Original Article

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Role of Mesenchymal Stem Cells in Osteoarthritis Knee

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Abstract

Background: Osteoarthritis of knee is a chronic and debilitating problem of the elderly to which a satisfactory cure is yet to be found. Mesenchymal stem cells have been used in preclinical studies and were found effective in treating osteoarthritis. In this study we aim to evaluate the role of mesenchymal stem cells in treatment of primary osteoarthritis of knee in humans and analyze the results in clinical terms. *Methods*: 60 patients of osteoarthritis knee were randomly grouped into two categories by simple randomization. 30 patients each were allocated to group A (cases) and group B (controls). Fifteen ml of bone marrow was aspirated from patient's sternum, concentrated stem cells solution was prepared and 1 ml of stem cell concentrate was injected into the knee joint of patients categorized as cases. Placebo injection of normal saline was given to controls. Patients were followed up at 1, 3, 6, 12, 18 and 24 months. In every follow up, patients were evaluated clinically using modified WOMAC index and compared with pre-treatment scores. The study was carried out in double blind manner. Statistical tests used were Mann Whitney test to compare the scores of cases and controls and Wilcoxon test to compare the paired data pre and post treatment within the case and control groups. *Results*: In group A mean [standard deviation [SD]] pre procedure pain score was 12.53 [1.187] and final score was 7.20 [1.474]. Mean [SD] pre procedure stiffness score in group A was 5.33 [0.976]. Final score was 2.47 [0.915]. Mean [SD] difficulty pre procedure score in Group A was 43.67 [5.394]. Final score was 26.00 [2.699]. Total pre procedure score in group A was 61.53 [6.300] and final score was 35.67 [4.806]. The reduction in all scores were statistically significant ($p<0.001$) compared to pre treatment values and compared to controls. There was no significant change pre and post-treatment in mean pain, stiffness, difficulty and total scores in group B. *Conclusion*: Mesenchymal stem cells are an effective treatment modality for osteoarthritis. The one-stage stem cell isolation and injection procedure is safe, quick and cost effective in managing osteoarthritis.

Keywords: Osteoarthritis; Stem Cells; WOMAC Index.

Introduction

Osteoarthritis (OA) is characterized by articular cartilage senescence, degeneration and consequent joint destruction. It is the most common joint disease worldwide and a cause of chronic debility and pain in the elderly population [1]. The osteoarthritis problem is a major economic burden on most nations and it will only increase in the near future with epidemic of obesity and decreased physical activity unfolding world over. The problem of OA is further accentuated by lack of adequate cure. Pharmacological treatment of present day for early osteoarthritis has seen limited success. The surgical therapies in vogue like arthroscopic debridement,

subchondral drilling, osteochondral autograft transfer, autologous chondrocyte transplantation etc have been successful in alleviating the pain temporarily, but eventually all fail [2]. Osteotomy for malaligned joints can provide pain relief for many years, but all these surgical options only function to delay the eventual need for a total knee replacement [3]. Lack of effective treatment options combined with increasing burden of osteoarthritis makes search for novel cartilage treatment strategies of prime importance. Mesenchymal Stem Cells [MSC] were first studied by Friedenstein and colleagues [4] and since then they have revolutionized the field of tissue engineering. Adult MSCs have the ability to differentiate into chondrogenic lineage and have come up as a major advancement in cell-based articular cartilage repair technologies. MSCs can be isolated from a variety of adult tissues, readily culture-expanded without losing their multilineage differentiation potential, and have been induced to undergo chondrogenic differentiation in vitro and in vivo [5-7]. MSCs have been used in animal models to grow cartilage tissue in vivo [8, 9] and for reduction of degeneration of the articular cartilage in an experimental model of knee osteoartritis [10]. Controversial results have been given by different authors about the relationship between osteorthritis and MSC chondrogenic activity [11]. The aim of the present study is to study the effectiveness of mesenchymal stem cells in the management of primary osteoarthritis of knee joint. The results were evaluated based on functional outcome of the treatment by modified WOMAC score and were compared with control subjects.

Methods

The present study was a double blind randomized control study conducted in collaboration with the research laboratory section of the blood bank. Informed written consent was taken from each patient. We studied a total of 60 patients of osteoarthritis knee. Patients were randomly grouped into two categories (30 patients each): Group A (cases) and Group B (controls) by simple randomization. The study was carried out in a double blind fashion in that neither the patients nor the person taking the clinical scores of the patients had the knowledge of who the cases or controls were. Inclusion criterion was primary osteoarthritis of knee with radiological confirmation of the diagnosis [Kellgren-Lawrence scale- grade 1 or 2] [12] and age group of 45 to 65 years. Exclusion criteria included patients with secondary osteoarthritis, co morbidities like diabetes mellitus, obesity [defined in our study by body mass index more than 30], and prior medical/surgical interventions for osteoarthritis.

