Efficacy of mesenchymal stem cells in healing of meniscus tear

Dr. Rama Kishan, Dr. Arjun RHH, Dr. Aditya Aggarwal and Dr. Mahesh Prakash

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Abstract

Background: The meniscus is a semilunar fibro-cartilaginous tissue performing various function including knee joint stability, shock absorption, load distribution, joint lubrication, and joint congruity. Meniscectomy often leads to articular cartilage degeneration and ends up in osteoarthritis of knee. Authors described use of mesenchymal stem cells for evaluation of healing of meniscus tear.

Purpose: To evaluate the efficacy of mesenchymal stem cells in healing of meniscal tear.

Study design: Prospective, randomised, case-control double blinded study.

Methods: A total number of 30 patients with meniscus tear of knee, were selected on the basis of predefined inclusion and exclusion criteria after informed written consent. Both cases and controls were 15 in numbers. No case were lost to follow-up. MRI was done twice pre and post intervention. In case group autologous bone marrow was aspirated, cultured and injected percutaneously under ultrasound guidance. Whereas in control group normal saline was injected as placebo. All patients received standard physiotherapy. Objective assessment was done by functional scoring systems (WOMAC, KOOS, VAS SCORE).

Results: There was significant reduction in pain scores (VAS scores) in case group (p=.001) as compared to control group (p=.166). There was improvement in functional outcome scores such as WOMAC in case group (P=0.001) as compared to control group (p=.419) and Global KOOS score in case group (p=.001) compared to control group (p=.910). Significant improvement was noted in MRI signals in case group as compared to control group (p=.013) depicting healing of meniscal tears with injection of mesenchymal stem cells.

Conclusions: Mesenchymal stem cells injection is efficacious for healing of meniscal tear which reduces pain, helps in integration of torn meniscus and improves functional outcome.

Keywords: Mesenchymal stem cells, meniscus tear, MRI, WOMAC, VAS and KOOS scores.

1. Introduction

The meniscus is a semilunar fibro-cartilaginous tissue present in each knee between the femoral and tibial joint surfaces. A functional intact meniscus is critical for the homeostasis of the knee joint, performing important biomechanical roles for knee joint stability, shock absorption, load distribution, joint lubrication, and joint congruity. Partial meniscectomy also leads to degenerative changes [4]. Successful surgical restoration of the damaged meniscus has been a challenge due to its poor healing potential. Due to the presence of a vascularized outer zone (10%–30% of the meniscal body), meniscal lesions in this zone heal spontaneously and can be sutured successfully with a high success rate [5]. However, majority of meniscal tears are in the inner avascular zone lacking spontaneous healing, which ultimately leads to permanent degenerative changes, including osteoarthritis [6, 7].

Meniscal repair is indicated in acute tears and peripheral tears <5 mm from the meniscosynovial junction with a stable knee [8-10]. Total meniscectomy is rarely performed nowadays because of direct relationship between meniscectomy and development of early osteoarthritis [11]. Further, the degree of osteoarthritis after meniscectomy is directly proportional to the amount of meniscus removed [12, 13]. Partial meniscectomy also leads to reduced contact areas in the joint and results in supra-physiological stresses on the articular cartilage which can lead to knee damage and osteoarthritis [14, 15].
Various other surgical options such as Fibrin sealant, Meniscal arrows, Laser welding, Cryopreserved allograft, Meniscal substitutes, Meniscal prostheses have been tried in the literature [16-19]. These techniques are effective only if meniscal tear in meniscal fibrocartilage [20]. Mesenchymal stem cells are of potential use as a source of repair cells. Mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells characterized in vitro by their extensive proliferative ability in an uncommitted state while retaining the potential to differentiate along various lineages of mesenchymal origin, including chondrocyte, osteoblast, and adipocyte in response to appropriate stimuli [21]. Mesenchymal stem cells have been shown to possess many potent paracrine effects through secretion of various soluble factors including Vascular Endothelial Growth Factor (VEGF), Monocyte Chemoattractant Protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, Interleukin-6 (IL-6), and monokine induced by IFN-γ (MIG) which can influence the local tissue environment and exert protective effects with an end result of effectively stimulating regeneration in situ [22]. The regenerative effects of MSCs in meniscus tear may be attributed to their ability to differentiate themselves into chondrocytes and thus structurally repair the meniscal cartilage. The aim of this study was to evaluate the effect of mesenchymal stem cells injection on repair of meniscal tear.

