bag was taken out and supernatant PRP was transferred in the transfer bag under strict aseptic precautions. This was followed by the sealing of the primary bag with tube sealed. About 15-20ml of PRP was collected and submitted for diagnostic evaluation with regard to the platelet count and relevant serological investigations before being injected into the joint with a 10ml syringe. The mean platelet count achieved in this method by us was around 8 times higher the normal platelet count of that patient.

PROCEDURE OF PRP INJECTION

In operation theatre, the patient in supine position, knee was thoroughly scrubbed, & painted after that sterile draping techniques followed. Then the patient's knee in slight flexion so that joint is opened for injection using lateral parapatellar approach.

Under sterile aseptic conditions, about 10 ml platelet concentrate was injected into knee joint using 18-gauge needle without using any local anesthetic. Post injection of PRP passive knee movements (flexion and extension) were performed.

After the procedure, Jone's compression bandage was applied and the knees were immobilized for ten minutes. Patients were then observed for thirty minutes for possible side effects like sweating, dizziness. During follow-up period, no analgesics were allowed.

Figure 33: Centrifuge used for PRP separation.

Figure 34: Vacutainer inside the centrifuge.

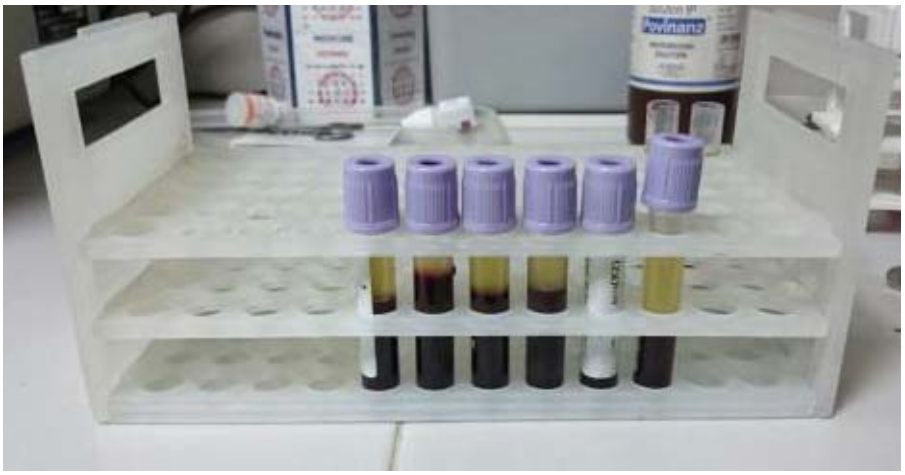


Figure 35: Vacutainers following 15 minutes of centrifuge with 1500 rpm used in single spin PRP.

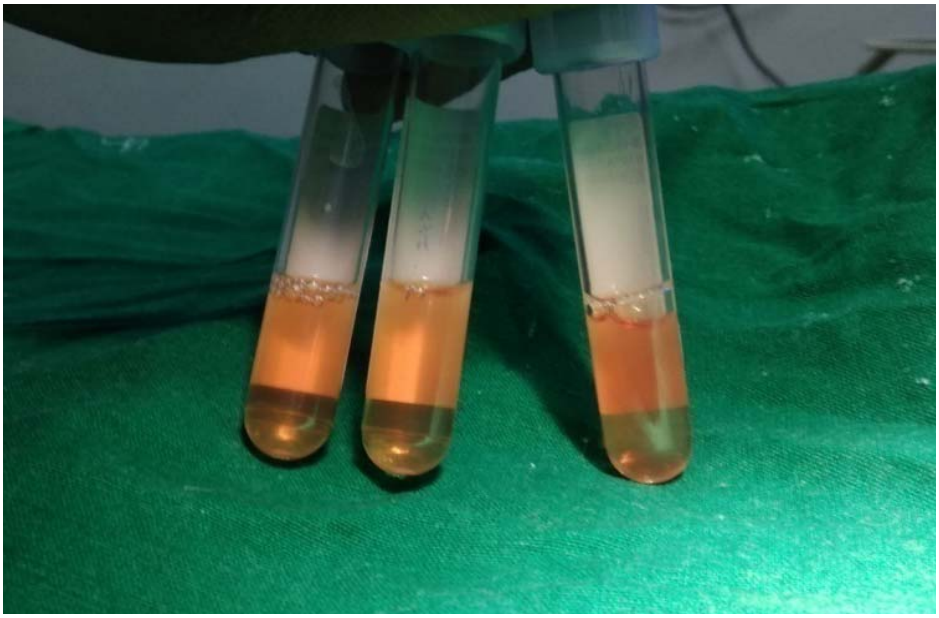


Figure 36: Vacutainers containing PRP prepared using single spin technique.

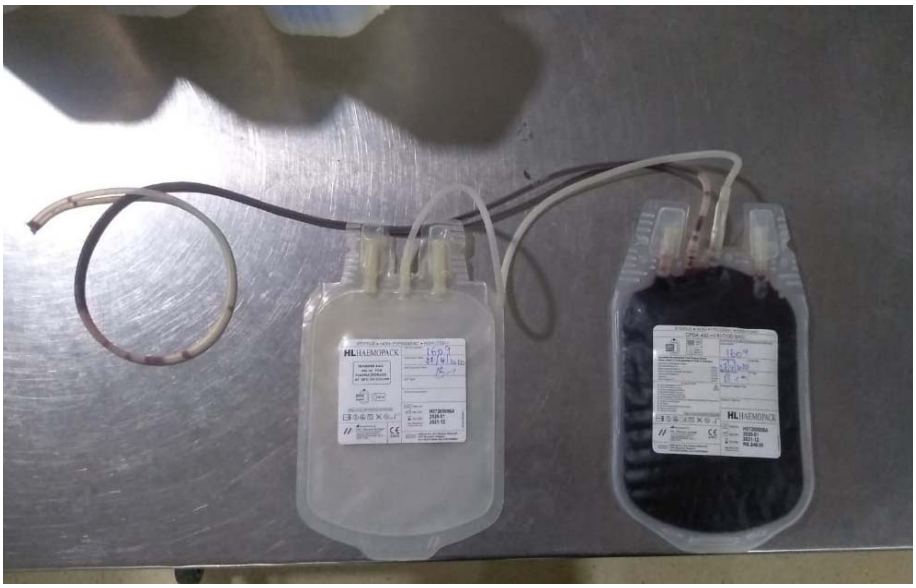


Figure 37: 150ml blood bag with transfer bag used in double spin PRP technique.

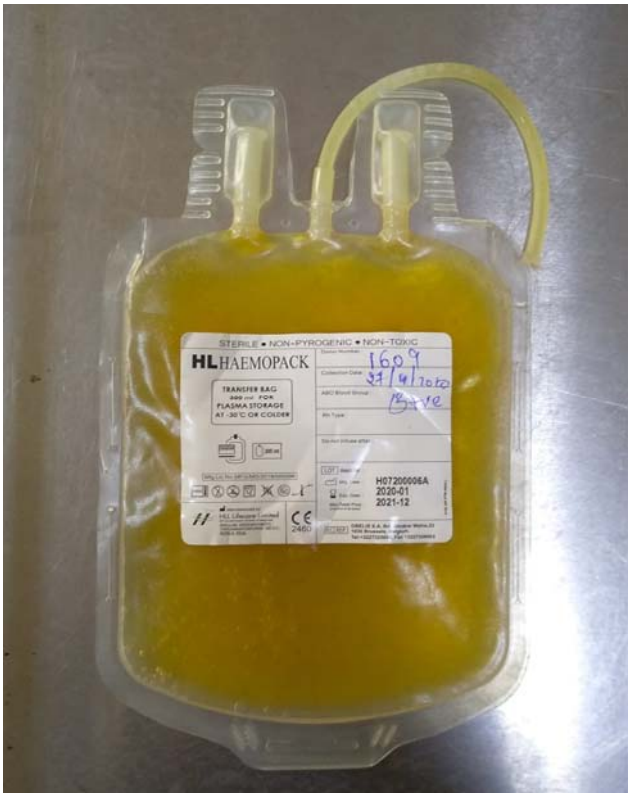


Figure 38: Transfer bag containing PRP prepared using double spinning technique.

Figure 39: PRP in 10ml syringe

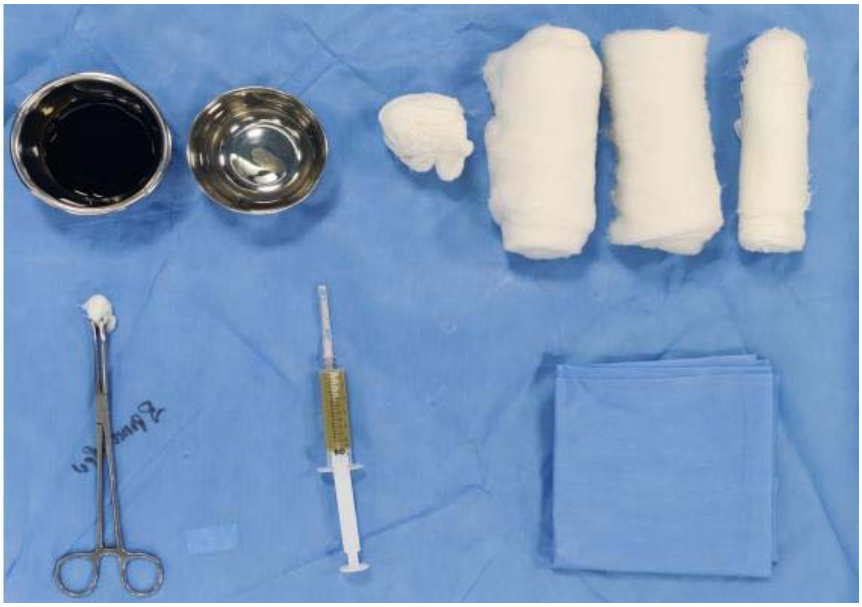


Figure 40: Preparation before injection

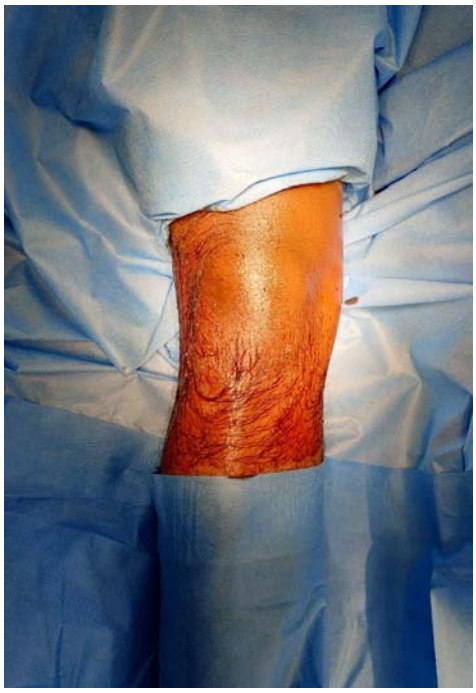


Figure 41: Knee painted and draped before injection.

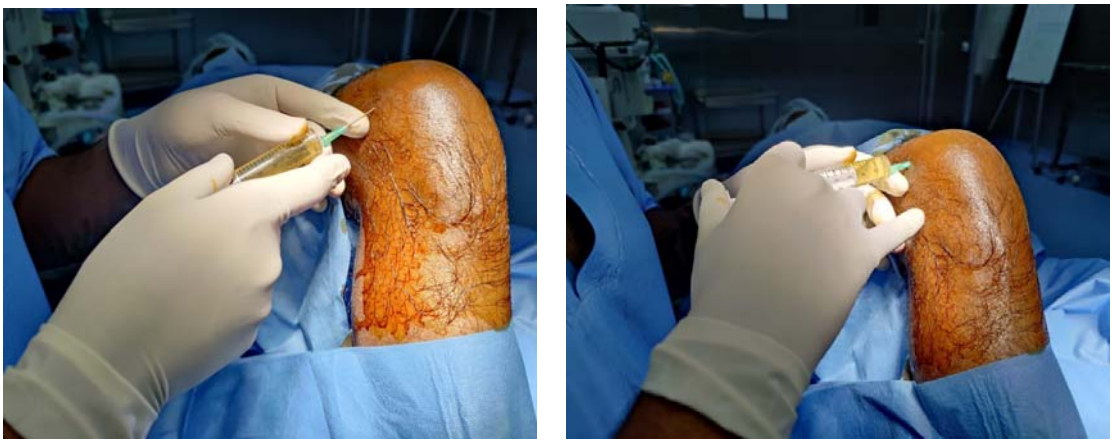


Figure 42: Infiltration of PRP into the joint.

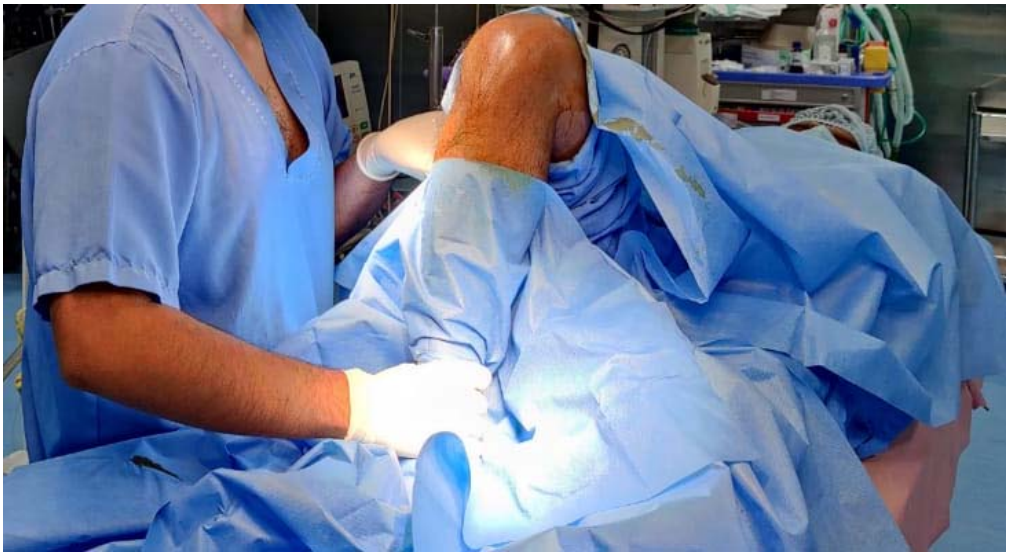


Figure 43: Performing flexion & extension of knee after infiltration of PRP.

