

Comparative study on efficacy of intralesional peripheral blood derived mesenchymal stem cell therapy and intralesional drug therapy for oral submucous fibrosis: A randomised control trial

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Abstract

Background: Oral Submucous Fibrosis is a premalignant condition that affects the entire oral cavity which sometimes causes a gradual reduction in mouth opening that may even extend up to the pharynx. The purpose of

this randomised control trial study was to assess the presence of stem cells in the peripheral blood and also to prove its efficacy in the better management of oral submucous fibrosis by comparing with the efficacy of intralesional steroids. A total of 20 subjects diagnosed

with OSMF were included in the study which were divided in 2 groups of 10 each. The subjects were selected for the study from HKE's S Nijlingappa Institute of Dental Sciences and Research, Kalaburagi for a time period of 2 years, by obtaining the patient's consent and with the approval of the institution's research ethical committee. Group A included 10 subjects who were treated with peripheral blood derived stem cells from patients own blood, the stem cells were injected at 1st, 15th and 30th day interval. Group B included 10 subjects who were treated with a combination of 2ml intralesional placentrex mixed with 1.5ml of dexamethasone, 1500IU of hyaluronidase and 2ml of 2% lignocaine Hcl weekly for 3 weeks. Subjects of both the groups were followed up at the intervals of 1st, 3rd and 6th month.

Conclusion: Clinically and Statistically better improvement was obtained with noticeable difference in burning sensation and mouth opening was noted to be significant in group A than in group B. Overall peripheral blood derived stem cells seems to be a better treatment regimen for improving the mucosal health and increasing the mouth opening as compared to intralesional steroids.

Keywords: OSMF, Burning Sensation, Blanching Of Mucosa, Dexamethasone, Fibrous Bands, Hyaluronidase, Intralesional Injections, Placentrex, Stem Cells.

Introduction

In the modern era, where biology and biotechnology have replaced the chemistry, there is a scope for exploring "biological solutions to biological problems." Stem cell therapy is a part of regenerative medicine that involves the use of undifferentiated cells in order to cure the disease. It is believed that stem cell treatment has the potential to change the face of human disease and reduce the suffering.¹

Amongst the many premalignant condition noticed, OSMF takes the first place in clinical practice. The Areca

nut, tobacco chewing, smoking, Chilies, Nutritional deficiency are the main factors that contribute to pathogenesis.² The risk increases with the frequency and the amount of its use.

Oral Sub mucous fibrosis is a premalignant condition of the oral cavity and a debilitating chronic disease. Pindborg and Sirsat defined OSMF (1966) as an insidious, chronic disease that affects any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by, and/ or associated with, formation of vesicles, it is always associated with a juxtraepithelial inflammatory reaction followed by fibro elastic changes of the lamina propria and epithelial atrophy that leads to stiffness of the oral mucosa and causes trismus and inability to eat. It is always associated with chewing of areca nut and tobacco.^{3,4}

The recent stem cell therapy for OSMF has given a new ray of hope for patients, which is principally aimed at neoangiogenesis by cytokine release and growth factors; it is also called as paracrine effect^{1,4}. Neoangiogenesis can also remove senescent cells from the affected area by supplying increased number of scavenging defense cell and reverses the state of hypoxia from the affected area. Stem cell therapy may induce the new fibroblast formation in the affected area which can be beneficial in discarding the altered collagen fibers.^{1,5,6} Studies have been conducted to assess the presence of stem cells in peripheral blood hence using this as the basis the present study has been conducted. There are studies conducted demonstrating the efficacy and effectiveness of stem cell therapy in OSMF patients. By injecting 0.5 to 1 ml of stem cells derived from bone marrow in to patients' buccal mucosa and tongue under the control of local anesthesia, positive results were found stating reduced blanching and decreased burning sensation on spicy food consumption with increased mouth opening within 5 to 6

months of therapy¹¹ but no research has been conducted so far to compare the peripheral Blood derived Stem cell and steroids with respect to prognosis of OSMF patient and no study has provided information with respect to the same. Hence this study was designed to assess the further treatment aspects of OSMF with stem cells. Objective of this study was to assess the presence of stem cells in the peripheral blood and also to prove its efficacy in the better management of oral submucous fibrosis by comparing with the efficacy of intralesional steroids.