Bone marrow was aspirated, concentrated stem cells were prepared and the stem cell concentrate was injected into the knee joint of patients categorized as cases and normal saline injection was given in controls.

Device Description and Preparation

Bone marrow aspirate was obtained by aspiration technique. Patients were made to lie supine comfortably and proper aseptic precautions were used. The sternum was injected with local anesthetic agent (2% xylocaine). After adequate effect of the anesthetic agent, Jamshidi type trocar needle [Figure 1] was introduced in the sternum. In the sternum, the trocar needle was directed at right angle to the horizontal plane and inserted approximately to a depth of 1cm [Figure 2]. 10 to 15 ml of Bone marrow aspirate was extracted while rotating and slowly withdrawing the needle towards the cortex using a 20 ml syringe which was pre-flushed with heparin (concentration of 1000 units / ml) .

Stem Cell Isolation [5]

15 ml Ficoll-Hipaque (sigma) solution with density of 1077 was taken in a sterile centrifuge tube. 10 ml of bone marrow aspirate was diluted with 20 ml of normal saline. Centrifugation was done at a spin speed of 1500 rpm and at a temperature of 22^0C for 30 minutes using Heraeus centrifuge device (6000i) [Figure 3]. After centrifugation [Figure 4] the plasma layer was carefully aspirated and discarded without disturbing the plasma-Ficoll interface [5]. Mono nuclear cell layer was transferred into another sterile tube using sterile pipette and phosphate buffered saline was added to the cell suspension and was washed thrice by centrifugation, at a spin speed of 1500 rpm at 22^0C temperature for 10 minutes. A final centrifugation in phosphate buffered saline was done and 1 ml of final stem cell suspension was obtained.

Procedure

After the preparation of stem cell concentrate, patient was shifted to operation theatre. After proper positioning of the patient, part preparation were done. About 3 to 5 ml of local anesthetic agent (2% xylocaine) was injected into the knee joint. 16 gauge needle and 10 ml syringe were used to inject the stem cells into the knee joint with the help of image intensifier [Figure 5].

Follow up Period

Patients were given a course of antibiotics for 7 days and were followed up at 1, 3, 6, 12, 18 and 24 months. In every follow up, patients were evaluated clinically using modified WOMAC [Western Ontario and McMaster Universities] scale [13,14]. Modified WOMAC scale is a clinical score based on 3 parameters- pain, stiffness and difficulty and consists of 24 questions. Each answer is scored from 0 to 4 with 0 being none and 4 meaning extreme. Maximum and minimum total scores are 96 and 0 respectively. Maximum pain, stiffness and difficulty scores are 20, 8 and 68 respectively and all minimum scores being 0.

Modified WOMAC Scale

How Much Pain Do You Have?

1. In walking on flat surface
2. Going up or down stairs
3. At night while in bed
4. Sitting or lying
5. Standing upright

How Much is Your Stiffness?

6. After first wakening in the morning
7. After sitting, lying or resting later in the day

How Much Difficulty do You Have?

8. Descending stairs
9. Ascending stairs
10. Standing up from a chair
11. While standing
12. Bending to floor (to pick up objects)
13. Walking on flat ground
14. Getting in and out of Auto Rickshaw / Bus / Car
15. Going shopping
16. On rising from bed
17. While lying on bed
18. While sitting on chair
19. Going on/off toilet –Indian/Western
20. Doing heavy domestic duties (moving heavy boxes, scrubbing floor, lifting shopping bags)
21. Doing light domestic duties (cleaning room/table/cooking/dusting)
22. While sitting cross legged on floor
23. Rising from cross legged position
24. While squatting on floor

Statistical tests used were Mann Whitney test to compare the scores of cases and controls and Wilcoxon test to compare the paired data pre and post treatment within the case and control groups.

Results

Majority of patients were between 50 and 60 years of age with mean age 55.6 years in group A and 56.8 years in group B. In a patient of osteoarthritis the primary concern of a patient is pain. That is why he first contacts any hospital that is what he's most worried about. So any treatment strategy devised for osteoarthritis should alleviate the pain component. In group A [stem cell group] the mean [standard deviation [SD]] pre procedure pain score was 12.53[1.187]. During follow up, mean [SD] score showed gradual reduction and final score was 7.20[1.474] at 2 years follow up. The reduction in score was statistically significant ($p<0.001$). There was no significant change in pain score in group B [control group].