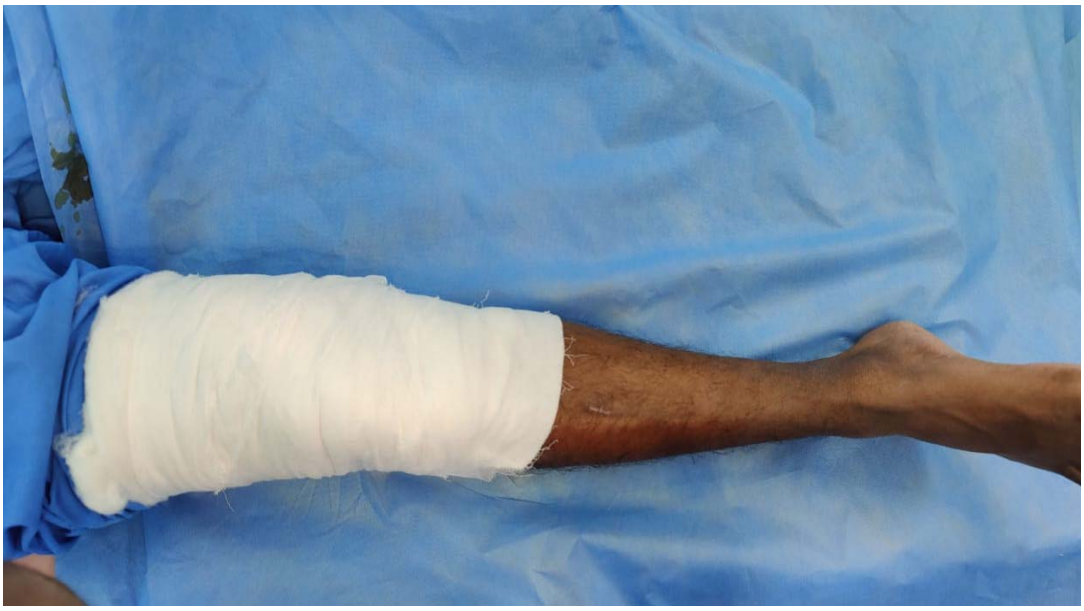


Figure 44: Application of Jone’s compression bandage.

OUTCOME MEASURES

Each patient was allotted a separate WOMAC, VAS and Oxford knee score chart till complete follow up. Initial WOMAC, VAS and Oxford knee score were recorded prior to the administration of PRP infiltration i.e. on day '0' and after the infiltration patients were asked to come for review on 1st, 3rd& 6th months. Decrease in WOMAC & VAS scores and increase in Oxford knee scores was considered as improvement in the patient's condition.

WOMAC SCORE

In 1982, Nicholas Bellamy had developed a health status questionnaire termed the Western Ontario and McMaster (WOMAC) Osteoarthritis Index. Between 1996 and 1999 the Index underwent significant refinement, a process that has been consolidated between 1999 and the present and has resulted in the 3.1 series of WOMAC questionnaires.[161]

The WOMAC consists of 24 items divided into 3 subscales(components):

- Pain (5 items): during walking, using stairs, in bed, sitting or lying, and standing
- Stiffness (2 items): after first waking and later in the day
- Physical Function (17 items): stair use, rising from sitting, standing, bending, walking, getting in / out of a car, shopping, putting on / taking off socks, rising from bed, lying in bed, getting in / out of bath, sitting, getting on / off toilet, heavy household duties, light household duties.
- In order to suite the WOMAC score with Indian rural population, we had replaced the item getting in/out of a car with getting in/out of auto and putting on/taking off socks with cleaning of ankles.

Each item mentioned in WOMAC scoring system was described in terms of - none, mild moderate, severe, and extreme. These correspond to scale of 0-4. Each component of the WOMAC score ranges between 0-20 for pain, 0-8 for stiffness and 0-68 for functionality. A total WOMAC score was obtained by adding the items for all three subscales, ranges from 0-96.[161]

As we have not found any literature for grading the results of WOMAC score, hence we have graded it to quantify the results. Outcome measured is quantified in percentage of improvement.

85-100% improvement – excellent

70-84% improvement – good

55-69% improvement – fair

< 55% improvement – poor.

The Western Ontario and McMaster Universities Osteoarthritis Index

(WOMAC)

Name:_____ Date:_____

Instructions: Please rate the activities in each category according to the following scale of difficulty: 0 = None, 1 = Slight, 2 = Moderate, 3 = Very, 4 = Extremely

Circle **one number** for each activity

Pain	1. Walking	0 1 2 3 4
	2. Stair Climbing	0 1 2 3 4
	3. Nocturnal	0 1 2 3 4
	4. Rest	0 1 2 3 4
	5. Weight bearing	0 1 2 3 4
Stiffness	1. Morning stiffness	0 1 2 3 4
	2. Stiffness occurring later in the day	0 1 2 3 4
Physical Function	1. Descending stairs	0 1 2 3 4
	2. Ascending stairs	0 1 2 3 4
	3. Rising from sitting	0 1 2 3 4
	4. Standing	0 1 2 3 4
	5. Bending to floor	0 1 2 3 4
	6. Walking on flat surface	0 1 2 3 4
	7. Getting in / out of car/auto	0 1 2 3 4
	8. Going shopping	0 1 2 3 4
	9. Putting on socks/cleaning of ankles	0 1 2 3 4
	10. Lying in bed	0 1 2 3 4
	11. Taking off socks	0 1 2 3 4
	12. Rising from bed	0 1 2 3 4
	13. Getting in/out of bath	0 1 2 3 4
	14. Sitting	0 1 2 3 4
	15. Getting on/off toilet	0 1 2 3 4
	16. Heavy domestic duties	0 1 2 3 4
	17. Light domestic duties	0 1 2 3 4

Total Score: _____ / 96 = _____ %

Comments / Interpretation (to be completed by therapist only):

Figure 45: Chart used to evaluate WOMAC score.[161]

VISUAL ANALOGUE SCALE (VAS)

This tool was first used in psychology by Freyd in 1923. The Visual Analogue Scale (VAS) consists of a straight line with the endpoints defining extreme limits such as 'no pain at all' and 'worst pain'. The patient is asked to mark his pain level on the line between the two endpoints. The distance between 'no pain at all' and the mark then defines the subject's pain.[162]

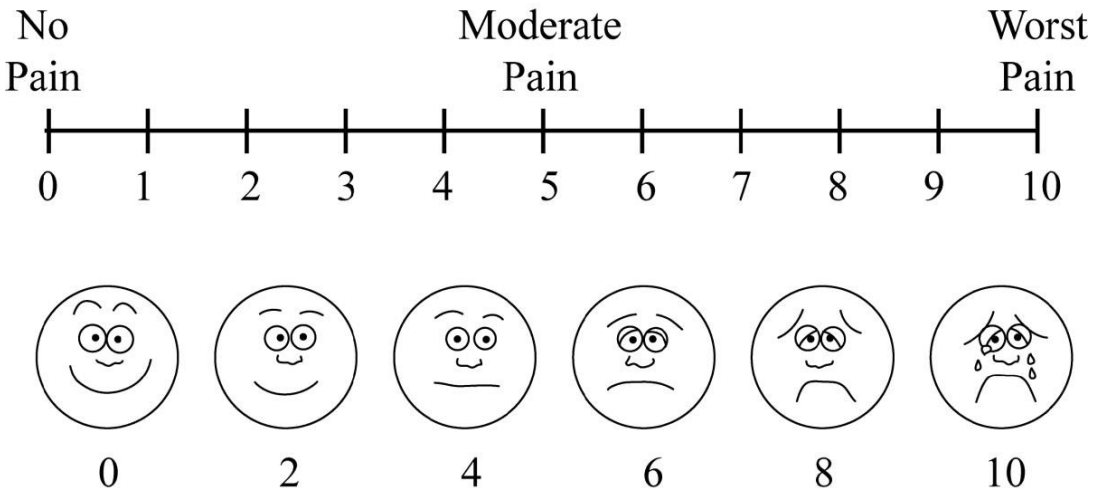


Figure 46: Chart used to evaluate VAS score.[162]

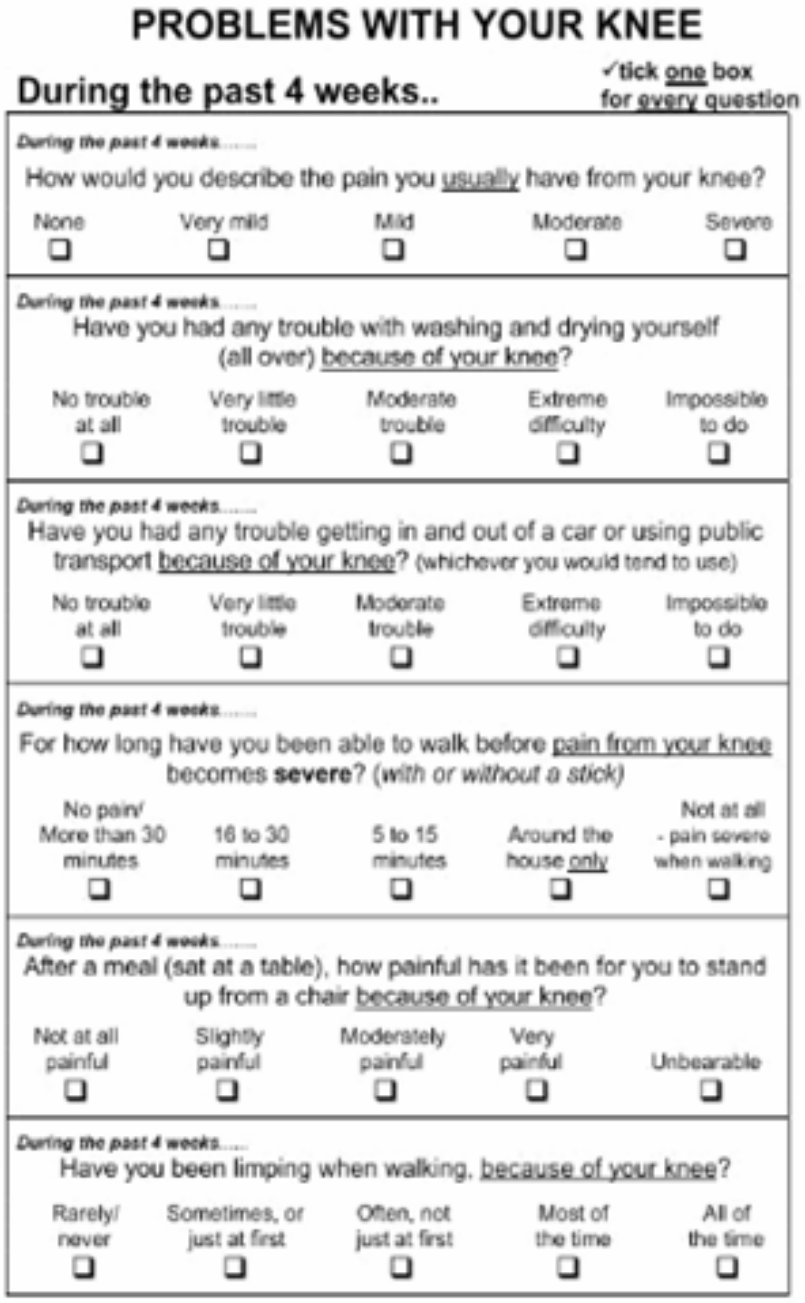
OXFORD KNEE SCORE (OKS)

The Oxford Knee Score (OKS) was a sequel to the Oxford Hip Score and the Oxford Shoulder Score. It is a 12 item self-completed patient based outcome score. The questionnaire was developed from patient interview and validated against two generic health scales, the SF-36 and Health Assessment Questionnaire (HAQ).

The OKS was designed to be short, practical, reliable, valid and sensitive to clinically important change, hence being more accurate than other patient based measures, such as the SF-36 or the Arthritis Impact Measurement Scale (AIMS). These other scales have been criticised for their length, difficulty in completion, unresponsiveness and lack of relevance for joint replacement patients. When compared with the Knee Society Score, SF-36 and HAQ, the OKS fared favourably in terms of reproducibility, internal consistency, validity and responsiveness.

Grading of the Oxford knee score:

1) Score 0 to 19- Poor
2) Score 20 to 29- Moderate
3) Score 30 to 39- Good
4) Score 40 to 48- Excellent. [163]



PROBLEMS WITH YOUR KNEE

During the past 4 weeks.. ✓tick one box for every question

1 *During the past 4 weeks.....*
How would you describe the pain you usually have from your knee?

None	Very mild	Mild	Moderate	Severe
❑	❑	❑	❑	❑

2 *During the past 4 weeks.....*
Have you had any trouble with washing and drying yourself (all over) because of your knee?

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

3 *During the past 4 weeks.....*
Have you had any trouble getting in and out of a car or using public transport because of your knee? (whichever you would tend to use)

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
❑	❑	❑	❑	❑

4 *During the past 4 weeks.....*
For how long have you been able to walk before pain from your knee becomes **severe**? (*with or without a stick*)

No pain/ More than 30 minutes	16 to 30 minutes	5 to 15 minutes	Around the house only	Not at all - pain severe when walking
❑	❑	❑	❑	❑

5 *During the past 4 weeks.....*
After a meal (sat at a table), how painful has it been for you to stand up from a chair because of your knee?

Not at all painful	Slightly painful	Moderately painful	Very painful	Unbearable
❑	❑	❑	❑	❑

6 *During the past 4 weeks.....*
Have you been limping when walking, because of your knee?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

Figure 47: The oxford knee score (questions 1 to 6). [163]

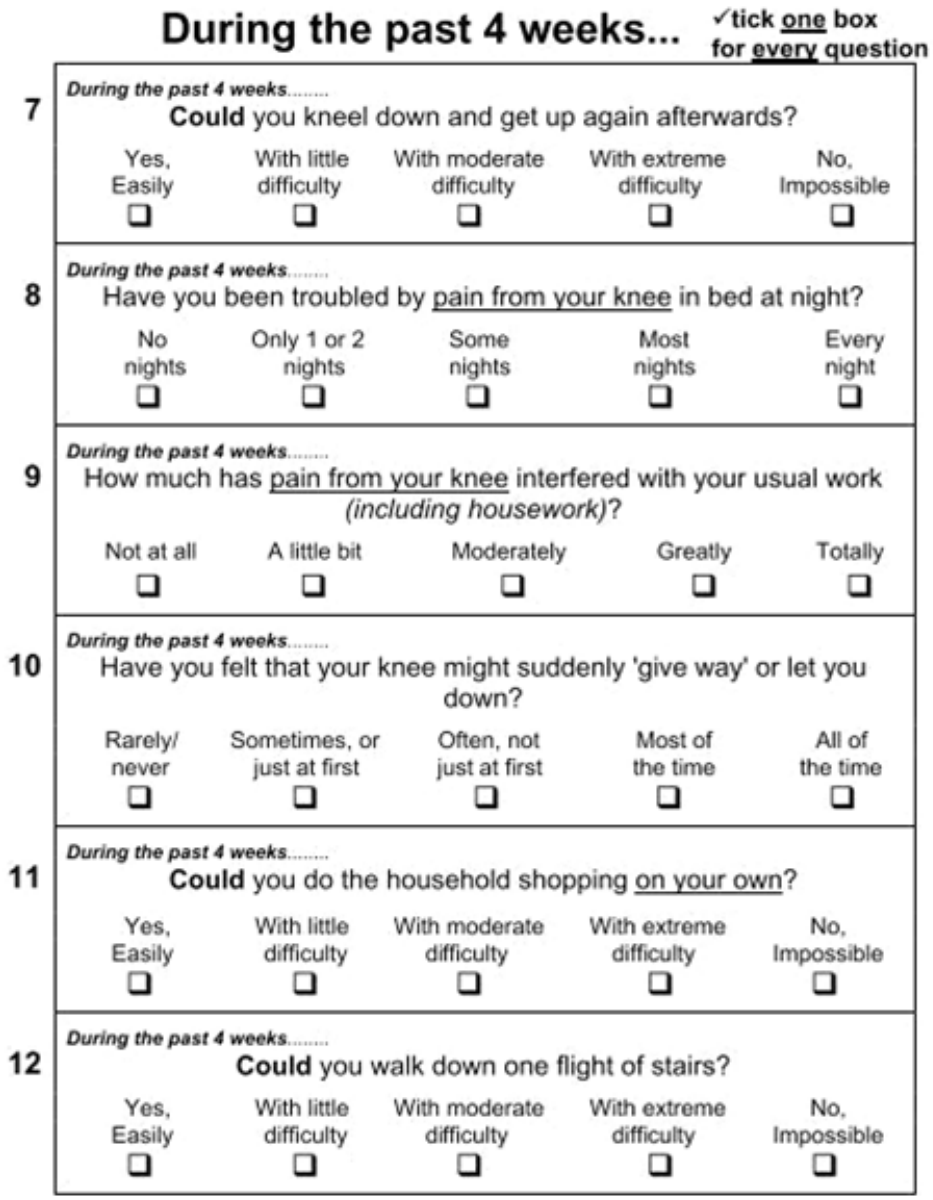


Figure 48: The oxford knee score (questions 7 to 12). [163]

STATISTICAL METHODS

VAS score (pre injection, 1st ,3rd and 6th month), WOMAC score (pre injection, 1st ,3rd and 6th month) and Oxford knee score (pre injection, 1st ,3rd and 6th month), were considered as primary outcome variables. Study group (SS-PRB and DS-PRS) was considered as primary explanatory variable. Demographic parameter like age, gender, knee affected, grade of osteoarthritis, platelet count (patient and platelet-rich-plasma), comorbidities (diabetes and hypertension), complication (pain and swelling) were also considered. All quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal distribution. Shapiro wilk test p value of >0.05 was considered as normal distribution. For normally distributed quantitative parameters the mean values were compared between study groups using independent sample t-test study group (SS-PRP and DS-PRP). For non-normally distributed quantitative parameters, medians and interquartile range (IQR) were compared between study groups using Mann Whitney u test study group (SS-PRP and DS-PRP)

Categorical outcomes were compared between study groups using Chi square test/Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.)