Materials And Methodology

The randomized control trial was conducted in the department of Oral and Maxillofacial Surgery at HKE's S. Nijlingappa college of dental sciences and research, Kalaburagi, Karnataka between the year 2018 to 2020. Ethical clearance was obtained from the Institutional Review Board. Informed written consent was obtained from all the participants of both the intralesional stem cell group and the intralesional steroid groups. Sample size of 20 participants was determined by statistical analysis who were divided in 2 groups each containing 10 subjects. A detailed case history of the subjects with emphasis on their habits and a thorough clinical examination were recorded. A clinical diagnosis of OSMF was made Based on the criteria of mouth opening as given by Ranganathan K et al (2001)⁷.

- Group A received peripheral blood derived stem cells which was isolated using density gradient centrifugation Ficoll Method.

Under group A - Preparation of stem cells: Collection of patient's own blood (8ml whole blood was withdrawn from cubital fossa through disposable 10ml syringe) into a vacuum tube containing a cell separator gel (FIG 1 a and b). The tube is then placed into a centrifuge and spun for 6 minutes at 3400 rpm (Remi R-8C, swing bucket rotor) to separate the blood

into a supernatant plasma/stem cell suspension. The red blood cells are located below the cell separator gel. The stem cells i.e., 0.5-1ml just above the gel. The concentrated solution containing the processed stem cells is mixed with equal amount of plasma and then was injected intraorally at various sites in the affected area under local anesthesia at the 1st, 15th and 30th day interval. Subjects were followed up at the intervals of 1st, 3rd and 6th month. At follow up appointments, mouth opening, reduction in burning sensation was assessed with the help of self-designed questionnaire and pre and post-operative photographs.

- Group B received steroid therapy

Under group B: Subjects received 2ml intralesional placentrex mixed with 1.5ml of dexamethasone, 1500IU of hyaluronidase and 2ml of 2% lignocaine HCl weekly for 3 weeks on patients with palpable fibrous bands on buccal mucosae bilaterally. All the intralesional drugs were given at various sites of fibrous bands and repeated injection were avoided to decrease mechanical insult of tissue. Subjects were advised to do mouth opening exercise for 30 minutes daily without any dropout.

The inter incisal mouth opening of every patient in each group were measured at every visit, follow up was done for 6 months. Two values of mouth opening; before and after the intralesional therapies of each individual were compared in order to identify whether the mouth opening was improved following stem cell therapy as well as steroid injection and to evaluate the degree of reduced burning sensation.



Fig 1: Merisis Prp Supercell + Glue Kit

Inclusion criteria

- Subjects diagnosed with OSMF with Grade I, Grade II, Grade III and Grade IV (with more than 10mm mouth opening)
- Subjects complaining of reduced mouth opening, blanching of the oral mucosa, ulceration, burning sensation and palpable fibrous bands.

Clinical outcome is evaluated by questionnaire by VAS SCALE. Functional outcome was evaluated by interincisal measurement which was done using Vernier caliper.

Results

In the study, maximum number of cases belonged to the age group of 31-40yrs with maximum age of the subjects were 46yrs and minimum being 25yrs. No statistically significant difference was noted in the mean age amongst both the Groups (Table 1).

Male cases were dominant 19 (95.0%) , female cases were 1 (5.0%), but there was no statistical significant difference of gender between the groups A and B ($P>0.05$)(Table 2). There was no notable clinical and statistical difference in mean mouth opening score pre-OP, at post-OP 1st visit and at post-OP 2nd visit between the group A and group B ($P>0.05$). Whereas there was moderate difference in clinical and statistical mean mouth opening score at post-OP 3rd visit, and at follow-up 1st month between the groups A and B ($P<0.05$) and there was statistically highly significant difference of mean mouth opening score at follow-up 3rd month and

follow-up 6th month between the groups A ($P<0.01$) and group B ($P<0.001$) respectively. On clinical examination of the palpable fibrous bands in between both the groups it was noted that the fibrous bands were noticeably reduced in Group A when compared with Group B which was mainly remarkable in the follow up 2nd and follow up 3rd visits in both the groups (Table 3).