2. Materials and Method

The study was conducted on patients attending either orthopaedics outpatient department or Trauma Ward in our institute from July 2012 to December 2013 who were diagnosed as meniscus tear clinically & radiologically. Sample size was estimated based on mean WOMAC score of 70. To detect a 30 % change in WOMAC score with S.D. of 18 our sample size came out to be 13 at 80% power and confidence interval of 95 % in each group [23]. This study was a prospective, randomized, controlled, double blinded study. Randomization was done by computer generated random number table. The patients were selected after going through the inclusion and exclusion criteria.

We included patients of 18 to 65 years with clinically and MRI evidence of meniscal tear and signal changes; unilateral or bilateral. Exclusion criteria were patient with flexion deformity of knee, unstable knee, locked knee and active infective or connective tissue disease (i.e. Lupus, Bacteremia, Fibromyalgia, RA). A written informed consent was obtained.

2.2 Pre-procedure data collection

Haemogram and Coagulation profile to rule out unknown medical condition. MRI of affected knee joint was obtained on 3.0 T Magnetom Verio Siemens medical system (Germany) in T1 and T2 in sagittal plane and Proton Density Fat Saturation (PDFS) in sagittal, coronal and axial planes. Grading of Meniscal signal changes was done as described by Crues et al. [24], Stoller et al. [25] (Table 1).

<table>
<thead>
<tr>
<th>Grading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal meniscus.</td>
</tr>
<tr>
<td>1</td>
<td>Central globular, irregular, marginated, intrameniscal, alteration not abutting/ communicating with an articular surface.</td>
</tr>
<tr>
<td>2</td>
<td>Linear signal not abutting/ communicating with an articular surface.</td>
</tr>
<tr>
<td>3</td>
<td>Linear alteration abutting/ communicating with an articular surface.</td>
</tr>
<tr>
<td>4</td>
<td>Multiple complex alterations.</td>
</tr>
</tbody>
</table>

2.3 Mesenchymal stem cell isolation, characterization

In those patients who gave consent to the study, the bone marrow sample was taken. Under all aseptic precautions 10 ml of bone marrow was aspirated under local anaesthesia (2% lignocaine) from the posterior superior iliac spine of the patient (Fig.1) by using sterile disposable Salah bone marrow aspiration needle (16 G) 2.0mm x 50mm. The sample was anticoagulated with preservative free heparin.

Collected bone marrow was transferred to stem cell research facility under sterile condition and was processed for Mesenchymal Stem Cells isolation as per standard protocols. Briefly, total mononuclear cells were separated by density gradient centrifugation (480g for 30 minutes). These mononuclear cells were inoculated at 1.5X10⁶cell/cm² in T-75 flasks (Corning, NY, USA), with 5% PHPL (pooled human plasma lysate) supplemented α-MEM media (minimum essential medium) containing antibiotics and were incubated in humid CO₂ chamber at 37 °C (Fig. 2). After 12 days of culture (Fig.3) spindle shaped cells appeared in the culture, and the number of cells increased by 15 to 18 day. These cells...
were thantrypsinized using 0.02% of trypsin-EDTA by incubating for 5 minutes at 37 °C. Isolated cells were characterized for the expression of MSC positive marker: >90% positivity for CD90, CD105 and CD 73; <5% for CD34 and CD45.