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis.

OBSERVATIONS AND RESULTS

68patients were included in the study following the inclusion and exclusion criteria. The patients were randomized into 2 groups using a standard randomization technique.

Out of the 68 patients, 4 patients were lost to follow-up, which gave a final sample size of 64 patients.

Table 5: Descriptive analysis of study group in the study population (N=64)

Study group	Frequency	Percentages
SS-PRP	32	50.00%
DS-PRP	32	50.00%

Among the study population, 32 (50%) were SS-PRP and 32 (50%) were DS-PRP. (Table 5& Figure 49)

Figure 49: Bar chart of study group in the study population (N=64)

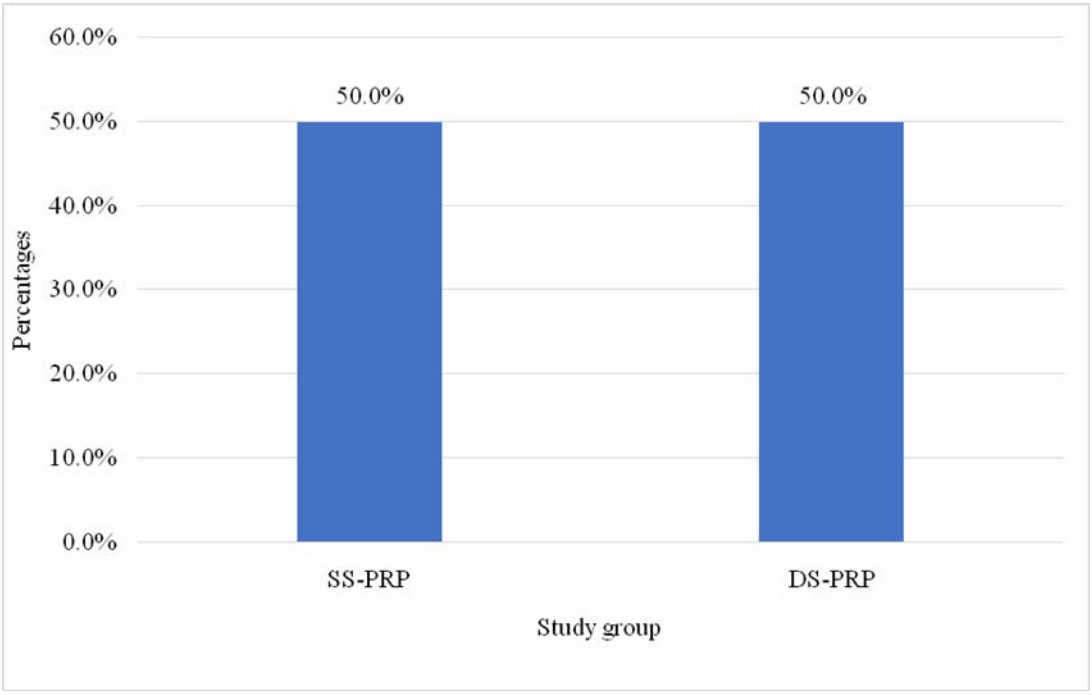


Table 6: Comparison of mean of age between study group(N=64)

Parameter	Study group (Mean± SD)		P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Age	57.63 ± 7.4	50.75 ± 7.33	<0.001

The mean age was 57.63 ± 7.4 in SS-PRP and it was 50.75 ± 7.33 in DS-PRP. The difference in mean age between the study groups was statistically significant. (P value <0.001) (Table 6& Figure 50)

Figure 50: Error bar chart of comparison of mean of age between study group (N=64)

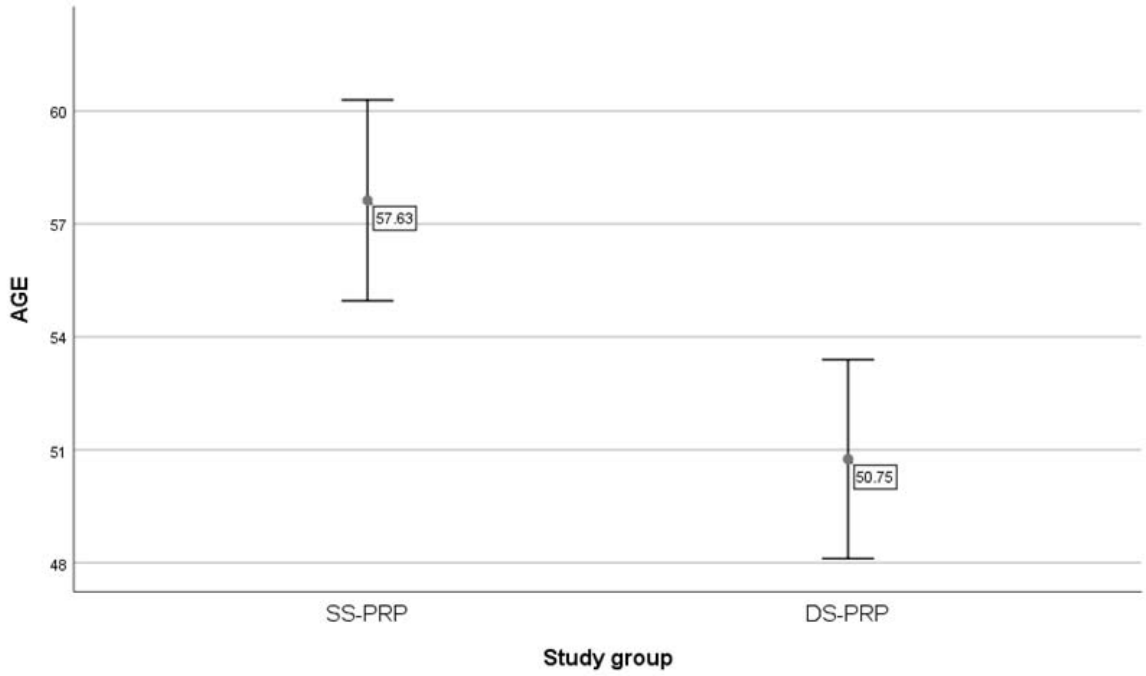


Table 7: Comparison of gender between study group (N=64)

Gender	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Male	8 (25%)	17 (53.13%)	5.317	0.021
Female	24 (75%)	15 (46.88%)		

Among SS-PRP 8 (25%) were male and 24 (75%) were female, among DS-PRP 17 (51.13%) were male and 15 (46.88%) were female. The difference in proportion of gender between the study groups was statistically significant. (P value 0.021) (Table 7& Figure 51)

Figure 51: Staked bar chart of comparison of gender between study group (N=64)

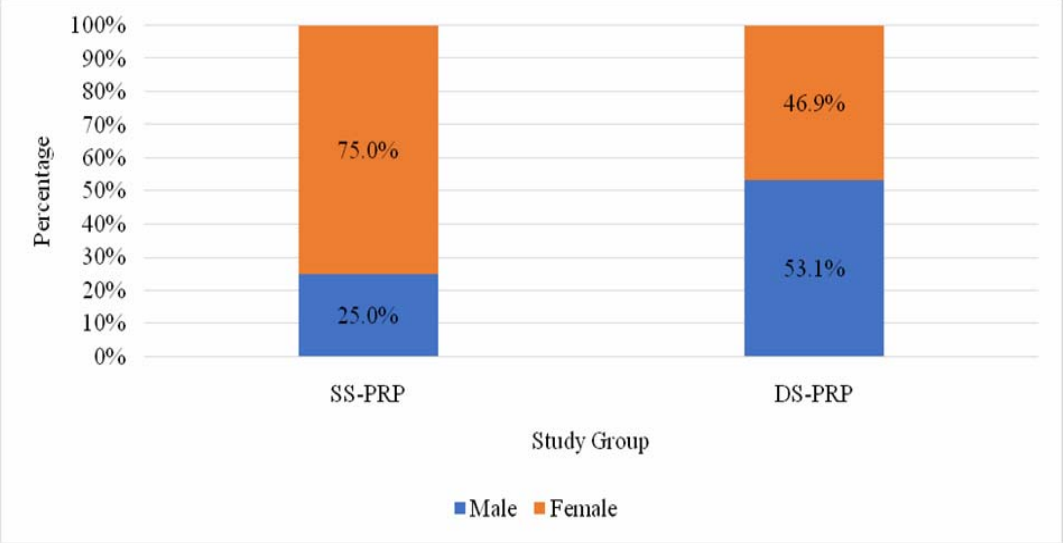


Table 8: Comparison of knee affected between study group (N=64)

Knee Affected	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Bilateral	23 (71.88%)	20 (62.5%)	0.700	0.705
Left	4 (12.5%)	6 (18.75%)		
Right	5 (15.63%)	6 (18.75%)		

Among SS-PRP, 23 (71.88%) had bilateral OA knee, 4 (12.5%) had left OA knee, 5 (15.63%) had right OA knee and among DS-PRP, 20 (62.5%) had bilateral OA knee, 6 (18.75%) had left OA knee, 6 (18.75%) had right OA knee. The difference in proportion of kneesaffected between the study groups was not statistically significant. (P value 0.705) (Table 4 & Figure 4)

Figure 52: Staked bar chart of comparison of knee affected between study group (N=64)

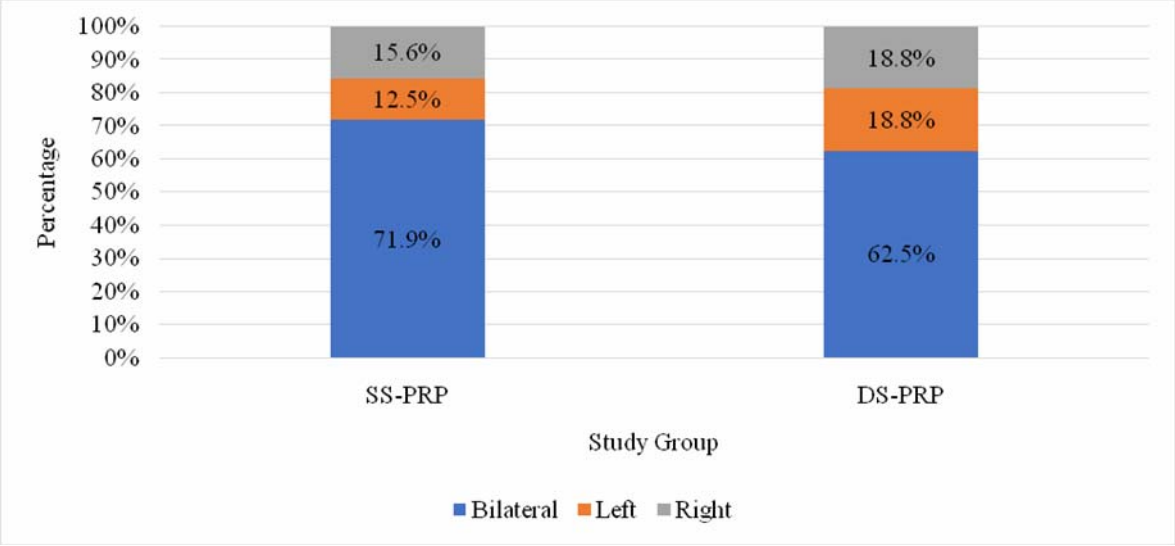


Table 9: Comparison of grade of osteoarthritis between study group (N=64)

Grade of osteoarthritis	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Grade 1	9 (28.13%)	15 (46.88%)	2.400	0.121
Grade 2	23 (71.88%)	17 (53.13%)		

Among SS-PRP, 9 (28.13%) were under Grade 1 OA, 23 (71.88%) were under Grade 2 OA and among DS-PRP, 15 (46.88%) were under Grade 1 OA, 17 (51.13%) were under Grade 2 OA. The difference in proportion of grades of OA between the study groups was not statistically significant. (P value 0.121) (Table 9& Figure 53)

Figure 53: Staked bar chart of comparison of grade of osteoarthritis between study group (N=64)

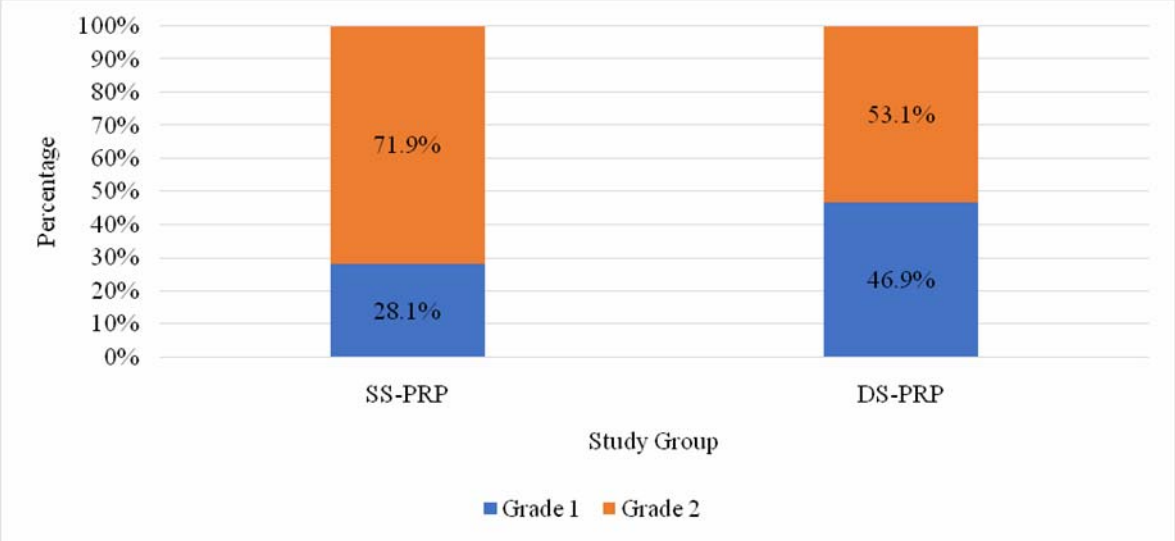


Table 10: Comparison of mean of platelet parameter between study group(N=64)