There was no clinical and statistical difference in the mean VAS score for pain due to burning sensation at pre op, 1st post op and 2nd post op visit between the Groups A and B ($P>0.05$), the pain due to burning sensation had gradually decreased during post-operative 3rd visit, post-operative 1st follow up between the groups A and B ($P<0.05$). It was noted that there was both clinical and statistical difference in VAS score. Meanwhile a highly significant clinical and statistical difference of mean VAS score at post-OP follow-up at 3rd month and follow-up at 6th month between the group A ($P<0.01$) and group B ($P<0.001$) respectively were noted. The mean VAS score was significantly low in Group A at post-OP 3rd visit, post-OP follow-up at 1st month, follow-up at 3rd month and follow-up at 6th month as compared to Group B. Hence in comparison between stem cell group and steroid group, the stem cell group showed better improvement by reduction of burning sensation as compared to steroid group (Table 4)

Table 1: Age wise distribution of cases

Age in years	Group A (Stem cell therapy)		Group B (Steroid therapy)	
	Number of cases	Percentage	Number of cases	Percentage
21-30	2	20.0	3	30.0
31-40	5	50.0	5	50.0
41-50	3	30.0	2	20.0
Total	10	100.0	10	100.0
Mean	36.30 \pm 6.12		34.10 \pm 5.66	

± SD		
t-test value and P-value	t = 0.834 P = 0.415 NS	

NS= not significant, S=significant, HS=highly significant, VHS=very highly significant

Table 2: Gender wise distribution of cases

Gender	Group A		Group B	
	Number of cases	Percentage	Number of cases	Percentage
Males	9	90.0	10	100.0
Females	1	10.0	0	0.0
Total	10	100.0	10	100.0
X ² -test value and P-value	$\chi^2_{\text{yates}} = 1.052$ P = 0.876 NS			

Table 3: Comparison of mouth opening score between the groups with different time interval

Time Interval	Group A	Group B	Unpaired t-test p-value and significance
	Mean ± SD	Mean ± SD	
Pre-OP	29.33 ± 1.75	27.48 ± 3.71	t = 1.352, P = 0.194, NS
Post-OP at 1 st visit	29.75 ± 1.73	27.50 ± 3.70	t = 1.639, P = 0.119, NS
Post-OP at 2 nd visit	29.85 ± 1.62	27.53 ± 3.71	t = 1.786, P = 0.098, NS
Post-OP at 3 rd visit	30.68 ± 1.52	27.65 ± 3.76	t = 2.238, P = 0.038, S
Follow-up at	31.34 ±	27.86 ±	t = 2.653, P =

1 st month	1.38	3.68	0.016, S
Follow-up at 3 rd month	31.95 ± 1.25	28.06 ± 4.00	t = 3.165, P = 0.005, HS
Follow-up at 6 th month	32.75 ± 1.31	28.33 ± 3.69	t = 3.432, P = 0.001, VHS

Table 4: Comparison of VAS score between the Group A and Group B

Time Interval	Group A	Group B	Unpaired t-test p-value and significance
	Mean ± SD	Mean ± SD	
Pre-OP	4.6 ± 0.48	4.5 ± 0.5	t = 0.429, P = 0.673, NS
Post-OP at 1 st visit	4.2 ± 0.60	4.3 ± 0.46	t = 0.397, P = 0.696, NS
Post-OP at 2 nd visit	3.9 ± 0.41	4.1 ± 0.44	t = 0.957, P = 0.484, NS
Post-OP at 3 rd visit	3.4 ± 0.48	3.9 ± 0.41	t = 2.611, P = 0.018, S
Follow-up at 1 st month	3.0 ± 0.63	3.7 ± 0.46	t = 2.689, P = 0.015, S
Follow-up at 3 rd month	2.5 ± 0.81	3.6 ± 0.49	t = 3.498, P = 0.003, HS
Follow-up at 6 th month	1.8 ± 0.75	3.5 ± 0.50	t = 5.667, P = 0.000, VHS

Fig. 2: STEM CELL GROUP, (A) Pre-Operative, (B) Intra Operative, (C) Follow up 1 visit, (D) Follow up 3rd visit





Fig 3: Steroid Group, (E) pre Operative, (F) Intra operative, (G) Follow up 1ST visit, (H) Follow up 3rd visit



