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Autologous Bone Marrow Derived Mesenchymal Stem Cells Enhanced with Platelet Rich Plasma Versus Platelet Rich Plasma in Osteoarthritis Knee: A Comparative Study

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Abstract

Introduction: Any synovial joint may be affected, but the most severe degeneration occurs in joints subjected to greatest compression.The disease is twice as prevalent in the obese and mainly affects the weight bearing joints, such as lower spine, knee and hip joints. *Methodology:* Our study was a prospective randomised double blinded comparative study between use of autologous mesenchymal stem cells with platelet rich plasma (PRP) and platelet rich plasma alone in early osteoarthritis knee. A total no. of 32 patients were studied and they were randomised into two groups group 1 mesenchymal stem cells with platelet rich plasma and group 2 platelet rich plasma alone by computer generated algrorithm table each group had 16 patients. *Results:* Mean cartilage thickness group1 at final followup right side 4.0619 (SD±0.38456) and left side 3.8469 (SD±0.4843) and group 2 3.772 right side (SD±0.3307) and left was 3.660 (SD±0.2761) and both groups are statistically significant with p value 0.030 for right side and 0.001 for left side. *Conclusion:* The probable mechanism involved was due to paracrine effects and differentiation of mesenchymal stem cell enhanced with platelet rich plasma.

Keywords: Mesenchymal Stem Cells; Platelet Rich Plasma; Osteoarthritis.

Introduction

The term ″osteoarthritis″ and ″osteoarthrosis″ (degenerative arthritis, chondromalacia arthrosis, arhtritis deformans,) is used to define idiopathic, slowly progressive disease of diarthrodial (synovial joints) joints, occuring late in life and characterised pathologically by focal degeneration of articular cartilage, subchondral bone thickening (sclerosis), marginal osteochondral outgrowths (osteophytes), and joint deformity, clinically by recurring episodes of pain, synovitis with effusion, stiffness, and progressive limitation of motion [1]. The present accepted view is that osteoarthritis is a degenerative process of unknown etiology affecting the articular cartilage of previously healthy joint [1].

The degenerative process appears to begin in the second decade of life, but does not become apparent until middle age, By 55 to 65 years of age, approximately 85% have roentgenographic evidence, to a variable degree of the disease. It is one of the leading cause of morbidity in the elderly age group [1].

Men and women are equally affected upto 54 yrs of age, and the pattern of involvement is similar in both, Thereafter the disease is more severe and more generalised in women [2].

Any synovial joint may be affected, but the most severe degeneration occurs in joints subjected to greatest compression.The disease is twice as prevalent in the obese and mainly affects the weight bearing joints, such as lower spine, knee and hip joints [3].

Osteoarthritis apart from involvement of articular

cartilage involves other articular tissues like synovial membrane, subchondral bone, and other connective tissues such as ligaments and tendons [4].The prevalence of symptomatic osteoarthritis after the age of 55 years ranges from about 30% to 50% in men and 40% to 60% in women [5,6].

Several risk factors have been identified, which includes age, female sex, obesity, occupation, biomechanics and increased dynamic loading of the joint and joint injury [7,8].

The treatment modality for the early osteoarthritis include physical exercise, bracing, anti-inflammatory drugs and intra articular injection of corticosteroids, hyaluronic acid reported to provide temporary relief, and ultimatly patient ends up with total replacement of knee in advanced disease [9,19].

The treatment of osteoarthritis using surgical means helpful in reducing the pain and improve mobility or function of joint, but the high cost and postsurgical complication divert the attention to find some alternate therapy for the disease, which can alter pathological pathway of disease in the early stages.

Recently researchers have focussed on use of mesenchymal stem cells in osteoarthritis. Mesenchymal stem cells (MSC) are bone marrow derived progenitor cells which can be differentiated to chondrogenic, adipogenic, osteogenic lineages in vitro and in vivo, having the capacity of limitless division and multi-lineage differentiation potential. They are reported to regenerate the various organs and their damaged cellular part to improve their functioning [11,12].