2.4 MSC preparation for therapeutic injection
MSCs cells of passage two were used for injection. Cells were washed with 1XPBS (Phosphate buffer saline), incubated with trypsin-ETDA for 5 minutes. Deadhered MSCs were collected in 50 ml falcon tube, and were washed with 1XPBS by centrifuging at 580g for 5 minutes. Finally, the cells were resuspended in 1XPBS having approximately 40-45x10^6 MSCs. Before injection the sample sterility for aerobic and anaerobic microbial growth was tested in department of microbiology as per standard protocols and cell viability and numbers were checked using trypan blue dye and haemocytometer.

2.5 Interventional procedure
The Patient was placed in supine position with knee in 30° flexion and under all aseptic precautions 15 ml of Mesenchymal stem cell (45x10^6) (Fig. 4) was injected under Ultrasound guidance (Fig.6) with 20G needle in the affected knee near the meniscus tear as depicted in figure 5. After 30 min of observation the patient was discharged.
2.6 Post procedure advice
The patients were advised to report in case of any adverse events like fever, erythema etc.
Non-steroidal Anti-Inflammatory Drugs (NSAIDS) were not allowed. In case of discomfort Paracetamol (dose of 500 mg) was used. Follow up clinical evaluation was done on twice first after 3 months and second followup with mean follow-up of 11.86 month (minimum 6.5 months, maximum 17 months) by an independent observer (blinded). Objective scoring done using VAS [20] ( Visual Analog Score), WOMAC [21] (Western Ontario and McMaster Universities Index of Osteoarthritis) and KOOS [22] (Knee injury and Osteoarthritis Outcome Score).
Post-procedure MRI was obtained at mean follow up of 11.86 months using same MRI machine, same sequences and grading was done. The results were charted, evaluated and statistically analyzed. Correlation of Clinical and Radiolgical (MRI) was done using same MRI machine, post procedure MRI was obtained at mean follow up of 11.86 month (minimum 6.5 months, maximum 17 months) and Mann Whitney U Test for Normally distributed data respectively. Between groups analysis, comparisons were performed using Student’s t test (unpaired) and Mann-Whitney U Test for Normally distributed data and Skewed data respectively.

A ‘P’value of < 0.05 was considered significant in all the tests. Analysis was done using statistical software package, SPSS (Statistical Package for the Social Sciences, version 20)

3. Results
In our study, the age ranged from 18 years to 54 years with a mean of 35.53±11.04 in Case group (Group I) and 36.07±9.60 in Control group(Group II). In Group I (Cases), out of 15 patients there were 13 males (86.7%) and 2 females (13.3 %). In Group II (Control), out of 15 patients there were 11 males (73.3 %) and 4 females (26.7 %)

Patients were comparable for demographic datas including age, sex, side of knee involved and meniscus involved. (Table 2)

Table 2: Comparison of Demographic Characteristics

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Group I (Case)</th>
<th>Group II (Control)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Mean ± S.D.)</td>
<td>35.53 ± 11.04</td>
<td>36.07± 9.60</td>
<td>.889</td>
</tr>
<tr>
<td>Sex Distribution (M/F)</td>
<td>13/2</td>
<td>11 /4</td>
<td>.361</td>
</tr>
<tr>
<td>Knee (R/L)</td>
<td>9/6</td>
<td>8/7</td>
<td>.589</td>
</tr>
<tr>
<td>Meniscus (MM/LM/Both)</td>
<td>15/6/6</td>
<td>14/12/11</td>
<td>.214</td>
</tr>
</tbody>
</table>

Baseline functional scores as WOMAC, VAS Score, KOOS score for both groups were tabulated (Table 3).

Table 3: Pre Injection Baseline Characteristics

<table>
<thead>
<tr>
<th>Pain Scores</th>
<th>Group I (Case)</th>
<th>Group II (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>WOMAC I</td>
<td>49.80</td>
<td>13.30</td>
</tr>
<tr>
<td>VAS1</td>
<td>68.67</td>
<td>17.67</td>
</tr>
<tr>
<td>KOOS Pain1</td>
<td>42.06</td>
<td>13.88</td>
</tr>
<tr>
<td>Symptom1</td>
<td>55.95</td>
<td>13.94</td>
</tr>
<tr>
<td>ADL1</td>
<td>49.80</td>
<td>11.68</td>
</tr>
<tr>
<td>Sport1</td>
<td>21.67</td>
<td>12.91</td>
</tr>
<tr>
<td>Life1</td>
<td>36.67</td>
<td>12.47</td>
</tr>
</tbody>
</table>

Patient were accessed clinicallyand objective parameter were derived twice first around 3 months and further at mean follow up of 11.86 months and MRI was done to acess healing radiologically.

