INTRALESIONAL AUTOGENOUS FAT INJECTION IN ORAL SUBMUCOUS FIBROSIS

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Keywords

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Abstract

Intralesional autogenous fat injection in oral submucous fibrosis

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Master in Science (MSc) - Maxillofacial and Oral Surgery

Introduction

Oral submucous fibrosis (OSMF) is a chronic disorder characterized by the fibrosis of the mucosal lining of the upper digestive tract involving the oral cavity, oropharynx, hypopharynx and the upper third of the oesophagus. Areca nut chewing has been implicated in the aetiology of this condition. This condition is prevalent in Kwa-Zulu Natal (KZN), South Africa, with many patients suffering from varying degrees of severity of this disease.

At Inkosi Albert Luthuli Central Hospital autogenous fat injections into the fibrous bands are being used as a means of treating OSMF. Anecdotal evidence suggested that this type of treatment modality helps to relieve the symptoms experienced by patients. There is no scientific data supporting this claim.

The aim of the study was to establish the effectiveness of intralesional autogenous fat injections in patients with oral submucous fibrosis.

The objectives of this study was to record the demographic details and medical information of the patients, evaluate the inter-incisal mouth opening, to assess the presence or absence of restricted tongue movements, record pain of the patients and record quality of life via a condition specific questionnaire prior to treatment and at six months post operatively.

Methodology

The design of the study was a prospective longitudinal observational study. It involved the recording of results via a condition specific quality of life questionnaire, visual analogue pain scale and clinical recording of mouth opening and tongue movements prior to treatment and six months post operatively.

Results

A sample population of 31 participants were screened and nine participants were selected for the study. The mean age was 51.67 years (SD 9.45 years). The age range was 34-67 years old with all participants being female. All participants are from KZN, South Africa and all were Pindborg stage II b OSMF patients. Absence or presence of restricted tongue movements were assessed and no changes in tongue movements were noted at six-month follow-up. No surgical complications from the treatment were noted in any of the participants. Mouth opening increased in all participants after treatment with a mean increase of 7.44mm (SD = 3.28mm)(95%CI = 4.92-9.97)(p = 0.0001). The range of mouth opening before and at six-month follow-up was 13-24mm and 18-33mm. There was a net decrease in the VAS pain scores of all participants, with a mean visual analogue pain score decrease of 7.33 points (SD = 2 points)(95%CI = 5.7-8.87)($p < 0.0001$). The range before and at six-month follow-up was six to ten points and zero to four points respectively. All participants showed an improvement in quality of life derived from their OPMDsQoL scores. The mean decrease was 44.99 points (SD = 17.35 points)(95%CI = 28.4-55.23)(p = 0.0001). The points range of the condition specific OPMDsQoL scores before treatment and at six-month follow-up was 60-99 points and 17-63 points scored.

Conclusion

This study has found that intralesional autogenous fat injection in oral submucous fibrosis has shown to be effective in increasing mouth opening, decreasing pain experienced by the participant and improving the participants' perception of quality of life. There were clinical and statistically significant improvements.

Declaration

I declare that ***Intralesional autogenous fat injection in oral submucous fibrosis*** at the University of the Western Cape Oral Health Care Centre is my own work, that it has not been submitted for any degree or examination in any other university and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Name: Tashen Gounden

Signature:

Date: November 2021

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Dedication

This thesis is dedicated to my loving parents for whom this would not be possible, my grandmother (Magha Lutchmee Karupen 1937-2021), my colleagues and to the patients suffering from oral submucous fibrosis.

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Glossary

The following terms will be clarified for purpose of this study:

Adipose derived stem cells: Cells found within the lipoaspirate

Adipose Tissue: Commonly known as fat, may be subcutaneous (under skin) or visceral (packed between internal organs)

Allicin: Organosulfur compound obtained from garlic

Anaemia: Deficiency of red blood cells or haemoglobin

Angiogenesis: Development of new blood vessels

Angiogenic Growth Factor: Class of molecules that have a pivotal role in the formation of blood vessels

Areca Nut: Also referred to as betel nut, is associated with the aetiopathogenesis of oral submucous fibrosis, contains a substance called arecoline

Autogenous Fat Graft: Adipose tissue from the same donor and recipient. In this study it represents the lipoaspirate preparation and not an en-bloc piece of adipose tissue

Epithelial atrophy: A hallmark feature of Oral Submucous Fibrosis and is a common histopathological finding OSMF cases.

Buccal mucosa: Inner cheek mucosa

B-Cells: Types of white blood cells that make antibodies, a part of immune system which develops from stem cells in the bone marrow

Centrifugation: A process whereby a centrifuge is used to separate fluids of different densities or liquids from solids

Centrifuge: A machine with a rapid rotating container that applies centrifugal force to its contents, typically to separate fluids from its different densities

Chi-square test: Is a statistical hypothesis test which compares two variables in a contingency table to see if they are related

Chymotrypsin: A digestive enzyme which breaks down proteins

Collagen: Main structural protein in the extracellular matrix and found in various connective tissues. Most abundant protein in mammals

Cytokines: A category of signalling molecules that mediate and regulate immunity, inflammation and haematopoiesis

Deposition: Placement of the lipoaspirate into buccal mucosa in the sub-epithelial plane

Dexamethasone: Corticosteroid medication

Distant Flap: A flap in which the donor site is distant from the recipient area

Dupuytren's Disease: A disease process whereby contractures may develop in the hands caused by gradual thickening of the connective tissue

Dysplasia: A term used to describe abnormal cells within a tissue or organ. Dysplasia is not cancer but it may in some cases develop into cancer

EAhy926 endothelial cell: Preserves the characteristics of endothelin converting enzyme from primary human umbilical vein endothelial cells

Fibrinolysis: The enzymatic breakdown of fibrin

Fibrosis: The thickening and scarring of connective tissue

Graft: A piece of living tissue that is transplanted surgically

Growth Factors: Diffusible signalling proteins that stimulate cell growth, differentiation, survival, inflammation and tissue repair

Harvest site: A site where tissue is removed to be transplanted to the donor site

Hyalinized: With reference to tissues that deteriorate into a homogenous translucent material

Hyperbaric oxygen therapy: A medical treatment in which the ambient pressure greater than sea level atmospheric pressure is a necessary component

Hypertrophic Scarring: A thickened, wide and often raised scar

Idiopathic thrombocytopaenic purpura: A condition when the immune system attacks platelets

Immunomodulatory function: Changes in the body's immune function that may be mediated by agents that activate or supress its function

Interferon-Gamma: A dimerized soluble cytokine

Ki67: A special stain that gives a sense of how aggressive a tumour is

Laser: Devices used in medicine/surgery that use precisely focused light sources to treat or remove tissues

Limited mouth opening: When the mouth opening is hampered due to a pathologic process or trauma

Lipoaspirate: Material removed via the process of lipoaspiration

Local Flaps: Are flaps that are transferred from an area adjacent to the defect or intended graft site

Metaplasia: Changes of cell form not typical to the region in which they are found

Mean: Statistical term referring to the average of the group

Pentoxifylline: A xanthine derivative, used as a drug to treat people with peripheral artery disease

Periumbilical: Adjacent to the navel (bellybutton)

Psychometric: Relating to psychometry or psychometrics, the field in psychology devoted to testing, measuring and assessing related activities

Quality of life: The standard of health, comfort and happiness experienced by an individual or group

Refractory pure red cell aplasia: Pure red cell aplasia is a rare cause of profound anaemia, characterized by very low reticulocyte count and virtual absence of erythroid precursors in bone marrow.

Regional Flap: Reconstructive tissue transfer where the donor tissue is transferred with an intact vascular pedicle from the site located outside of the general area of the recipient defect

Scar Contracture: The result of a contractile wound-healing process occurring in a scar that has already re-epithelialized and adequately healed. Examples: keloids/ hypertrophic scars

Scleroderma: A chronic hardening and contraction of the skin and connective tissue, either locally or throughout the body

Standard deviation: A statistical term measuring the amount of variation or dispersion of a set of values

Stromal vascular fraction: A cellular extract made in a laboratory from fat

Triamcinolone acetonide: Synthetic corticosteroid medication

T-test: Any statistical hypothesis test in which the test statistic follows a student's distribution under the null hypothesis

T-Cells: Types of white blood cells, a part of the immune system that focuses on specific foreign particles

Tumescent solution: A mixture of saline and a local anaesthetic

Velopharyngeal incompetence: When the soft palate does not close tightly against the back of throat, leading to air coming out the nose (characterised by hypernasality and/or nasal air emission) during speech

95% Confidence Interval: A statistical term referring to the range of values you can be 95% confident contains the true mean of the study population

List of Abbreviations

Abbreviation	Terms
AFG	Autogenous Fat Graft
AFT	Autologous Fat Transfer
ASC	Adipose derived stem cell
BCM	Basement Cell Membrane
BDNF	Brain-Derived Neurotrophic Factor
BFP	Buccal Fat Pad
BQ	Betel Quid
COMDQ	Chronic Oral Mucosal Diseases Questionnaire
DD	Difficulties of diagnosis
ECM	Extracellular Matrix
GA	General Anaesthetic
GCSF	Granulocyte Colony Stimulating Factor
GPCA	Gastric Parietal Cell Antibody
IALCH	Inkosi Albert Luthuli Central Hospital
IL-10	Interleukin-10
IL-4	Interleukin-4
IL-6	Interleukin-6
KZN	Kwa-Zulu Natal
LA	Local Anaesthetic
MSC	Mesenchymal Stem Cell

OL	Oral Leukoplakia
OLP	Oral Lichen Planus
OPMDsQoL	Oral Potentially Malignant Disorders Quality of Life
OSMF	Oral Submucous Fibrosis
PGE-2	Prostaglandin E2
PIF	Physical Impairment and Functional limitations
PSB	Psychological and social wellbeing
QOL	Quality of Life
RBC	Red Blood Cell
RPM	Rotations per Minute
SD	Standard Deviation
SSG	Split Skin Graft
SVF	Stromal vascular fraction
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumour Necrosis Factor Alpha
TRE	Effectiveness of treatment
T0	Prior to treatment (on admission)
T1	Six-month follow up visit after treatment
VAS	Visual Analogue Scale
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation
95% CI	95% Confidence interval

Chapter 1

Introduction

Oral submucous fibrosis (OSMF) is a chronic disorder characterized by the fibrosis of the mucosal lining of the upper digestive tract involving the oral cavity, oropharynx, hypopharynx and the upper third of the oesophagus. Areca nut chewing has been implicated in the aetiology of this condition. OSMF is prevalent in Kwa-Zulu Natal (KZN), South Africa, with many patients suffering from varying degrees of severity of this disease.

OSMF causes detrimental effects on the quality of life of the patient and has proven to be a challenge to treat. At Inkosi Albert Luthuli Central Hospital (IALCH) in KZN, approximately one to two new patients per a month present with this condition.

Patients commonly present with the feeling that they are unable to open their mouth as wide as before. Their buccal mucosa 'feel tight' and they experience varying symptoms such as a burning sensation inside their mouth. It has a debilitating effect on their daily functioning and many complained that they 'can't eat properly'. The patient's quality of life has been altered and they complain 'my lifestyle has changed because of this'.

Current management protocols include laser treatment, flap surgery and numerous grafting techniques. Much of this requires prolonged theatre times, surgical expertise and are expensive. Due to a limited budget, theatre time and available resources it is necessary to look at additional modalities which are more cost and time effective.

At IALCH autogenous fat injections into the fibrous bands is being used as a means of treating OSMF. Anecdotal evidence suggests that this type of treatment modality helps to relieve the symptoms experienced by patients. However, there is no scientific data supporting this claim.

The importance of this study is that it will provide a time efficient, low-cost and minimally invasive surgical treatment in a Pindborg stage I/II a and b OSMF patient. The treatment will aid in improving their quality of life, decrease their pain experienced and increase their mouth opening. This is the first study and treatment for OSMF of its kind nationally and internationally. It has introduced the use of autogenous fat injections in oral pathology and the head and neck region as a whole.

This study was a prospective study which reviewed patients being managed with autogenous fat injections as a treatment modality for OSMF and to discuss the clinical outcomes of these findings. These findings may increase interest in this kind of treatment for OSMF, not only within South Africa but also in Asia where the burden of disease is high.

Through the literature review OSMF will be discussed as a disease process, with reference to the various current treatment options. Fibrosis as a pathological condition will be discussed to draw correlation to OSMF fibrosis. Fat grafting will be discussed with its historical background, en-block and lipoaspirate techniques with particular reference to the Coleman technique for fat harvesting and handling of lipoaspirate. Adipose derived stem cells (found within adipose tissue) will be discussed with its effects on donor tissue regions and pathologies that it is being used for currently, with special reference to scar and fibrotic pathologies. Quality of life will be discussed as an important clinical outcome marker, with interest in the condition specific oral potentially malignant disorder questionnaire used in this study.

The aim of the study was to prospectively identify the effectiveness of intralesional autogenous fat injections in patients with oral submucous fibrosis.

The objectives were to record the demographic details and medical information of the patients and evaluate the inter-incisal mouth opening (millimetres- mm), assess the presence or absence of restricted tongue movements, record pain of patients (Visual Analogue Scale-VAS scores), to record quality of life via a patient derived questionnaire (OPMDsQoL) prior to treatment (T0- on admission) and six month (T1) post-operatively.

Chapter 2

Literature Review

2.1 Oral Submucous Fibrosis (OSMF)

2.1.1 Introduction and Historical Background

This condition was first described in five Indian females from Kenya and East Africa by Schwartz in 1952. Schwartz initially proposed the term '*atrophica idiopathica mucosae oris'* and was later replaced by the term currently being used.

In 2005 the World Health Organization (WHO) introduced the terminology oral potentially malignant disorders (OPMDs) which conveys that all clinical presentations carry a risk of malignant transformation. OPMDs comprises both of 'oral precancerous lesions' and 'oral precancerous conditions' (Warnakulasuriya *et al*., 2007). OSMF falls in the category of OPMDs.

Oral submucous fibrosis (OSMF) is a chronic disorder characterized by fibrosis of the mucosal lining of the upper digestive tract involving the oral cavity, oropharynx, hypopharynx and the upper third of the oesophagus (Namboodiripad, 2014).

2.1.2 Prevalence

In a stratified random sample amongst South African Indians living in Durban it was estimated that five percent of the population were areca nut (also referred to as betel nut) chewers. If only established OSMF (the presence of palpable fibrous bands in the mouth) was considered then the calculated crude prevalence of the disease in the total population was 1.2% and 1.6% in people older than 10 years (Seedat *et al*., 1988).

OSMF has been reported most commonly amongst people living in the Indian subcontinent, and neighbouring Asian countries, who have a habit of chewing areca nut. The chewing of betel quid (BQ), including areca catechu, the betel leaf and lime is a habit of antiquity in south and Southeast Asia. It is estimated that 600 million people chew BQ worldwide (Reichart, 2005).

In a study done by Methora *et al*. in 2016 in Uttar Pradesh, India 450 000 people were surveyed and it was found that the prevalence of oral submucous fibrosis had tremendously

increased. Leukoplakia that was once believed to be the most common oral potentially malignant disorder had then been outnumbered by oral submucous fibrosis.

2.1.3 Aetiopathogenesis

South Africans of Indian descent show a certain uniqueness in their chewing constituents compared to other countries. In South Africa the areca nut habit is more common amongst women. Only 60% of chewers prefer the betel quid while the rest like the nut by itself, the majority of chewers prefer the baked (black) nut variety and a minority add tobacco to their chew. Compared to chewers without OSMF, OSMF subjects are younger and have shorter histories of chewing. The profile of systemic diseases was similar among subjects with and without OSMF (Seedat *et al*., 1988).

OSMF is mainly caused by the habit of chewing paan (a combination of smokeless tobacco and other ingredients including a substance called Arecoline). Arecoline is a chemical substance found in the areca nut and is responsible for the progressive fibrotic change of the oral mucosa. Restricted mouth opening due to the fibrotic changes within the oral mucosa may become severe and can sometimes leave the patient with a grossly decreased mouth opening (Angadi, 2010).

Figure 1: Commercially available areca nut found in a local store in Verulam, KZN

A clear dose-dependent relationship was observed for both frequency and duration of chewing areca nut in the development of OSMF. The commercially available freeze-dried products such as paan masala, guthka and mawa (areca and lime) have high concentrates of areca nut per chew and appear to cause OSMF more rapidly than self-prepared conventional betel quid (Tilakaratne *et al*., 2006).

According to Tilakaratne *et al*. in 2006 it was suggested that current evidence implicates collagen-related genes in susceptibility and pathogenesis of OSMF.

In a study done by Tseng *et al*. in 2012 whereby EAhy926 endothelial cells were cultured and exposed to various concentrations of arecoline for 24 hours, a decrease in cellular viability was found. EAhy926 endothelial cells showed marked morphological changes and cellular migration decreased after 24 and 48 hours of exposure to arecoline. In conclusion it was found that arecoline impaired vascular endothelial cells by inhibiting their growth and migration as well as their adhesion to U937 mononuclear cells. These results reveal that arecoline may contribute to the pathogenesis of OSMF and cardiovascular diseases by affecting endothelial cell function in BQ chewers (Tseng *et al*., 2013).

A complete blood count, serum iron, vitamin B12, folic acid, homocysteine levels and serum GPCA titers are usually examined to ascertain whether patients have a microcytic/normocytic or macrocytic anaemia, deficiencies of haematinics, abnormally high serum homocysteine level and serum GPCA positivity (Sun *et al*., 2015).

OSMF is basically a collagen disorder. Hydroxyproline is an amino-acid found only in collagen which is present in its hydroxylated form of 4- hydroxyl proline and requires ferrous iron and ascorbic acid for reaction. The decreased iron levels may be due to utilization in the fibrotic process (Gupta, 2015).

2.1.4 Clinical Features

Early onset clinical features of OSMF include a burning sensation that is aggravated by spicy food, ulceration or vesiculation of the oral mucosa, blanching of the mucosa and a "leathery" mucosa which shows thickened and firm tissue with a wrinkled surface (Warnakulasuriya *et al*., 2007).

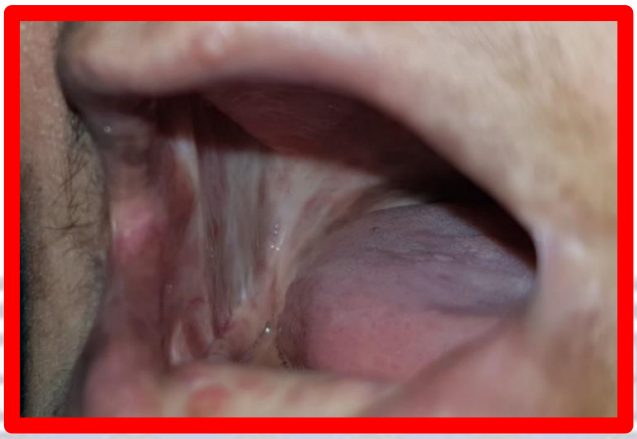


Figure 2: Clinical photograph showing fibrosis of the buccal mucosa- Pindborg stage II (b)

Late onset clinical features show fibrous bands within the mucosa, limitation of mouth opening, difficulties with mastication and phonation, narrowing of the oropharyngeal orifice with distortion of the uvula and may cause changes to the mucosa and tongue (Warnakulasuriya *et al*., 2007).

Figure 3: Clinical photograph showing late form – Pindborg stage III

Anaemia and haematinic deficiencies may cause or aggravate oral mucosal diseases such as atrophic glossitis, burning mouth syndrome, oral lichen planus, recurrent aphthous ulcerations or OSMF.

In a study done by Sun *et al*. in 2015 they found that there were deficiencies of haemoglobin (7.4%), iron (20.6%), vitamin B12 (50.0%) and folic acid (41.2%) in a group of 68 male OSMF patients.

Various staging systems were put forward, such as by Pindborg *et al*. in 1975, Mathur *et al*. in 1993 and Haider *et al*. in 2000 which can routinely be used in the clinical practice which can help in early diagnosis and treatment:

Table 1 - Clinical Stages of OSMF according to Pindborg et al. in 1975

STAGE	CLINICAL DESCRIPTION
I	Stomatitis
II	Fibrosis (a) Early lesion, blanching of the oral mucosa (b) Older lesions, vertical and circular palpable fibrous bands in and around the mouth or lips, resulting in a mottled, marble-like appearance of the buccal mucosa
III	Sequelae of OSMF (a) Leukoplakia (b) Speech and hearing deficits (c) Oral squamous cell carcinoma

Table 2 - Clinical and functional staging of OSMF according to Haider et al. in 2000

CLINICAL STAGING	
Stage 1	Faucial bands only
Stage 2	Faucial and buccal bands
Stage 3	Faucial, buccal and labial bands
FUNCTIONAL STAGING	
Stage A	Mouth opening > 20mm
Stage B	Mouth opening 11-19mm
Stage C	Mouth opening < 10mm

Table 3 - OSMF staging based on clinical presentation according to Mathur et al. in 1993

Stage 1- Early OSMF	Stage 2- Moderate OSMF	Stage 3- Severe OSMF
a) Mild blanching	a) Moderate to severe blanching	a) Burning sensation very severe, patient unable to do day-today work
b) Mouth opening normal	b) Mouth opening reduced by 33%, tongue protrusion reduced by 33%, flexibility also demonstrably decreased	b) More than 66% reduction in the mouth opening, cheek flexibility and tongue protrusion. In many, the tongue may appear fixed.
c) No restriction on tongue protrusion	c) Burning sensation even in the absence of stimuli	c) Ulcerative lesions may appear in cheek
d) Burning sensation-only on taking spicy food or hot temperature liquids	d) Palpable bands felt	d) Thick palpable bands
	e) Lymphadenopathy either unilateral or bilateral	e) Lymphadenopathy bilaterally present
	f) Demonstrable anaemia on haematological examination	

2.1.5 Histopathology

2.1.5.1 Histology

Definitive diagnosis of OSMF is based on histology. A clinical diagnosis of OSMF can be readily obtained from clinical examination, presenting symptoms and a known habit of areca nut chewing.