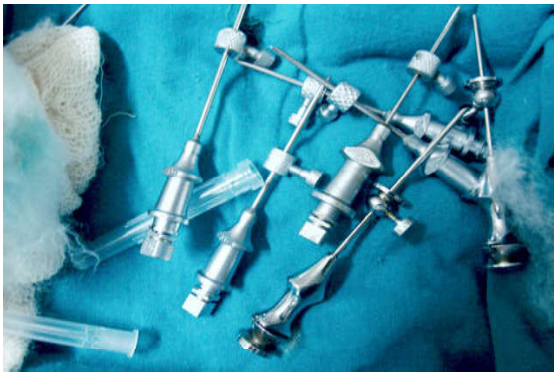


Fig. 1: Jamshedi type bone marrow aspiration needle.

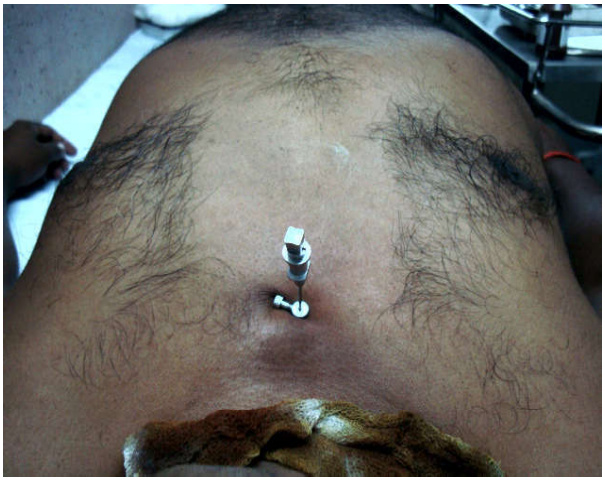


Fig. 2: Bone marrow aspiration being done from sternum
Legend: The procedure can be done on outpatient basis with conscious sedation and local anaesthesia with proper asceptic precautions.

Fig. 3: Centrifugation device

Fig. 4: Post centrifugation appearance
Legend: The supernatant straw coloured viscous fluid is the plasma. Lower red layer is Ficoll solution with the red blood cells and the middle white layer is the buffy coat with stem cells and white blood cells

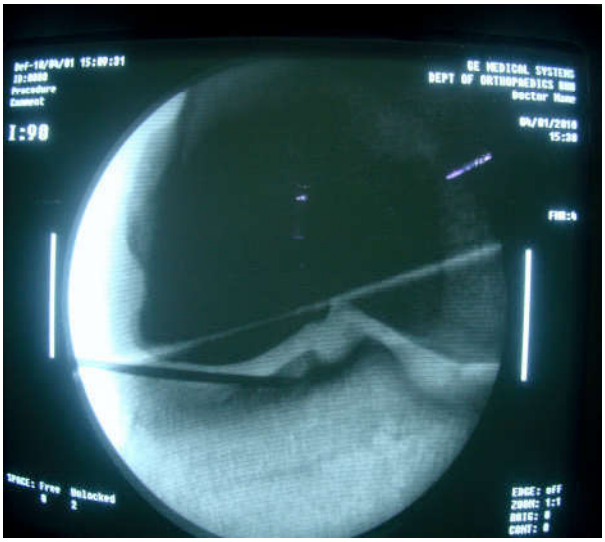
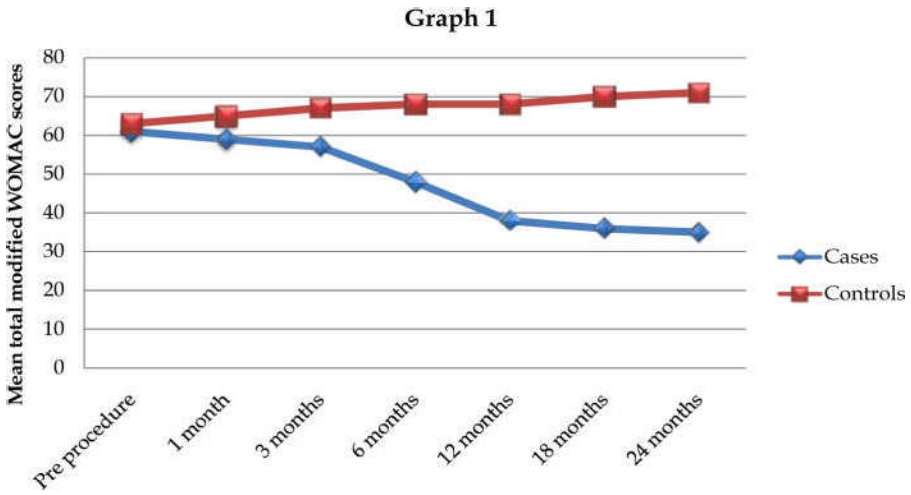


