Autologous Bone Marrow Concentrate (Mononuclear Stem Cell) Therapy in the Treatment of Oral Submucous Fibrosis

Sankaranarayanan Seshadri, S Kailasam, S Elangovan, VR Ravi, Sonali Sarkar

ABSTRACT

Oral submucous fibrosis (OSMF) is a well-recognized, chronic debilitating and potentially malignant disease of the aerodigestive tract, with initial inflammation followed by progressive fibrosis of the underlying connective tissues which can be treated by stem cell therapy.

AIMS AND OBJECTIVES: To assess the effectiveness of stem cell therapy in the treatment of OSMF by evaluating the improvement in function and to assess the sustainability of the result with 5 years follow-up. This study also assessed the safety of stem cell therapy for OSMF.

MATERIALS AND METHODS: Seven patients with OSMF were selected. Out of seven patients, three were treated with stem cells obtained by Ficoll method and four patients were treated with stem cells obtained by point of care delivery system. Post-treatment improvement in the clinical presentation was assessed and confirmed by histopathological features. The range of follow-up of cases is from 6 months to 5 years.

RESULTS: Reduction in blanching, improved/better suppleness of the mucosa, decrease in the burning sensation while consuming spicy food, increase in the mouth opening was observed. The above mentioned results were found to be sustained in the follow-up period.

CONCLUSION: From the results obtained, it is evident that the stem cell therapy has played a role in the improvement of the signs and symptoms probably through neoangiogenesis, anti-inflammatory effects and antifibrinolytic effect. However, as this is only a preliminary attempt to assess the safety of stem cell therapy in OSMF, further randomized control trials with larger sample size are required for using this as a possible alternative therapeutic approach.

KEYWORDS: Autologous bone marrow, Oral submucous fibrosis, Harvest technology, Ficoll method, Point of care delivery system.


SOURCE OF SUPPORT: Nil

CONFLICT OF INTEREST: None declared

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, insidious disease caused by areca nut use, and is associated with both significant morbidity (including pain and reduced oral opening) and an increased risk of malignancy. A multifactorial model for the pathogenesis of OSMF, such as iron and nutritional deficiencies, chronic candidiasis, tobacco, lime, betel quid, genetic abnormalities, herpes simplex virus (HSV), human papilloma virus (HPV), autoimmunity, etc. have been postulated and are known to have either direct effect in causing OSMF or an indirect effect by mediating the immune system which is compromised in OSMF. Areca nut chewing is known to cause local trauma and injury to the oral mucosa due to its abrasive nature. This could be more severe in users of pan masala and gutka due to their fine particulate nature, with the high probability of particle adhesion to the traumatized mucosa, leading to morphological changes and membrane damage. This continuous local irritation by pan masala, gutka or areca nut can lead to injury-related chronic inflammation, oxidative stress and cytokine production. Oxidative stress and subsequent reactive oxygen species (ROS) generation can induce cell proliferation, cell senescence or apoptosis, depending upon the level of ROS production. During chronic exposure, these events can lead to preneoplastic lesions in the oral cavity and subsequently to malignancy.

No known treatment for OSMF is effective, although some conservative and surgical interventions may result in improvement. Intralresional steroids, mouth-opening exercises, excision of fibrotic band, hyaluronidase injection, placentaex injection, use of micronutrients and minerals, carbon dioxide laser, pentoxifylline, lycopene, immunized milk, interferon gamma, turmeric, chymotrypsin and collagenase have all been used in the treatment of OSMF with minimal success with no complete regression of the lesion. There has been no previous study conducted to assess the effective of autologous bone marrow stem cells in the treatment of OSMF as per published literature in English.
blanching of the oral mucosa with burning sensation while eating spicy food. The clinical diagnosis was confirmed with biopsies. The patients were divided into two groups. Three patients were treated with stem cells obtained by Ficoll method (group A) and four patients were treated with stem cells obtained by point of care delivery system (group B), either by Sepax or by SmartPReP Harvest Technology. The patients were advised to quit areca nut chewing, alcohol consumption and smoking habits. All the seven patients had been treated earlier with other modalities which did not yield any successful outcome. The patients were explained about the stem cell therapy procedure and a specific written consent was obtained prior to the treatment. This study was approved by the Institutional Ethical Committee for new experimental clinical trials as per the ICMR-DBT Guidelines for Stem Cells 2012.

**Bone Marrow Aspiration for Point of Care Delivery System**

Four patients were thoroughly assessed for spinal anesthesia and physician’s opinion for bone marrow aspiration was obtained. Under strict aseptic conditions 60 ml of bone marrow blood from the superior iliac crest was aspirated by the orthopedic surgeon using three 20 ml syringes each containing 0.3 ml of heparin. A total of 6 ml of concentrated bone marrow cells (BMCs) were prepared using Sepax® System (Biosafe America Inc; TX, USA) or with Harvest Technology. Once the stem cells were prepared; they were ready for injection into the fibrotic areas. Concentrated bone marrow with stem cell was injected in the patient’s mouth as 0.5 to 1 ml in each fibrotic site in the labial and buccal mucosa and also in tongue under local anesthesia.