Discussion

Oral Submucous Fibrosis is most commonly found in Asian countries^{1,11}. Many authors have suggested a variety of etiological factors including capsaicin, betel nut, alkaloids, autoimmunity, hypersensitivity, genetic predisposition and malnutrition. Ramanathan has suggested that OSMF may be a mucosal change secondary to chronic iron deficiency calling it an Asian analogue of sideropenic dysphagia. The disease can be classified clinically into two phases: 1) an eruptive phase, characterized by formation of vesicles, erythema, and a burning sensation. The vesicles rupture to form small ulcers, which leads to further increase in the burning sensation. 2) The fibrosis induction phase, it is characterized by disappearance of the vesicles and healing of ulcers which results in decreased burning sensation, which further leads to blanching and stiffness of the oral and oropharyngeal mucosa owing to healing by fibrosis. These two phases appear in a cyclic manner.^{9,26,30}

Various treatment options for the management of OSMF can be classified under the headings: medical, surgical and physical treatments^{6,8,10}. Medical treatment includes dietary supplements such as vitamins and antioxidants, the use of anti-inflammatory drugs mainly corticosteroids, use of proteolytic agents such as hyaluronidase, anti-cytokines have been given orally, topically or by submucosal injections. Surgical treatment is used mainly to manage trismus, which involves incising and releasing the fibrotic areas and leads to scarring and fibrosis. Physical treatment aimed to influence the remodeling of tissue by using movement which includes exercises and physiotherapy, various splints or other devices to improve mouth opening or localized heat such as with microwave diathermy. Physical treatment measures combined with other interventions are usually

used for treating OSMF and surgery is reserved only for established cases.^{5,7}

In the present study of 20 patients, the patients were divided into two groups. The first group was given peripheral blood derived mesenchymal stem cells. Second group was given submucosal injection which consisted of dexamethasone, hyaluronidase, placentex and local anesthetic agent lignocaine 2% with adrenaline 1:80000.

According to the study conducted by Hazarey et al, it has been found that male to female ratio was 5:1^{11,15}. The present study depicted that male were dominant which was in agreement with studies conducted by Pindborg and Sirsat; 1966 and Wahi et al., 1966 males have dominated over females. The present study reveals that the male to female ratio to be 19:1, which accounts for 95% of male and 5% of female. Prevalence of OSMF is more in male when compared to female because of illiteracy, unemployment and easy accessibility. The study results are not in concordance with the survey conducted by J. J. PINDBORG et al in the year 1968 female: male ratio of 3:1 is surprising in the light to various studies conducted and also Shear et al. (1967) found a predominance of females among unselected Indians in South Africa. Cultural disapproval prohibits women from smoking so they prefer smokeless tobacco.

The younger the age, the more rapid the progression of the disease, and the more likely the recurrence of symptoms^{1,7}

The present study shows that the peak incidence of OSMF at the age group of 20-40 years which is in concordance with the study conducted by Panday et al in the year 2009. Kumar et al found similar results from Chennai^{6,8}. Hazarey et al from Nagpur also reported that most of their patients were in the younger age group (< 30 years). Most prevalence is seen in younger generation all because of selling of tobacco and areca nut products in different attractive pouches. Other influences that affect

youths for the use of tobacco include: lower socioeconomic status including lower income or education, not knowing how to say no to tobacco products, lack of support or involvement from parents, accessibility, availability and price of tobacco products, seeing tobacco product advertising in stores on television, the internet movies or in the magazines and newspaper.

Total 20 subjects aged from 20-50 years were selected for the study, the age group was stratified into three groups for data analysis 20-30yrs, 31-40yrs and 41-50yrs. Study observes that, maximum number of cases belonged to the age group of 31-40yrs, these findings are similar to More et al and Parag et al. this age group accounts for 10 subjects (50.0%) amongst the two groups, followed by 5 subjects (25.0%) belonging to the age groups of 21-30 years and 41-50 years each. The maximum age of the subjects was 46 years and minimum age of the subjects was 25 years. The mean age of the cases in group A was 36.30 ± 6.12 and the mean age of the cases in group B was 34.10 ± 5.66 . There was no statistically significant difference of mean age between the Group A and Group B. In present study we have observed that most of the patients had a habit of chewing tobacco, pan masala and gutka, it is also observed that there is increased severity as the age advances due to the duration of habit. Factors attributed for this are illiteracy, unemployment and easy accessibility for tobacco and gutka products.