Histopathological changes in the oral mucosa of betel quid chewers may vary according to variability of chewing habits, betel quid consistency, constituents of betel quid, duration of exposure differences in grade of disease severity and individual susceptibility to disease. Betel chewer's mucosa an oral condition induced either by a direct action of the quid or traumatic injury of chewing, shows a tendency toward desquamation of oral epithelium. The underlying area shows pseudomembranous or wrinkle like appearance (Reichart *et al*., 1998).

According to Shahid in 2008 biopsy often reveals an inflamed and atrophied oral mucosa with fibrosis of the submucosa and normal deeper skeletal muscle. According to Rajendren *et al*. in 2005 vascular dilatation may occur in the affected mucosa as an adaptive response to compensate for tissue ischaemia/ hypoxia.

A *B*

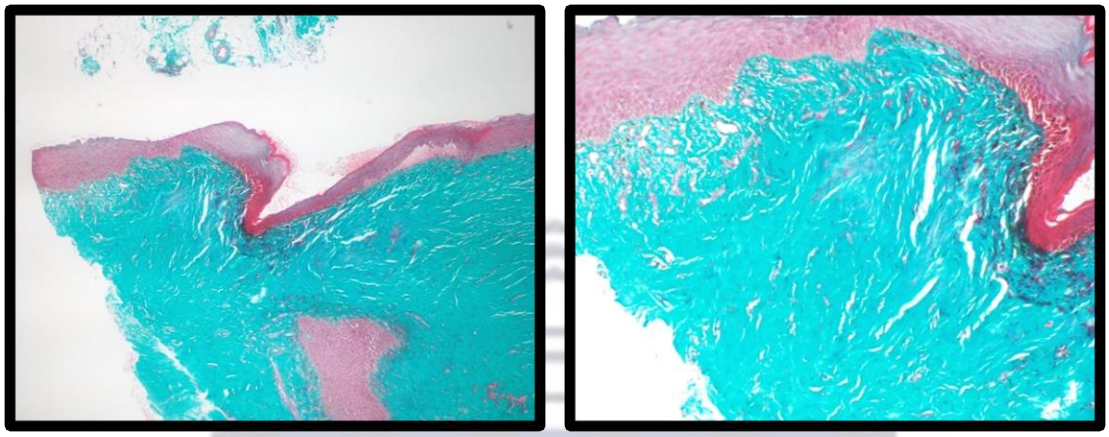


Figure 4 Histopathological features of one of the participants highlighting fibrosis, A (x40 magnification) and B (x100 magnification). Masson's trichrome stain was used.

A *B*

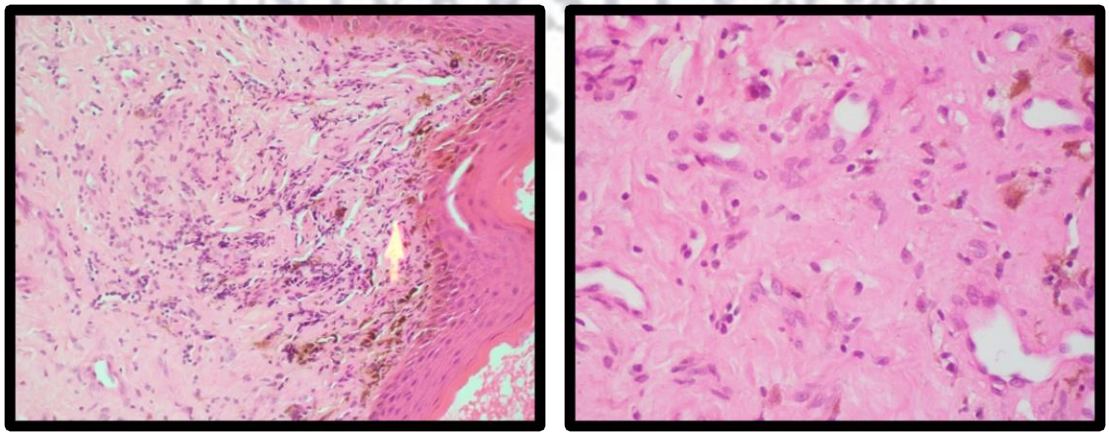


Figure 5 Histopathological features of one of the participants showing ectatic vascular channels, background chronic inflammation, lymphocytes, plasma cells, histiocytes and fibrosis, A (x200 magnification) and B (x400 magnification). Hematoxylin and Eosin staining was used.

Early changes in connective tissue have been reported to be marked oedema, a strong fibroblast response, inflammatory cell infiltration, dilated and congested blood vessels. Subsequently, subepithelial tissue reveals early signs of hyalinization, the presence of thick collagen bundles and a moderate number of fibroblasts and the infiltration of chronic inflammatory cells such as lymphocytes, eosinophils and plasma cells (Pillai *et al*., 1992).

In advanced stages of OSMF, juxta-epithelial hyalinization of connective tissue with a markedly reduced fibroblast response is frequently noted often with concomitant fibrosis of the lamina propria. Blood vessels are usually narrowed or obliterated and are relatively few in number (Pillai *et al*., 1992).

A reduction in Type III and IV collagen with a concomitant increase in type I collagen in the connective tissue is observed by immunohistochemistry in OSMF specimens. Other common histopathological findings may include vacuolization of the prickle-cell layer, increased mitotic activity, nuclear pleomorphism, subepithelial inflammatory cell infiltration and epithelial atypia (Reichart *et al*., 1998).

Occasionally some superficial epithelial cells in the BCM show a ballooning appearance with fine intra-cellular and extra-cellular granular materials as observed by light and electron microscope. Surface epithelial hyperplasia with marked rete peg and subepithelial inflammatory cell infiltration may be observed in long standing lesions (Reichart *et al*., 1998).

Metaplasia of non-keratinized areas may be seen including para-keratinization or ortho-keratinization with varying degrees of dysplasia. Thickening of the basement membrane is common and there is marked reduction in vascularity of the connective tissues in inverse proportion to increased density of collagen, which appears hyalinised (Warnakulasuriya *et al*., 2007).

2.1.5.2 Fibrosis as a pathology in OSMF, Regeneration and Repair

Fibrosis is defined as the deposition of collagen as part of normal wound healing. However, the term fibrosis is used more broadly to denote the excessive deposition of collagen and other ECM components (Kumar *et al.*, 2010).

Fibrosis is associated with quantitative and qualitative alterations of collagen deposition within the subepithelial layer of the oral mucosa. This is partly due to marked deficiencies in

collagen and fibronectin phagocytosis by fibroblasts caused by areca nut alkaloids such as arecoline and arecaidine (Wollina *et al*., 2015).

The term scar and fibrosis are used interchangeably, but fibrosis most often indicates the deposition of collagen in chronic diseases. The basic mechanisms that occur in the development of fibrosis associated with chronic inflammatory diseases are generally similar to the mechanisms of skin wound healing. Injury to cells and tissues sets in motion a series of events that contain the damage and initiate the healing process. This process can be broadly separated into regeneration and repair. Regeneration refers to the proliferation of cells and tissues to replace lost structures and repair most often consists of a combination of regeneration and scar formation by deposition of collagen (Kumar *et al*., 2010).

According to Kumar *et al*. in 2010 repair by connective deposition includes the following basic features inflammation which include angiogenesis, migration and proliferation of fibroblasts, scar formation and connective tissue remodelling.

Tissue repair and regeneration depend not only on the activity of soluble factors, but also on interactions between cells and the components of the extracellular matrix (ECM). The ECM regulates the growth, proliferation, movement and differentiation of the cells living with it. It is constantly remodelling and its synthesis and degradation accompanies morphogenesis, regeneration, wound healing, chronic fibrotic processes, tumour invasion and metastasis (Kumar *et al*., 2010).

Areca nut aggravates the increase of pro-inflammatory cytokines and reduced anti-fibrotic IFN- γ in the lamina propria which suggests that OSMF is an altered version of wound healing. This may be because the expression of various ECM molecules is similar to those seen in maturation of granulation tissue (Tilakaratne, 2006).

2.1.6 Malignant Transformation

The precancerous nature of OSMF was first mentioned by Paymaster in 1956. Epithelial dysplasia in OSMF tissues appeared to vary from 7-26% depending on the population. The malignant transformation rate of OSMF to oral cancer was reported as 7.6% in a long-term follow-up study of 17 years in India (Murti *et al*., 1985).

Some of the chemical components of the areca nut and betel quid are mutagenic, they have the capacity to cause DNA breaks or DNA-protein cross links in keratinocytes, which may in turn create an epithelial field of genetically altered precancerised keratinocytes (which may show cellular atypia) but are in relation to their neighbouring normal keratinocytes in terms of metabolic activity and proliferation rate. TNF- α, IL-6 and PGE-2 within the inflamed microenvironment of the submucous fibrosis may favour malignant transformation of the precancerised keratinocytes by driving their clonal expression (Jeng *et al*., 2003).

According to Rajendren in 2007 various authors have reported the ischaemic atrophy of the overlying epithelium in OSMF due to stromal changes, which undergoes progressive hyalinization, decrease in vascularity and cellularity, therefore epithelium becomes more prone to oral carcinogenesis and predisposed to malignant transformation.

According to Gupta in 2015 unlike other potentially malignant disorders, OSMF is irreversible, it either remains stationary or becomes severe, with a high risk of oral cancer development due to the denuded atrophic oral mucosa making the oral mucosa vulnerable to carcinogens.

2.1.7 Treatment

Treatment is focused on cessation of the areca nut habit and also in improving the quality of life for these individuals.

According to Haider *et al*. in 2000 conventional therapies in the treatment of OSMF are empirical and symptomatic in nature. The major therapeutic objectives are either anti-inflammatory, oxygen radical-scavenging or antifibrotic in nature.

Treatment is based on severity of disease. Once trismus has developed, the disease is considered mild to moderate. The goal of medical and surgical therapy at this stage is to maintain oral function and limit progression of disease as well as encourage cessation of the betel nut chewing habit. Physical therapy combined with medical treatment is often utilized (Aziz, 2008).

Most treatment modalities in OSMF have centered on relief of the burning sensation and release of the fibrotic bands to assist oral opening. Predictability of the release of the fibrotic bands has been the basis of all surgical techniques employed (Kamath, 2015).

2.1.7.1 Non-surgical/ minimally invasive techniques

There are various non-surgical/minimally invasive approaches used in the treatment of OSMF:

Cessation of habit

According to Neville *et al*. in 2009 stopping the habit is pivotal in the treatment of OSMF, unlike disease processes such as tobacco pouch keratosis, OSMF does not regress with habit cessation. According to Kumar *et al*. in 2010 the relative contributions of repair and regeneration are influenced by the resolution or chronicity of the injury and inflammation therefore enforcing the need to stop the habit.

Steroids

Corticosteroids are immunosuppressive agents which are believed to decrease inflammation and collagen formation, thereby reducing the symptoms of OSMF and resulting in increased mouth opening. Corticosteroids such as hydrocortisone, triamcinolone, dexamethasone and betamethasone have been used in the treatment of OSMF. Steroids suppress inflammatory reactions, thereby preventing fibrosis by decreasing fibroblastic proliferation and deposition of collagen (Gupta *et al*., 2018).

Physiotherapy

Cox *et al*. in 2009 conducted a clinical trial of physiotherapeutic treatment to improve oral opening in oral submucous fibrosis patients in a Nepalese population. Their study was designed with 54 Nepali OSMF patients which were managed for four months in three randomly assigned groups. Group one had received physiotherapy five times daily by inter-positioning of tongue spatulas between the teeth and adding a new spatula every five to ten days, group two had local injections of hyaluronidase with steroids and group three had no active treatment. They suggested that physiotherapy is effective for increasing the oral

opening and can be readily used to improve OSMF in communities with otherwise limited health resources. Physiotherapy had improved mouth opening ($p < 0.0005$), but not oral pain.

Hyaluronidase and dexamethasone

Hyaluronidase acts by breaking down hyaluronic acid (the ground substance in connective tissue) lowers the viscosity of intercellular cement substance.

In a study done by James *et al*. in 2015 a total of 28 patients diagnosed with OSMF were treated over nine months. They were treated by administering an intralesional injection of dexamethasone 1.5 ml and hyaluronidase 1500 IU with 0.5 ml lignocaine hydrochloride injected intralesionally twice a week for four weeks. Improvement was noted in the patient's mouth opening with a net gain of 6mm (SD=2 mm), the range being four to eight millimetres. Their conclusion was injection of hyaluronidase with dexamethasone is an effective method of managing Grade III.

Chymotrypsin

Lavina *et al*. in 2007 suggested chymotrypsin an endopeptidase, hydrolyses ester and peptide bonds, thus acting as a proteolytic and anti-inflammatory agent.

Pentoxifylline

Gupta *et al*. in 2018 suggested peripheral vasodilators like pentoxifylline have vasodilating properties and hampered mucosal vascularity in OSMF could be increased by the use of pentoxifylline. Pentoxifylline suppresses leucocyte function and alters fibroblast physiology and stimulates fibrinolysis.

Iron and multivitamin supplement including lycopene

According to Gupta *et al*. in 2018 vitamins A, B, C, D, E and minerals like copper, iron and magnesium stabilize and deactivate the free radicals before they attack cells.

In a study by Kumar in 2007 oral lycopene therapy showed improvement in the signs and symptoms of OSMF. Fifty-eight patients with OSMF were randomly divided into three groups, evaluated weekly over a two-month period. Patients of group A (n=21) received 16 mg of lycopene, those of group B (n=19) received 16 mg of lycopene along with twice a week intralesional steroid injections and those of group C (n=18) were given a placebo. They

found that the mouth-opening for the patients showed an average increase of A=3.4 mm, B=4.6 mm and C=0mm. In conclusion they found these values were statistically highly significant and that the observed effects suggest that lycopene can and should be used as a first line of therapy in the initial management of oral submucous fibrosis.

Allicin and Triamcinolone Acetonide

A randomized clinical trial was performed by Jiang *et al*. in 2015 whereby triamcinolone acetonide (TA) or allicin was injected intralesionally weekly for 16 weeks. Improvements in mouth opening, burning sensation and oral health-related quality of life were evaluated. Forty-eight subjects completed the study without obvious adverse reactions. At 40 weeks, the net gain in mouth opening was 2.27mm (0.84 mm) in the TA group and 5.16mm (1.04 mm) in the allicin group. Burning sensation improved by 2.79 (0.87) in the TA group and by 4.33 (1.04) in the allicin group. The OHIP-14 score improved by 4.67 (2.94) in the TA group and by 12.58 (9.82) in the allicin group.

Hyperbaric oxygen therapy

Hyperbaric oxygen treatment as a supplementary treatment to improve or cure disorders involving hypoxia was hypothesized by Xiaojing in 2014. It was hypothesized that due to the nature of hyperbaric oxygen treatment, it may have a supplemental therapeutic role in the management of potentially oral malignant diseases but more evidence-based, randomized and controlled studies need to be conducted.

Interferon- Gamma

In an open uncontrolled study conducted by Haque *et al*. in 2001 with 29 participants it was found that intra-lesional IFN-γ treatment gave a net gain of eight mm (SD= four mm) and a range four to 15 mm. This plays a role in treatment of patients with OSMF because of its immune-regulatory effect. Interferon gamma is also known as an anti-fibrotic cytokine, patients treated with an intralesional injection of interferon gamma experienced improvement of symptoms.

Tumeric

Administration of turmeric powder offers protection against benzopyrene induced increase in micro nuclei in circulating lymphocytes and is an excellent scavenger of free radicals in vitro. Turmeric oil and turmeric oleoresin both acts synergistically in vivo to offer protection against DNA damage (Hastak *et al.*, 1997).

2.1.7.2 Surgical techniques

According to Kamath in 2015 most treatment modalities in OSMF have centered on relief of the burning sensation and release of the fibrotic bands to assist oral opening. Predictability of the release of the fibrotic bands has been the basis of all surgical techniques employed.

Numerous surgical interventions are available for the treatment of OSMF which include laser therapy, local/ regional or distant flaps and grafts (autogenous, xenograft, allograft) placement.

There are various surgical treatment modalities which exist, such as:

Simple excision

According to Herbst in 2021 simple excision can result in contracture of the tissue and exacerbation of the condition.

Lasers

According to Azadgoli *et al*. in 2016 lasers are being increasingly utilized for treatment of a variety of pathologies as interest in less invasive treatment modalities intensifies.

In modern dentistry, the main concern remains the bloodless surgical field, minimum operating time and the least amount of patient discomfort with effective long term post-operative results, both for the surgeon as well as the patient. Although a wide variety of surgical aids are in use in the medical and dental field, laser has proven to be a non-invasive surgical technique with fewer limitations. In the premalignant conditions such as OSMF, lasers can be used as a reliable, reproducible method preventing further morbidity (Gupta *et al*., 2021).

The use of laser therapy has proven to provide effective long-term results in the treatment of all stages of OSMF without any complications and defects that are usually demonstrated during conventional surgeries. This technique can be used even as a chairside procedure under local anaesthetic minimizing the need for a large operating field. However, different lasers used till date have their specific properties, functioning, wavelengths, effects on tissues, benefits and limitations (Gupta *et al*., 2021).

In a systematic review by Gupta *et al*. in 2021 it was found that all the studies reported favourable results regardless of the type of laser used. One of the major points highlighted in the review is that for the successful long-term effects of laser in OSMF patients, this therapy has to be followed by some post-operative adjunctive aids such as physiotherapy, cessation of habit, other nutritional supplements and regular follow up to evaluate the improvement in oral symptoms. Despite limitations such as high cost, need for advanced training and safety measures and delayed wound healing, laser has become indispensable in the management of OSMF.

Advantages of lasers

Minimal operating time, bloodless surgical field, can be carried out under local anaesthetic, non-contact type technique, monochromatism, coherence and collimation, inhibition of inflammatory mediators of pain such as histamine, potassium, hydrogen and adenosine triphosphate. No need for sutures or any grafting except in a few cases, inhibition of factors such as TGF-B and connective tissue growth factor two resulting in less collagen deposition. Proliferation of myofibroblasts leading to collagen deposition and haemostatic property to sealing of blood vessels and lymphatics (Gupta *et al*., 2021).

Disadvantages of lasers

There is a need for special safety measures and special training as well as knowledge about technique on how to use them. Patient co-operation is required, high cost of maintenance, poor thermal properties of some lasers, dependence on electric source, long time for re-epithelialisation and delayed wound healing (Gupta *et al*., 2021).

In a systematic review of literature by Gupta *et al*. in 2021 it was found that one of the major limitations of their study was the lack of an exact scientific basis of selection and applied criteria in using lasers for the management of OSMF. In the literature reviewed, only a few specific lasers had been used although a variety of other types are also available.

Local Flaps

Tongue flap:

Advantages of this flap may include that it is accessible, has good muscular bulk and adequate pedicle vascular supply based on lingual vessels. Disadvantages include dysphagia, limited amount of donor tissue due to inadequate reach, lack of stability and dehiscence due to uncontrolled tongue movements and requires a second procedure for detachment (Kamath, 2015).

Palatal flap:

Advantages of this flap may include that it is accessible and has adequate vascular supply of pedicle based on the greater palatine vessels. Disadvantages of this flap may include limited reach and coverage, second molar extraction required at times to extend reach, fibrotic involvement of the graft site and may require a second procedure for detachment (Kamath, 2015).

Buccal fat pad:

Advantages of this flap may include that the buccal fat pad is unaffected by disease process in the mouth, adequate tissue available for coverage of posterior areas, limited morbidity to patient in terms of aesthetics, limited expertise needed and it may be a chairside procedure that can be done under local anaesthetic. Disadvantages of this flap may include anterior reach inadequate and regions anterior to canines have to be left uncovered, inadequate harvesting of BFP due to atrophy in chronic and severe cases of OSMF and may include excessive fear of breakdown and loss due to lack of protection tissue (Kamath, 2015).