Parameter	Study group (Mean± SD)		P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Platelet count	361781.25 ± 69903.96	299487.5 ± 78632.64	0.001
Platelet-rich plasma	665718.75 ± 88607.96	853281.25 ± 135644.28	<0.001

The mean platelet count was 361781.25 ± 69903.96 in SS-PRP and it was 299487.5 ± 78632.64 in DS-PRP. The difference in mean patient platelet countbetween the study groups was statistically significant. (P value 0.001)

The mean platelet count of patient-rich plasma was 665718.75 ± 88607.96 in SS-PRP and it was 853281.25 ± 135644.28 in DS-PRP. The difference in mean platelet count of patient-rich plasma between the study groups was statistically significant. (P value 0.001) (Table 10& Figure 54)

Figure 54: Error bar chart of mean of platelet count Patient between study group(N=64)

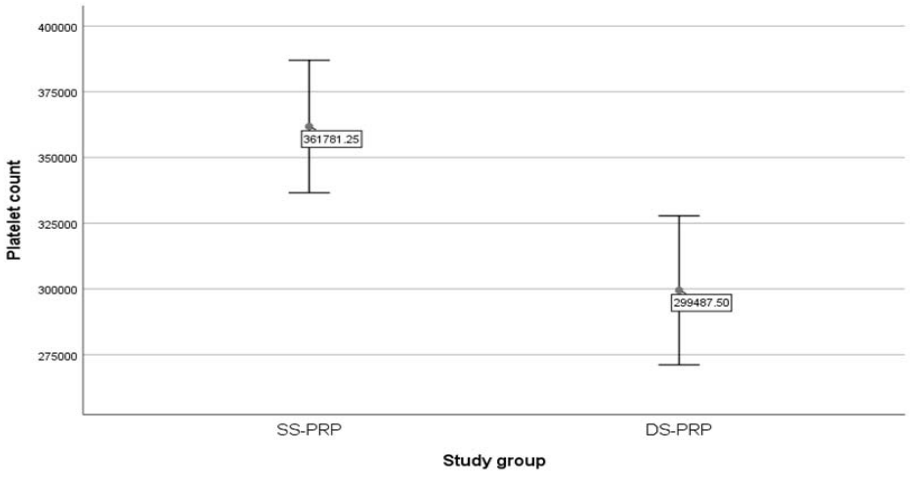


Figure 55: Error bar chart of mean of Platelet-rich plasma between study group(N=64)

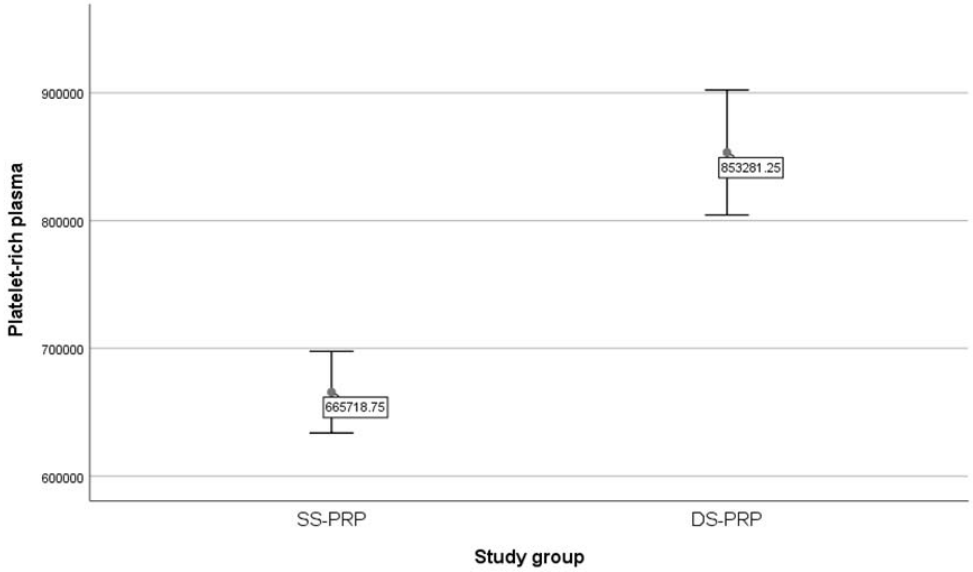


Table 11: Comparison of diabetes between study group (N=64)

Diabetes	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Yes	7 (21.88%)	11 (34.38%)	1.237	0.266
No	25 (78.13%)	21 (65.63%)		

Among SS-PRP, 7 (21.88%) patients had diabetes and among DS-PRP, 11 (34.38%) patients had diabetes. The difference in proportion of diabetes between the study groups was not statistically significant. (P value 0.266) (Table 11& Figure 56)

Figure 56: Staked bar chart of comparison of diabetes between study group (N=64)

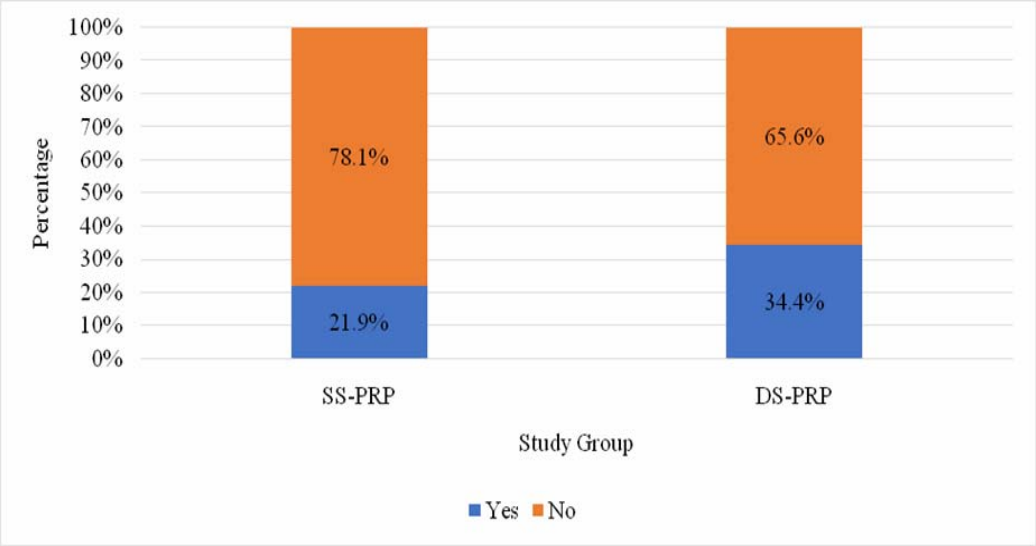


Table 12: Comparison of hypertension between study group (N=64)

Hypertension	Study Group		Fisher exact P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Yes	3 (9.38%)	2 (6.25%)	1.000
No	29 (90.63%)	30 (93.75%)	

Among SS-PRP, 3 (9.38%) patients had hypertension and among DS-PRP, 2 (6.25%) patients had hypertension. The difference in proportion of hypertension between the study groups was not statistically significant. (P value 1.000) (Table 12& Figure 57)

Figure 57: Staked bar chart of comparison of hypertension between study group (N=64)

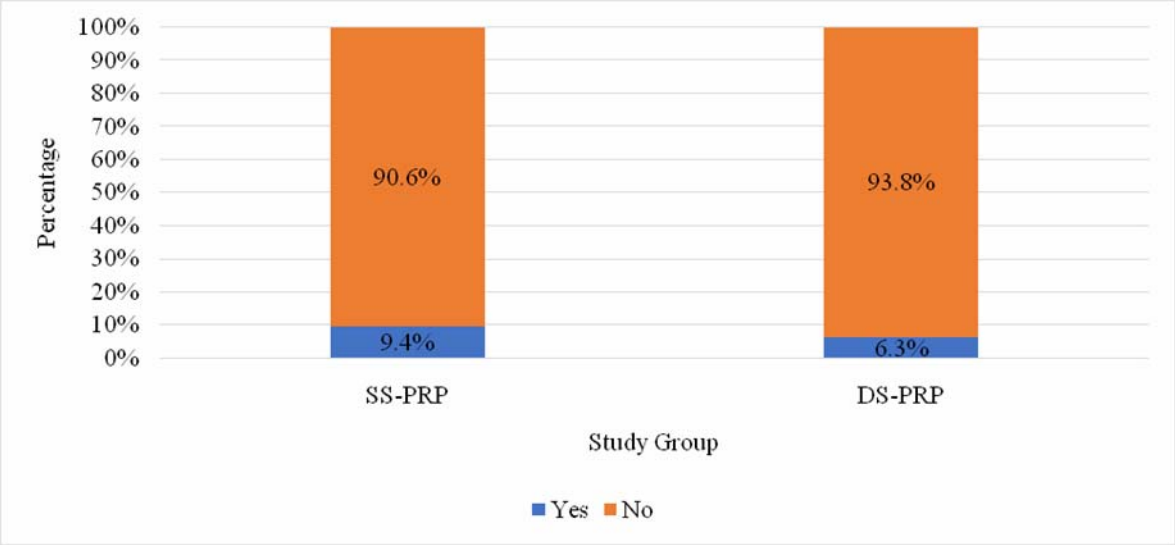


Table 13: Comparison of mean of VAS score between the two groups at different follow-up time periods (N=64)

Parameter	Study group (Mean± SD)		P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Pre injection	8.25 ± 0.62	8 ± 0.51	0.083
1 month	6.28 ± 0.73	5.88 ± 0.61	0.018
3 months	5.16 ± 0.57	4.63 ± 0.61	<0.001
6 months	4.28 ± 0.58	3.34 ± 0.65	<0.001

The mean pre-injection VAS score was 8.25 ± 0.62 in SS-PRP, it was 8 ± 0.51 in DS-PRP. The difference in mean pre-injection VAS score between the study groups was not statistically significant. (P value 0.083)

The mean VAS score at 1 month was 6.28 ± 0.73 in SS-PRP, while it was 5.88 ± 0.61 in DS-PRP. The difference in mean VAS score at 1 month between the study groups was statistically significant. (P value 0.018)

The mean VAS score at 3 months was 5.16 ± 0.57 in SS-PRP, it was 4.63 ± 0.61 in DS-PRP. The difference in mean VAS score at 3 months between the study groups was statistically significant. (P value <0.001)

The mean VAS score at 6 months was 4.28 ± 0.58 in SS-PRP and 3.34 ± 0.65 in DS-PRP. The difference in mean VAS score at 6 months between the study groups was statistically significant. (P value <0.001) (Table 13)

Table 14: Comparison of mean of WOMAC score between the two groups at different follow-up time periods (N=64)

Parameter	Study group (Mean± SD)		P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Pre injection	76.75 ± 3.62	74.84 ± 3.02	0.026
1 month	67.28 ± 4.46	61.25 ± 2.75	<0.001
3 months	60.66 ± 5.38	52.72 ± 3.09	<0.001
6 months	54.78 ± 5.95	44.53 ± 3.03	<0.001

The mean pre-injection WOMAC score was 76.75 ± 3.62 in SS-PRP, it was 74.84 ± 3.02 in DS-PRP. The difference in mean pre-injection WOMAC score between the study groups was statistically significant. (P value 0.026)

The mean WOMAC score at 1 month was 67.28 ± 4.46 in SS-PRP and it was 61.25 ± 2.75 in DS-PRP. The difference in mean WOMAC score at 1 month between the study groups was statistically significant. (P value <0.001)

The mean WOMAC score at 3 months was 60.66 ± 5.38 in SS-PRP, it was 52.72 ± 3.09 in DS-PRP. The difference in mean WOMAC score at 3 months between the study groups was statistically significant. (P value <0.001)

The mean WOMAC score at 6 months was 54.78 ± 5.95 in SS-PRP, it was 44.53 ± 3.03 in DS-PRP. The difference in mean WOMAC score at 6 months between the study groups was statistically significant. (P value <0.001) (Table 14)

Table 15: Comparison of oxford knee score between the two groups at different follow-up time periods (N=64)

Parameter	**Study Group**		**Mann Whitney U test (P value)**
	SS-PRP Median (IQR)	**DS-PRP** Median (IQR)	
Pre injection (N=64)	20 (18,21)	19 (18,20)	0.713
1 month (N=64)	24 (21.25,25)	24 (23,25)	0.184
3 months (N=64)	26.5 (25,28)	27.5 (27,28)	0.028
6 months (N=64)	29 (28,30)	30 (29,31)	0.013

The median pre-injection Oxford knee score was 20 (IQR 18,21) in SS-PRP, it was 19 (IQR 18,20) in DS-PRP. The difference in median pre-injection knee score between the study groups was not statistically significant. (P value 0.713)

The median Oxford knee score at 1 month was 24 (IQR 21.25,25) in SS-PRP, it was 24 (IQR 23,25) in DS-PRP. The difference in median Oxford knee score at 1 month between the study groups was not statistically significant. (P value 0.184)

The median Oxford knee score at 3 months was 26.5 (IQR 25,28) in SS-PRP, it was 27.5 (IQR 27,28) in DS-PRP. The difference in median Oxford knee score at 3 months between the study groups was statistically significant. (P value 0.028)

The median Oxford knee score at 6 months was 29 (IQR 28,30) in SS-PRP, it was 30 (IQR 29,31) in DS-PRP. The difference in median Oxford knee score at 6 months between the study groups was statistically significant. (P value 0.013) (Table 15)

Range of motion:

Table 16: Comparison of pre injection range of motion between study group (N=64)

Pre-Injection	**Study Group**	
	SS-PRP (N=32)	**DS-PRP (N=32)**
Right		
0-110	12 (37.5%)	3 (9.38%)
0-115	0 (0%)	1 (3.13%)
0-120	14 (43.75%)	19 (59.38%)
0-125	0 (0%)	1 (3.13%)
0-130	4 (12.5%)	2 (6.25%)
0-135	1 (3.13%)	3 (9.38%)
0-140	1 (3.13%)	3 (9.38%)
Left		
0-110	6 (18.75%)	1 (3.13%)

0-120	7 (21.88%)	11 (34.38%)
0-125	1 (3.13%)	1 (3.13%)
0-130	18 (56.25%)	9 (28.13%)
0-135	0 (0%)	8 (25%)
0-140	0 (0%)	2 (6.25%)

*No statistical test was applied- due to 0 subjects in the cells

Among SS-PRP, pre-injection, 12 (37.5%) patients hada range of motionof 0-110 in the right knee and 14 (43.47%) patients had a range of motion of 0-120 the right knee. Among DS-PRP, pre-injection, 3 (9.38%) patients had range of motionof 0-110 in right knee and 19 (59.38%) patientshad range of motionof 0-120 in right knee. Among SS-PRP, pre-injection, 6 (18.75%) patientshad range of motionof 0-110 in left knee, 18 (56.25%) patients had range of motion between 0-130 in left knee and among DS-PRP, pre-injection, 11 (34.38%) patients had range of motion 0-120 in left knee, 9 (28.13%) patientshad range of motionof 0-130 in left knee. (Table 16)