**Ficoll Method**

In three patients, 40 ml of bone marrow was aspirated from posterior iliac crest, transported in acid citrate dextrose and was processed for mononuclear cells by Ficoll density gradient centrifugation, following the cGMP protocols. The CD34 and CD45 mononuclear cells were mixed with normal saline and made into 5 ml solution. The 5 ml of mononuclear concentrate containing 294 million cells was injected intraorally at various sites in the affected area under local anesthesia.

Assessment of clinical findings in the immediate postoperative period was done. The patients were discharged the following day itself. The patients were reviewed every week for the first 4 weeks and then every month for the next 6 months. Apart from clinical findings, the outcome of the treatment was also studied by histopathology after 3 months in few cases. In all, the cases were assessed from 6 months for the latest case to maximum of 5 years for the first case.

**RESULTS**

Out of seven cases one in group A and one in group B showed no appreciable improvement in mouth opening. Improvements in all other parameters were significant. Cases started showing improvement from the fourth week onward. The overall follow-up (Table 1) ranges from 6 months for the latest case to minimum of 5 years for the first case. None of the cases showed any sign of untoward alterations.

**DISCUSSION**

OSMF has always been a challenging disease with high prevalence in India. OSMF is predominantly seen in males. The disease is characterized by epithelial atrophy and subepithelial inflammatory reaction followed by fibroblastic changes in the submucosa. In the recent past the incidence of OSMF has increased many folds and has shown high risk for malignant transformation.

In addition to the established theories of pathogenesis including, increased fibroblast proliferation, reduction in collagen degeneration, damage by reactive oxygen species and hypoxia, there is growing evidence that features like increased population of senescent cells, marked reduction in the growth potential and altered morphology of fibroblast also play an important role in the pathogenesis of OSMF. Mamta Singh et al in their research communication have reported that the mean blood vessel area and mean blood vessel diameter has increased in grade II OSMF and measurably decreased in grades III and IV.

<p>| Table 1: Details of OSMF patients with respect to burning sensation, mouth opening, salivary secretion, suppleness and follow-up |
|-----------------------------------------------|------|------------|-----------|---------|---------|---------|</p>
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Methods</th>
<th>Burning sensation</th>
<th>Mouth opening</th>
<th>Salivary secretion</th>
<th>Suppleness</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group A</td>
<td>Decreased</td>
<td>Increased</td>
<td>Improved</td>
<td>Good</td>
<td>5 years</td>
</tr>
<tr>
<td>2</td>
<td>Decreased</td>
<td>Increased</td>
<td>Improved</td>
<td>Good</td>
<td>3 years</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Decreased</td>
<td>Not increased</td>
<td>Improved</td>
<td>Good</td>
<td>3 years</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Group B</td>
<td>Decreased</td>
<td>Increased</td>
<td>Improved</td>
<td>Good</td>
<td>2 years</td>
</tr>
<tr>
<td>5</td>
<td>Decreased</td>
<td>Increased</td>
<td>Improved</td>
<td>Good</td>
<td>2 years</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Decreased</td>
<td>Increased</td>
<td>Improved</td>
<td>Good</td>
<td>6 months</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Decreased</td>
<td>Not increased</td>
<td>Improved</td>
<td>Good</td>
<td>6 months</td>
<td></td>
</tr>
</tbody>
</table>
The luminal diameter in grade IV OSMF showed marked obliteration. It was also reported that the number of endothelial cells per low power field consistently decrease with increasing grades of OSMF.

Stem cell-based therapy is becoming a promising new approach in almost every medical specialty. In recent years there has been tremendous scientific activity focused on this area of research (basic, preclinical, as well as clinical). There is rapidly growing evidence to support the growing therapeutic potential of stem cells in tissue engineering and in wound healing.17

Stem cell therapy is primarily aimed at neoangiogenesis by releasing cytokines and growth factors (paracrine effect).18 This may result in increased free radical
scavenging by antioxidants (either naturally occurring or extraneous); neoangiogenesis may also facilitate the removal of senescent cells from the lesions by supplying more scavenging defense cells, and reversal of hypoxia in the diseased tissue.

Stem cell therapy may help to stimulate resident tissue stem cells to transform into new fibroblasts, which may help in removal of disintegrated biochemically and morphologically altered collagen fibers.

In our study the post stem cell therapy histopathological examination sections have demonstrated a good wavy pattern of collagen fibers which is a sign of the function of healthy fibroblasts. This may be considered as the evidence for the above hypothesis.

CONCLUSION

From the results shown, it is evident that the stem cell therapy has played a role in the improvement of the signs and symptoms probably through neoangiogenesis (Figs 1A and B), anti-inflammatory effects and fibrinolytic effect (Figs 2A to D). However as this is only a preliminary attempt to assess the safety of stem cell therapy in OSMF, further randomized control trials with larger sample size are required for more definite conclusions and using this as a possible alternative therapeutic approach.

REFERENCES


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