Regional Flaps

Nasolabial flap:

Advantages of this flap may include that it is an accessible flap and is unaffected by disease process in mouth. Disadvantages of this flap may include that it has limited reach even when extended nasolabial flaps are used, limited width of flap material for coverage and requires a second procedure for detachment (Kamath, 2015).

Temporalis fascia:

Advantages of this flap may include that it is accessible and is unaffected by disease process in mouth. Disadvantages of this flap may include limited reach, coverage of posterior areas of mouth only possible, hollowing of temporal region of face can sometimes occur and aesthetic morbidity in terms of post-operative scar and requires a second procedure for detachment (Kamath, 2015).

Distant flaps

Radial Forearm Free Flaps:

Advantages of this flap may include that it is accessible and is unaffected by disease process in mouth. Disadvantages of this flap may include these flaps are hairy, requires high surgical expertise, not cost effective, limitations of flap survival due to dependence on microvascular anastomoses, requires second procedure for detachment and in cases of failure anastomoses and extraction of third molar required to avoid flap inclination between teeth (Kamath, 2015).

Anterolateral Thigh Flaps:

Advantages of this flap may include adequate tissue bulk and can be used for coverage of large defects and unaffected by disease process in mouth. Disadvantages of this flap may include that it requires high surgical expertise, not cost effective, limitations of flap survival due to dependence on microvascular anastomoses and secondary procedures to debulk and in cases of failure of anastomoses sometimes required (Kamath, 2015).

Grafts

These include split skin grafts (SSG), collagen membranes, artificial dermis, human placenta/amnion grafts and platelet-rich membrane. Advantages of these grafts include that they are commercially available, least morbidity to donor site and adequate protection to host site ensuring healing. Disadvantages include increased incidence of post-operative contractures, common failure of SSGs due to lack of vascular supply, best used as immediate coverages (Kamath, 2015).

According to Kamath in 2015 the use of SSG and allogenic materials is purely supportive and protective in the post-operative healing period. The difficulties in harvesting placental grafts

and human amnion and their ethical problems have largely rendered the use of this graft redundant.

For cases in which initial surgical intervention is unsuccessful, resulting in recurrent trismus usually due to a lack of compliance with physical therapy or less commonly from shrinkage of the skin graft or alloplastic graft, a more aggressive surgical therapy is indicated. Excision of any fibrous bands intraorally may require repeated masticatory muscle myotomy. Often in this situation a larger soft tissue buccal defect is created needing large soft tissue reconstruction. This can include a temporalis pedicled flap, superficial temporalis fascia pedicled flap, or a radial forearm free flap combined with split thickness skin graft coverage (Lee, 2007).

MAFT® gun (Microautologous fat transfer gun), stripping off the temporalis muscle and tendon from the ramus

In an isolated abstract published by the international Journal of Oral and Maxillofacial, KO *et al*. in 2017 had done coronoidotomies, injection of fat by using a MAFT® gun (1/30–1/240 mL), stripping of the temporalis muscle and tendon from the ramus, mouth opening exercises and postoperative acupuncture were used as adjunctive treatment modalities. KO *et al*. in 2017 had found that five patients had an average increase in mouth opening of 18mm (SD= 4 mm) at six-month follow-up. Visual analogue pain scale scores also reduced significantly within few days following surgery. This graft technique also showed increased elasticity and hence with high affinity to the recipient sites. Increased elastin was found within the postoperative mucosa. No full publication of this study could be found.

Coronoidectomies/muscle myotomies

Are often used as adjunctive surgical procedures or own their own.

2.2 Fat Grafting

2.2.1 Introduction and historical background

According to Pu *et al*. in 2015 fat which is readily available, was considered the ideal solution to fill depressions and contour deformities.

White adipose tissue, composed of adipocytes with a single large lipid inclusion and large peripherally located nucleus, represents the predominant type of fat in humans. It is involved in a variety of physiological roles including the storage of energy rich triglycerides, cushioning of vital structures and organs, metabolic homeostasis, immune regulation, reproduction and angiogenesis (Haque *et al*., 2004).

In 1893, the German surgeon Gustav Neuber (1850-1932) first harvested adipose tissue from the arm and transferred it to the orbital region to correct adherent scar sequelae from osteomyelitis. In 1895, Viktor Czerny (1842-1916), transferred a lipoma to the breast to re-establish symmetry, following unilateral partial mastectomy for fibrocystic mastitis.

Initially surgeons enthusiastically favoured the technique of en-bloc fat grafting, alone or in combination with skin flaps, as it often represented the sole way to solve major problems in a simple way. In the 1930s, with growing experience, clinicians realized that the encouraging early results worsened over the long term because of an unpredictable reabsorption rate and a tendency to form oily cysts. The pliable fat graft gradually modified, becoming hard and fibrotic (Pu *et al*., 2015).

At the end of the 1980s, Argentinean Abel Chajchir published favourable long-lasting results using fat injection. He considered cautious manipulation of the adipocyte to reduce potential rupture of its fragile cell, rinsing the lipoaspirate in saline to eliminate dead cells and debris and finally grafting fat into close contact with a well vascularized tissue, crucial steps to minimize reabsorption. In the 1990s, Sydney Coleman standardised the procedure.

Until the introduction of syringes for placing adipose tissue, as it is done currently, fat was always transplanted en-bloc, often with the dermis (the so-called dermal fat graft). The healing potentials of fat was empirically noticed by surgeons who were confronted with the management of the terrible disfigurements caused by World War I. Fat was inserted into the wounds either to promote the healing process or to correct uneven scars from gunshot wounds of soldiers injured in the battlefields (Pu *et al*., 2015).

2.2.2 Coleman technique

The use of fat grafts to correct congenital deformities and complex traumatic injuries with soft tissue loss after radical oncological surgery was first performed in 1893 by Neuber and in 1987 Coleman introduced a new technique to decrease traumatic handling of fat during liposuction (Billings *et al*., 1989). His technique consisted of three steps: manual lipoaspiration under low pressure, centrifugation for three minutes at 3400 rpm (rotations per a minute) and reinjection in 3D (three-dimensionally).

The harvesting of fat grafts can be performed via a wet or dry method. In 1993, Klein *et al*. described the 'wet' method, which involves the injection of the donor site with fluid solution containing 0.9 % NaCl, epinephrine and local anaesthetic. The most commonly used methods to prepare fat grafts are sedimentation, filtering, washing and centrifugation. In animal experiments no significant differences have been observed in the weight or architecture of the fat grafts obtained using the centrifugation, filtration or sedimentation methods. In contrast, studies conducted in patients have demonstrated more favourable outcomes with centrifugation rather than gravity separation.

Coleman in 2006 suggested a processing method that has gained popularity and has been since integrated in many fat-transfer clinical protocols. Aspirated fat in syringes is spun at 3000 rpm for three minutes to isolate the fat.

Coleman in 2006 described a technique for fat harvesting that minimized trauma to the adipocytes. With a three millimetre, blunt-edged, two- hole cannula connected to a ten-millilitre syringe fat is suctioned manually by withdrawing the plunger. The cannula is pushed through the harvest site as the surgeon uses digital manipulation to pull back on the plunger of the syringe and create a gentle negative pressure. A combination of slight negative pressure and the curetting action of the cannula through the tissues allows parcels of fat to move through the cannula and Luer-Lok aperture into the barrel of the syringe. When filled, the syringe is disconnected from the cannula, which is replaced with a plug that seals the Luer-Lok end of the syringe. The plunger is removed from the syringe before it is placed into a centrifuge.

2.2.3 Autogenous fat grafting (lipoaspirate) and technique

Autogenous fat transfer has become a well-established method of soft tissue augmentation for both cosmetic and reconstructive indications. Fat naturally fulfills many of the characteristics required of a soft tissue filler. It is autogenous, non-toxic, biocompatible, easily available in most patients and potentially removable and long-lasting (Bucky *et al*., 2008).

Fat injection to improve healing in various types of wounds is most likely caused by several factors in the adipose-derived stem cells (ASCs) themselves and by growth factors already present in the injected fat, contributing to diminishing fibrosis, inflammation and favouring healing processes (Sultan *et al*., 2011). Caviggiloi *et al*. in 2011 reported frequently that hypertrophic scarring and scar contracture are softer and that skin hyperpigmentation disappears after fat grafting.

Klinger *et al*. in 2008 and Bruno *et al*. in 2013 both studied the changes that occur within scar tissue following autogenous fat grafting (AFG) treatment in a total of 96 patients. Both studies reported morphological appearance post treatment, displaying features closer to normal tissue, compared with pre-treated scars. Lipofilling induced organised collagen deposition, vascularisation and reappearance of papillary dermis.

Complications at the donor-site appear to be minimal and more so related to the liposuction technique. Possible complications that may arise include bruising, swelling, haematoma formation, paraesthesia or donor-site pain, infection, hypertrophic scarring, contour irregularities and damage to underlying structures such as intra-peritoneal or intra-muscular damage via penetration of the cannula.

Bruno *et al*. in 2013 reported an altered presence of antibodies including reduced presence of P63, which is responsible for diffuse differentiation and proliferation in the epithelium. Reduced P63 expression inhibits these processes. Alternatively, Ki67 expression is increased, linked with stem cell induced cellular proliferation.

According to Riyat *et al*. in 2017 AFG is a minimally invasive and safe approach to treating scars, a promising alternative to surgical excision. The technique of blunt cannula insertion optimises the release of scar retraction, which contributes to the analgesic effect of this treatment method. The evidence supports current theories of mesenchymal stem cells' regenerative and anti-inflammatory properties responsible for scar healing.

Riyat *et al*. in 2017 found the documented description of AFG benefits in improving scar tissue was promising, indicating its ability to reduce functional limitations and enhance cosmetic appearance. Analgesic effects are caused by nerve repair (mediated by BDNF) and scar entrapment release. The injection procedure itself is responsible by making space under the scar tissue and it is hypothesised that grafts containing TGF-β play a role in immunosuppression by acting on T- cells, resulting in an analgesic effect.

According to Riyat *et al*. in 2017 they had suggested that fat contains specialised stem cells that possess properties which improve the quality of scars. Fat injection has been shown to decrease tension which softens scar tissue and provides pain relief. Analgesic effects are caused by nerve repair and scar entrapment release. AFG is a safe treatment method with few complications. This method poses promising benefits in the future treatment of scar-related conditions.

Fat grafting technique

Aseptic environment/Sterile conditions

Sterile conditions are maintained throughout the procedure and the lipoaspirate is to be handled with care to avoid contamination. Harvesting sites are cleansed with an aseptic medium.

Donor site selection

A variety of body areas that uniformly have abundant or excess fat is suitable as donor sites for harvest of fat grafts, such as the abdomen, flanks, buttocks, medial and lateral thighs, or knees. As a general rule, donor sites are selected so that enhance the body contour and are easily accessible in supine position, which is the position that is used for almost all facial and body augmentation procedures (Pu *et al*., 2015).

Although there is no evidence of a favourable donor site for harvest of fat grafts because viability of adipocytes within the fat grafts from different donor sites may be considered equal, a higher concentration of ASCs is found in the lower abdomen and inner thigh in one study (Padoin *et al*., 2008).

Anaesthesia

Anaesthesia for harvesting of fat grafts can be performed under general anaesthesia, epidural anaesthesia, or local anaesthesia with or without sedation. The tumescent solution used for donor site analgesia or haemostasis should contain the lowest concentration of lidocaine possible because its high concentration may have a detrimental effect on adipocyte function and viability (Keck *et al*., 2010).

Pu *et al*. in 2015 often used 0.01 % to 0.02% of lidocaine in ringers' lactate if the fat grafting procedure is performed under general anaesthetic and 0.004% if performed under local anaesthetic with or without sedation. The tumescent solution also contains epinephrine with a concentration of 1:1000000. Epinephrine can precipitate vasoconstriction in the donor sites, which may decrease blood loss, bruising, haematoma and the possibility of intra-arterial injection of the transplanted fat especially when injecting around periorbital areas or the face.

Harvesting

According to Pu *et al*. in 2008 syringe aspiration is a relatively less traumatic method for the harvesting of fat grafts and should be considered as the standardized technique of choice for harvesting.

Centrifugation

Attention should be made to avoid prolonged exposure of fat grafts to air and to avoid bacterial contamination. After being centrifuged, lipoaspirates within the syringe are divided into three layers: the oil content in the upper layer, fatty tissue in the middle layer and the fluid portion at the bottom (Pu *et al*., 2008).

The oil can be decanted from the Luer-Lok syringe. The residual oil is wicked with a cotton strip or swab. The fluid at the bottom can be easily drained out once the plug at the Luer-Lok aperture is removed. Transfer to delivery syringe is then done with an adapter carefully to avoid damage to cells within the graft. A higher content of stem cells or angiogenic growth factor positively correlated with fat graft survival both in experimental and clinical studies (Phillips *et al*., 2013).

Centrifugation at 3000rpm for three minutes seems to offer more benefits for effectively concentrating adipocytes and ASCs and should be a valid method of choice for processing fat grafts, especially for small volume fat grafting (Boscher *et al*., 2002).

Deposition

The key to a successful fat graft injection is to achieve an even distribution of fat grafts in the recipient site. By doing so, the injected fat grafts may have a maximal amount of contact with the tissue in the recipient site for better fat graft survival through plasmatic imbibition and neovascularisation. Not only can grafting with a small volume in each pass get better surgical outcomes but complications such as fibrosis, oil cyst formation, calcification or even infection with large-bolus grafting, can be avoided. Slow injection of 0.5 to 1ml/s should be injected during the withdrawal phase (Pu *et al*., 2015).

Post-operative care

Swelling in the recipient site is expected for one to two weeks and the grafted areas can be become firm or hard in the first few weeks. Patients should be informed about this normal healing process after fat grafting and some reassurance to the patient may be given where necessary.

During the post-operative period and recovery time, ice packing, tight compression and massaging of grafted region is indicated. A soft diet is indicated to avoid trauma to the grafted site.

Additional fat grafting procedure

Kanchwala *et al*. in 2009 suggested that the timing of an additional fat grafting session should be deferred until six months post operatively to diminish the inflammatory response in the grafted area. Pu *et al*. in 2015 observed that transplanted fat gradually loses its volume with time and usually stabilises at three months post operatively if surgical recovery is uneventful. The timing of subsequent fat grafting procedures should be deferred to at least three months after previous transplantation.

According to Pu *et al*. in 2015 common uses of lipofilling include but not limited to breast reconstruction/ augmentation, scleroderma, velopharyngeal incompetence, scars, ulcers, Dupuytren's disease and cosmetic procedures.
Fat injection has evolved dramatically and it ranks amongst the most popular procedures, for it provides the physician with an incredible range of aesthetic and reconstructive clinical applications with amazing regenerative effects on the surrounding tissues. New research has

been made all over the world to demonstrate the role of ASCs, present in adipose tissue, in the repair of damaged or missing tissues (Pu *et al*., 2015).

2.2.4 Adipose derived stem cells (ASCs)

2.2.4.1 History and discovery of adipose derived stem cells

At the beginning of the new millennium, a Pittsburgh team of plastic surgeons and researchers made a crucial discovery. They demonstrated that adipose tissue is the greatest source of adult mesenchymal stem cells, the so-called adipose- derived stem cells (ASCs), capable of differentiating into other types of tissue. They also identified the stromal vascular fraction (SVF) a source of ASCs, endothelial (progenitor) cells, T cells, B cells, mast cells and adipose tissue macrophages with repair and regenerative potential, obtained from the lipoaspirates, once the adipose and fluid portion has been removed and processed. This may explain the role of fat grafting in accelerated healing process and in replacing damaged or missing cells (Zuk *et al*., 2002).

According to Zuk *et al*. in 2002 adipose tissue is a valuable source of stem cells with multipotency as well as angiogenic and immunomodulatory properties that facilitate tissue repair.

The most abundant and accessible source of adult stem cells is adipose tissue. MSCs have been obtained by liposuction of human adipose tissue under physiological and pathological conditions (Kim WS *et al*., 2009).

Adipose tissue contains various cells such as ASCs, endothelial progenitor cells and immune cells which act together for tissue repair and regeneration. The abundant supply of fat tissue, the ease of isolation, the ability to secrete angiogenic growth factors and the abundance of stem/progenitor cells make adipose-based therapy ideal for ischaemic or non-healing wounds (Lee *et al*., 2015).

2.2.4.1 Adipose derived stem cells and its' applications

According to Smahel in 1986 adipose tissue and ASCs obtained from liposuction were shown to have potential for regenerative therapeutic use. Although differentiation specific uses have great potential and use tissue regeneration, ASCs also exert significant immunomodulatory effects. They have been shown to increase secretion of IL-6, IL-10, IL-4 and GCSF and to stimulate proliferation of regulatory and helper T-cell phenotypes (Kim I *et al*., 2000).

According to Rehman *et al*. in 2004 hypoxia amplifies the paracrine effects of MSCs by enhancing the secretion of certain growth factors. According to Kinnaird *et al*. in 2004 ASCs improved perfusion in hind limb ischemia induced by the ligation of the femoral arteries, a function that was enhanced by hypoxic culture conditions.

Adipose tissue has many types of cells other than adipocytes, which can be extracted as a cell pellet called stromal vascular fraction (SVF) through collagenase digestion of aspirated adipose tissue. SVF contains adipose- derived stem/ stromal cells (ASCs), vascular endothelial cells, pericyte, adipose resident macrophages, lymphocytes (Yoshimura *et al*., 2006).

According to Coleman in 2006 fatty tissue has the highest percentage of adult stem cells of any tissue in the body, with as many as 5000 ASCs per gram of fat compared with 100-1000 stem cells per millimeter of bone marrow.

In biological systems the normal processes of oxidation produce highly reactive free radicals, which may continue to damage cells. Anti-oxidants play a housekeeping role, scavenging free radicals before they get a chance to do harm to the body. Recent evidence has supported the protective role of ASCs against skin oxidative damage, most of which is mediated by secretory factors (Baregamian *et al*., 2006).

The regenerative effects of fat grafting are appreciated by many clinicians. Stem cell-depleted tissues such as irradiated tissue, chronically inflamed tissue and ischaemic fibrosis are improved by fat grafting in quality, vascularity and healing and expanding capacity (Salgrello *et al*., 2012) (Rigotti *et al*., 2007).

Stem cell therapy may stimulate resident tissue stem cells to transform into new fibroblasts, which may help in the removal of disintegrated biochemically and morphologically altered collagen fibers (Sankaranarayanan, 2007).

ASCs have the potential to modulate or suppress immunoreaction (De Miguel *et al.*, 2012), differentiate into adipocytes, vascular endothelial cells, or other cells and release angiogenic growth factors, such as hepatocyte growth factor and VEGF especially under hypoxic conditions (Suga *et al*., 2009). According to Si *et al*. in 2019 the ASCs have the following differentiation capabilities into adipocytes, muscle cells, keratinocytes, glial lineages, hepatocytes, chondrocytes, osteoblasts, neuronal lineages, Beta-islet cells and endothelial cells.

It is hypothesised that ASCs that are present in high concentrations in lipoaspirates, mediate complex tissue remodelling (Brown *et al*., 2010).

The same properties that make ASCs useful for tissue healing and regeneration also create the potential for the stimulation of tumour cell growth when the cells are used for cancer reconstruction (Donnenberg *et al.*, 2010).

ASCs are prevalent surrounding the blood vessels and within the connective tissue of human adipose tissue. These non-lipid-laden stromal cells can be isolated either by suction-aspirated adipose tissue or from excised human fat by enzymatic collagenase digestion (Lee *et al*., 2015). The appeal of ASC incorporation in lipoinjection is mostly because of the growth factor release of the stem cells, especially angiogenic growth factors such as vascular endothelial growth factor (Lee *et al*., 2015).

Stem cell therapy is primarily aimed at neo-angiogenesis by releasing cytokines and growth factors (via paracrine effect). This may result in increased free radical scavenging by antioxidants (either naturally occurring or extraneous). Neo-angiogenesis may also facilitate the increase of senescent cells from the lesions by supplying a greater number of scavenging defense cells and reversal of hypoxia in the diseased tissue (Suma *et al*., 2015).

ASCs are a subset of mesenchymal stem cells (MSCs) that can be obtained easily from adipose tissues and possess many of the same regenerative properties as other MSCs. ASCs easily adhere to plastic culture flasks, expand in vitro and have the capacity to differentiate into multiple cell lineages, offering the potential to repair, maintain, or enhance various

tissues. Since human adipose tissue is ubiquitous and easily obtained in large quantities using a minimally invasive procedure, the use of autologous ASCs is promising for both regenerative medicine and organs damaged by injury and disease. ASCs are effective for the treatment of severe symptoms such as atrophy, fibrosis, retraction and ulcers induced by radiation therapy. Moreover, ASCs have been shown to be effective for pathological wound healing such as aberrant scar formation. Additionally, ASCs have been shown to be effective in treating severe refractory acute graft-versus-host disease and haematological and immunological disorders such as idiopathic thrombocytopenic purpura and refractory pure red cell aplasia, indicating that ASCs may have immunomodulatory function (Si *et al*., 2019).