Table 17: Comparison of range of motion at 1 month between study group (N=64)

Range of motion at 1 month	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP N=32)		
Right				
0-120	13 (40.63%)	3 (9.38%)	10.393	0.034
0-125	4 (12.5%)	6 (18.75%)		
0-130	13 (40.63%)	15 (46.88%)		
0-135	1 (3.13%)	4 (12.5%)		
0-140	1 (3.13%)	4 (12.5%)		
Left				
0-120	5 (15.63%)	1 (3.13%)	*	*
0-125	4 (12.5%)	2 (6.25%)		
0-130	18 (56.25%)	16 (50%)		
0-135	5 (15.63%)	10 (31.25%)		
0-140	0 (0%)	3 (9.38%)		

*No statistical test was applied- due to 0 subjects in the cells

Among SS-PRP,1 month post-injection, 13 (40.63%) patients had range of motion of 0-120 in right knee, 13 (40.63%) patients had range of motionof 0-130 in right knee and among DS-PRP, 6 (18.75%) patients had range of motionof 0-125 in right knee, 15 (46.88%) patients had range of motionof 0-130 in right knee. The difference in proportion at 1 month for right knee between the study groups was statistically significant. (P value 0.034) Among SS-PRP, 5 (15.63%) had range of motionof 0-120 in left knee, 18 (56.25%) had range of motionof0-130 in left knee and among DS-PRP, 16 (50%) were had range of motionof 0-130 in left knee, 10 (31.25%) had range of motionof 0-135 in left knee. (Table 17)

Table 18: Comparison of range of motion at 3 months between study group (N=64)

Range of motion at 3 months	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Right				
0-125	2 (6.25%)	3 (9.38%)	2.259	0.520
0-130	20 (62.5%)	15 (46.88%)		
0-135	8 (25%)	9 (28.13%)		
0-140	2 (6.25%)	5 (15.63%)		
Left				
0-120	2 (6.25%)	0 (0%)	*	*
0-125	3 (9.38%)	1 (3.13%)		
0-130	12 (37.5%)	10 (31.25%)		
0-135	12 (37.5%)	15 (46.88%)		
0-140	3 (9.38%)	6 (18.75%)		

*No statistical test was applied- due to 0 subjects in the cells

Among SS-PRP, at 3 months post-injection, 2 (6.25%) patients had range of motionof 0-125 in right knee, 20 (62.5%) patients had range of motionof 0-130 in right knee and among DS-PRP, 15 (46.88%) patients hada range of motion of 0-130 in the right knee, 9 (28.13%) patients had range of motion of 0-135 in the right knee. The difference in proportion at 3 months post-injection of the right knee between the study groups was not statistically significant. (P value 0.520). Among SS-PRP, at 3 months post injection, 2 (6.25%) patientshad range of motion of 0-120 in the left knee, 12 (37.5%) patientshad range of motionof 0-130 in the left knee and among DS-PRP, 10 (31.25%) patients had range of motionof 0-130 in left knee, 15 (46.88%) patientshad range of motionof 0-135 in left knee. (Table 18)

Table 19: Comparison of range of motion at 6 months right between study group (N=64)

Range of motion at 6 months	Study Group		Chi square	P value
	SS-PRP (N=32)	DS-PRP (N=32)		
Right				
0-125	0 (0%)	1 (3.13%)	*	*
0-130	8 (25%)	2 (6.25%)		
0-135	17 (53.13%)	18 (56.25%)		
0-140	7 (21.88%)	11 (34.38%)		
Left				
0-130	10 (31.25%)	3 (9.38%)	8.625	0.013
0-135	16 (50%)	13 (40.63%)		
0-140	6 (18.75%)	16 (50%)		

*No statistical test was applied- due to 0 subjects in the cells

Among SS-PRP, 6 months post injection, 8 (25%) patientshad range of motionof 0-130 of the right knee, 17 (53.13%) patients had range of motion of 0-135 of the right knee and among DS-PRP, 18 (56.25%) patientshad range of motionof 0-135 of the right knee, 11 (34.38%) patients had range of motionof 0-140 of the right knee.

Among SS-PRP, 6 months post injection, 10 (31.25%) patients had range of motion of 0-130 of the left knee, 16 (50%) patients had a range of motion of 0-135 of the left knee and among DS-PRP, 13 (40.63%) patients had a range of motion of 0-135 of the left knee, 16 (50%) patients had a range of motion of 0-140 of the left knee. The difference in proportion 6 months post-injection of the left knee between the study groups was statistically significant. (P value 0.013) (Table 19)

Table 20: Comparison of pain between study group (N=64)

Pain	Study Group		Fisher exact P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Pain	3 (9.38%)	6 (18.75%)	0.474
No Pain	29 (90.63%)	26 (81.25%)	

Among SS-PRP, 3 (9.38%) had pain and among DS-PRP 6 (18.75%) had pain. The difference in proportion of pain between study group was not statistically significant. (P value 0.474) (Table 20& Figure 58)

Figure 58: Staked bar chart of comparison of pain between study group (N=64)

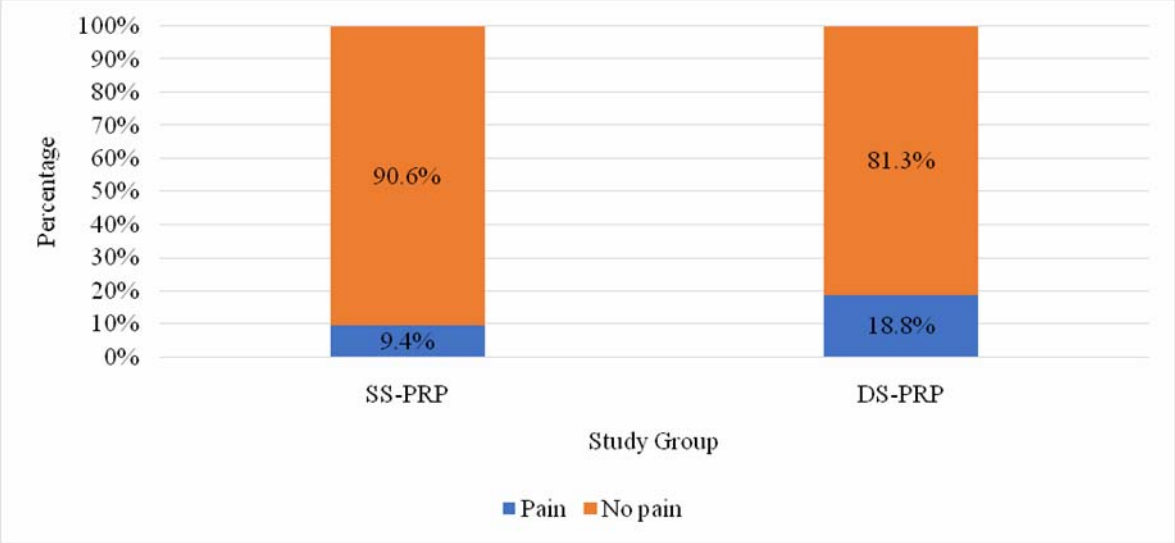
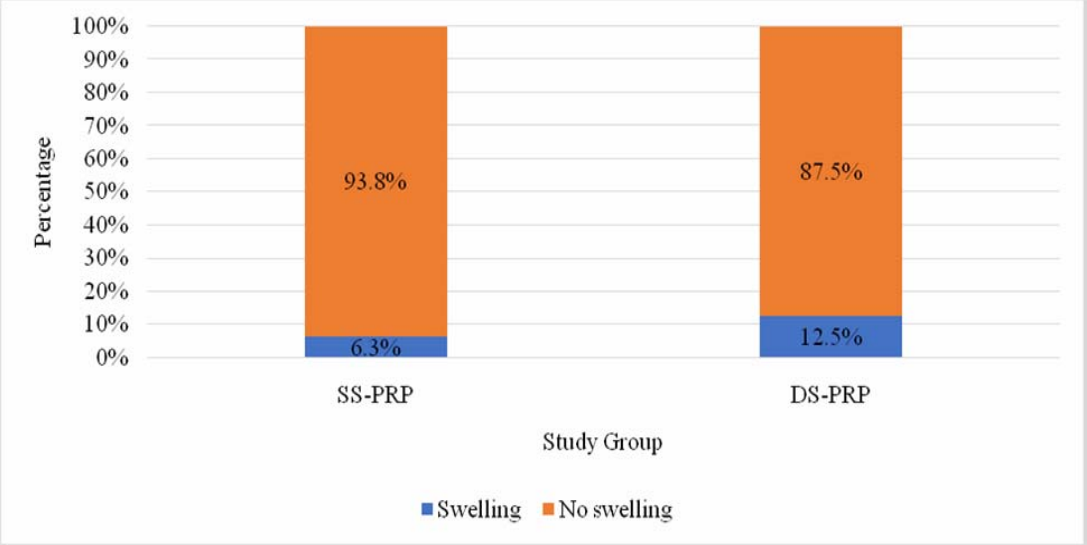


Table 21: Comparison of swelling between study group (N=64)

Swelling	Study Group		Fisher exact P value
	SS-PRP (N=32)	DS-PRP (N=32)	
Swelling	2 (6.25%)	4 (12.5%)	0.672
No Swelling	30 (93.75%)	28 (87.5%)	

Among SS-PRP, 2 (6.25%) had swelling and among DS-PRP 4 (12.5%) had swelling. The difference in proportion of swelling between study group was not statistically significant. (P value 0.672) (Table 21& Figure 59)

Figure 59: Staked bar chart of comparison of swelling between study group (N=64)



DISCUSSION

Osteoarthritis of the knee is a degenerative condition involving softeningand focal disintegration of articular cartilage. The prevalence of osteoarthritis is more in developing countries like India. Studies have showed OA is more common in lower socioeconomic than higher socioeconomic population and also suggested that more active individuals have a lower risk of OA. The various modifiable risk factors are repetitive movement of joints, obesity, infection and injuries. The occupational physical activities include monotonous motions and great forces such as kneeling, squatting on joints, climbing and heavy weight lifting. Treatment of knee osteoarthritis is difficult because of avascular and aneural nature of adult knee cartilage. These characteristics render low regenerative capacity and low healing potential to knee cartilage. There are many nonpharmacological and pharmacological treatment options for knee osteoarthritis which help in delaying or avoiding surgical treatment option which is total knee arthroplasty. Total knee arthroplasty though effective is not preferred treatment option because of many factors like high costs involved, its invasive procedure and a reported poor outcome rate of 20% which make it a less considered treatment option. It is also estimated that one-third of total knee arthroplasty patients have chronic postoperative pain.[4]

Platelet rich plasma (PRP) injections are one of the four main injection therapies used for treatment of osteoarthritis.[164]

PRP injections are very convenient, efficient and simple to administer. They can be administered in simple outpatient clinical setting.[4]

The effect of PRP in osteoarthritis is mainly due to growth factors released from platelet activation. It has been observed that along with growth factors PRP also

enhances type II collagen production, stimulates endogenous hyaluronic acid production and helps in mesenchymal cell survival and proliferation.[165]

PRP is safe as it is an autologous blood product and hence will not cause any immunological reactions and disease transfer.[149]

Though the rationale for use of PRP is strong there are still concerns regarding its clinical efficacy, mainly due to the heterogeneity of preparation methods and resulting products.[166]Single-spinning centrifugation results in platelets up to three times that of baseline level whereas double-spinning centrifugation results in platelets up to eight times the baseline level with a high leucocyte content. However, there are very few studies which compare the use of the two techniques of preparation of PRP in early OA knee.[15]

The aim of this study is to compare the functional outcomes of two methods of preparation of PRP, single spinning method and double spinning method for grade 1 and grade 2 OA knee in an Indian population.

Study group (SS-PRP and DS-PRP) was considered as primary explanatory variables. 68 patients were included in the study, however, 4 patients were lost for follow-up, giving a total of 64 subjects that were included in the final analysis. The subjects were divided into two groups of 32 each and group A was given single spin PRP and group B was given double spin PRP injection. The mean age was 57.63 ± 7.4 in SS-PRP and it was 50.75 ± 7.33 in DS-PRP. The participants in both groups are above 40 years of age.

The mean pre-injection VAS score was 8.25 ± 0.62 in SS-PRP, it was 8 ± 0.51 in DS-PRP. The mean VAS score at 1 month was 6.28 ± 0.73 in SS-PRP, it was 5.88 ± 0.61 in DS-PRP. The mean VAS score at 3 months was 5.16 ± 0.57 in SS-PRP, it was 4.63 ± 0.61 in DS-PRP. The mean VAS score at 6 months was 4.28 ± 0.58 in SS-

PRP, it was 3.34 ± 0.65 in DS-PRP. From above observations it can be deduced that PRP injections helps in reduction of pain in osteoarthritis over a period of 6 months which is evident by the reduction in VAS scores. This finding is supported by different studies which include study by Sanchez et al. in which effectiveness of intra-articular injections of autologous PRP for knee OA treatment in an observational retrospective cohort study on 30 patients was studied and results suggested the safety and usefulness of this treatment approach.[167]

In another study by Wang-Saegusa et al. single-spinning procedure PRP was used to treat knee OA, and the evaluation of 261 patients showed a significant increase in all the clinical scores applied, where 73.4% of patients had an improvement at 6 months' follow-up. [15]

In a study by Sampson et al.single-spinning procedure was used for the treatment of a small group of patients affected by primary and secondary knee OA and reported a favourable outcome in the majority of the patients and maintained those positive results for at least 12 months.[168]

Kon et al.published a pilot study of 100 patients treated with intraarticular injections of PRP obtained with a double-spinning procedure. The results of this study indicated that the treatment with PRP injections is safe and has the potential to reduce pain and improve knee function and quality of live in younger patients with low degree of articular degeneration.[14]

Another observation that can be made from the results of our study is that the reduction in VAS scores is slightly higher for group injected with double spin PRP at 1,3 and 6 months follow up. This observation is not reported in a similar study by Filardo, G., et al.in which both treatment groups presented a similar statistically significant improvement in all the scores evaluated at all the follow-up times.[6]

The mean pre-injection WOMAC score was 76.75 ± 3.62 in SS-PRP, it was 74.84 ± 3.02 in DS-PRP. The mean WOMAC score at1 month was 67.28 ± 4.46 in SS-PRP, it was 61.25 ± 2.75 in DS-PRP. The mean WOMAC score at 3 months was 60.66 ± 5.38 in SS-PRP, it was 52.72 ± 3.09 in DS-PRP. The mean WOMAC score at 6 months was 54.78 ± 5.95 in SS-PRP, it was 44.53 ± 3.03 in DS-PRP. Improvement in WOMAC scores after administration of PRP injections was noted in two studies one by Sandeep Patel, et al.in which effect of single and two PRP injections on two different groups was studied and statistically significant improvement in all WOMAC parameters was noted in groups A and B within 2 to 3 weeks and lasting until the final follow-up at 6 months.[2]

In another study by Spakova, T., et al. in which effect of PRP injections and hyaluronic acid was compared between two groups. Statistically significantly better results in the WOMAC and Numeric Rating Scale scores were recorded in a group of patients who received PRP injections after a 3 and 6month follow-up. The difference in WOMAC scores between single spin PRP and double spin PRP groups was again similar to VAS scores.[169]

There was slightly higher reduction in WOMAC scores in DS-PRP group than in SS-PRP group which is different from the observation noted in similar study by Filardo, G., et al. in which same outcome was reported in both groups.[6]

The median pre-injection Oxford knee score was 20 in SS-PRP, it was 19 in DS-PRP. The median Oxford knee score at 1 month was 24 in SS-PRP, it was 24 in DS-PRP. The median Oxford knee score at 3 months was 26.5 in SS-PRP, it was 27.5 in DS-PRP. The median Oxford knee score at 6 months was 29 in SS-PRP, it was 30 in DS-PRP. From above observations it can be deduced that the improvement noted in knee scores was same for both single spin and double spin PRP groups.