The advance in Mesenchymal stem cell (MSC) research and its therapeutic importance hope to be a significant contributor to deal with osteoarthritis and prove a alternative therapy for the disease [13].

The ability of MSCs to repair musculoskeletal defect was first time documented by Quarto et alin 2001 in three patients with large bone defects. Similar kind of study was reported by Murphy et al [14]. In 2003 which reported the use of MSCs in caprine knee joint injury. Yan et al [15] in 2007 demonstrated the use of MSCs to repair the full thickness cartilage defect in rabbit model. They demonstrated the regeneration of hyaline like cartilage tissue which was better than that of fibroblast treated group.

Lee et al [16] in 2007 demonstrated the beneficial effect of injected MSCs to stimulate the repair of chondral defects in pig models. Kuroda et al [1] indicated that the transplantation of autologous bone-marrow stromal cells can promote the repair of large focal articular cartilage defects in young, active patients.

Centeno et al [17] in 2008 reported the efficacy of mesenchymal stem cells in osteoarthritis of knee which was due to their potential differentiation to chondrocytes.

In the recent years combination of both mesenchymal stem cells and platelet rich plasma has been used as platelets enhance the differentiation of mesenchymal stem cells into chondrogenic tissue.

Platelet Rich Plasma (PRP) is defined as the plasma fraction of autologous blood having a platelet concentration above baseline Normal platelet concentration is 200,000 platelets/ul. Studies have shown that clinical efficacy can be expected with a minimum increase of 4 times baseline (1million platelets/microlitre). Platelets play an instrumental role in the normal healing response via the local secretion of growth factors and recruitment of reparative cells. Its use in orthopaedic began early in this decade as PRP was used with bone grafts to augment spinal fusion and fracture healing. Anitua et al hypothesized that the delivery of a natural mixture of biologically active molecules incorporated in a forming fibrin matrix within the joint compartment may target synovial fibroblasts, thus inducing positive changes in the whole joint micro-environment. A major finding of their work was the ability of PRGF to stimulate hyaluronic acid (HA) synthesis driving the secretion of hyaluronic acid by the synovial fibroblasts. Tissue growth factor-1 (TGF-1) up-regulates the hyaluronan synthase isoform-1 while Platelet derived growth factor primarily stimulates hyaluronan synthase isoform-2. By regulating the endogenous hyaluronic acid synthesis Platelet releasing growth factor (PRGF) would restore hyaluronic acid levels, thereby enhancing cartilage protection and joint lubrication.

Only a few studies are reported in the literature regarding the use of mesenchymal stem cells and platelet rich plasma in osteoarthritis of knee.Thus keeping in view the encouraging results of synnergistic effect of platelet rich plasma on mesenchymal stem cells in repairing the cartilage defects and paucity of literature, the present study is proposed to find out the effect of autologous mesenchymal stem cells plus platelet rich plasma compared with that of platelet rich plasma only in osteoarthritis of knee.

Methodology

Our study was a prospective randomised double

blinded comparative study between use of autologous mesenchymal stem cells with platelet rich plasma (PRP) and platelet rich plasma alone in early osteoarthritis knee. A total no. of 32 patients were studied and they were randomised into two groups group 1 mesenchymal stem cells with platelet rich plasma and group 2 platelet rich plasma alone by computer generated algrorithm table each group had 16 patients .

Exclusion Criteria

- Osteoarthritis secondary to joint inflammatory diseases (eg- rheumatoid arthritis, ankylosing spondylitis etc)
- Patients with co-existing low back ache or any other hip joint disease.
- Patients with other diseases, affecting the knee joint like crystal arthropathy, symptomatic chondrocalcinosis, acute synovitis, excessive joint effusion(>100ml), cystic disease around the knee joint(eg-popliteal cyst)
- Advanced stage of osteoarthritis
- Bone marrow suppression
- Co morbidities like pregnancy, cancer, immunosuppression
- All patients suitable for inclusion into the study were informed of the nature of the study in detail and a written consent was obtained.