Krastev *et al*. in 2020 did a meta-analysis of 45 studies (3033 patients), they had found that autologous fat injection appeared to be a safe and effective treatment for fibrosis and scar-related conditions as demonstrated by significant improvement in patient and surgeon satisfaction, scar quality, pain, sequelae of radiotherapy and function after a mean follow up of 1.4 years. Treated patients underwent on average 1.4 sessions to achieve desired result and in only 4.8% of procedures minor complications were reported.

2.3 Quality of Life

2.3.1 Introduction

One of the main goals of the health care professional is to improve the patient's quality of life (QoL). It is now widely considered an important indicator of health outcome and a valuable adjunct to clinical evaluation, particularly in chronic conditions (Asadi-Lari *et al*., 2004).

Montazeri in 2009 defined QoL as a multidimensional construct that includes, at a minimum, physical, functional, psychological and social well-being. Other dimensions include spirituality, sexuality, occupational functioning and treatment satisfaction.

According to Kerr *et al*. in 2010 such patient-reported perceptions of QoL are usually quantified using questionnaires.

According to Kumar *et al*. in 2015 oral potentially malignant disorders can significantly impair quality of life. OSMF not only physically debilitates a patient, it has repercussions on the social, physical and psychological domains of the patient as well. Apart from trismus which is the most common and evident symptom associated with OSMF, a substantial portion of the suffering that ensues is also due to the ulcerations and burning sensation in the oral cavity and worsening of dental health (Chaudry *et al*., 2021).

2.3.2 Condition specific OPMDs questionnaire

Tadakamadla *et al*. in 2017 developed a condition-specific QoL instrument for patients with OPMD's to evaluate subjective perceptions of the impact of these disorders on everyday aspects of life.

With chronic disorders such as oral submucous fibrosis and other OPMD's, QoL and its assessment of it has becoming increasingly important in evaluating disease outcomes. A clinicians' duty is to improve the QoL of their patients, with this in mind a questionnaire was developed by Tadakamadla *et al*. in 2017.

The questionnaire designed by Tadakamadla *et al*. in 2017 was the questionnaire of choice for this study as it was specifically designed for the use of epidemiological and treatment studies for OSMF. The questionnaire exhibited good discriminant and convergent validity.

The questionnaire involved four main steps, 1- item generation, 2- item reduction, 3- formatting and 4- pretesting of the developed items (Tadakamadla *et al*., 2017).

1- Item generation- used inputs from patients and expert clinicians in the field of oral medicine. Patients suffering from oral submucous fibrosis/ oral leukoplakia and oral lichen planus undergoing treatment were invited to participate. Existing head and neck cancer QoL questionnaires were reviewed and potential items were generated with duplicate/ redundant items deleted. The Delphi technique was then used whereby ten clinicians with specialization in oral medicine and expertise in the area of OPMDs took part. The clinician's opinions on suitability were given and then a final list of items that achieved consensus including their ratings were sent around.
2- Item reduction- the pilot questionnaire was then tested on five participants who were asked if they found in understandable and then followed by administration to a further 15 not involved in item generation. Each item was reviewed by the patient and they were asked to report if they had experienced that item and rate its importance on a 5-point Likert scale, from 1- not important at all to 5- very important. Items that had an impact score of more than 1.5 and those endorsed by 50% or more of the individuals were considered for inclusion in the final questionnaire.
3- Formatting of the questionnaire- chronological order with questions related to diagnosis placed first, followed by items related to the physical, psychological and social implications of the disease and items related to treatment at the end
4- Pretesting the questionnaire- administered to 15 patients (five of each patient with OL, OSMF or OLP) who had not participated in item generation or item reduction. Each patient was interviewed to determine whether the patient could comprehend and accept each question. They were also invited to make suggestions for improvement and explore any problems experienced.

Validity and reliability-

The psychometric properties of the questionnaire were evaluated via 150 OPMD patients (50 patients of each OSMF,OL or OLP) undergoing treatment were recruited during October 2014 to May 2015.

Discriminant validity of the OPMD QoL questionnaire was evaluated by taking an equal number of healthy patients matched for age and gender.

The questions from this questionnaire allows for a holistic review of the patients QoL and has addressed things such as symptoms related to OPMDs, as well as issues related to psychological and social well-being in these patient's lives.

For item reduction they used the clinical impact method rather than a psychometric technique as it allows inclusion of items of particular importance to patients.

In their conclusion, it was found that the QoL questionnaire was the first condition-specific questionnaire to evaluate QoL in patients with OPMD's. The questionnaire compromises of 20 items measuring four domains and was found to be simple and comprehensive by the target population. The factor structure of the questionnaire conformed to the four hypothesized domains. The overall scale demonstrated good concurrent validity, with strong correlations with the COMDQ and global self-ratings. It also had good discriminant validity, with patients with OPMD's reporting poorer QoL compared with healthy individuals. The internal consistency of the overall scale and the domains was acceptable and the reliability on repeated administrations was excellent. They recommended the use of the questionnaire in epidemiologic and treatment studies of this group of disorder.

Chapter 3

Aim and Objectives

3.1 Aim

To identify the effectiveness of intralesional autogenous fat injections in patients with oral submucous fibrosis.

3.2 Objectives

1. To record the demographic details and medical information of the patients
2. To evaluate the inter-incisal mouth opening prior to treatment and six months post-operatively
3. To assess the presence or absence of restricted tongue movements prior to treatment and six months post-operatively
4. To record the pain score of patients prior to treatment and six months post-operatively
5. To record quality of life via a patient derived questionnaire prior to treatment and six month post-operatively

Chapter 4

Methods and Materials

4.1 Study Design

The design of the study was a prospective longitudinal observational study.

4.2 Study Population

The study population included patients with a clinical and histopathological diagnosis of OSMF seen at the Inkosi Albert Luthuli Central Hospital (IALCH) in Kwa-Zulu Natal (KZN) and treated with autogenous fat injections, provided they met the inclusion criteria. Since 01 May 2021 to 01 September 2022.

4.3 Study sample

4.3.1 Inclusion Criteria

- Class I/II Pindborg OSMF cases
- Age >18 with consent
- Patients fit for general anaesthesia
- OSMF patients that have stopped areca nut chewing.

4.3.2 Exclusion Criteria

- Patients that have received other forms of treatment for their OSMF
- Mentally challenged patients
- Class III Pindborg
- Patients on homeopathic or alternative medications.
- Patients with trismus caused by other reasons e.g., pericoronitis, radiation therapy or abscesses.

4.4 Surgical and medical protocol

Presurgical protocol:

- Baseline blood tests: including a full blood count, iron studies and vitamin B12
- Cessation of areca nut chewing

- Iron and B12 supplementation if indicated

Harvesting protocol:

- Stat dose of prophylactic antibiotics intra-operatively: Augmentin® (1.2g)
- Periumbilical region to be exposed and cleaned with an aseptic medium
- Inject local anaesthetic solution- 0.5% Marcaine® and saline solution to create a wet medium (20ml 0.5% Marcaine® mixed with 80ml saline, 40ml of mixed solution used in region of harvesting, fan insertion with spinal needle)
- Coleman technique to be used for harvesting
- Removal of aspirate via negative pressure from periumbilical area with 20ml syringes

Fat injection protocol:

- Placement of aspirate into a centrifuge at 3000 rpm for three minutes
- Removal of centrifuged material and drain excess fluid and oil
- Place into 10ml syringes
- Use of tulip cannula to insert fat into fibrous bands using negative aspiration before injection
- Five ml to be inserted bilaterally

Post-operative protocol:

- Analgesics: paracetamol 1000g every eight hours / ibuprofen 400mg every six hours
- Mouth rinse: chlorhexidine 0.2% twice daily 15ml
- Diet: soft diet

4.5 Study Instruments

Visual analogue scale (pain) – zero to ten (*appendix 1*), quality-of-life questionnaire (*appendix 2*) and a data capturing sheet (*appendix 6*).

4.6 Data Analysis and Management

A standardized data collection sheet was used to collect information from each patient record. All data collected was entered onto a 2010 Microsoft Excel sheet. The data that was recorded

included the patient's age (in years), gender and ethnic background. The signs and symptoms of the patient as well as the history of the lesion was collected.

Data was analysed using chi-square test to draw correlations between various parameters Microsoft Excel spreadsheet version 2010 was used to process the data digitally and compute means, percentages and other statistics. StataCorp, 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC. Software was used to perform statistical analysis. Statistical significance was set at $p < 0.05$. Sample size = all the patients treated with autogenous fat injections for OSMF.

Patient details recorded as part of this study did not include identifiable information such as their name, address or date of birth. Their file numbers were recorded as part of a data collection sheet for reference purposes only. All electronic data was saved on a password protected Excel spread sheet and computer. All physical data collection sheets is kept in a secure office. Clinical photographs are included in the study, but the patient's identity is hidden and written consent of the patient was obtained.

4.7 Ethics

Permission from the patients was obtained via informed consent (*appendix 3*). The research purpose and objectives of the study was explained to each patient by using an information sheet (*appendix 4*). Consent for clinical pictures and videos of the patients were taken as well (*appendix 5*). Confidentiality was maintained and participants had the right to withdraw from the study at any time without deprivation of their rights and future treatment. Procedures for confidentiality of data was adopted. Permission was obtained from the CEO of IALCH -Dr T Khanyile (*appendix 7*). This thesis was presented to the Faculty of Dentistry's Research Committee at the University of the Western Cape and approval was given by the Biomedical Research Ethics Committee, University of the Western Cape (*appendix 8*). Hospital permission from medical management was obtained (*appendix 9*). Provincial permission was obtained from KZN (*appendix 10*).

4.8 Declaration of Interest

The researcher has no commercial or associative interest that represents a conflict of interest in connection with the study.

Chapter 5

Results

Thirty-one patients were screened for the study, 22 were excluded out of which 19 were Pindborg stage III, two declined treatment and one patient was lost to follow up. Nine participants were included after meeting the inclusion criteria (*Table 4).*

Table 4: Exclusion/ Inclusion participants

Exclusion reason	
Pindborg Stage III	19
Declined treatment	2
Lost to follow up	1
Total excluded	**22**
Included in study	**9**
Total screened	**31**

The sample had range of 34-67 years old. Mean age was 51.67 years old with a standard deviation of 9.45. All study participants were female.

All participants were Indian from urban housing in Kwa-Zulu Natal, South Africa. Six participants were married and three were single.

History of lesions according to participants revealed a range of six months to seven years with mean of 3.1 years (standard deviation= 1.31 years).

One patient had no co-morbidities, seven had one co-morbidity and one had two co-morbidities. Five participants (56%) were diagnosed anaemics- one whom had hypertension as their second co-morbidity, one had hypothyroidism and one suffered from osteoarthritis. All were on chronic medications for their varying ailments.

Table 5: Demographic, Medical and OSMF details of Participants

Participant (*n* = 9)	**Age (years)**	**Sex**	**Residential region**	**Marital Status**	**Pindborg classification**	**History of lesions**	**Medical History (on treatment)**
1	34	Female	Phoenix, KZN, RSA	Single	Stage II b	3 years	Nil
2	51	Female	Mayville, KZN, RSA	Married	Stage II b	3 years	Anaemic
3	59	Female	Isipingo, KZN, RSA	Single	Stage II b	4 years	Anaemic
4	49	Female	Stanger, KZN, RSA	Married	Stage II b	6 months	Anaemic
5	67	Female	Isipingo, KZN, RSA	Married	Stage II b	4 years	Hypothyroidism
6	49	Female	Marianhill, KZN, RSA	Married	Stage II b	3 years	Hypertension/ Anaemic
7	52	Female	Umkomaas, KZN, RSA	Single	Stage II b	7 years	Anaemic
8	45	Female	Stanger, KZN, RSA	Married	Stage II b	4 years	Osteoarthritis
9	59	Female	Chatsworth, KZN, RSA	Married	Stage II b	4 years	Anaemic
Mean (SD)	51.67 (9.45)					3.1 (1.31)	
Minimum	34					6 months	
Maximum	67					7 years	

Two participants showed a presence of restricted tongue movements and seven showed an absence of restricted tongue movements at T0. There were no changes at six-month (T1) follow-up. No complications were noted by any of the participants or clinician. No other treatments were given besides the intralesional fat injection and all participants stopped the habit of chewing areca nut prior to the study.

Table 6: Presence or absence of restricted tongue movements before (T0) and after treatment (T1) with net change / complications encountered (T1) and any other forms of treatment for OSMF (besides AFG)/ cessation of habit

Participants (*n*=9)	**Restricted tongue movements (T0)**	**Restricted tongue movements (T1)**	**Net change**	**Complications encountered from procedure (T1)**	**Any other forms of treatment for OSMF (besides AFG)**	**Cessation of habit**
1	Present	Present	Nil	Nil	Nil	Yes
2	Absent	Absent	Nil	Nil	Nil	Yes
3	Present	Present	Nil	Nil	Nil	Yes
4	Absent	Absent	Nil	Nil	Nil	Yes
5	Absent	Absent	Nil	Nil	Nil	Yes
6	Absent	Absent	Nil	Nil	Nil	Yes
7	Absent	Absent	Nil	Nil	Nil	Yes
8	Absent	Absent	Nil	Nil	Nil	Yes
9	Absent	Absent	Nil	Nil	Nil	Yes

Mouth opening Data

Before treatment (T0) there was mean of 17.33mm (standard deviation= 3.87mm) interincisal mouth opening. Six participants were within one standard deviation away and three were within two standard deviations away. There was a mouth opening range of 13mm to 24mm. The 95% confidence interval was 14.36-20.31mm, (*Table 7*).

After treatment (T1) there was a mean of 24.77mm (standard deviation= 5.26mm) interincisal mouth opening. Six participants were within one standard deviation away and three were within two standard deviations. There was a mouth opening range of 18mm to 33mm. One participant increased between zero to five mm, six increased between five to ten mm and two increased between ten to 15mm. All participants had an increase in interincisal mouth opening. The 95% confidence interval was 20.73-28.82mm, (*Table 7*).

The net difference (T1-T0) mean increase in mouth opening was 7.44mm (standard deviation= 3.28mm). A range of five to 15mm increase in mouth opening was observed. There were seven participants within one standard deviation and two were within two standard deviations. The range of mouth opening had increased from 13mm-24mm to 18mm-33mm showing improvement in mouth opening. The 95% confidence interval was 4.92-9.97mm, (*Table 7*).

Zero participants increased by 0-25%, seven increased by 25-50%, one increased by 50-75% and one by 75-100%. A net difference percentage mean in mouth opening of 44% (standard deviation= 20%) was established. Eight participants were within one standard deviation from the mean and one was within two standard deviations. The range in net difference increase in mouth opening was 38-83%, (*Table 7*).

Table 7: Inter-incisal mouth opening (mm) before (T0) and at six month follow up (T1) with percentage difference and summary statistics

Participants (*n*=9)	**T0**	**T1**	**Difference (mm) (T1-T0)**	**Difference (%)**
1	13	18	5	39
2	18	33	15	83
3	15	25	10	67
4	15	22	7	47
5	24	30	6	25
6	15	22	7	46
7	22	29	7	31
8	20	26	6	30
9	14	18	4	28
Mean (SD)	17.33 (3.87)	24.77 (5.26)	7.44 (3.28)	44 (20)
95% Confidence Interval	14.36-20.31	20.73-28.82	4.92-9.97	29-59
Minimum	13	18	5	38
Maximum	24	33	15	83

Figure 6: Interincisal mouth opening (mm) at T0 and T1 and net difference in mm

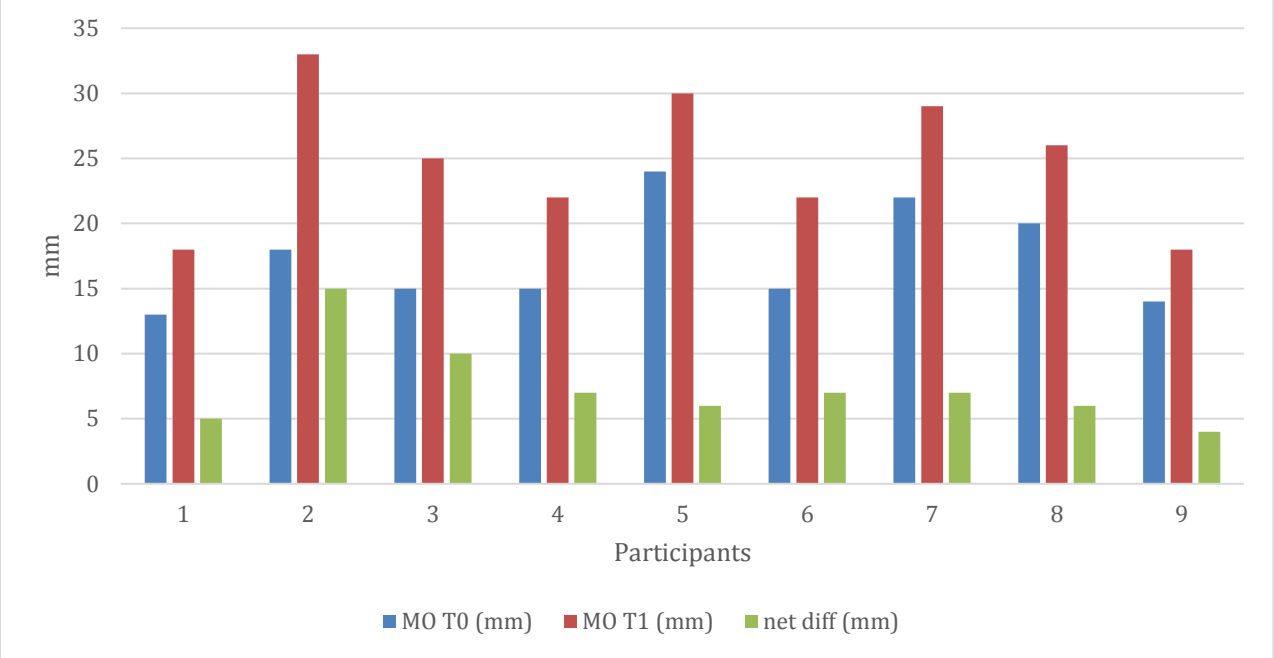


Figure 7- Percentage increase in interincisal mouth opening in participants

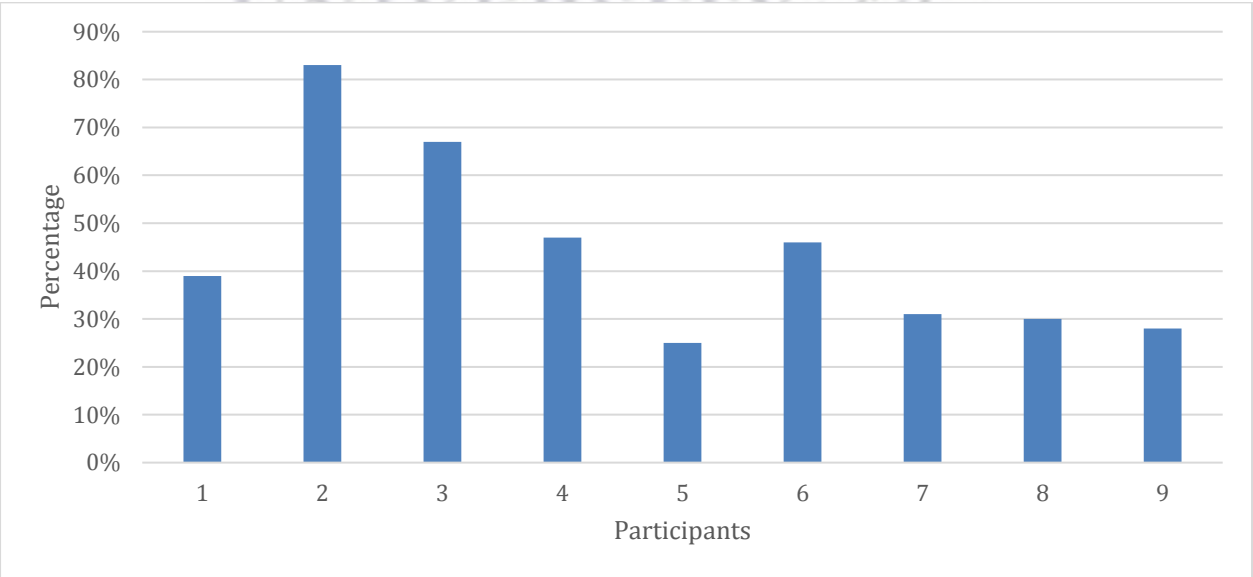


Figure 8: Box and whisker graph of interincisal mouth opening (mm) before treatment (T0) and at six month follow up (T1)