PRP can be prepared using single centrifugation, double centrifugation or blood selective filtration procedures. PRPs prepared by different methods differ in terms of proportion of platelets in PRP to platelets in whole blood which is called platelet enrichment factor, presence of absence of WBC and method of activation.[166]

Different studies have controversial results on effect of leucocyte concentration in PRP. In a study by Pifer et al. it was shown that PRP with leucocytes contain MMP-2, 3 and 9 which can have deleterious effect.[170]

Braun et al.concluded in their study that treatment of synovial cells with leucocyte rich PRP resulted in significant cell death and proinflammatory mediator production.[171]

In the only study by Filardo G, et al. available in literature which compared the outcomes of single spin PRP and double spin PRP in knee osteoarthritis reported similar outcomes for both types of PRPs with only difference in that double spin PRP group (leucocyte rich) suffered from more swelling and pain reaction immediately after the injections.[6]

Comparing the results of present study with the above study and other studies on effect of single spin and double spin PRP on knee osteoarthritis it can be deduced that outcomes resulting from both PRPs are almost similar though small incremental reductions have been observed in VAS and WOMAC scores in double PRP group. These results cannot be used to conclude that double spin PRP will result in better outcomes because they are small and the sample size is also small and moreover not much difference was noted in knee scores between two groups. VAS and WOMAC scores are obtained by questionnaires and is dependent on patient's response and hence slight variations in outcomes between single spin and double spin PRP groups cannot be considered for concluding that double PRP will give better outcomes as

these variations may be due to differences in individual perceptions. It has been reported by many studies in literature that higher concentration of platelets within PRP may not result in enhanced tissue healing effect as it is observed that after certain threshold amount of concentration of platelets there will be inhibitory effect on tissue healing. In a study by Seyed Ahmad Raeissadat, et al.it was reported that PRP with 4 to 6 times concentration of platelets is only effective and concentration of more than 8 or less than 4 will not have any enhancing effect.[172]

COMPLICATIONS

Immediate post infiltration, few patients complained of pain and swelling, but no local or systemic complications were noted during our study. These complains of pain and swelling were seen more in the patients of the double spin PRP injection group as compared to the single spin PRP injection group.

LIMITATIONS

The limitations of this study are short term follow-up period, small sample size, no control group. However, further studies on a larger population and longer follow up is recommended. Radiographic follow-up investigation methods such as magnetic resonance imaging may be considered for evaluating cartilage regeneration (if any) in subsequent research efforts.

CONCLUSIONS

This study concludes that PRP can be used for treatment of osteoarthritis as it results in reduction of pain and increase in range of movement. Regarding superiority of single spin or double spin PRP both can be considered to give similar outcomes in terms of pain reduction and range of movement and preference must hence be based availability of single of double spin PRP and physician's choice. Double spin PRP preparation requires a haematology unit whereas single spin PRP can be easily prepared in normal clinical setting and hence the choice should be made accordingly depending on resource availability as there is not much difference in outcomes.

The findings of this study are:

- PRP is a safe and effective for treatment of osteoarthritis.
- PRP can be easily administered in normal clinical settings.
- Double spin PRP has slightly better outcomes in terms of VAS and WOMAC scores.
- The outcome difference is not much higher and hence both single and double spin PRPs can be used for treatment of knee OA and selection must be based on availability.

Further prospective and larger studies, including randomised control studies are required to confirm better outcomes of double spin PRP over single spin PRP in knee osteoarthritis.

SUMMARY

PRP is a safe and new treatment for early osteoarthritic knee joints with increasing number of studies showing promising results.

It is a prospective, comparative, observational, time bound, hospital-based study conducted from November 2018 to May 2019, after obtaining institutional Ethical committee approval. 68 patients were included in the study, however, 4 patients were lost to follow up, therefore, 64 patients with primary OA knee were included in this study following the inclusion and exclusion criteria. Patients were randomized into 2 groups using a standard randomization technique (odd and even method). Group A (odd number) received single spin PRP intra-articular injection and group B (even number) received double spin PRP intra-articular injection. Patients were selected from R L Jalappa Hospital and Research centre, Department of Orthopaedics, Kolar, on outpatient and in-patient basis who meets inclusion criteria.

For single spin PRP injection, 36-ml venous blood sample was collected in 4 tubes which were centrifuged at 1500rpm for 15 minutes. Total amount of sample infiltrated was 10 ml, into each knee joint under aseptic conditions in operation theatre

For double spin PRP injection, 150ml of whole blood was collected in a double bag having 63 ml of Citrate phosphate dextrose adenine (CPDA). Two centrifugations (the first at 1,800 rpm for 15 min to separate erythrocytes, and a second at 3,500 rpm for 10 min to concentrate platelets) was done. About 10ml of PRP was collected and injected into the joint under aseptic precautions in the operating theatre.

Patients were assessed with VAS score for pain, WOMAC scoring,Oxford knee scoring, before giving the PRP injection & after giving the injection at periods of 1, 3 & 6 months.

On assessing the results, there is a significant improvement in VAS, WOMAC and Oxford knee score for patients in both the groups with sustained results throughout the follow-up period of 6 months, which was confirmed by significant change of p value. Greater reduction of VAS and WOMAC scores were seen in the double spinning PRP group.

There were no long term local systemic complications noted through the course of the study except acute pain at the site of inject for 10-15 minutes immediately after the infiltration

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ANNEXURES-I

PROFORMA

Name	:	Case no	:
Age	:	Ip/op no	:
Sex	:	DOB	:
Address	:	Date	:

Phone no:

Chief complaints :

History of presenting illness:

Past history:

Family history:

Personal history:

General physical examination:

Vital signs:	Systemic examination:
BP -	CVS -
RR -	RS -
PR -	PA -
Temp -	CNS -

LOCAL EXAMINATION OF BILATERAL KNEE:

	RIGHT	LEFT
• Deformity		
• Swelling		
• Mid joint line Tenderness		
• Lateral joint line Tenderness		
• Retro Patellar Tenderness		
• Retro patellar crepitus		
• Patellar tap		
• Synovial thickness		
• ROM		

X ray of knee (both) :-

Kellergen and Lawrence grading :-

Routine:

Hb%	RBS
TC	Blood urea
DC	Serum creatinine
ESR	Blood group
BT	CT

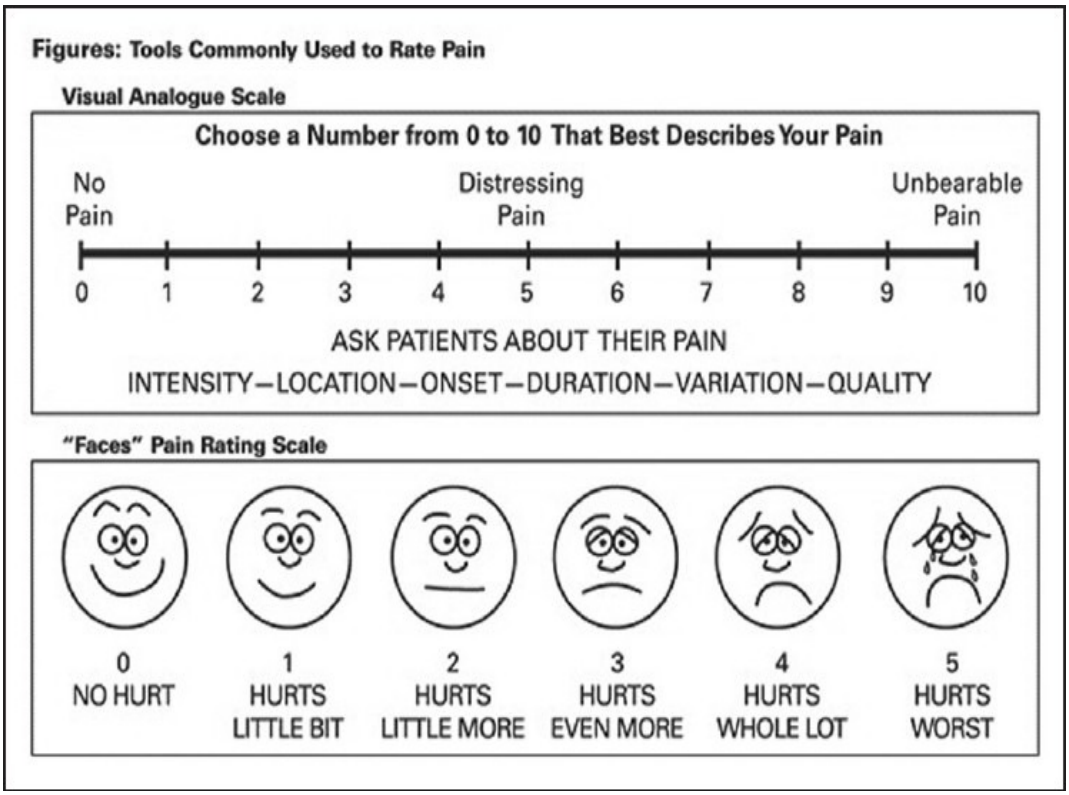
Chest X-ray

LAB INVESTIGATION OF PRP COLLECTED:

Volume: platelet count: sterility: serological tests: HIV
HBsAg
HCV

Diagnosis:

VISUAL ANALOGUE SCALE



WOMAC, OKS AND VAS scoring during follow ups:

IA-PRP Injection	PRE-OP			1 MONTH			3MONTHS			6MONTHS		
	VAS	OKS	WOMAC	VAS	OKS	WOMAC	VAS	OKS	WOMAC	VAS	OKS	WOMAC
SS-PRP												
DS-PRP												

ASSESSMENT OF RESULT:

Signature of candidate: **Signature of Guide:**

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

Name:_____Date:_____

Instructions:

Please rate the activitiesineachcategoryaccording to thefollowingscale ofdifficulty:

0=None, 1=Slight, 2 =Moderate, 3=Very, 4=Extremely

Circle one numberfor each activityPain

PAIN						
1.	Walking	0	1	2	3	4
2.	Stair Climbing	0	1	2	3	4
3.	Nocturnal	0	1	2	3	4
4.	Rest	0	1	2	3	4
5.	Weight Bearing	0	1	2	3	4
STIFFNESS						
1.	Morning stiffness	0	1	2	3	4
2.	Stiffness later in the day	0	1	2	3	4
PHYSICAL FUNCTION						
1.	Descending stairs	0	1	2	3	4
2.	Ascending stairs	0	1	2	3	4
3.	Rising from sitting	0	1	2	3	4
4.	Standing	0	1	2	3	4
5.	Bending to floor	0	1	2	3	4
6.	Walking on a flat surface	0	1	2	3	4

7.	Getting in /out of car	0	1	2	3	4
8.	Going Shopping	0	1	2	3	4
9.	Putting on socks	0	1	2	3	4
10.	Lying in bed	0	1	2	3	4
11.	Taking off socks	0	1	2	3	4
12.	Rising from bed	0	1	2	3	4
13.	Getting in / out of bath	0	1	2	3	4
14.	Sitting	0	1	2	3	4
15.	Getting on/off toilet	0	1	2	3	4
16.	Heavy domestic duties	0	1	2	3	4
17.	Light domestic duties	0	1	2	3	4

Total score: /96= %

OXFORD KNEE SCORE

PROBLEMS WITH YOUR KNEE

During the past 4 weeks.. ✓tick one box for every questio

1 *During the past 4 weeks.....*

How would you describe the pain you usually have from your knee?

None	Very mild	Mild	Moderate	Severe
☐	☐	☐	☐	☐

2 *During the past 4 weeks.....*

Have you had any trouble with washing and drying yourself (all over) because of your knee?

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
☐	☐	☐	☐	☐

3 *During the past 4 weeks.....*

Have you had any trouble getting in and out of a car or using public transport because of your knee? (whichever you would tend to use)

No trouble at all	Very little trouble	Moderate trouble	Extreme difficulty	Impossible to do
☐	☐	☐	☐	☐

4 *During the past 4 weeks.....*

For how long have you been able to walk before pain from your knee becomes **severe**? (*with or without a stick*)

No pain/ More than 30 minutes	16 to 30 minutes	5 to 15 minutes	Around the house only	Not at all - pain severe when walking
☐	☐	☐	☐	☐

5 *During the past 4 weeks.....*

After a meal (sat at a table), how painful has it been for you to stand up from a chair because of your knee?

Not at all painful	Slightly painful	Moderately painful	Very painful	Unbearable
☐	☐	☐	☐	☐

6 *During the past 4 weeks.....*

Have you been limping when walking, because of your knee?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
☐	☐	☐	☐	☐

During the past 4 weeks...

✓tick one box for every questio

7 *During the past 4 weeks.....*

Could you kneel down and get up again afterwards?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

8 *During the past 4 weeks.....*

Have you been troubled by pain from your knee in bed at night?

No nights	Only 1 or 2 nights	Some nights	Most nights	Every night
❑	❑	❑	❑	❑

9 *During the past 4 weeks.....*

How much has pain from your knee interfered with your usual work *(including housework)*?

Not at all	A little bit	Moderately	Greatly	Totally
❑	❑	❑	❑	❑

10 *During the past 4 weeks.....*

Have you felt that your knee might suddenly 'give way' or let you down?

Rarely/ never	Sometimes, or just at first	Often, not just at first	Most of the time	All of the time
❑	❑	❑	❑	❑

11 *During the past 4 weeks.....*

Could you do the household shopping on your own?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

12 *During the past 4 weeks.....*

Could you walk down one flight of stairs?

Yes, Easily	With little difficulty	With moderate difficulty	With extreme difficulty	No, Impossible
❑	❑	❑	❑	❑

INFORMED CONSENT FORM

I/we the patient attenders have been explained about outpatient's condition i.e., osteoarthritis knee and the need for the procedure i.e., single spin versus double spin intra articular platelet rich plasma (PRP) injections in the treatment of osteoarthritis knee.

The procedure and complications associated with this procedure i.e., single spin versus double spin intra articular platelet rich plasma (PRP) injections, have been explained to me in my own understandable language. I am willing to pay for the procedure and the treatment.

I have been explained regarding the study design and I am participating in the study with my willful consent in group I (single spin)/group II (double spin). I have been also explained by the investigator that I am free to participate in the study, I can withdraw from the study at any point of time and I would continue to receive the standard care and treatment in this hospital as long as I wish to receive the treatment.

I/we the patient and the patient attenders hold the full responsibility for the procedure and the further consequences. I will not hold any treating doctor, nursing staff and hospital management for any untoward consequences.

I hereby give my consent for the same.

SIGNATURE OF THE PATIENT: SIGNATURE OF DOCTOR:

WITTNESS:

1.

2.