Table 4: Change in various outcome scores after final follow up

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in WOMAC (Baseline – Final Follow up)</td>
<td>37.7333</td>
<td>12.0971</td>
<td>3.12207</td>
<td>.000</td>
</tr>
<tr>
<td>Change in VAS (Baseline – Final Follow up)</td>
<td>3.1333</td>
<td>9.57576</td>
<td>2.47245</td>
<td>.000</td>
</tr>
<tr>
<td>Change in KOO P (Baseline – Final Follow up)</td>
<td>-39.7927</td>
<td>17.84043</td>
<td>4.60638</td>
<td>.000</td>
</tr>
<tr>
<td>Change in KOO SYM (Baseline – Final Follow up)</td>
<td>-39.5280</td>
<td>13.73154</td>
<td>3.54547</td>
<td>.000</td>
</tr>
<tr>
<td>Change in KOO ADL (Baseline – Final Follow up)</td>
<td>-35.8813</td>
<td>13.34951</td>
<td>3.44683</td>
<td>.000</td>
</tr>
<tr>
<td>Change in KOO SPORT (Baseline – Final Follow up)</td>
<td>-0.940</td>
<td>9.07282</td>
<td>2.34259</td>
<td>.000</td>
</tr>
<tr>
<td>Change in KOO LIFE (Baseline – Final Follow up)</td>
<td>-30.5833</td>
<td>12.19875</td>
<td>3.14970</td>
<td>.000</td>
</tr>
</tbody>
</table>

Table 5: Comparison of Improvement in MRI in the two groups

<table>
<thead>
<tr>
<th>MRI</th>
<th>Group I (Case) No. Of Pt. (%)</th>
<th>Group II (Control) No. Of Pt. (%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
<td>7 (46.67%)</td>
<td>1 (6.67%)</td>
<td>.013</td>
</tr>
<tr>
<td>Not Improved</td>
<td>8 (53.33%)</td>
<td>14 (93.33%)</td>
<td>.013</td>
</tr>
</tbody>
</table>

In Case group, radiological (MRI) improvement was seen in 7 patients (46.67%) but in control group improvement was seen in only 1 patient (6.67%). The improvement in case group was statistically significant (p=.013). The improvement in MRI
signals indicates meniscal tear healing and is attributed to tissue regeneration by mesenchymal stem cells.

The changes in MRI signal in terms of healing of meniscal tear after injection of MSCs were noted in Case group (Group I) which are shown in Fig 7-8.

![Fig 7: Pre - injection MRI showing grade II horizontal tear of medial meniscus posterior horn and grade III horizontal tear lateral meniscus as depicted by arrow in group-I (Case Group)](image)

![Fig 8: Post-injection MRI showing improvement in healing of meniscus tear showing grade I signal changes of medial meniscus posterior horn and grade II signal changes in body and posterior horn of lateral meniscus as depicted by arrow in group-I (Case Group)](image)

In Control group (Group II) no significant changes in MRI signal were seen as shown in Fig 9-10.

![Fig 9: Pre - injection MRI showing complex tear of medial meniscus posterior horn as depicted by arrow in group II (Control Group)](image)

![Fig 10: Post- injection MRI showing complex tear of medial meniscus posterior horn (no improvement) as depicted by arrow in group II (Control Group)](image)

4. Discussion

Healing is problematic in inner 2/3 zone of meniscus so called as red-white and white-white zone which has variable or no vascularity [6, 7].