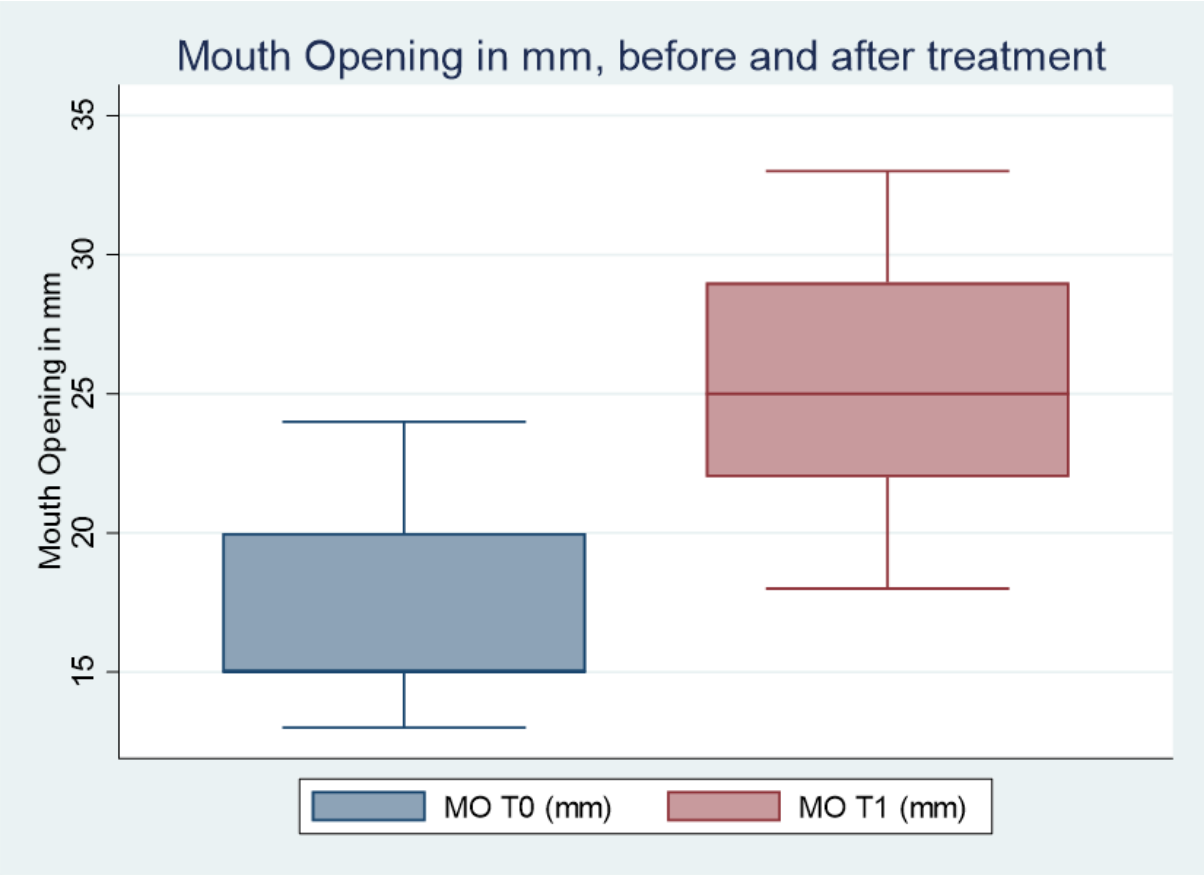
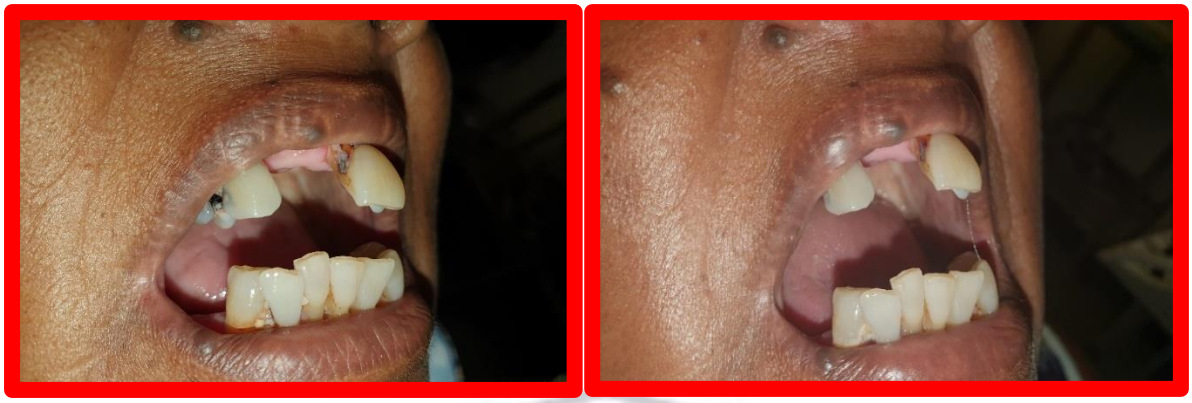


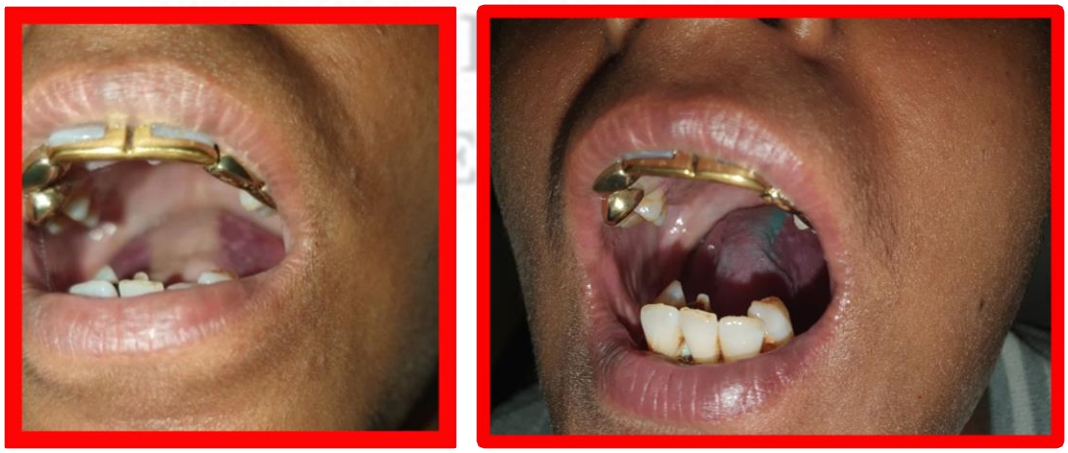
Figure 9: Picture showing T0 and T1 mouth opening



T0 **T1**

Fig. 9. Clinical pictures to illustrate interincisal mouth opening before treatment (T0) and at six month follow up (T1).

Figure 10: Pictures showing mouth opening at T0 and T1



TO **T1**

Fig 10. Clinical pictures to illustrate interincisal mouth opening before treatment (T0) and at six month follow up (T1).

VAS Pain score

Before treatment (T0) there was mean of 8.67 (standard deviation= 1.41 points). The 95% confidence interval was 7.60-9.75 points. Seven participants were within one standard deviation away and one within two standard deviations. There was a VAS range of six to ten points at T0, (*Table 8*).

At six month follow up (T1) there was a mean of 1.33 (standard deviation= 1.41 points). The 95% confidence interval was 0.25-2.42 points. Seven participants were within one standard deviation away and one within two standard deviations away. All study participants had a decrease in VAS. The VAS range had improved from prior to treatment (T0) six to ten points to zero to four points (T1) showing a decrease in pain experienced by the participants. The VAS range at six month follow up (T1) was zero to four points, (*Table 8*).

The net difference (T1-T0) mean decrease in VAS was 7.33 points (standard deviation= two points). The 95% confidence interval was 5.7-8.87 points. A net difference range of four to ten points was observed. There were six participants within one standard deviation and three were within two standard deviations, (*Table 8*).

One participant had a net decrease of pain between 25-50% at six month follow up, whilst three recorded a decrease of pain between 50-75% and five recorded a decrease of 75%-100% on the VAS pain scale, (*Table 8*).

Table 8: VAS pain score before treatment (T0) and at six month follow up (T1) with summary statistics

Participants	**T0**	**T1**	**Difference (decrease) (T1- T0)**	**Difference (decrease) (%)**
1	10	2	8	80
2	10	0	10	100
3	8	0	8	80
4	10	4	6	60
5	8	2	6	60
6	8	0	8	80
7	8	2	6	60
8	10	0	10	100
9	6	2	4	40
Mean (SD)	8.67 (1.41)	1.33 (1.41)	-7.33 (2)	-73.33 (20)
95% Confidence Interval	7.60-9.75	0.25-2.42	5.7-8.87	5.80-8.87
Median	8 [8 to 10]	2 [0 to 2]	-8 [-8 to -6]	-80 [-80 to -60]
Minimum	6	0	-4	-40
Maximum	10	4	-10	-100

(-) indicates decrease

Figure 11:VAS Pain scale decrease per participant between T0 and T1

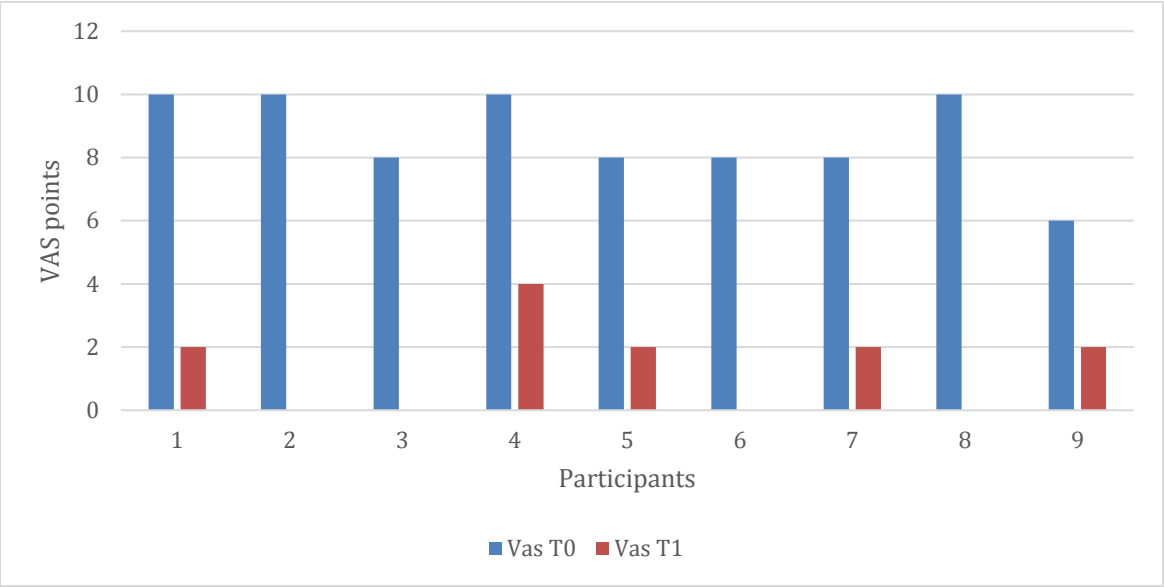


Figure 12: Percentage Decrease in VAS pain scores between T0 and T1

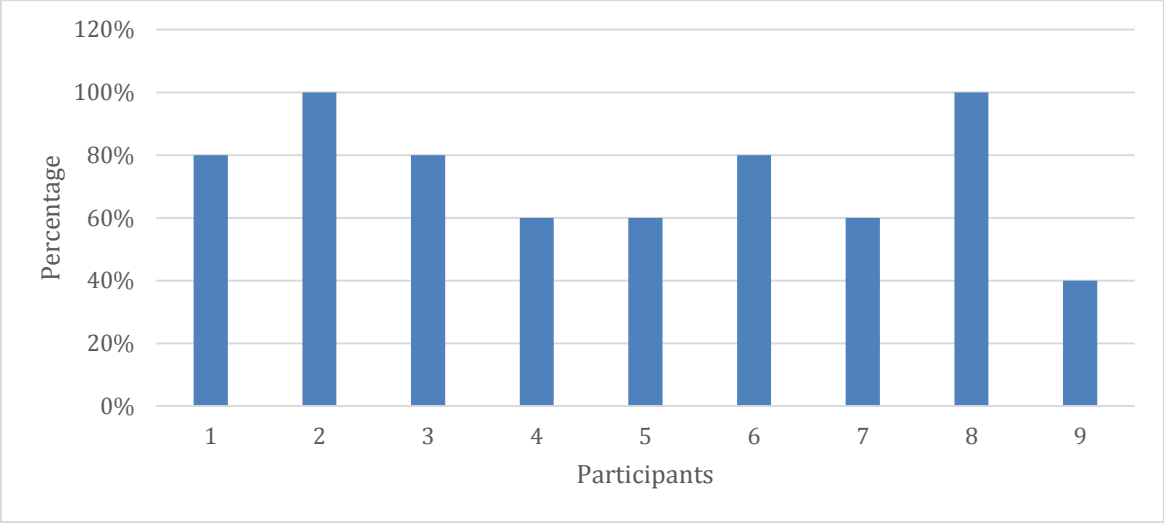
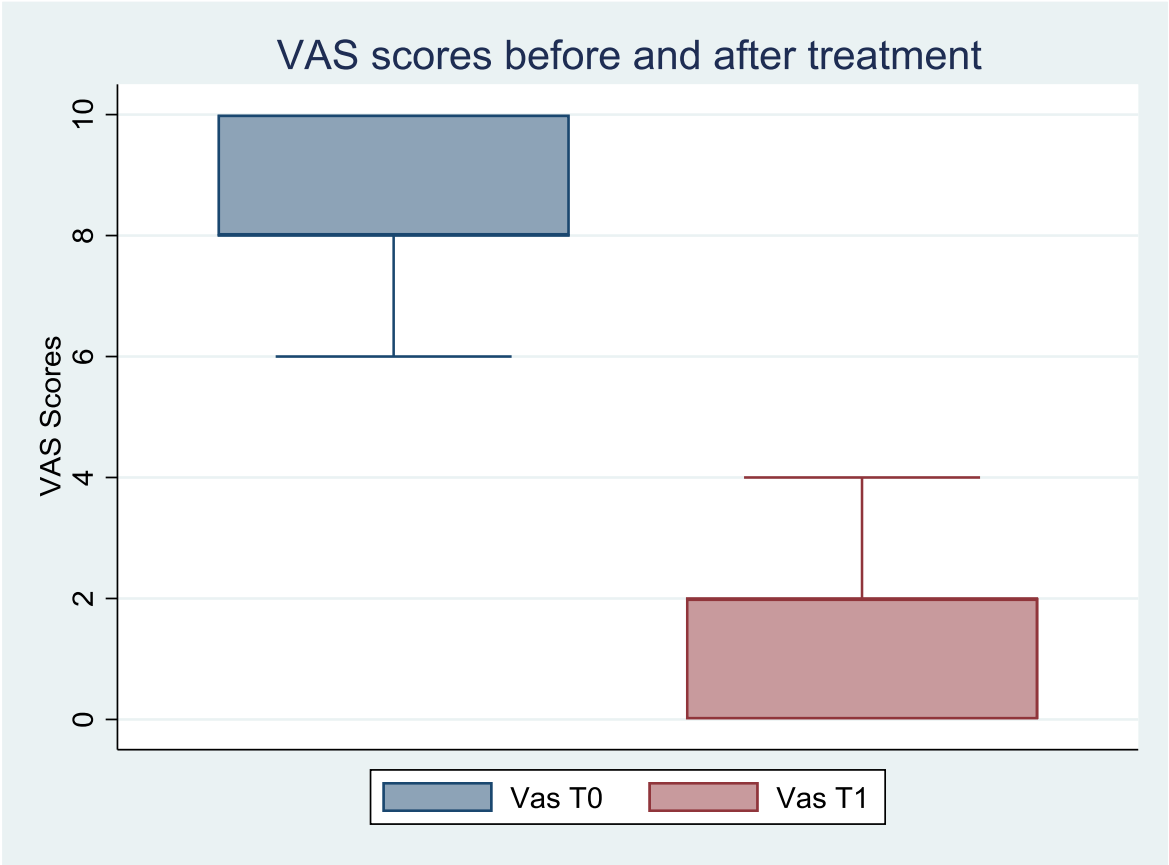


Figure 13: Box and Whisker graph of VAS scores before (T0) and after treatment (T1)



OPMDS QoL Questionnaire

There were 20 questions which were designed holistically to look at the quality of life of the participants. There were five possible answers for each question and each group of questions assessed a specific domain. The four domains are: DD- Difficulties with diagnosis/ PIF- Physical impairment and functional limitations/ PSB- Psychological and social wellbeing/ TRE- Effect of treatment on daily life. Nineteen of the questions regarded 'very much' as the negative sentiment with the exception of one question which had 'very much' as a positive sentiment. Each question and answer were given a numerical value one to five and these numbers were interpreted. The overall oral health related quality of life (OPMDsQoL) score was determined by adding the four domain scores together.

DD - Difficulties with diagnosis, (*Table 9*).

Before treatment (T0) there was a mean of 12.78 (standard deviation= 2.59 points). The 95% confidence interval was 10.79-14.77 points and a range of eight to 15 points. Seven participants were within one standard deviation away and two were within two standard deviations.

At six month follow up (T1) there was a mean of 11.67 (standard deviation= 2.55 points). The 95% confidence interval was 9.7-13.62 points and a range of eight to 15 points. Six participants were within one standard deviation and three were within two standard deviations.

PIF- Physical impairment and functional limitations, (*Table 9*).

Before treatment (T0) there was mean of 30.11 points (standard deviation= 7.11 points). The 95% confidence interval was 26.64-33.58 points. Seven participants were within one standard deviation and two were within two standard deviations away. There was a range of 20-35 points scored.

At six month follow up (T1) there was a mean of 12.33 points scored (standard deviation= 4.74 points). The 95% confidence interval was 6.87-17.80 points. There was a range of seven to 26 points scored. Seven participants were within one standard deviation away from the mean and two were within two standard deviations.

PSB- Psychological and social wellbeing, (*Table 9*).

Before treatment (T0) there was mean of 31.67 points (standard deviation= 4.74 points). The 95% confidence interval was 28.02-35.31 points. Eight participants were one standard deviation away with one within two standard deviations. There was a range of 20-35 points scored.

At six month follow up (T1) there was a mean of 16.78 (standard deviation= 6.48 points). The 95% confidence interval was 11.80-21.76 points. There was a range of seven to 30 points scored. Seven participants were within one standard deviation away from the mean (T1), one was within two standard deviations and one which most likely an outlier was within three standard deviations.

TRE- Effect of treatment of daily life, (*Table 9*).

TRE- Before treatment (T0) there was mean of 12.22 points (standard deviation= 1.78 points). The 95% confidence interval was 10.85-13.59 points. Seven patients were within one standard deviation away with two within two standard deviations. There was a range of nine to 15 points scored.

At six month follow up (T1) there was a mean of 4.11 points (standard deviation= 1.27 points). The 95% confidence interval was 3.13-5.09 points. There was a range of three-seven points scored. Eight participants were within one standard deviation away from the mean, one was within two standard deviations.

Table 9 : Individual participant scores across the domains before treatment (T0) and at six month follow up (T1), with summary statistics

Participants	**DD T0**	**DD T1**	**PIF T0**	**PIF T1**	**PSB T0**	**PSB T1**	**TRE T0**	**TRE T1**
1	13	10	32	9	35	13	12	4
2	15	14	35	7	34	12	15	3
3	15	12	30	8	33	17	14	3
4	10	9	34	23	35	17	11	5
5	8	8	27	12	32	22	11	4
6	11	11	20	9	20	16	9	4
7	13	11	30	10	30	17	12	4
8	15	15	33	7	31	7	13	3
9	15	15	30	26	35	30	13	7
Mean (SD)	12.78 (2.59)	11.67 (2.55)	30.11 (4.51)	12.33 (7.11)	31.67 (4.74)	16.78 (6.48)	12.22 (1.78)	4.11 (1.27)
95% Confidence Interval	10.79-14.77	9.7-13.62	26.64-33.58	6.87-17.80	28.02-35.31	11.80-21.76	10.85-13.59	3.13-5.09
Minimum	8	8	20	7	20	7	9	3
Maximum	15	15	35	26	35	30	15	7

DD- Difficulties in diagnosis, (*Table 10*).

There was a net difference decrease mean (T0-T1) score of 1.11 points (standard deviation= 1.27 points) and a net difference range of 0-3 points decrease. The 95% confidence interval was 0.1-2.09 points. Seven participants were within one standard deviation and two were within two standard deviations. The range before treatment (T0) and at six month follow up (T1) remained the same at eight to 15 points.

PIF- Physical impairment and functional limitations, (*Table 10*).

The net difference decrease in mean was 17.78 points (standard deviation= 8 points) and a range of 4-28 points decrease. The 95% confidence interval was 11.63-23.92 points. There were six participants within one standard deviation and three were within 2 standard deviations.

PSB - Psychological and social wellbeing, (*Table 10*).