Group 1: Once the mesenchymal stem cell are ready, the PRP was prepared from the patient blood on the day of interventions. The subject was placed in supine position with knee in slight flexion and under full aseptic precautions 8-10 ml PRP was mixed with around 2-3 ml of autologous cultured mesenchymal stem cell and injected by lateral approach with an 18-20 G needle followed by 2ml of calcium chloride injected. After 30 min of observation the patient was discharged, advised three day antibiotics and paracetamol for analgesia.

Group 2: The subject was placed in supine position with knee in slight flexion and under full aseptic precautions 8-10 ml of PRP was injected by lateral approach with an 18-20 G needle followed by 2ml calcium chloride. After 30 min of observation the patient was discharged, and advised three day course of antibiotics and analgesia.

Post Procedure Advice

1. The patient was advised to report in case of any adverse events
2. Non-steroidal Anti-inflammatory Drugs (NSAIDS) were not be allowed. In case of discomfort Paracetomol (dose of 500 mg tds) can be used.
3. The Subject was advised to stop paracetomol (if consuming) 48 hrs prior to follow up for assessment.

Results

KOOS consists of 5 subscales: Pain (nine items); Symptoms (seven items); ADL Function (17 items); Sport and Recreation Function (five items); Quality of Life (four items).

Pain at baseline in Group 1 mean was 11.75 (SD±4.074) and in Group 2 mean was 9.94 (SD±1.652) were insignificant at baseline and comparable with p=0.112.

At 1st follow mean 4.19 (SD±1.55) in Group 1 while in Group 2 mean was 4.19 (SD±2.167). It was statistically insignificant with p value 0.727.

At 2nd follow up Group1 mean was 3.63 (SD±1.025) Group 2 had mean 4.44 (SD±1.094). It was statistically significant with p value of 0.032.

At final follow up Group1 mean was 2.81(SD±0.981) Group 2 had mean 3.00(SD±1.15), At final follow up womac pain was insignificant with p value of 0.745.

Table 1: Pain score of KOOS in two groups

Pain	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	11.75	4.074	9.94	1.652	.112
Follow up 1	4.19	1.559	4.19	2.167	.727
Follow up 2	3.63	1.025	4.44	1.094	.032
Final Follow up	2.81	.981	3.00	1.155	.745

Group 1 had mean 6.06 (SD±2.594) and group 2 had mean 5.75(SD±2.569) both groups are comparable at KOOS symptoms at baseline with insignificant with p value of 0.605.

At 1st follow up mean was 5.19 (SD±2.344) in group1, while group 2 had mean 4.88 (SD±2.630).It was statistically insignificant p value of 0.467.

At 2nd followup group 1 had mean 5.06 (SD±1.482), and group 2 had mean 3.94 with (SD±2.175).It was statistically insignificant with p value of 0.064.

At final followup group 1 mean was 4.13 (SD±1.204) and group 2 had mean 2.44 (SD±1.672) which were statistically significant (p=0.002).

A t baseline the group 1 had mean 14.88 (SD±6.228) and group 2 had mean 11.44 (SD±2.658) The two groups were comparable and was insignificant with p value 0.218.

At 1st follow up mean 10.75 in group 1 with (SD±4.626) while group 2 had mean 10.19 (SD±2.509) and it insignificant p value0.879.

At 2nd follow up 6 months mean was 12.38 in group 1(SD±3.181)and in group 2 had mean 9.81 (SD±2.073) which was statistically significant with p value 0.016.

Table 2: Symptoms

Symptoms	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	6.06	2.594	5.75	2.569	.605
Follow up 1	5.19	2.344	4.88	2.630	.467
Follow up 2	5.06	1.482	3.94	2.175	.064
Final Follow up	4.13	1.204	2.44	1.672	.002

Table 3: ADL

ADL	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	14.88	6.228	11.44	2.658	.218
Follow up 1	10.75	4.626	10.19	2.509	.879
Follow up 2	12.38	3.181	9.81	2.073	.016*
Final Follow up	10.75	3.804	7.50	1.461	<0.000**

At final followup group 1 had mean 10.75 (SD±3.804) Group 2 had mean 7.50 (SD±1.461)

At baseline group 1 had mean 5.69 (SD±1.922) and group 2 had mean 5.75 (SD±2.595) both groups were comparable It was statistically insignificant (p=1.000).