DATE

ರೋಗಿಯತಿಳಿವಳಿಕೆಯಸಮ್ಮತಿನಮೂನೆ

ಅಧ್ಯಯನದಶೀರ್ಷಿಕೆ:ಮೊಣಕಾಲಿನಪ್ರಾರಂಭಿಕಸಂಧಿವಾತದಲ್ಲಿಕೀಲಿನನುಡುಭಾಗಕ್ಕೆಪ್ಲೇಟ್ಲೆಟ್ಸ್ (ಕಿರುಬಿಲ್ಲೆಗಳು) ಭರಿತಪ್ಲಾಸ್ಮಾಚುಚ್ಚುಮದ್ದನ್ನುಒಮ್ಮೆಅಥವಹಲವಾರುಬಾರಿನೀಡುವುದರಕ್ರಿಯಾತ್ಮಕಫಲಿತಾಂಶದಒಂದುತುಲನಾತ್ಮಕಅಧ್ಯಯನ.

ಅಧ್ಯಯನದಸ್ಥಳ :ಆರ್ ಎಲ್ ಜಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಶ್ರೀ ದೇವರಾಜ್ ಉರ್ಸ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ತಮಕ, ಕೋಲಾರ.

ವಿವರಗಳು -

ಆರ್. ಎಲ್. ಜಲಪ್ಪಆಸ್ಪತ್ರೆಗೆಆರ್ಥೋಪೆಡಿಕ್ಸ್ಭಾಗಕ್ಕೆಭೇಟಿನೀಡುವಅಸ್ಥಿಸಂಧಿವಾತಮೊಣಕಾಲುಇರುವ 40 ರಿಂದ 60 ವರ್ಷವಯಸ್ಸಿನರೋಗಿಗಳನ್ನು ಈ ಅಧ್ಯಯನದಲ್ಲಿಎರಡುಗುಂಪುಗಳಲ್ಲಿಸೇರಿಸಿಕೊಳ್ಳಲಾಗುವುದು.ಗುಂಪು ಎ ಏಕ ಅಂತರ-ಕೀಲಿನ ಪಿ.ಆರ್.ಪಿ ಚುಚ್ಚುಮದ್ದನ್ನು ಸ್ವೀಕರಿಸುತ್ತದೆ, ಗುಂಪು ಬಿ ಆಪರೇಷನ್ ಥಿಯೇಟರ್‌ನಲ್ಲಿ ಕಟ್ಟುನಿಟ್ಟಾದ ಅಸೆಪ್ಟಿಕ್ ಮುನ್ನೆಚ್ಚರಿಕೆಗಳ ಅಡಿಯಲ್ಲಿ ಅನೇಕ ಪಿ.ಆರ್.ಪಿ ಚುಚ್ಚುಮದ್ದನ್ನು ಸ್ವೀಕರಿಸುತ್ತದೆ ಮತ್ತು ಇದು ಅಸ್ಥಿಸಂಧಿವಾತ ಮೊಣಕಾಲಿಗೆ ಒಂದು ಹೊಸ ಚಿಕಿತ್ಸೆಯಾಗಿದ್ದು, ಇದು ತನಿಖಾ ಹಂತದಲ್ಲಿದೆ, ಇನ್ನೂ ಪ್ರಮಾಣೀಕರಿಸಲಾಗಿಲ್ಲ, ಆದರೂ ಈ ಚುಚ್ಚುಮದ್ದಿನ ಮಾದರಿಯನ್ನು ವೈಜ್ಞಾನಿಕ ಸಾಹಿತ್ಯದಲ್ಲಿ ಮೂಳೆಚಿಕಿತ್ಸೆಯ ಬಳಕೆಯಲ್ಲಿಪಿ.ಆರ್.ಪಿ ಚುಚ್ಚುಮದ್ದಿನಿಂದ ಉತ್ತಮಮಾದರಿಸ್ಥಾಪಿತವಾಗಿದೆ.ಈ ಅಧ್ಯಯನದ ರೋಗಿಗಳು ಎಪಿ ವೀಕ್ಷಣೆ ಮತ್ತು ಪೀಡಿತ ಮೊಣಕಾಲುಗಳ ಪಾರ್ಶ್ವ ವೀಕ್ಷಣೆಗಾಗಿ ನಿಂತಿರುವ ಸ್ಥಾನದಲ್ಲಿ ದಿನನಿತ್ಯದ ತನಿಖೆ ಮತ್ತು ಮೊಣಕಾಲಿನ ಎರಡೂ ಎಕ್ಸರೆಗಳಿಗೆ ಒಳಗಾಗಬೇಕಾಗುತ್ತದೆ. ಈ ಅಂತರ್-ಕೀಲಿನ ಪಿ.ಆರ್. ಪಿ. ಚುಚ್ಚುಮದ್ದಿನಿಂಧಾ
ಇ ಕೆಳಗಿನತೊಡಕುಗಳನ್ನುಹೊಂದಬಹುದು - ಮೊಣಕಾಲುಉಥಾ, ಎರಿಥೆಮಾ, ಚಲನೆಯ ಮೊಣಕಾಲಿನ ವ್ಯಾಪ್ತಿಯಲ್ಲಿ ತೊಂದರೆ, ಸೋಂಕು, ಸೆಪ್ಟಿಕ್ ಸಂಧಿವಾತ ಇತ್ಯಾದಿ.

ದಯವಿಟ್ಟು ಈ ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ.
ಅಧ್ಯಯನಕ್ಕೆಸಂಬಂಧಿಸಿದಂತೆನೀವುಯಾವುದೇಪ್ರಶ್ನೆಯನ್ನುಕೇಳಬಹುದು.
ಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನೀವುಒಪ್ಪಿದರೆನಾವುನಿಮ್ಮಿಂದಅಥವಾನಿಮ್ಮಇಬ್ಬರಜವಾಬ್ದಾರಿಯುತವ್ಯಕ್ತಿಯಿಂದಮಾಹಿತಿಯನ್ನು (ಪ್ರೊಫಾರ್ಮಾದಪ್ರಕಾರ) ಸಂಗ್ರಹಿಸುತ್ತೇವೆ.ಸಂಗ್ರಹಿಸಿದ ಈ
ಮಾಹಿತಿಯನ್ನುಪ್ರಬಂಧಮತ್ತುಪ್ರಕಟಣೆಗೆಮಾತ್ರಬಳಸಲಾಗುತ್ತದೆ.

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ.ನಿಮ್ಮಗುರುತುಬಹಿರಂಗಗೊಳ್ಳುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವನ್ನು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯು ಪರಿಶೀಲಿಸಿದೆ ಮತ್ತು ನೀವು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯ ಕಾರ್ಯದರ್ಶಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ.ಈ ಅಧ್ಯಯನವನ್ನು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಬಲವಂತವಿಲ್ಲ.

ನೀವುಭಾಗವಹಿಸಲುಬಯಸದಿದ್ದರೆನೀವುಪಡೆಯುವಕಾಳಜಿಬದಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಕೊಂಡರೆ ಮಾತ್ರ ನೀವು ಹೆಬ್ಬೆರಳು ಅನಿಸಿಕೆಗೆ ಸಹಿ / ಒದಗಿಸುವ ಅಗತ್ಯವಿದೆ.

ರೋಗಿಯಸಹಿ / ಹೆಬ್ಬೆರಳಿನಗುರುತು-

ಹೆಸರು:

ಸಾಕ್ಷಿಸಹಿ(ರೋಗಿಗೆಸಂಬಂಧ):

ಹೆಸರು:

ಸಾಕ್ಷಿಸಹಿ(ರೋಗಿಗೆಸಂಬಂಧ):

PATIENT INFORMATION SHEET

EVALUATION OF FUNCTIONAL OUTCOME OF INTRA-ARTICULAR PLATELET RICH PLASMA (PRP) INJECTION FOR EARLY OSTEOARTHRITIS OF KNEE: A COMPARISON BETWEEN SINGLE VERSUS DOUBLE SPINNING TECHNIQUE.

Study location: R L Jalappa Hospital and Research Centre attached to Sri DevarajUrs Medical College, Tamaka, Kolar.

Details-

Patients aged between 40 and 70 years diagnosed having osteoarthritis knee who visit to the department of Orthopaedics to R.L.Jalappa Hospital will be included in this study in one of two groups. Group A will receive single spin intra-articular PRP injection, group B will receive double spin PRP injections under strict aseptic precautions in operation theater and this a novel treatment for osteoarthritis knee which is under investigational stage not yet standardized ,yet the sampling of this injection has been well established, by the PRP injection for orthopedic usage in scientific literatures. Patients in this study will have to undergo routine investigations and x ray of both knee in standing position for AP view and lateral view of affected knees. This intra-articular PRP injection can have the following complications like increase pain in the knee, swelling, erythema, difficulty in knee range of motions, infection, might leads to septic arthritis etc .

Please read the following information and discuss with your family members. You can ask any question regarding the study. If you agree to participate in the study we will collect information (as per proforma) from you or a person responsible for you or both. Relevant history will be taken. This information collected will be used only for dissertation and publication.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. This study has been reviewed by the Institutional Ethics Committee and you are free to contact the secretary of the Institutional Ethics Committee. There is no compulsion to agree to this study. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

For further information contact

DR. SOURADEEP MITRA

Post graduate,

Department of ORTHOPAEDICS,

SDUMC, Tamaka,Kolar.

CONTACT NO:9972869275

ರೋಗಿಯಸಮ್ಮತಿಪತ್ರ

ಕ್ರಮಸಂಖ್ಯೆ :

ರೋಗಿಯಹೆಸರು :

ಮೊಬೈಲ್ಸಂಖ್ಯೆ:

ಶೀರ್ಷಿಕೆ :ಮೊಣಕಾಲಿನಪ್ರಾರಂಭಿಕಸಂಧಿವಾತದಲ್ಲಿಕೀಲಿನನುಡುಭಾಗಕ್ಕೆಪ್ಲೇಟ್ಲೆಟ್ಸ್ (ಕಿರುಬಿಲ್ಲೆಗಳು) ಭರಿತಪ್ಲಾಸ್ಮಾಚುಚ್ಚುಮದ್ದನ್ನುಒಮ್ಮೆಅಥವಹಲವಾರುಬಾರಿನೀಡುವುದರಕ್ರಿಯಾತ್ಮಕಫಲಿತಾಂಶದಒಂದು ತುಲನಾತ್ಮಕಅಧ್ಯಯನ.

ಈಕೆಳಗೆರುಜುಮಾಡಿರುವನಾನು, ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲು, ಅಧ್ಯಯನನಡೆಸಲುಮತ್ತು ಈಸಮ್ಮತಿನಮೂನೆಯಅಂಶಗಳಂತೆನನ್ನವೈಯಕ್ತಿಕಮಾಹಿತಿಯನ್ನುಬಹಿರಂಗಪಡಿಸುವಒಪ್ಪಿಗೆನೀಡಿರುತ್ತೇನೆ.ನನಗೆಈಅಧ್ಯಯನದಉದ್ದೇಶಹಾಗೂಗೋಪ್ಯತೆಯವಿಚಾರಗಳಬಗ್ಗೆನನ್ನಭಾಷೆಕನ್ನಡದಲ್ಲಿವಿವರಿಸಲಾಗಿದೆ.

ಈಅಧ್ಯಯನಕುರಿತಾದನನ್ನಎಲ್ಲಾಪ್ರಶ್ನೆಗಳಿಗೂಸಮಾಧಾನಕರಉತ್ತರನನಗೆದೊರಕಿರುತ್ತದೆ.

ಎಲ್ಲಾಮಾಹಿತಿಗಳುಸಂಶೋಧನೆಗಾಗಿಯೇಬಳಸಲಾಗುವುದು.

ಎಲ್ಲಾಮಾಹಿತಿಯನ್ನುಗೌಪ್ಯವಾಗಿಇಡಲಾಗುತ್ತದೆಮತ್ತುಇವನ್ನುಹೊರಗಿನವರಿಗೆಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ಈಅಧ್ಯಯನದಿಂದನನ್ನಜೀವಕ್ಕೆಯಾವುದೇರೀತಿಯಹಾನಿಆಗುವುದಿಲ್ಲಮತ್ತುಹೆಚ್ಚುಅನುಕುಲಕರವಗುತಡಎಂದುನನಗೆಅರ್ಥವಾಗಿರುತ್ತದೆ.

ನಾನುಯಾವಾಗಬೇಕಾದರೂಈಅಧ್ಯಯನದಿಂದಹೊರನಡೆಯಬಹುದುಮತ್ತುನನಗೆಇದರಿಂದಯಾವುದೇರೀತಿಯಅಧಿಕಖರ್ಚಾಗಿರುವುದಿಲ್ಲವೆಂದುನಾನುಒಪ್ಪಿಕೊಂಡಿರುತ್ತೇನೆ.

ರೋಗಿಯಹೆಸರುಮತ್ತುರುಜು/ಬೆರಳುಗುರುತು

ಸಾಕ್ಷಿಗಳಹೆಸರುಮತ್ತುರುಜು

1.

2.

ಪ್ರಮುಖಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ರುಜು: ಡಾ: ಪರಮಾನಂತಂಮಾಧವನ್.