The net difference decrease in mean was 14.89 points (standard deviation= 7.41 points) and a range of four to 24 points decrease. The 95% confidence interval was 9.20-20.58 points. There were six participants within one standard deviation and three were within two standard deviations.

TRE- Effect of treatment of daily life, (*Table 10*).

The net difference decrease in mean was 8.11 points (standard deviation= 2.42 points) and a range of five to 12 points decrease. The 95% confidence interval was 6.25-9.97 points. There were seven participants within one standard deviation and two were within two standard deviations.

Table 10: Difference between DD/PIF/PSB/TRE amongst all the participants

Variable	Observed	Mean	Standard Deviation	95% Confidence Interval	Minimum	Maximum
Difference in DD (T1-T0)	9	-1.11	1.27	0.1-2.09	0	-3
Difference in PIF (T1-T0)	9	-17.78	8	11.63-23.92	-4	-28
Difference in PSB (T1-T0)	9	-14.89	7.41	9.20-20.58	-4	-24
Difference in TRE (T1-T0)	9	-8.11	2.42	6.25-9.97	-5	-12

(- indicates decrease)

Total OPMDsQoL scores, (*Table 11*).

Before treatment (T0) there was mean of 86.78 (standard deviation= 11.61 points). The 95% confidence interval was 77.86-95.70. Seven patients were within one standard deviation away with two within two standard deviations. There was a range of 60-99 points scored, *Table* 11. At six month follow up (T1) there was a mean of 44.89 (standard deviation= 13.97 points). 95% confidence interval= 34.15-55.62 points. There was a range of 32-78 points scored. Eight participants were within one standard deviation away from the mean (T1), one was within three standard deviations, a suspected outlier. The mean (T1) 44.89 points (standard deviation = 17.35), *Table* 11. The net difference mean (T1-T0) was 41.89 points (standard deviation=17.35 points). The 95% confidence interval was 28.54-55.23 points. A range of 15-63 points decrease. There were six participants within one standard deviation and three were within two standard deviations.

Table 11: Individual total OPMDsQoL score before (T0) and after (T1) treatment with summary statistics. (-) indicates decrease

Participants	**OPMDsQoL T0**	**OPMDsQoL T1**	**Difference (T1-T0)**
1	92	36	-56
2	99	36	-63
3	92	54	-52
4	90	54	-36
5	78	46	-32
6	60	40	-20
7	85	42	-43
8	92	32	-60
9	93	78	-15
Mean (SD)	86.78 (11.61)	44.89 (13.97)	-41.98 (17.35)
95% Confidence interval	77.86-95.70	34.15-55.62	-(28.54-55.23)
Minimum	60	32	-15
Maximum	99	78	-63

Figure 14: Box and whisker showing OPMDsQoL scores before treatment (T0) and at six month follow up (T1)

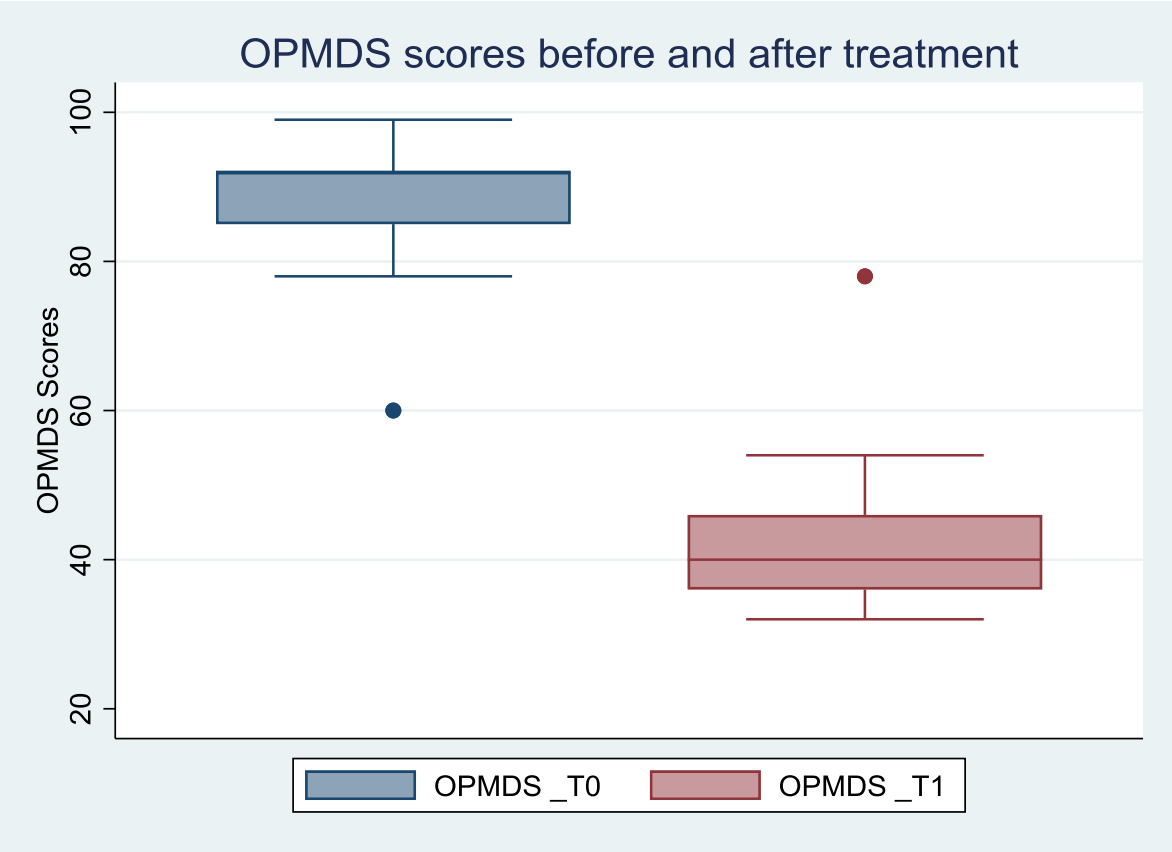
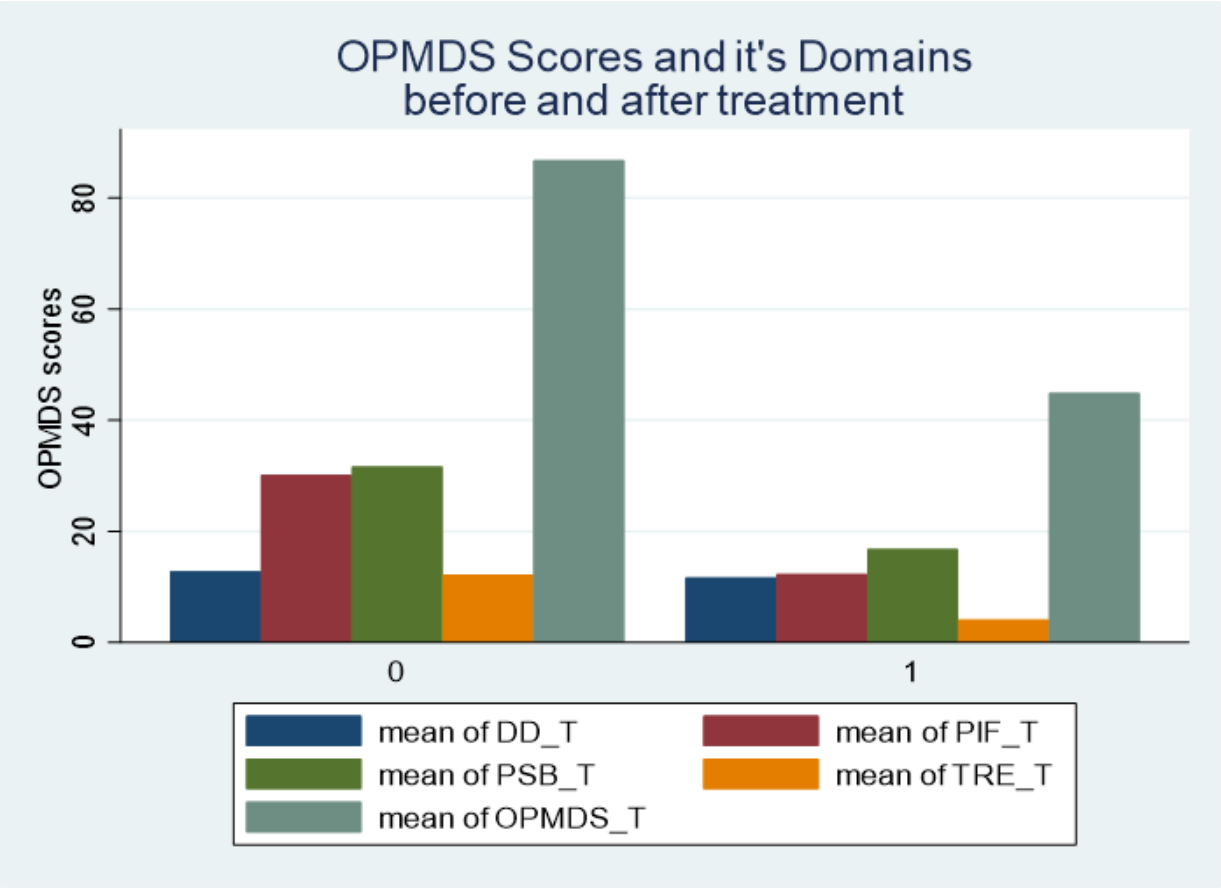


Figure 15: Bar graphs showing total OPMDsQoL Scores and its domains at T0 and T1



P-Values, (*Table 12*).

The difference between interincisal mouth opening at six month follow up (T1), 24.78mm (SD 5.26mm) was statistically significantly different from before treatment (T0), 17.33mm (SD 3.87mm), $p = 0.0001$.

The difference between VAS pain score at T1, 1.33 (SD 1.41) was statistically significantly different from T0, 8.67 (SD 3.87), $p = 0.0001$.

The difference between DD at T1, 11.67 (SD 2.54 points) was statistically significantly different from T0, 12.78 (SD 2.58 points), $p = 0.0304$.

The difference between PIF at T1, 12.33 points (SD 7.11 points) was statistically significantly different from T0, 30.11 points (SD 4.51 points), $p = 0.0002$.

The difference between PSB at T1, 16.78 points (SD 6.48 points) was statistically significantly different from T0, 31.67 points (SD 4.74 points), $p = 0.0003$.

The difference between TRE at T1, 4.11 (SD 1.27 points) was statistically significantly different from T0, 12.22 (SD 1.78 points), $p = < 0.0001$.

The difference between OPMDsQoL (Total) at T1, 44.88 points (SD 13.97 points) was statistically significantly different from T0, 86.78 points (SD 11.61 points), $p = 0.0001$.

Table 12 :P values (set at P<0.05 for statistical significance)

Variable	**T0 Mean (SD)**	**T1 Mean (SD)**	***p*- value (<0.05= statistically significant)**
Mouth opening (mm)	17.33 (3.87)	24.78 (5.26)	*p* = 0.0001
VAS (points)	8.67 (1.41)	1.33 (1.41)	*p* = <0.0001
OPMDSQoL -total (points)	86.78 (11.61)	44.89 (13.97)	*p* = 0.0001
Domain DD (points)	12.78 (2.59)	11.67 (2.55)	*p* = 0.03
Domain PIF (points)	30.11 (4.51)	12.33 (7.11)	*p* = 0.0002
Domain PSB (points)	31.67 (4.74)	16.78 (6.48)	*p* = 0.0003
Domain TRE (points)	12.22 (1.79)	4.11 (1.27)	*p* = <0.0001

Pearson's Correlation, (*Table 13*).

There is a moderate negative correlation between VAS percentage difference and net difference in mouth opening, r = -0.5199 (p = 0.1514).

There is a moderate positive correlation between VAS percentage difference and difference in OPMDsQoL, r = 0.4776 (p = 0.1935).

There is a moderate negative correlation between net difference in mouth opening and difference in OPMDsQoL, r = -0.4683 (p = 0.2036).

Table 13: Pearson's Correlation (r- value) and p- value when correlating mouth opening/Vas and OPMDsQoL scores

	Decrease in MO	**VAS % decrease**	**Difference in OPMDsQoL score**
Decrease in MO	1		
VAS % decrease	r = -0.52 / p = 0.1514	1	
Difference in OPMDsQoL score	r = -0.47 / p = 0.2036	r = 0.48 / p = 0.1935	1

Chapter 6

Discussion

Oral submucous fibrosis may in some cases be a challenge to treat. It has a profound effect on the oral cavity and neighbouring tissues. According to Angadi in 2010, OSMF may become severe and can sometimes leave the patient with grossly decreased mouth opening. Grossly decreased mouth opening impacts the patient's oral health and quality of life. Besides effecting oral tissues, the ensuing reduced mouth opening makes dental treatment difficult due to lack of mouth opening. OSMF has a progressive nature and carries a certain risk of malignant transformation, which provides the treating clinician the task of addressing the patient's current main complaint and necessitating the foresight to identify potential complications in the future.

According to Namboodiripad in 2014, the damage to soft tissue is not only limited to the oral cavity, it may affect the mucosal lining of the upper digestive tract involving the oral cavity, oropharynx and hypopharynx and the upper third of the oesophagus. To prevent further worsening of the condition that is initially presented to the clinician, cessation of the habit and offering a treatment that prevents progression of disease process is important.

When constructing treatment plans and techniques to combat OSMF, local geographical factors such as health infrastructure, staffing expertise, cost effectiveness and availability of certain medications must be taken into consideration.

When reviewing the literature, it is evident that there are various different treatment regimens ranging from surgical to non-surgical options with or without adjunctive therapies. Injectable medications suggested are in conjunction with or without other medications and are not well defined in large randomised control trials, but rather as single isolated studies.

In the case of surgical treatments as seen in the study by Kamath in 2015 who did a meta-analysis and reviewed 56 articles (total of 995 cases surgically treated). He found that there were very few controlled clinical trials, most being random trials on surgical procedures in small groups of patients with short follow ups. In his conclusion he stated, there exist no definite protocols for the adoption of a particular treatment mode in OSMF, indicating the need for definitive protocols and controlled clinical trials to be done.

This trend is further corroborated by Gupta *et al*. in 2021 who did a systematic review of laser treatments in OSMF treatments with 20 articles and 250 patients treated (1952-2019). In their conclusion they had found that large scale studies are required to investigate the efficacy and other effects of this treatment.

Laser treatment as a treatment modality for OSMF is worth mentioning as it is being increasingly utilized for the treatment of a variety of pathologies, as interest in less invasive treatment modalities intensifies as suggested by Azadgoli *et al*. in 2016.

According to Gupta *et al*. in 2021 it was found that one of the major limitations of their study was the lack of an exact scientific basis of selection and applied criteria in using lasers for the management of OSMF. In the studies, only few specific lasers have been used, although a variety of other types are also available. The use of lasers in OSMF is a developing field.

It was suggested by Gupta *et al*. in 2021 that in premalignant conditions such as OSMF, lasers can be used as a reliable, reproducible method preventing further morbidity. The use of laser therapy has proven to provide effective long-term results in the treatment of all stages of OSMF without any complications and defects that are usually demonstrated during conventional surgeries. However also according to Gupta *et al*. in 2021, some of the disadvantages of lasers such as a high cost of maintenance, a long time for re-epithelialisation and delayed wound healing would not be favourable in treatment planning in certain clinics. These disadvantages mentioned are not found when using intralesional autogenous fat injection in OSMF, as found in this study.

Invasive and current surgical options as outlined by Kamath in 2015 such as flaps and grafts come with their own advantages and disadvantages, dependent on the type of flap or graft in use. They are generally highly dependent on skilled surgeons, high costs, prolonged theatre time, well equipped hospitals, general anaesthesia and have a certain degree of associated morbidity and mortality rates attached to them. In geographical regions, as seen in certain regions of South Africa and in under developed countries, there are constraints such as theatre time/ infrastructure, lack of highly specialised surgeons and busy theatre lists. There is a need for minimally invasive, effective and efficient theatre usage and cost-effective alternative therapies.

Unlike contemporary OSMF treatments where the treatment is focussed on either removal of the fibrosis or changing the micro-cellular environment, intralesional autogenous fat injections has both elements of treatment to it.

The aim of this study was to establish the effectiveness of intralesional autogenous fat injections in patients with oral submucous fibrosis.

The objectives were to record the demographic details and medical information of the patients and evaluate the inter-incisal mouth opening (mm), assess the presence or absence of restricted tongue movements, record pain of patients (Visual Analogue Scale scores), to record quality of life via a patient derived questionnaire (OPMDsQoL) prior to treatment and six month post-operatively.

6.1 Data interpretation

6.1.1 Demographic/medical/ OSMF related data

All participants had exhibited Pindborg stage II b OSMF, (*Table 5*). Interestingly out of the total study population, 19 were Pindborg stage III and nine were stage II b (*Table 4*), indicating a delay of presentation of the patients. History of when the participants noticed the lesions in the oral cavity showed there was a mean of 3.1 years (SD= 1.31 years) and a range of six months to seven years, therefore also indicating a degree of delay in presentation. No other treatments besides intralesional autogenous fat injections were given to these participants.

The sample size had range of 34-67 years old. Mean age was 51.67 years old with a standard deviation of 9.45, the mean age roughly in keeping with what Seedat *et al*. in 1988 found in Durban with majority of sufferers with signs of OSMF were between 45-54 years old. All study participants were female also in keeping with Seedat *et al*. in 1998 findings, that established OSMF had a ratio of 70:1 female to male and that OSMF was more commonly found in women in Durban, which is seen by the female only participant group in this study.

One patient had no co-morbidities, seven had one co-morbidity and one had two co-morbidities. Five participants (56%) were diagnosed anaemics- one whom had hypertension as their second co-morbidity, one had hypothyroidism and one suffered from osteoarthritis. All were on chronic medications for their varying ailments, (*Table 5*). Fifty-six percent of the

participants in this study were diagnosed anaemics, this may be in keeping with what was proposed by Sun *et al*. in 2015, that there may be a relationship between anaemia, haematinic deficiencies and OSMF, their findings recorded various anaemias within a study group. Gupta in 2015 suggested that decreased iron levels may be due to utilization in the fibrosis process.

Therefore, indicating that OSMF patients and their disease is not limited to the oral cavity and patients must be reviewed holistically. Treatment planning may be centered on the dental and facial region, however the patient's medical status needs to be taken into account.

All participants were Indian from urban housing in KZN, South Africa and six participants were married and three were single, (*Table 5*). Nil complications from the procedure were noted by the participants or clinician, (*Table 6*). No other adjunctive treatments were given besides the fat injection and all participants stopped the habit of chewing areca nut prior to the study, (*Table 6*).

Two participants showed a presence of restricted tongue movements and seven showed an absence of restricted tongue movements at T0. There were no changes at six-month (T1) follow-up, (*Table 6*). Indicating that in this study treatment did not affect tongue movements.

6.1.2 Mouth Opening (MO) data

Mouth opening mentioned in this study is a measurement of interincisal distance with the use of a calliper measuring in millimetres.

Figure 16: Calliper for mouth opening measurement (comes in varying sizes)

When measuring mouth opening, using a standardised tool for recording is important as it helps to prevent erroneous recordings. Using fingers and rulers maybe ineffective and may lead to inaccurate readings when recording inter-incisal distance.

The participants in this study all presented with restricted mouth opening, (*Table 7*). Restricted mouth opening is a general term given when mouth opening is impaired due to any cause. Interincisal mouth opening as a measurement allows for an objective review of the treatment.

It is difficult to compare the mouth opening results in this study directly to another study of this nature as there is no other study available that has this protocol. However, there are other minimally invasive treatment strategies that may be reviewed in the treatment of OSMF such as:

Physiotherapy as a treatment option was suggested by Cox *et al*. in 2009 whereby physiotherapy had improved oral opening ($p < 0.0005$). Intralesional autogenous fat injection in OSMF had a *p*-value = 0.0001 for mouth opening between T0 and T1, indicating when using intralesional autogenous fat injections findings were more statistically significant than physiotherapy. Interestingly the study done by Cox *et al*. in 2009 had found no clear improvement in the other groups receiving intralesional hyaluronidase/ steroid injections and a group receiving no active treatment which contradicts James *et al*. in 2015 findings.

In a retrospective study done by James *et al*. in 2015, 28 patients with OSMF had intralesional injections of dexamethasone 1.5ml, hyaluronidase 1500 IU with 0.5 ml lignocaine hydrochloride injected intralesionally twice a week for 4 weeks. They found a mean increase of 6mm (SD= 2mm), range of four to eight millimetres in mouth opening. Intralesional autogenous fat injection in OSMF had found a mean increase of 7.44mm (SD= 3.28mm) and a range increase of five to 15 mm in mouth opening. Which indicated a higher mean increase and range increase when using intralesional autogenous fat injections as compared to the study conducted by James *et al*. in 2015.