The aim of the present study was to compare the effectiveness of intralesional stem cell therapy and steroid therapy as a treatment modality of OSF by evaluating the degree of improvement of inter incisal mouth opening and to identify the group of patients who respond well to this particular treatment. One intralesional injection per week for three weeks, each intralesional injection contained dexamethasone combined with hyaluronidase, placentex and local anesthesia with 2% lignocaine. In the present

study it has been observed an average of increase in mouth opening by 2mm, which was statistically significant. Chetan et al reported an average of 4.0mm improvement in mouth opening in the group which they administered dexamethasone with hyaluronidase intralesional along with physiotherapy exercises^{11,13}. The study by Cox and Zoellner states that there was no significant mouth opening by injecting of steroids and hyaluronidase. Hyaluronidase acts by breaking down hyaluronic acid (the ground substance in connective tissue) and lowers the viscosity of intercellular cement substance which results in improved reduction with respect to trismus and fibrosis. Hydrocortisone appears to be a better regimen to enhance the mucosal health and increase the mouth opening as compared to placentex as a treatment regimen^{16,17}

The prime mechanism of action of corticosteroids is immune modulation. Steroids act through its anti-inflammatory activity by inhibiting the generation of inflammatory factors and increasing the apoptosis of inflammatory cells by suppressing inflammation and resulting fibrosis and furthermore it upregulates immune mediated fibrolytic pathways. In order to partially relieve the patients of their symptoms at an early stage of OSMF. Steroids are useful in controlling symptoms, and as an adjunct therapy.¹

Placentex is an aqueous human placental extract that contains nucleotides, enzymes, vitamins, amino acids, and steroids. Extract contains growth factors and anti-inflammatory activity and when administered result in significant improvement in mouth opening, color of mucosa, burning sensation, and reduction of fibrotic bands.^{15,1}

Collagenase which is a lysosomal enzyme is capable of degrading phosphate esters, proteins, glycosides, polysaccharides and sulphate esters¹⁶ Hyaluronidase also showed a much quicker effect in reducing the burning

sensation and painful ulceration than did dexamethasone, though the effect was short-term. Hyaluronidase acts by depolymerizing hyaluronic acid, which is the ground substance in connective tissue and acts by lowering the viscosity of the intercellular cemental substance, and decreasing collagen formation²⁶

In present study, intralesional steroids are used as treatment modality. A combination of Chymotrypsin (5000 HJ), hyaluronidase (1500 HJ) and dexamethasone (4 mg), twice weekly submucosal injections for 10 weeks are injected into the fibrotic bands along with mouth-opening exercises. Treatment with submucosal injections using variety of drugs leads to aggravated fibrosis, pronounced trismus, dysphagia, and increased morbidity after a certain period of time. The studies attribute this to the mechanical insult to the tissue from the needle sticks that were repeated at multiple sites, chemical irritation.^{11,17}

Stem cells are unspecialized cells in the human body that have the ability of becoming specialized cells, each with new specialized cell functions. The first stem cells studied by researchers were derived from adult tissues, and more recently, scientific breakthroughs have enabled research on stem cells that are removed from one of the earliest human cellular formations, the blastocyst (Stem cells are basic cells of all multicellular organisms having the potency to differentiate into wide range of adult cells).

Several studies demonstrate that MSCs from PB maintain similar characteristics and have similar chondrogenic differentiation potential to those derived from BM. The results obtained by the study on autologous bone marrow concentrate in the treatment of OSMF¹¹ has concluded that burning sensation in stem cell is comparatively better than the intralesional steroids therapy and the study also presented that intralesional stem cell therapy has shown improvement in mouth opening and there was gradual decrease in burning sensation and blanching of mucosa.¹¹

²⁸, 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. Sankaranarayan et al. injected 175 million Bone Marrow derived Mesenchymal stem cells into the area affected.^{21,23} The parameters such as blanching, fibrous band which improved, 4 weeks after the injection and a significant improvement in mouth opening correlates with our findings in which we infiltrated the peripheral blood derived mesenchymal stem cells.

Conclusion

This study concluded that intralesional stem cell therapy showed better clinical results than intralesional Steroid injections in patients with Oral Submucous Fibrosis of grade I, II, III and IV, with no side effects. However, both intralesional steroid therapy and intralesional stem cell therapy were effective in reduction of burning sensation and as well as reduction of the fibrous bands and improving the mouth opening.

Further studies are required with a large sample size, for longer duration with molecular level - in vitro, on cell lines or in animal models and increasing the quantity of stem cells obtained by adding growth factors.

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