At 6 weeks first followup group1 had mean 3.69 (SD±1.493) while group 2 had mean was 3.94 (SD± 1.652) which was statistically insignificant at 0.685.

At 6 months 2nd followup group 1 mean was 3.06 (SD±1.181) and group 2 mean was 3.44 (SD±1.504). It was statistically insignificant (p= 0.625).

Table 4: Sports and recreation

Sports N Recreation	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	5.69	1.922	5.75	2.595	1.000
Follow up 1	3.69	1.493	3.94	1.652	.685
Follow up 2	3.06	1.181	3.44	1.504	.625
Final Follow up	2.75	1.844	2.81	1.223	.596

Table 5: Knee related quality of life

Knee related QOL	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	6.69	.704	6.38	.885	.506
Follow up 1	6.50	1.713	5.31	.946	.008**
Follow up 2	6.56	1.825	5.25	1.000	.007**
Final Follow up	4.88	1.360	3.56	1.153	0.008**

At final follow up group 1 mean was 2.75(SD± 1.844) and group 2 mean was 2.81 (SD±1.223) which was statistically insignificant (p= 0.596).

At baseline mean was 6.69 in group 1 (SD±0.704) and group 2 mean was 6.38 (SD±0.885). Both the groups are comparable and statistically insignificant (p= 0.564).

At follow up after 6 weeks group 1 mean was 6.50 (SD±1.713) and group 2 mean was 5.31 (SD±0.946). This was statistically significant with p value 0.008. At 2nd follow up after 6 month group 1 had mean 6.56 (SD±1.825) and group 2 had mean 5.25 (SD±1.00). It was significant statistically with p value 0.007.

Table 5: Knee related quality of life

Knee related QOL	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	6.69	.704	6.38	.885	.506
Follow up 1	6.50	1.713	5.31	.946	.008**
Follow up 2	6.56	1.825	5.25	1.000	.007**
Final Follow up	4.88	1.360	3.56	1.153	0.008**

Table 6: Global KOOS score

Global Koos	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	44.75	10.517	39.56	3.915	.054
Follow up 1	30.31	9.127	29.31	6.161	.970
Follow up 2	30.69	6.129	27.75	4.810	.192
Final Follow up	25.312	6.007	19.312	3.280	.001

At final follow up group 1 had mean 4.88 (SD±1.360) and group 2 had mean 3.56 (SD±1.153) significant with p value 0.008.

The group 1 had mean 44.75 at baseline with (SD±10.517) and while group 2 had mean 39.56 (SD±3.915) which were comparable and statistically insignificant p =0.054.

At 1st follwup at 6 weeks group 1 had mean of 30.31(SD±9.127) and group 2 had mean of 29.31 (SD±6.161) this was not significant statistically with p value of 0.970.

At 2nd follow up mean was 30.69 in group 1 (SD±6.129) and group 2 mean was 27.75 (SD±4.810) it was statistically insignificant p value of 0.192.

At final follow-up mean was 25.312 in group 1 (SD± 6.00) and in group 2 mean was 19.31 (SD±3.28) this was statistically significant with p value of 0.001.

The group 1 had mean baseline thickness right side 3.625 (SD±0.5604), and left side 3.465 (SD±0.5685) and group 2 had baseline mean 3.616 right side with (SD±0.3270) and left side 3.421 (SD±0.30712) and both groups statistically significant at baseline with p value for right side was 0.025 and left was 0.046 .

Mean cartilage thickness group1 at final followup right side 4.0619 (SD±0.38456) and left side 3.8469

Table 7: Mean cartilage thickness

Mean cartilage thickness	Group 1		Group 2		p-value
	Mean	S.D.	Mean	S.D.	
Baseline Right	3.625	.5604	3.616	.3270	.025*
Baseline Left	3.465	.568	3.421	.307	.046*
Follow up Right	4.061	.384	3.772	.3307	.030*
Follow up Left	3.846	.484	3.660	.276	<.001**

(SD±0.4843) and group 2 3.772 right side (SD±0.3307) and left was 3.660 (SD±0.2761) and both groups are statistically significant with p value 0.030 for right side and 0.001 for left side.