MASTER CHART : SS- PRP GROUP

UHID number	AGE	SEX	GROUP	KNEE AFFECTED		GRADE OF OA	DATE OF INJECTION	PLATELET COUNT		CO-MORIDITIES		VAS SCORES				WOMAC SCORES				OXFORD KNEE SCORE				RANGE OF MOTION								COMPLICATIONS
																								RE-INJECTIO		1 MONTH		3 MONTH		6 MONTH		
								PATIENT	PRP	DIABETES	HYPERTENSION	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	
793024	62	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	11/18/2019	405,000	810,000	NO	NO	8	6	5	5	76	67	64	61	19	20	24	25	0-120	0-120	0-125	0-125	0-130	0-130	0-130	0-130	PAIN
791690	60	F	SS-PRP	RIGHT		GRADE 2	11/18/2019	190,000	450,000	NO	NO	9	7	6	5	86	75	70	63	14	19	24	29	0-120	0-110	0-120	0-120	0-125	0-120	0-130	0-130	
791689	70	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	11/18/2019	375,000	743,000	NO	NO	9	8	6	5	82	78	70	64	16	19	21	24	0-120	0-130	0-130	0-130	0-130	0-130	0-130	0-130	
794464	45	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	11/21/2019	422,000	710,000	NO	NO	9	8	6	5	84	78	70	66	16	19	23	25	0-130	0-130	0-130	0-130	0-130	0-130	0-130	0-130	PAIN
737647	60	F	SS-PRP		LEFT	GRADE 2	11/24/2019	425,000	645,000	YES	NO	9	7	6	5	76	70	67	64	20	22	24	25	0-120	0-130	0-125	0-130	0-130	0-135	0-130	0-135	
848132	50	M	SS-PRP	RIGHT	**LEFT**	GRADE 2	11/25/2019	390,000	630,000	NO	NO	8	7	6	5	73	70	68	65	19	21	24	26	0-130	0-110	0-130	0-120	0-135	0-125	0-140	0-130	
846097	70	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	11/28/2019	410,000	625,000	YES	NO	9	7	6	5	74	68	64	59	19	23	25	28	0-120	0-120	0-130	0-120	0-135	0-135	0-135	0-135	
408047	57	F	SS-PRP	RIGHT	**LEFT**	GRADE 2	12/9/2019	330,000	710,000	YES	NO	8	6	5	4	70	66	62	58	23	25	27	28	0-120	0-110	0-130	0-125	0-135	0-130	0-135	0-130	
846071	60	F	SS-PRP	RIGHT		GRADE 2	12/17/2019	419,000	655,000	NO	NO	8	6	5	4	75	70	64	59	20	23	25	27	0-110	0-130	0-120	0-130	0-130	0-130	0-130	0-135	
847689	50	M	SS-PRP		LEFT	GRADE 1	12/20/2019	218,000	540,000	NO	NO	8	6	5	4	74	67	62	57	20	23	26	28	0-135	0-120	0-135	0-130	0-140	0-130	0-140	0-135	
845543	62	F	SS-PRP	**RIGHT**	LEFT	GRADE 1	1/18/2020	390,000	540,000	YES	NO	8	6	5	4	72	67	63	58	21	24	27	30	0-120	0-130	0-130	0-130	0-130	0-130	0-130	0-130	
832922	70	M	SS-PRP	**RIGHT**	LEFT	GRADE 1	1/23/2020	438,000	680,000	NO	NO	8	7	6	5	76	70	63	60	20	23	26	28	0-120	0-130	0-130	0-130	0-135	0-135	0-135	0-135	
830502	70	M	SS-PRP	RIGHT	**LEFT**	GRADE 2	2/3/2020	275,000	590,000	YES	NO	9	6	5	4	77	69	61	57	19	24	27	30	0-120	0-110	0-130	0-120	0-130	0-120	0-140	0-130	
827511	60	F	SS-PRP	**RIGHT**	**LEFT**	GRADE 2	2/20/2020	373,000	730,000	NO	NO	8	6	5	4	81	70	62	54	14	19	25	30	0-110	0-120	0-120	0-130	0-130	0-130	0-140	0-140	
827508	55	F	SS-PRP	RIGHT	**LEFT**	GRADE 2	2/20/2020	288,000	640,000	NO	YES	9	6	5	4	80	69	60	48	14	20	26	30	0-120	0-110	0-130	0-130	0-135	0-135	0-140	0-135	
836525	56	M	SS-PRP	**RIGHT**	LEFT	GRADE 2	3/10/2020	380,000	740,000	YES	NO	8	6	5	5	78	68	60	57	15	21	26	28	0-110	0-130	0-120	0-135	0-135	0-140	0-140	0-140	SWELLING
836690	46	M	SS-PRP	**RIGHT**	LEFT	GRADE 1	3/12/2020	427,000	680,000	NO	NO	8	6	5	5	73	60	50	48	18	23	27	30	0-120	0-130	0-125	0-130	0-130	0-135	0-135	0-140	
836560	60	F	SS-PRP	RIGHT	**LEFT**	GRADE 1	3/14/2020	316,000	620,000	NO	NO	7	5	4	4	71	60	51	48	18	24	29	30	0-130	0-120	0-130	0-125	0-130	0-125	0-135	0-130	
838456	56	M	SS-PRP	RIGHT		GRADE 2	3/16/2020	414,000	810,000	NO	NO	9	7	5	4	76	62	50	47	21	26	30	31	0-110	0-130	0-120	0-130	0-130	0-130	0-130	0-130	
833438	52	F	SS-PRP	RIGHT		GRADE 2	4/19/2020	340,000	710,000	YES	NO	8	6	5	4	74	62	51	45	21	25	29	30	0-110	0-130	0-120	0-130	0-125	0-130	0-135	0-135	
849536	55	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	4/20/2020	319,000	630,000	NO	YES	8	6	5	4	79	65	58	53	18	24	26	28	0-110	0-130	0-120	0-135	0-130	0-140	0-135	0-140	
892977	62	F	SS-PRP	**RIGHT**	**LEFT**	GRADE 2	4/28/2020	390,000	745,000	NO	NO	8	6	5	4	80	67	60	54	21	26	30	31	0-110	0-130	0-120	0-130	0-130	0-135	0-135	0-140	
845543	62	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	5/4/2020	288,000	590,000	NO	NO	9	7	5	4	76	68	60	51	20	24	26	28	0-110	0-130	0-120	0-135	0-130	0-135	0-135	0-135	
847174	61	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	5/13/2020	323,000	620,000	NO	NO	8	6	5	4	74	65	60	49	18	23	26	29	0-110	0-130	0-120	0-130	0-130	0-130	0-135	0-135	
783218	60	F	SS-PRP	**RIGHT**		GRADE 2	5/20/2020	290,000	590,000	NO	NO	7	5	4	3	75	64	59	49	20	25	27	30	0-110	0-130	0-120	0-135	0-130	0-140	0-135	0-140	
848193	52	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	5/21/2020	390,000	710,000	NO	NO	8	6	5	4	77	61	56	48	21	26	28	31	0-110	0-125	0-120	0-130	0-130	0-135	0-135	0-135	
849142	65	F	SS-PRP	RIGHT	**LEFT**	GRADE 2	5/28/2020	467,000	820,000	NO	NO	9	7	6	4	77	66	59	52	22	26	28	30	0-120	0-110	0-130	0-120	0-130	0-125	0-135	0-135	
849232	44	M	SS-PRP		LEFT	GRADE 1	5/28/2020	319,000	580,000	NO	YES	8	6	5	3	76	67	63	54	22	25	28	29	0-140	0-120	0-140	0-130	0-140	0-130	0-140	0-135	
849233	59	F	SS-PRP	**RIGHT**	LEFT	GRADE 1	5/31/2020	383,000	685,000	NO	NO	8	6	5	4	78	69	60	52	19	24	27	29	0-120	0-130	0-130	0-130	0-135	0-135	0-135	0-135	
849436	56	F	SS-PRP	**RIGHT**	**LEFT**	GRADE 1	5/31/2020	336,000	625,000	NO	NO	8	6	5	4	79	68	59	51	20	26	29	30	0-120	0-130	0-125	0-135	0-130	0-135	0-135	0-135	PAIN, SWELLING
849231	54	F	SS-PRP		LEFT	GRADE 1	5/31/2020	330,000	610,000	NO	NO	7	5	4	4	76	67	60	53	22	25	28	31	0-130	0-120	0-130	0-125	0-135	0-135	0-135	0-135	
850748	43	F	SS-PRP	**RIGHT**	LEFT	GRADE 2	6/8/2020	517,000	840,000	NO	NO	9	6	5	5	81	60	55	49	20	26	29	30	0-110	0-130	0-120	0-130	0-130	0-135	0-135	0-135	

MASTER CHART : DS-PRP GROUP

UHID number	AGE	SEX	GROUP	KNEE AFFECTED		GRADE OF OA	DATE OF INJECTION	PLATELET COUNT		CO-MORIDITIES		VAS SCORES				WOMAC SCORES				OXFORD KNEE SCORE				RANGE OF MOTION								COMPLICATIONS
																								PRE-INJECTION		1 MONTH		3 MONTH		6 MONTH		
								PATIENT	PRP	DIABETES	HYPERTENSION	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	PRE-INJECTION	1 MONTH	3 MONTH	6 MONTH	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	
853666	45	M	DS-PRP		LEFT	GRADE 2	11/25/2019	290,000	730,000	YES	NO	7	4	3	2	69	53	47	40	23	26	29	32	0-140	0-125	0-140	0-130	0-140	0-130	0-140	0-135	
853615	45	M	DS-PRP	**RIGHT**	LEFT	GRADE 1	11/28/2019	256,000	780,000	NO	NO	8	5	4	3	71	59	50	41	20	25	29	31	0-120	0-130	0-130	0-130	0-130	0-135	0-135	0-140	PAIN
853624	60	F	DS-PRP	RIGHT	**LEFT**	GRADE 2	12/16/2019	367,000	1,010,000	NO	NO	8	6	4	3	76	60	52	43	20	23	27	29	0-125	0-110	0-130	0-120	0-130	0-125	0-135	0-130	
853634	50	F	DS-PRP	RIGHT		GRADE 1	12/26/2020	437,000	945,000	NO	NO	7	5	4	3	71	58	48	40	21	25	27	29	0-120	0-135	0-125	0-135	0-130	0-135	0-140	0-140	
853641	46	F	DS-PRP	**RIGHT**	LEFT	GRADE 1	1/27/2020	340,000	925,000	NO	NO	8	6	4	3	74	60	48	39	20	24	27	30	0-120	0-135	0-130	0-135	0-130	0-135	0-135	0-140	
852846	58	M	DS-PRP	**RIGHT**	LEFT	GRADE 1	1/30/2020	265,000	930,000	NO	NO	8	5	4	3	72	60	51	45	19	24	26	28	0-120	0-130	0-130	0-135	0-140	0-140	0-140	0-140	PAIN
854317	40	M	DS-PRP		LEFT	GRADE 2	1/31/2020	256,000	670,000	NO	NO	8	6	4	3	74	62	53	44	22	25	28	30	0-135	0-120	0-135	0-125	0-135	0-130	0-140	0-130	
836552	40	M	DS-PRP	RIGHT		GRADE 1	3/11/2020	288,000	930,000	YES	NO	7	5	4	3	76	61	52	43	19	24	28	30	0-120	0-140	0-130	0-140	0-135	0-140	0-140	0-140	
836599	59	M	DS-PRP	**RIGHT**	LEFT	GRADE 1	3/13/2020	295,000	848,000	NO	YES	8	6	5	4	79	62	53	46	19	23	26	28	0-120	0-130	0-130	0-140	0-135	0-140	0-140	0-140	
834981	59	M	DS-PRP		LEFT	GRADE 2	3/13/2020	502,000	1,032,000	NO	NO	8	6	5	4	77	65	57	48	20	25	28	30	0-135	0-120	0-140	0-130	0-140	0-135	0-140	0-140	SWELLING
837884	41	F	DS-PRP		RIGHT	GRADE 2	3/15/2020	195,000	860,000	YES	NO	8	6	5	4	72	60	52	45	15	22	26	29	0-120	0-135	0-135	0-135	0-135	0-135	0-135	0-140	
837759	60	F	DS-PRP	**RIGHT**	**LEFT**	GRADE 2	3/15/2020	366,000	1,160,000	YES	NO	9	7	5	4	72	61	50	42	19	24	26	29	0-115	0-130	0-125	0-135	0-130	0-135	0-135	0-135	
851851	57	F	DS-PRP	**RIGHT**	LEFT	GRADE 2	4/4/2020	169,000	820,000	YES	NO	9	7	5	4	76	62	51	42	18	23	27	29	0-120	0-130	0-125	0-130	0-130	0-135	0-135	0-135	
851870	53	F	DS-PRP	**RIGHT**	LEFT	GRADE 1	4/8/2020	210,000	880,000	NO	NO	8	6	5	4	78	60	53	46	20	26	29	30	0-120	0-120	0-130	0-130	0-135	0-140	0-140	0-140	PAIN, SWELLING
851858	50	M	DS-PRP	RIGHT	**LEFT**	GRADE 1	4/11/2020	310,000	900,000	NO	NO	7	5	4	3	75	60	49	42	22	26	28	30	0-130	0-120	0-135	0-130	0-125	0-130	0-130	0-135	SWELLING
851842	49	M	DS-PRP		LEFT	GRADE 1	4/15/2020	286,000	885,000	YES	NO	8	6	4	3	77	60	52	46	22	27	29	31	0-140	0-120	0-140	0-130	0-140	0-130	0-140	0-135	
851832	49	F	DS-PRP	**RIGHT**	**LEFT**	GRADE 1	4/18/2020	328,000	890,000	NO	NO	8	6	5	4	72	60	51	45	23	27	30	31	0-120	0-135	0-125	0-135	0-130	0-140	0-135	0-140	
853091	54	F	DS-PRP	**RIGHT**	LEFT	GRADE 2	4/21/2020	234,000	830,000	YES	NO	8	6	5	4	77	60	53	48	18	24	27	29	0-110	0-120	0-120	0-130	0-125	0-130	0-125	0-130	
851880	56	M	DS-PRP	**RIGHT**	LEFT	GRADE 1	4/26/2020	260,000	910,000	NO	NO	8	6	5	4	76	61	52	44	17	23	27	29	0-120	0-130	0-125	0-130	0-130	0-130	0-135	0-135	
838403	57	M	DS-PRP	RIGHT	**LEFT**	GRADE 1	4/28/2020	391,000	1,010,000	YES	NO	8	6	5	4	80	64	57	49	18	24	28	30	0-130	0-120	0-130	0-130	0-135	0-135	0-135	0-135	
550185	60	M	DS-PRP	**RIGHT**	LEFT	GRADE 2	4/30/2020	237,500	695,000	NO	NO	8	6	4	3	77	61	54	46	19	24	26	29	0-110	0-130	0-120	0-130	0-125	0-135	0-130	0-140	
845535	64	M	DS-PRP	**RIGHT**	**LEFT**	GRADE 2	5/4/2020	242,500	840,000	YES	NO	9	7	6	4	80	68	60	51	17	22	26	29	0-120	0-120	0-125	0-130	0-130	0-135	0-135	0-135	
853652	42	M	DS-PRP	RIGHT		GRADE 1	5/6/2020	213,000	695,000	NO	YES	8	6	5	3	72	60	51	42	18	23	27	31	0-120	0-140	0-130	0-140	0-130	0-140	0-135	0-140	
853594	44	M	DS-PRP		LEFT	GRADE 1	5/9/2020	232,600	730,000	NO	NO	8	6	5	4	71	60	52	44	18	23	28	30	0-135	0-120	0-135	0-130	0-135	0-130	0-135	0-135	PAIN
854309	45	F	DS-PRP	RIGHT		GRADE 2	5/13/2020	245,000	815,000	NO	NO	8	6	5	3	72	60	54	47	18	25	27	29	0-120	0-135	0-130	0-135	0-130	0-135	0-135	0-140	PAIN

MASTER CHART : DS-PRP GROUP

854349	45	F	DS-PRP		LEFT	GRADE 2	5/21/2020	360,000	660,000	NO	NO	8	6	5	3	73	60	53	48	18	24	28	30	0-140	0-120	0-140	0-125	0-140	0-130	0-140	0-135	
854431	42	F	DS-PRP	RIGHT		GRADE 2	5/25/2020	385,000	845,000	NO	NO	8	6	5	4	74	62	51	43	18	23	28	32	0-120	0-135	0-130	0-135	0-135	0-135	0-140	0-140	
854326	56	F	DS-PRP	**RIGHT**	LEFT	GRADE 2	5/26/2020	327,000	920,000	NO	NO	9	6	4	2	72	61	53	42	19	24	28	31	0-110	0-120	0-120	0-130	0-130	0-130	0-135	0-135	
843990	48	F	DS-PRP	**RIGHT**	LEFT	GRADE 2	5/30/2020	197,000	424,000	YES	NO	8	6	5	4	79	66	58	49	18	24	27	30	0-120	0-130	0-130	0-130	0-130	0-130	0-135	0-135	
849432	40	M	DS-PRP	**RIGHT**	**LEFT**	GRADE 1	5/30/2020	303,000	911,000	NO	NO	8	6	5	3	76	64	58	48	20	24	28	32	0-120	0-135	0-130	0-135	0-135	0-135	0-135	0-135	
849434	50	M	DS-PRP	**RIGHT**	LEFT	GRADE 2	5/30/2020	420,000	945,000	NO	NO	8	6	5	3	79	66	57	46	20	25	28	31	0-120	0-135	0-130	0-135	0-130	0-135	0-135	0-140	PAIN, SWELLING
849435	60	F	DS-PRP	**RIGHT**	LEFT	GRADE 2	5/31/2020	376,000	880,000	YES	NO	8	6	5	2	76	64	55	41	19	24	27	30	0-120	0-130	0-130	0-130	0-130	0-135	0-135	0-140	