In a randomised control study by Kumar in 2007 where 21 patients, were exposed to 16mg oral lycopene they found a mean increase= 3.4mm in mouth opening, in another group (19 patients) exposed to 16 mg oral lycopene and twice a week intralesional steroid injections a mean increase= 4.6mm and the placebo group (18 patients) a mean of zero millimetres. Groups were reviewed over two months. Intralesional autogenous fat injections had found a

mean increase of 7.44mm (SD = 3.28mm) indicating a higher mean increase when using intralesional autogenous fat injections in mouth opening than all three groups.

In a randomised clinical trial by Jiang *et al*. in 2015 where triamcinolone acetonide (TA) or allicin was injected intralesionally weekly for 16 weeks, they found TA had a mean increase = 2.7mm (SD = 1.04mm) in mouth opening. The allicin group had a mean increase = 4.33mm (SD = 9.82mm) in mouth opening. Intralesional autogenous fat injections had found a mean increase of 7.44mm (SD = 3.28mm) in mouth opening, indicating a higher mean increase when using intralesional autogenous fat injections than the TA and allicin groups in the study by Jiang *et al*. in 2015.

In an open uncontrolled study by Haque *et al*. in 2001 (n = 29), interferon gamma intralesional injections had a mean increase of eight millimetres (SD= four mm), range of four to 15mm of mouth opening. Intralesional autogenous fat injections had found a mean increase of 7.44mm (SD= 3.28mm), a range increase of five to 15 mm in mouth opening. The mean increase from the Haque *et al*. in 2001 was marginally higher by 0.66 mm than intralesional autogenous fat injections and the mean range increase was relatively the same.

In an isolated abstract published by the international Journal of Oral and Maxillofacial, KO *et al*. in 2017 treated patients by injection of fat grafting by using MAFT® gun (1/30-1/240 mL), coronoidectomies and stripping off the temporalis muscle and tendon from the ramus with mouth opening exercise and postoperative acupuncture. Five patients had a mean increase of eight millimetres (SD= four mm) at six-month follow-up. There is no full published study. Intralesional autogenous fat injections had found a mean increase of 7.44mm (SD= 3.28mm) indicating that the mean increase in the study conducted by KO *et al*. in 2017 was marginally higher by 0.66 mm than using intralesional autogenous fat injections.

Mouth opening (net difference between T1 and T0) exhibited a moderate negative correlation with VAS percentage, r = -0.5199. Indicating that as mouth opening improved, pain score decreased. This however was not statistically significant (p = 0.1514), (*Table 13*).

There was also a moderate negative correlation between net difference in mouth opening and difference in OPMDsQoL, r = -0.4683, suggesting that as mouth opening improved, QoL improved as well, however this was not statistically significant (p = 0.2036), (*Table 13*).

6.1.3 Visual analogue scale (VAS) pain score

A visual analogue pain scale was used with scores zero (no pain) to ten (worst possible pain). Pain is subjective and is generally difficult to quantify, therefore a standardised tool for recording was used in this study. OSMF has a unique presentation of burning mouth, burning mouth was to be considered apart of overall pain experienced by the participant. The burning sensation/ pain experienced, becomes a separate entity apart from the reduced mouth opening requiring treatment in OSMF patients.

Seven participants saw more than 60% (six-point) reduction in pain. Four participants saw a decline to zero on the VAS pain scale including two who noted a ten (100%) point decline on their VAS score, (*Table 8*). These changes seen are extremely promising and the treatment of intralesional autogenous fat injection in this study has had a profound effect on the pain/ burning sensation experienced by the participants.

The data from this study suggests a significant decrease in pain according to the VAS scores difference between T0 and T1. The difference between VAS pain score at T1 was statistically significantly different from T0, $p = 0.0001$, (*Table 12*).

The r value= 0.4776 which indicated there is a moderate positive correlation between VAS pain scale decrease and difference in OPMDsQoL, meaning that as the participants' pain decreased their QoL increased, this was not statistically significant $p = 0.1935$, (*Table 13*).

The decrease in VAS pain scores in this study could be attributed to what Riyat *et al*. in 2017 found, that the analgesic effects are caused by nerve repair (mediated by BDNF) and scar entrapment release. They had also suggested that the injection procedure itself is responsible by making space under the scar tissue and it is hypothesised that grafts containing TGF-B play a role in immunosuppression by acting on T- cells, resulting in an analgesic effect.

When treating OSMF patients, pain/ burning sensation is generally a part of the presenting complaint and even improving mouth opening may not necessarily improve pain as seen in the study done by Cox *et al*. in 2009, whereby physiotherapy improved mouth opening but not the burning sensation experienced by patient.

6.1.4 Questionnaire - OPMDsQoL

When drawing up treatment plans for OSMF patients, QoL is always a key consideration. In essence when trying to address any functional or aesthetic concerns of a patient, it will inevitably have a reflective effect on QoL. According to Asadi-Lari *et al*. in 2004 the main goal of health care professionals is to improve the patient's QoL, all participants had an improvement in quality of life.

The 20-question questionnaire used in this study which was developed by Tadakamadla *et al*. in 2017 was invaluable, as it not only gave an overall QoL but also had four domains within it demonstrating which area of the participants' life was affected.

The other advantage of using this specific questionnaire is that the questions were made for OMPDs, which OSMF belongs to.

DD- Difficulties with diagnosis (Questions 1-3)

Four participants had no change indicating they still felt the same with difficulties of diagnosis and five had negligible change, (*Table 9*).

The difference between T0 and T1 indicated that there was a negligible change in what patients felt with difficulties of diagnosis. The range before treatment (T0) and at six month review (T1) remained the same. Four participants had no change indicating they still felt the same with difficulties of diagnosis and five had negligible change, (*Table 10*).

These three questions in the questionnaire may not be suitable when comparing treatment outcomes but is useable when reviewing the difficulties in diagnosis that a patient has experienced.

PIF- Physical impairment and functional limitations (Questions 4-10)

There were seven questions with a point scoring range of seven to 35 points. Thirty-five points being the highest possible negative sentiment and seven being the highest possible positive sentiment.

Before treatment (T0) there was a range of 20-35 points scored. The mean= 30.11 points out of a maximum of 35 points which indicates that the participants were experiencing substantial physical impairment and functional limitations. At six month follow up (T1), the mean decreased to 12.33 points, moving in the direction of the minimum of 7 points (positive

sentiment) for this domain, suggesting that the participants had experienced less physical impairment and functional limitation after treatment, (*Table 9*).

The overall net difference mean decrease of 17.78 points is suggestive that the participants had experienced less physical impairment and functional limitation after treatment, (*Table 10*).

All participants had shown they had experienced less physical impairment and functional limitation after treatment.

PSB- psychological and social wellbeing (Questions 11-17)

There were seven questions with a point scoring range of seven to 35 points. Thirty-five points being the highest possible negative sentiment and seven being the highest possible positive sentiment.

Before treatment (T0), the mean = 31.67 points out of a maximum of 35 points (negative sentiment) which indicated that the participants were experiencing substantial disturbances to psychological and social wellbeing before treatment. At six month follow up (T1), the mean decreased to 16.78 points which was moving in the direction of the minimum of seven points (positive sentiment) for this domain, suggesting that the participants had improved psychological and social wellbeing after treatment, (*Table 9*).

The overall net difference mean decrease of 14.89 points is suggestive that the participants had improved psychological and social wellbeing after treatment, (*Table 10*).

All participants showed they had improved psychological and social wellbeing after treatment.

TRE- Effect of treatment on daily life (Question 18-20)

There were three questions with a point scoring range of three to 15 points. Fifteen points being the highest possible negative sentiment and three being the highest possible positive sentiment. Question 19 scoring was reversed due to nature of question and answer.

Before treatment (T0), the mean= 12.22 points out of a maximum of 15 points (negative sentiment) which would indicate that the participants had a negative outlook on the effect of treatment on daily life. At six month follow up (T1), the mean decreased to 4.11 points which was moving in the direction of the minimum of three points (positive sentiment) for this

domain, suggesting that the participants' treatment had a positive effect on daily life, (*Table 9*).

The overall net difference mean decrease of 8.11 points is suggestive that the participants' treatment had a positive effect on daily life, (*Table 10*).

All participants showed that the treatment had a positive effect on daily life.

Complete OPMDsQoL- Oral potentially malignant disorder Quality of Life score (Questions 1-20)

There were a total of 20 questions with a point scoring range of 20 to 100 points. A hundred points being the highest possible negative sentiment and 20 being the highest possible positive sentiment. Question 19 scoring was reversed in the statistical analysis due to nature of question and answer.

Before treatment (T0), the mean was 86.78 points out of a maximum of 100 points (negative sentiment) which would indicate that the participants had an overall poor quality of life. At six month follow up (T1), the mean decreased was 44.98 points scored, which is closer in the direction of the minimum of 20 points (positive sentiment), suggesting that the participants treatment had a positive impact on their entire quality of life, covering all four domains, (*Table 11*).

The overall net difference mean decrease of 41.98 points is suggestive that the participants' treatment had a positive impact on their entire quality of life.

All participants saw an improvement in QoL scores.

6.2 Safety and complications

No complications occurred in this study, but this could be due to the sample size. Riyat *et al*. in 2017 concluded that AFG is a minimally invasive and safe approach to treating scars, a promising alternative to surgical excision.

Krastev *et al*. in 2020 found in a meta-analysis of 45 studies (3033 patients) that AFT (autologous fat transfer- lipoaspirate) appears to be a safe and effective treatment for fibrosis and scar-related conditions, thereby indicating a relatively good safety profile when using AFT.

Bucky *et al*. in 2008 suggested that autologous fat transfer has become a well-established method of soft tissue augmentation for both cosmetic and reconstructive indications. Fat naturally fulfills many of the characteristics required of a soft tissue filler. It is autogenous, non-toxic, biocompatible, easily available in most patients and potentially removable and long-lasting thereby fulfilling the needs for an ideal grafting material.

OSMF is an extremely dehabilatating disease process and if left unchecked can have a detrimental effect on the patient's life and lifestyle. When considering treatment options for OSMF, a holistic approach must be utilised and the risk of developing oral CA must be discussed with the patient at length to ensure adequate follow up. Prior to any intervention, cessation of the areca nut habit is a necessity. If intralesional autogenous fat grafting is considered for the treatment of OSMF, the earlier in the disease process the better, but moderate-severe cases may be considered.

The progressive nature of OSMF fibrotic bands and regions of fibrosis must be discussed with the patients and follow-up mouth opening measurements are important. Treatments centered around improving mouth opening aid in oral cancer surveillance. The risk of developing oral cancer must also be discussed and a timeous appearance in the clinic should any abnormal tissues appear.

The effect of these positive results may not be as appreciated in this continent as much as it may be appreciated in Asia, as the burden of this disease is greatest on the Asiatic continent.

When correlating intralesional autogenous fat injection in OSMF to the current uses of AFG in hypertrophic scars, burns and keloids it is important to note that OSMF falls in the category of OMPDs. According to Murti *et al*. in 1985 OSMF had a malignant transformation

rate of 7.6% in a long-term follow-up study of 17 years in India. Therefore, Stage III Pindborg cases would not be a suitable for AFG due to the unstable nature of the oral cavity mucosa and the fact, that lipoaspirates contain ASCs that are involved in neo angiogenesis could be detrimental.

According to Donnenberg *et al*. in 2010 the same properties that make ASCs useful for tissue healing and regeneration also create the potential for the stimulation of tumour cell growth. Therefore, in regions of occult signs of possible malignancy such as erythroplakia, severely dysplastic tissues and frank malignancy, intralesional autogenous fat injections should be avoided.

6.3 Hypothesis on the subclinical and clinical therapeutic effects of intralesional autogenous fat injection in the background of OSMF pathology

Intralesional autogenous fat injection in OSMF is a minimally invasive surgical treatment option, it mechanically releases the fibrotic bands and treats locally compromised tissue at the cellular level via the adipose tissue and adipose-derived stem cells (ASCs- cells found in adipose tissues). The target of treatment being the fibrotic bands and the local tissues. OSMF is looked within this study as a disease of fibrosis/ scar tissue with underlying subclinical and histological complications such as epithelial atrophy/ inflammation and a hypoxic micro-environment.

When reviewing key histological findings of the participants' biopsies, mixed acute and chronic inflammation with mild to moderate inflammatory cell infiltration (lymphocytes, plasma cells and neutrophils), hyperplastic stratified squamous epithelium, epithelial and skeletal atrophy (hyalinised in some) and margination of blood vessels were some of the more consistent findings through the reports. No dysplasia or malignancy was seen in any of the reports. Reports suggested a diagnosis of OSMF with clinical correlation advised. However due to the histopathology being reviewed by more than one pathologist, it has led to an inconsistent style of reporting of the findings.

In keeping with some of this study's histopathological findings, Pillai *et al*. in 1992 had found histological findings that suggested fibroblast and collagen dysfunction at the cellular level drives the fibrotic pathogenesis, OSMF subepithelial tissue reveals early signs of hyalinization, the presence of thick collagen bundles and a moderate number of fibroblasts and the infiltration of chronic inflammatory cells such as lymphocytes, eosinophils and

plasma cells. These histopathological findings allows us to correlate the effects seen in other pathologies and theorize the effects of ASCs and adipose tissue in OSMF tissues.

Fibrosis

Fibrosis can be considered as one of the hallmarks of OSMF, in essence treatments are centred around removing the fibrosis or addressing the fibrosis at a cellular level.

Fibrosis even though it is occurring submucosal in the oral cavity is still fibrosis and occurs in OSMF due to arecoline (by-product of the areca nut). Kumar *et al*. in 2010 defined fibrosis as, the deposition of collagen as part of normal wound healing. However, the term fibrosis is used more broadly to denote the excessive deposition of collagen and other ECM components. According to Tilakaratne *et al*. in 2006 current evidence implicates collagen-related genes in susceptibility and pathogenesis of OSMF.

Sankaranarayanan in 2007 suggested that stem cell therapy may stimulate resident tissue stem cells to transform into new fibroblasts, which may help in the removal of disintegrated biochemically and morphologically altered collagen fibres. This correlates the literature on abnormal collagen function in OSMF tissues and fibrosis as a general pathology. OSMF at a clinical and histopathological level is grossly characterised by fibrosis as a pathological entity. The use of adipose tissue to treat OSMF is paralleled to the therapeutic effects seen when using adipose tissue to treat scars and other fibrotic pathologies. It also brings into question the exact role of the ASC in treating or removing altered collagen fibres, which may be beneficial to OSMF treatment. In a study by Klinger *et al*. in 2008 whereby fat injections were used in severe burns they found histological changes that showed patterns of new collagen deposition, local hypervascularity and dermal hyperplasia in the context of new tissue, with high correspondence to the original.

According to Kumar *et al*. in 2010 the term scar and fibrosis are used interchangeably, but fibrosis most often indicates the deposition of collagen in chronic diseases as seen in OSMF. This semantics allow us to correlate findings of studies using AFG in varying scar treatments and understand the possible therapeutic effects of intralesional autogenous fat injections in OSMF. It is with this in mind that the success of intralesional autogenous fat injection treatment in OSMF was paralleled to AFG treatments seen in breast reconstruction/

augmentation, scleroderma, velopharyngeal incompetence, scars, ulcers, Dupuytren's disease and cosmetic procedures (Pu *et al*., 2015).

Within this current study, the action of blunt dissection via a tulip cannula and deposition of adipose tissue within the site, may create adipose bridges between the fibrosis thereby diminishing its mouth opening reducing effect. This may be mechanical and may either aid the role of adipose tissue and its constituents or be a separate pathway to treatment success.

Inflammation

According to Shahid in 2008 biopsy often reveals an inflamed and atrophied oral mucosa, with a fibrosis of the submucosa and normal skeletal muscle deeper. Inflammation being another key feature of the OSMF microenvironment.

Jeng *et al*. in 2003 established a link between the inflammatory environment found in OSMF which may favour malignant transformation. Then in theory, mild-moderate OSMF cases where the submucosa has an acute or chronic inflammatory environment, may benefit from the intralesional autogenous fat grafting. The reason for this is that ASCs in the fat injections and growth factors may contribute to diminishing inflammation, which may in turn help prevent an environment favourable for malignant transformation.

As Lee *et al*. in 2015 explained adipose tissue contains various cells such as ASCs, endothelial progenitor cells and immune cells act together for tissue repair and regeneration. Furthermore, Sultan *et al*. in 2011 stated that fat injections improved healing in various types of wounds is most likely caused by several factors in ASCs themselves and by growth factors already present in the injected fat, contributing to diminish fibrosis and inflammation and favour healing processes. This brings into question whether or not OSMF malignant transformation risk can be reversed via the role of ASCs and adipose tissue constituents.

Hypoxic micro-environment

According to Rajendren *et al*. in 2005 vascular dilatation may occur in the affected mucosa as an adaptive response to compensate for tissue ischaemia/ hypoxia. This local feature will cause a hypoxic microenvironment.

Haque *et al*. in 2004 had suggested that white adipose tissue is involved in angiogenesis making it a powerful assistant in combating a hypoxic microenvironment which is caused by a lack of localized vascularity. OSMF tissues may have a hypoxic microenvironment and this physiological role of adipose tissue would be beneficial for treating the microenvironment.

The cellular constituents of fat may in fact be of benefit in mild to moderate OSMF cases, where localised hypoxia is present, due to the ASCs inherent characteristics to allow for neo-angiogenesis, it may reverse localised hypoxia. According to Suga *et al*. in 2009 ASCs may differentiate into adipocytes, vascular endothelial cells, or other cells and release angiogenic growth factors, such as hepatocyte growth factor and VEGF especially under hypoxic conditions.

When looking at stem cell therapy as a whole, it is primarily aimed at neo-angiogenesis by releasing cytokines and growth factors. This may result in increased free radical scavenging by antioxidants (either naturally occurring or extraneous). Neo-angiogenesis may also facilitate the removal of senescent cells from the lesions by supplying a greater number of scavenging defence cells and reverse the hypoxia in the diseased tissue (Suma *et al*., 2015).

According to Rehman *et al*. in 2004 hypoxia amplifies the paracrine effects of MSCs by enhancing the secretion of certain growth factors. This would assist in preventing secondary epithelial atrophy and death of localised cells. In a study done by Kinnaird *et al*. in 2004 it was found that ASCs improved perfusion in hind limb ischaemia induced by the ligation of the femoral arteries, a function that was enhanced by hypoxic culture conditions, thereby correlating hypoxic micro-environments and ASC interactions.

Epithelial Atrophy

Rajendren in 2007 had reported that ischaemic atrophy of the overlying epithelium in OSMF is due to stromal changes, which undergoes progressive hyalinization, decrease in vascularity and cellularity, therefore epithelium becomes more prone to oral carcinogenesis and predisposed to malignant transformation. Thereby suggesting that if the ischaemic atrophy can be stopped or reversed, predisposition to malignant transformation can be stopped.

According to Si *et al*. in 2019 ASCs are effective for the treatment of severe symptoms such as atrophy. Epithelial atrophy may in fact be a consequence of the hypoxic micro-environment. Therefore, indicating that ASCs may reverse the degree of epithelial atrophy seen.

Gupta in 2015 had suggested that OSMF may become stationary or become severe with high risk of oral cancer development due to atrophic oral mucosa vulnerable to carcinogens. If ASCs can improve epithelial integrity within the oral cavity, it may have an effect on the ability of carcinogenic penetration and carcinogenesis within OSMF tissues. Leading to a decreased risk of malignant transformation within the tissues.

6.4 Suggested procedural steps when using intralesional autogenous fat injections in OSMF

Intralesional autogenous fat injection procedural protocol

Aseptic environment/Sterile conditions

General anaesthetic or local anaesthetic (with or without sedation). Sterile conditions to be maintained throughout the procedure. Harvesting site to be cleansed with an aseptic medium and consider a pre procedural rinse of chlorhexidine 0.2 % of the oral cavity.

Donor site selection

In this study only abdominal fat was used. The abdomen/ periumbilical region would be the first site of choice but may not always have the required amount of fat.

Pu *et al*. in 2015 had suggested that a variety of body areas that uniformly have abundant or excess fat are suitable as donor sites for harvest of fat grafts such as the abdomen, flanks, buttocks, medial and lateral thighs or knees. Donor sites are selected that enhance the body contour and are easily accessible in supine position.

Padoin *et al*. in 2008 suggested that although there is no evidence of a favourable donor site for harvest of fat grafts, viability of adipocytes within the fat grafts from different donor sites may be considered equal, a higher concentration of ASCs is found in the lower abdomen and inner thigh in one study.

Anaesthesia

General anaesthesia/ epidural anaesthesia or local anaesthesia (with or without sedation) is up to the experience or discretion of the operator. In this study's protocol, patients were treated under general anaesthesia with a tumescent solution of Marcaine® and saline solution to create a wet medium. According to Keck *et al*. in 2010 the tumescent solution used for donor site analgesia or haemostasis should contain the lowest concentration of lidocaine possible because its high concentration may have a detrimental effect on adipocyte function and viability. In this study the tumescent solution used was 20ml of 0.5% Marcaine® mixed with 80ml saline, 40ml of the mixed solution was used in site of harvest via fan insertion inferiorly peri-umbilical with a spinal needle.