Discussion

- The delivery of a natural mixture of biologically active molecules incorporated in a forming fibrin matrix within the joint compartment may target synovial fibroblasts, inducing positive changes in the whole joint micro-environment. Ability of PRGF to stimulate HA synthesis driving the secretion of HA by the synovial fibroblasts [18].
- TGF-1 up-regulates the hyaluronan synthase isoform-1 while PDGF primarily stimulates hyaluronan synthase isoform-2.
- PRGF will provide an exogenous source of TIMPs and Alfa 2-macroglobulin, natural inhibitors of activeproteases.
- The synergy between HGF andVEGF, which possibly can act cooperatively in normal physiology suggesting a role in non-inflammatory as opposed to inflammatory angiogenesis.
- PRGF can modify the angiogenic balance by triggering the secretion of HGF as demonstrated by Anitua et al in two studies
- PRGF therapy is also a relevant source of anabolic factors for cartilage including IGF-I and TGF-1 and might influence cartilage metabolism.

Marx RE et al [19] in 2001 used platelets as vehicles for the delivery of a balanced pool of healing factors

has become a new therapeutic treatment. At that time, platelet-rich plasmas (PRPs) were introduced as autologous modifications of potent adhesives known as fibrin glues.

Later many studies were conducted both animal and human studies were made regarding the use of PRP in bone and tendon healing, periodontal and oral surgery, plastic surgery, tendinopathies and muscle injuries, ligament reconstruction and in joint arthroplasty.

Sanchez et al [20] in 2003 used PRP to augment Kirschner wire fixation of a large (>2 cm) articular cartilage avulsion in a young soccer player and reported excellent results. The patient returned to full competition and on MRI had complete healing of the cartilage lesion at 18 weeks.

Anitua et al [18] in 2004 hypothesized that the delivery of a natural mixture of biologically active molecules incorporated in a forming fibrin matrix within the joint compartment may target synovial fibroblasts, thus inducing positive changes in the whole joint micro-environment. A major finding of their work was the ability of PRGF to stimulate HA synthesis driving the secretion of HA by the synovial fibroblasts. TGF-1 up-regulates the hyaluronan synthase isoform-137 while PDGF primarily stimulates hyaluronansynthase isoform-238. By regulating the endogenous HA synthesis PRGF would restore HA levels, thereby enhancing cartilage protection and joint lubrication.

Filardo G et al in 2010 used PRP injections and reported to show a transient effect to reduce the pain and improving the knee functioning, this effect end for longer time inchondral degenerative lesion or early osteoarthritis and in younger patients.

Centeno et al [21] in 2008 studied MSCs can be differentiated to chondrogenic, adipogenic, osteogenic lineages in vitro and in vivo and their beneficial effect on osteoarthritis of knee, showed that this improved effect of MSCs might be due to their potential differentiation to chondrocytes and structurally repair the articular cartilage.

Uccelli A et al in 2007, Kan I et al in 2007 show that MSCs show their beneficial effect by of its paracrine action to modify the function and activity of neighbouring cells, which include with the activation and stimulation of various signalling molecules bound the cellular surfaces .

Yan et al [22] in 2007 use of MSCs to repair the full thickness cartilage defection in rabbit model MSCs isolated from the bone marrow of rabbits was able to repair the femoral trochlear grooves of both knee in 36 adult NewZeland white rabbits. This repair suggested with regeneration of hyaline like cartilage tissue.

Lee et al [23] in 2007 demonstrated the beneficial effect of injected MSCs to stimulate the repair of chondral defects in pig models. Autologous MSCs were injected into 27 pigs and histological analysis was done after the completion of study period,12 weeks with full healing.