Fig. 5: Stem cell injection being given to knee joint under image intensifier guidance

The mean [SD] pre procedure stiffness score in group A was 5.33 [0.976]. Final score was 2.47 [0.915]. The reduction in score was statistically significant ($p<0.001$). There was no significant change in stiffness score in group B.

Osteoarthritis does not only cause pain but also secondarily lead to loss of work, difficulty in day to day life activities, depression etc. It is a major healthcare burden in the elderly. A holistic approach to osteoarthritis should relieve the patient off the difficulties he/she faces due to this condition. The mean [SD] difficulty pre procedure score in Group A was 43.67[5.394]. Final score was 26.00[2.699]. The reduction in score was statistically significant ($p<0.001$). There was no significant change in difficulty score in group B.



Graph 1: Shows the change in mean total modified WOMAC scores with increasing follow up among the cases and the control groups

Total pre procedure score in group A was 61.53 [6.300] and final score was 35.67 [4.806]. The reduction in score was statistically significant ($p<0.001$). There was no significant change in total score in group B.

Discussion

Several studies, mostly in mice, have demonstrated that the transplantation of mesenchymal progenitors found in bone marrow aspirates to be useful in OA. Pre clinical studies in larger animals like goat have also demonstrated the usefulness of MSCs in OA [10]. This had inspired us to study the effect of mesenchymal stem cells in osteoarthritis knee in humans.

Stem cells can be obtained from three different sources- embryo, umbilical cord and adult stem cells [MSCs] [15]. Adult stem cells are limited in their regenerative capability in comparison to embryonal and umbilical cord stem cells. Inspite of that they have their own distinct advantages. These MSCs are readily available and are amenable to harvesting and isolation from the bone marrow and other tissues of mesodermal origin. Since they have limited potential of differentiation there is less chances of teratoma formation [a fear associated with use of embryonal stem cells]. MSCs are already pre-programmed to differentiate into musculoskeletal tissue types. The challenge would be to expand them in adequate numbers and ensure that they are able to differentiate into the correct phenotype of tissue that they are intended to repair [15].

MSCs have been identified in umbilical cord blood, placenta, bone marrow, skin, fat, eyes and brain. Among these the bone marrow seems to be the most obvious clinically useful source of stem cells. It is easily accessible, has the highest multilineage potential amongst all adult MSCs and because they are autologous they are non-immunogenic and safe. MSCs also have drawbacks including diminished availability with age and cell senescence with loss of multilineage differentiation capability after about 34–50 population doublings as a result of telomere shortening [16].

Approximately one of every 100,000 nucleated cells aspirated from the bone marrow is a stem cell. Hence several protocols have been developed for isolation and expansion of MSCs from bone marrow so far. These include using density-gradient centrifugation, specific cell surface antibody [17], selective adhesion to laminin- coated plate Hoechest dye exclusion, and size-sieved culture etc. Potential disadvantages of these methods in terms of clinical applications are the heterogeneity of cultured cells, high risk of contamination, and high cost of production. A simpler method is centrifugation of the bone marrow aspirate as described by Pittenger et al [5]. We have used this method in our study[see material and methods] and when compared with other isolation and culture techniques this method is relatively much more cost effective and less time consuming and can be performed on outpatient basis also.