For tissue repair, transplantation of bone marrow derived MSCs is a promising alternative. MSCs can differentiate into various lineage including osteoblast, chondroblast and adipoblast tissues [21]. In addition MSCs secrete various paracrine factors including Vascular Endothelial Growth Factor (VEGF), Monocyte Chemo attractant Protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, Interleukin-6 (IL-6), and monokine induced by IFN-γ (MIG) which can influence the local tissue environment and exert protective effects with an end result of effectively stimulating regeneration in situ [22].

Centeno et al. [29], reported single case using MSC in human. They showed a significant improvement in volume of injured meniscus and cartilage by magnetic resonance imaging (MRI) however they didn’t take functional outcome scores such as WOMAC, KOOS in account and they also not considered grading of meniscal tear. In our study we followed our patients with clinical examination and we used functional outcome scores WOMAC, KOOS in addition to pain score (VAS score).

In our study there was statistically significant decrease in VAS Score at follow ups in case group as compared to control group (p=0.000). In case group the decrease in VAS score from baseline at 1st and final follow up was 54.38 % and 72.81% respectively. On the other hand in Control group the decrease in VAS score was significantly less which was 14.07% at 1st follow up and 7.04% from baseline at final follow up.

The mean WOMAC score in case group (Group I) at baseline was 49.80, and the mean WOMAC score at 1st follow up was 19.73 and at final follow-up was 12.07 which was a decrease of 60.38% and 75.76% respectively over the baseline. Whereas in control group (Group II), the mean WOMAC score at baseline was 38.07, and the mean WOMAC score at 1st follow up was 30.13 and at final follow-up was 34.93 which was a decrease of 20.86% and 8.25% respectively over the baseline. In case group there were statistically significant decrease in all three domains of WOMAC score (pain, stiffness and physical...
function) as compared to control group (p=.000).

The KOOS is a useful, reliable, valid and responsive instrument for assessment of patient-relevant outcomes in subjects with meniscus injury. 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). Traditionally in orthopedics, KOOS score of 100 indicates no problems and KOOS score of 0 indicate extreme problems and so an increase in KOOS score in subsequent follow up indicates symptomatic improvement. In case group the mean global KOOS at baseline was 41.23, and the mean global KOOS at 1st follow up was 73.64 and at final follow up was 81.65 which was an increase of 78.6% and 98.04% respectively over the baseline. In control group the mean global KOOS at baseline was 56.20 and the mean global KOOS at 1st follow up was 60.62 and at final follow up was 56.56 which was an increase of 7.86% and 0.64% respectively over the baseline. In case group there were statistically significant increase in global KOOS score as compared to control group (p=0.000).

5. Conclusion
Thus it can be concluded that MSCs therapy have potential for meniscal tear as depicted clinically as well as radiologically in this study. Complications: In our study we did not encounter any complications immediately after the knee injection or at the first follow up at 3 months or at the final follow up. Even in the previous studies by Centeno et al. no complications were observed. Even though the sample size in our study and the previous studies is small, grossly it could be stated that there are no obvious side effects with the mesenchymal stem cells atleast in the short term. Strength of study: Our study was a Randomized, prospective, double blinded, case- controlled study. The patients encountered less difficulty as the procedure (of both bone marrow aspiration and knee injection) was simple. The number of visits to the hospital was also less as the knee injection of MSCs was given only once. The most important strength of the study was that it was conducted in a clinical situation in 30 patients including 15 controls. This was only second of this kind as previous studies were performed in mostly in animals which could never be equivalent to a human study, MSCs were isolated, cultured and sufficient quantity (45 x 10^6 cells) and were used for injection directly at the site of meniscus injury under ultrasound guidance. We used MRI to assess the healing response of meniscus tear as MRI is most sensitive non-invasive method available to know about the knee meniscus. Two excellent outcome scoring systems (WOMAC and KOOS) were used for evaluation.

5.1 Limitations of the study
Every research work is subjected to certain limitations and this study is also not an exception. There are a few limitations observed in the study, which may be brought out for the benefit of researchers planning similar studies in future. In our study relatively small sample size may be potential limitation and longer follow up is required for better assessment of MRI changes of meniscal cartilage healing.

6. Reference