Kuroda et al [24] in 2007 indicated that the transplantation of autologous bone-marrow stromal cells can promote the repair of large focal articular cartilage defects in young, active patients.

Quarto et al [25] in 2001 reported the ability of mesenchymal stem cells to repair a large musculoskeletal defect with successful healing of a large bone defect in three patients. This was the first human trial assessing the ability of MSCs to repair a musculoskeletal defect. For each patient, osteoprogenitor cells were isolated from bone marrow and expanded in vitro,These cells were placed on macroporous hydroxyapatite scaffolds, and implanted at the lesion sites. External fixation was provided initially for mechanical stability and was subsequently removed, All patients recovered limb function.

In 2002, Wakitani et al. [26] studied autologous culture expanded bone marrow mesenchymal stem cell transplantation for the repair of articular cartilage defects in humans. Mesenchymal stem cells from bone marrow aspirates were culture expanded and transplanted into the articular cartilage defect in the medial femoral condyle and covered with autologous periosteum at the time of high tibial osteotomies. 42 weeks after transplantation, hyaline cartilage like tissue was observed in the defects. Although the clinical improvement was not significantly different, histological grading score was better in the cell-transplanted group than in the control group.

Pittenger et al [27] in 2009 described some of the paracrine effects which were (1) modulating the immune response, (2) mobilising or promoting host cell survival, (3) recruiting and inducing mitosis of endogenous tissue progenitor cells at the site of injury while stimulating an angiogenic response, or (4) preventing an inappropriate fibrotic response.

Ortiz et al in 2007 reported that the factors produced by MSCs in response to the injured environment include interleukin (IL)-10, IL-1 receptor antagonist (IL-1Ra), and transforming growth factor (TGF)-b. They reported a bleomycin-induced lung injury model in which murine MSCs, administered after exposure to the bleomycin, homed to the injured lung and reduced not only the inflammatory

response but also collagen deposition. IL-1Ra, secreted by the MSCs, was found to be integral to this process and acted by blocking tumor necrosis factor (TNF)-alpha and IL-1 in the lung .

Centeno et al., reported one case in which they showed that isolated and expanded autologous mesenchymal stem cells when percutaneously injected into a knee with symptomatic and radiographic osteoarthritis, resulted in significant cartilage growth, decreased pain and increased joint mobility. This is the first report of a human clinical trial of direct injection of mesenchymal stem cells in Knee osteoarthritis. By means of pre- and post-procedure MRI analysis they were able to show a significant increase in meniscus and cartilage volume. At 3-month followup, modified VAS pain scores decreased by 95%. Range of motion in extension increased from -2 degrees to +3 degrees with an associated decrease in VAS pain score. Although no conclusion can be derived from one case report, yet it underlines the necessity for further research involving this aspect.

Friedenstein et al in1995 first isolated mesenchymal stem cells from rat bone marrow. These cells have been experimented in diseases as diverse as myocardial infarction and lung fibrosis. However, as of date, only isolated case reports, describe the clinical use of stem cells in patients of knee osteoarthritis. Davatchi et al studied a human clinical trial for knee OA, showing that intra-articular injection of expanded MSCs is a safe procedure without any complication.

Lian Z et al [28], in 2014 Studied the synergistic effect of bone marrow-derived mesenchymal stem cells and platelet-rich plasma in streptozotocin-induced diabetic rats in diabetic wound healing showed that the expression of platelet/endothelial cell adhesion molecule 1, proliferating cell nuclear antigen, and transforming growth factor-â1 increased significantly in the BMSC plus PRP group compared to the other treatment groups.

Kruger et al [29] in 2013 studied the use of this combination in vitro they reported that platelet-rich plasma facilitated chondrogenic differentiation of subchondral progenitor cells in polyglycolic acid-hyaluronanscaffolds.Thus it may be beneficial in scaffold-assisted cartilage repair involving stem and progenitor cells.

Conclusion

Mesenchymal stem cells with platelet rich plasma was more efficacious than platelet rich plasma alone in promoting the regeneration of articular cartilage in knee as indicated by increase in cartilage thickness as shown in MRI which was statistically significant.

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