In this study the total amount injected for bone marrow aspirate is 15 ml and that of stem cell is less than 1 ml. Though the volume injected is less on analysing with the staining procedures the stem cell concentration was 2- 3 million cells/ml. There are various ways used to deliver mesenchymal stem cells, like cultured scaffolds, gel foams, with auto grafts, systemic delivery and local site injection. The local site injection route was used in the present study. Since aspiration, concentration and injection were all performed on the same day the costs were significantly reduced. It was performed on outpatient basis and patients were allowed full activity following the procedure, thus reducing the patient morbidity associated with admission and hospital stay. Site specific delivery also has the advantage of delivering large number of cells directly to the site of requirement. An immediate autologous transplantation of bone marrow concentrate can prevent complications related to the reduced quality of the transplanted cells such as pre-aging (telomere shortening), reduced viability or dedifferentiation / reprogramming that is associated with in-vitro cultivation. In addition, the risk for infection is reduced by decreasing the ex-vivo time period. When injected locally, MSCs have been shown to differentiate into linage-specific cells under the micro environment into which they are transplanted. This has been demonstrated by the detection of various markers of the differentiated cells utilizing reverse transcription polymerase chain reaction and immune staining [18]. Site specific delivery is not without problems. Local tissue damage, septic arthritis and inability to deliver multiple doses are known complications associated with it. Bone marrow aspiration from the sternum did not limit rehabilitation and did not cause delay in discharge from hospital, this procedure had done in outpatient basis and the patient is discharged on the same day of injection and the inpatient stay is shortened.

In stem cell group there was a lag period from the procedure to 3 months post procedure when there was no significant reduction in scores. From 3 months to 12 months there was gradual reduction in scores. After 1 year the improvement in pain scores was not

that marked. This shows that stem cell therapy for osteoarthritis starts acting after a lag phase of 3 months and gives maximum benefits by 1 year and from then on the benefits are not that significant.

Currently, it is difficult to know the exact mechanism that follows once the injection is given. The mechanism by which stem cells act is a relevant matter for future studies.

Possible Mechanisms

1. Tissue-specific differentiation
2. Immunomodulatory effects
3. Paracrine effect.
4. Stimulation of angiogenesis

Data suggest that the therapeutic potential of these cells, at least in some applications, is related to paracrine effects such as the release of factors that (1) modulate the immune response, (2) mobilize or promote host cell survival, (3) recruit and induce mitosis of endogenous tissue progenitor cells at the site of injury while stimulating an angiogenic response, or (4) prevent an inappropriate fibrotic response [10].

Immunomodulatory Effects

Factors produced by MSCs in response to the inflammatory environment include interleukin (IL)-10, IL-1 receptor antagonist (IL-1Ra), and transforming growth factor (TGF)-b. Upregulation of IL-10 in the brain has been associated with the therapeutic potential of MSCs in reducing neuronal injury after an ischemic injury in rats and increased IL-10 production by host macrophages in response to MSCs has been described to occur through a PGE2-dependent mechanism in treating sepsis in mice. Such mechanism may be in action in treatment of OA by MSCs.

Paracrine Effects

The beneficial paracrine effects of MSCs have been demonstrated in the articular joint via administration of cells after surgically induced injury. Murphy et al [10] observed that local delivery of autologous caprine MSCs in a solution of hyaluronan to a meniscectomized joint resulted in significantly better regeneration of meniscal tissue and chondroprotection in comparision with meniscectomized joints treated with hyaluronan alone. However, it was readily apparent that the green fluorescent protein (GFP)-labeled MSCs used in the study colonized just a small proportion of the regenerated meniscus. It was therefore concluded that the implanted MSCs induced a host repair response through the release of paracrine factors to replace the resected medial meniscus.

Stimulation of Angiogenesis

Increased angiogenesis has been shown to occur in late OA. MSCs can promote early angiogenic events, a mechanism known to contribute to tissue repair, by increasing endothelial cell (EC) proliferation and migration in vitro as well as significantly increasing the stability of vessels formed by ECs through a cell contact-mediated mechanism.

Extensive search of literature yields only few human studies that present preliminary clinical data showing successful treatment of osteorthritis by bone marrow derived MSCs.

Although there was significant improvement in all components of WOMAC index in patients treated with MSCs there was no radiological change in the degree of degenerative change as visible in radiographs. By 2 years follow up the radiological picture was same as that when the study had started. This was probably because radiological improvement lags behind clinical improvement by many years. However in the control group there was further progression of the disease in radiographs at 2 years follow up.

In conclusion, mesenchymal stem cells are safe and effective treatment modality for management of osteoarthritis. One stage aspiration, concentration and injection of bone marrow stem cells is an effective and cost effective treatment strategy with low patient morbidity. Improvement in clinical scores may not correlate with the radiological stage of the disease. The procedure of harvesting autologous bone marrow stem cells is safe and not associated with any major complication. Further research in this field is necessary to evaluate the exact mechanism of action of these stem cells, their long term effectiveness and safety. For now is shall be safe to conclude that the mesenchymal stem cells in treatment of osteoarthritis is a modality which is here to stay.

List of abbreviations used

OA: Osteoarthritis

MSCs: Mesenchymal Stem Cells

SD: Standard Deviation

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