Pu *et al*. in 2015 often used 0.01 % to 0.02% of lidocaine in ringers' lactate if the fat grafting procedure is performed under general anaesthetic and 0.004% if performed under local anaesthetic with or without sedation. The tumescent solution also contains epinephrine with a concentration of 1:1000000. Epinephrine can precipitate vasoconstriction in the donor sites, which may decrease blood loss, bruising, hematoma and the possibility of intra-arterial injection of the transplanted fat especially when injecting around periorbital areas or the face.

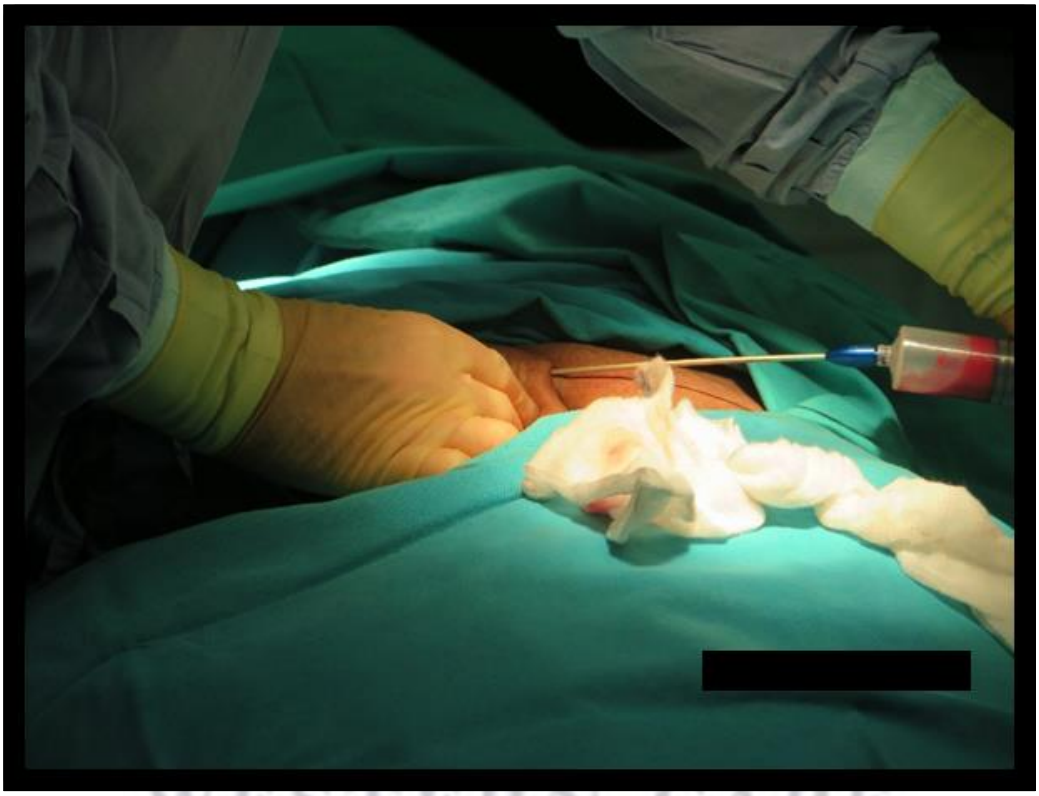


Figure 17 Harvesting of fat from abdomen via negative pressure

Negative aspiration with 20ml syringes was used in this technique. Pu *et al*. in 2008 suggested that syringe aspiration as a relatively less traumatic method for harvesting of fat grafts and should be considered as a standardized technique of choice for harvesting fat.

Figure 18 Harvested Adipose Tissue

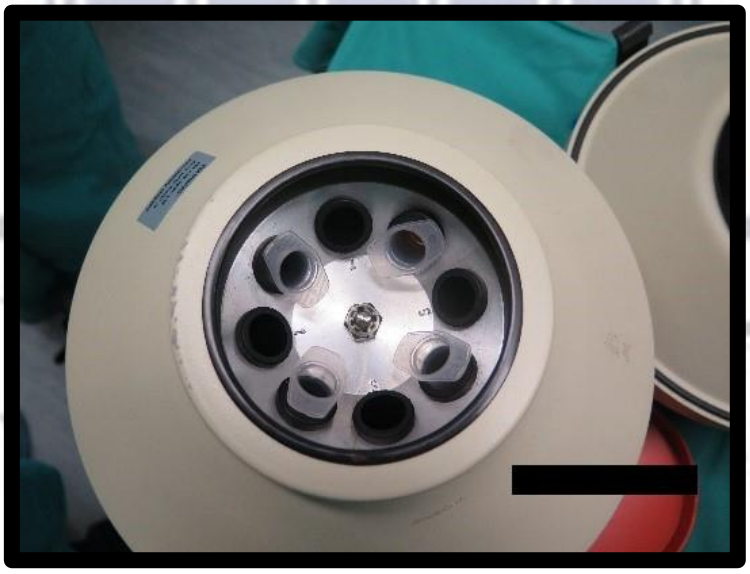


Figure 19 Centrifuge

The Coleman technique was used in this study, placement of aspirate into a centrifuge at 3000rpm for three minutes. Boscher *et al*. in 2002 makes reference to centrifugation at 3000rpm for three minutes which seems to offer more benefits for this effectively concentrating adipocytes and ASCs and should be a valid method of choice for processing fat grafts, especially for small volume fat grafting.

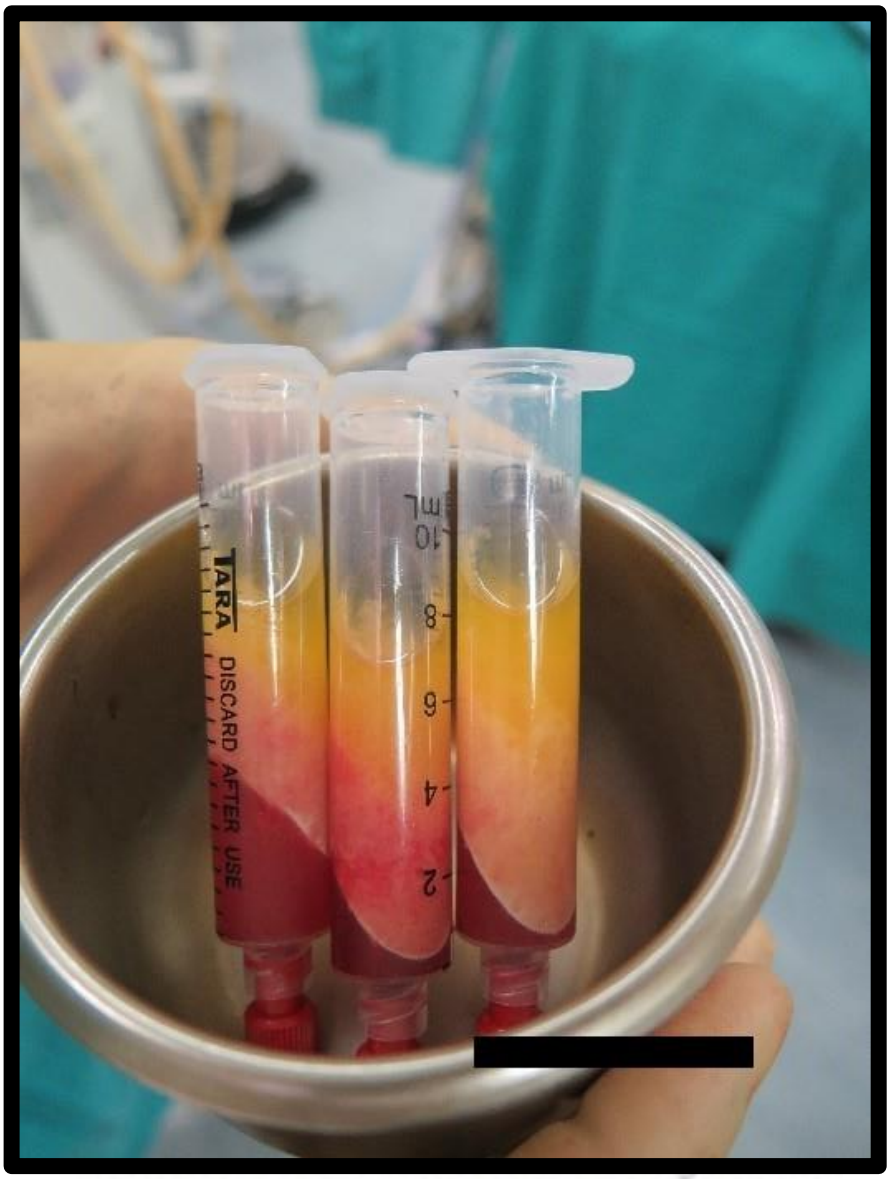


Figure 20 Post centrifugation of lipoaspirate

In this technique removal of oil content with a wicked swab, fluid portion was drained and the fatty tissue remaining was utilised. According to Pu *et al*. in 2008 attention should be made to avoid prolonged exposure of fat grafts to air and to avoid bacterial contamination.

Figure 20 shows three layers: the oil content in the upper layer, fatty tissue in the middle layer and the fluid portion at the bottom.

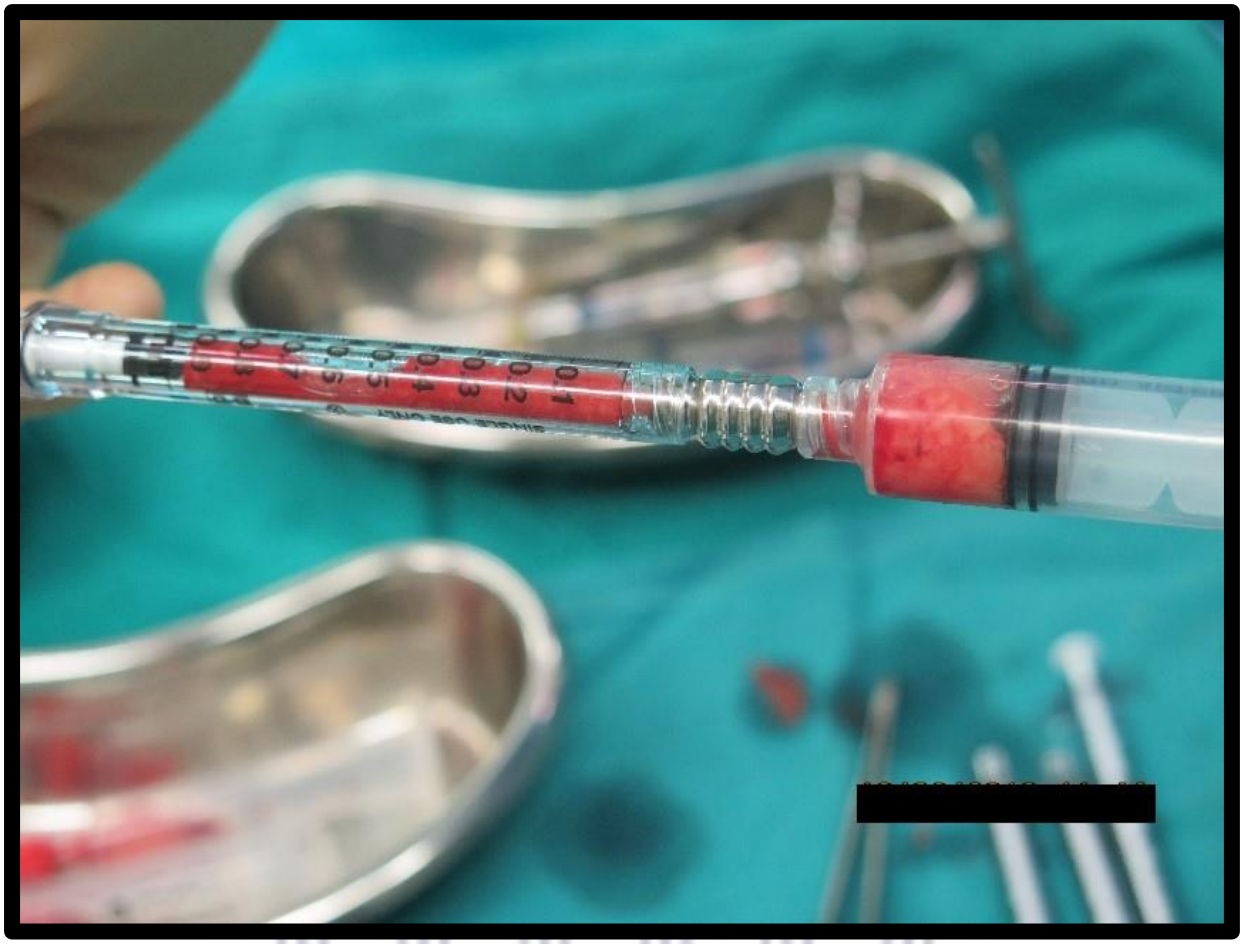


Figure 21 Transfer of lipoaspirate from large volume syringe to smaller volume syringe

Placement into ten ml syringes was used in this study. Smaller volume syringes are more desirable to allow for controlled deposition.

According to Phillips *et al*. in 2013 transfer to delivery syringe is done with an adapter carefully to avoid damage to cells within the graft. A higher content of stem cells or angiogenic growth factor positively correlated with fat graft survival both in experimental and clinical studies.

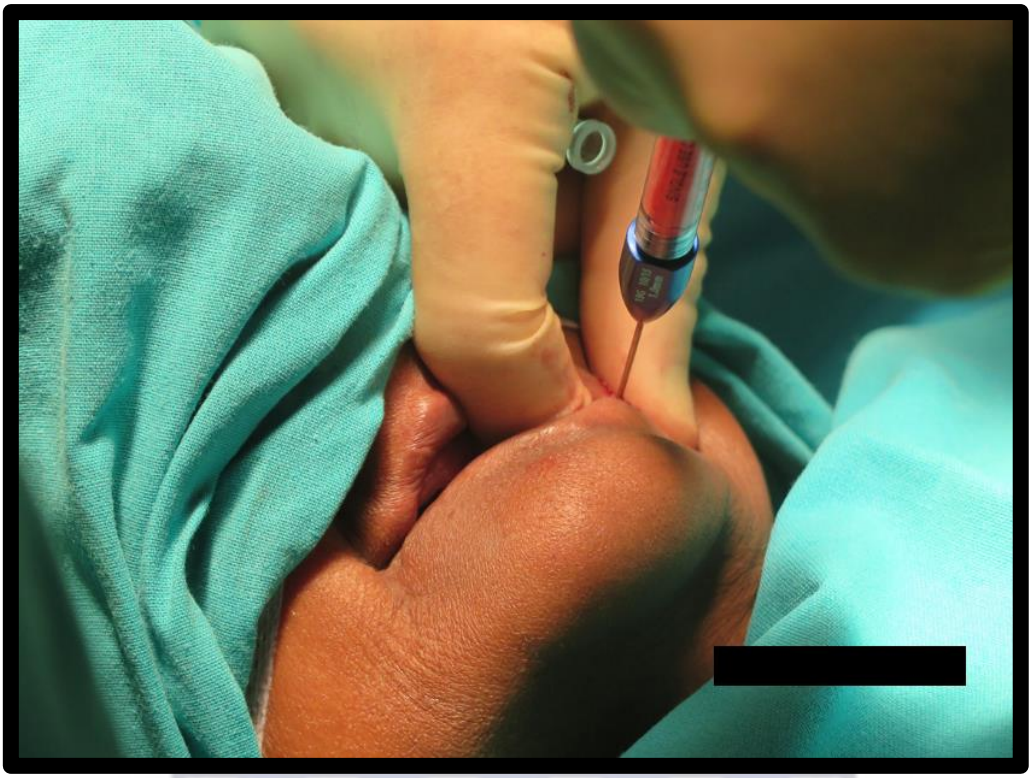


Figure 22 Deposition of post centrifuged lipoaspirate

Five millilitres were inserted bilaterally in this technique.

Enter anteriorly just passed the commissure of the lip, a single puncture is to be made with the blunt tulip cannula and then fan insertion of the lipoaspirate, always remain subepithelial and parallel to the buccal mucosa but do not penetrate into the deeper layers. Fibrous bands will be encountered, try to dissect gently with the blunt cannula as inserting. Pull back on syringe to ensure there is no intra-vascular deposition. Deposit the lipoaspirate gently as the syringe is withdrawn and do not perforate the buccal mucosa when depositing. Avoid opening the mouth vigorously intra-operatively as tears in the thinned out buccal mucosa may occur.

As suggested by Pu *et al*. in 2015 the key to a successful fat graft injection is to achieve an even distribution of fat grafts in the recipient site. Thereby allowing the injected fat grafts to have a maximal amount of contact with the tissue in the recipient site for better fat graft survival through plasmatic imbibition and neovascularisation. Not only can grafting with a small volume in each pass get better surgical outcomes but complications such as fibrosis, oil cyst formation, calcification or even infection with large bolus grafting can be avoided. Slow injection of 0.5 to 1mL/s should be injected during the withdrawal phase.

Chapter 7

Limitations of study

The study was limited by a small sample size. A larger sample size may have allowed for more variations in statistical analysis, which may have added benefit to the study. However, the current data through the objectives were sufficient to conclude statistical significance in p–values. The limited sample size may be attributed to the fact that, OSMF is fairly uncommon in most ethnicities except for those of Asiatic descent with areca nut chewing habits (Indians are a minority population in South Africa) and this treatment option is novel, so a small sample size was anticipated prior to starting this study. Through the inclusion/exclusion data, (*Table 4*), it is seen that patients appeared later in the disease process and were excluded from this study, affecting the sample size.

The COVID-19 pandemic caused dysfunction in the hospital environment and referral pathways were not referring patients as per usual, theatre times were negatively affected. Which may have contributed to limited sample size.

All study participants were of one ethnicity and were female. Having male participants would have been beneficial to establish if any gender differences to treatment existed.

Chapter 8

Recommendations

An international multicentre study where there is a set protocol for intralesional fat injections in OSMF, to evaluate the results with a larger study sample size to review the reproducibility of the treatment outcomes found in this study.

Follow up histology of grafted regions to review the changes at a histological level.

A comparative study for the results obtained from this study against other treatment options.

When using the condition specific questionnaire suggested by Tadakamadla *et al*. in 2017, consider removing questions one to three as they may not be of diagnostic value when reviewing treatment outcome perceptions.

Improve OSMF education amongst patients and clinicians alike.

Immediate referral of OSMF patients to referral centres (even when trismus has not occurred) to be evaluated, these patients need to be educated and put on review programmes even if they require treatment or not.

Suggested treatment protocol for treating OSMF patients with Intralesional autogenous fat injection:

1- Cessation of beetlenut habit
2- Dental management as a priority, all dental work to be done as soon as possible with considerations for elective removal of molars, case and severity dependent.
3- Medical work up- baseline bloods with particular attention to RBC baseline/ vitamin B12 and iron studies to be included. All chronic illnesses, examples such as: Diabetes Mellitus and hypertension to be well controlled
4- Fat grafting protocol

    Harvesting protocol:

    - Stat dose of prophylactic antibiotics: Augmentin® 1.2g intravenously
    - Periumbilical region to be exposed and cleaned with an aseptic medium

- Inject local anaesthetic solution- Marcaine® and saline solution to create a wet medium (20ml of 0.5% Marcaine® mixed with 80ml saline, 40ml of mixed solution injected into harvesting region with a spinal needle)
- Coleman technique to be used for harvesting
- Removal of aspirate via negative pressure from periumbilical area with 20ml syringes

Fat injection protocol:

- Placement of aspirate into a centrifuge at 3000 rpm for three minutes
- Removal of centrifuged material and drain excess fluid
- Place into 10ml syringes
- Use of tulip cannula to insert fat into fibrous bands using negative aspiration before injection
- Five ml to be inserted bilaterally

5- Periodic reviews of the oral cavity for sites of malignancy suspicion
6- Consideration for more than one session of autogenous fat grafting, depending on patient satisfaction

Indications

- Pindborg stage I/II with distinct palpable bands in OSMF patients

Contra-indications

- Pindborg stage III
- Poorly managed co-morbidities examples: poorly controlled Diabetes Mellitus, Aids, chemotherapy, metastatic disease
- Malignancy in the oral cavity
- Patients with signs of unstable mucosa example: multiple sites of previous malignancy or lesions or mucosa under review
- Clinician unable to visualise grafting region adequately due to trismus
- Fat injections into regions of leukoplakia or erythroplakia

Chapter 9

Conclusion

This study has found that intralesional autogenous fat injection in oral submucous fibrosis has shown to be effective in increasing mouth opening, decreasing pain experienced by the participants and improving the participants' perception of quality of life. There were clinical and statistically significant improvements.

Intralesional autogenous fat injections in OSMF has also brought into question whether or not OSMF is truly an irreversible disorder as suggested by Gupta in 2015. It also suggests that adipose tissue and its inherent ASCs could possibly reverse the potentially malignant microenvironment found in OSMF tissues. It may even decrease the malignant transformation risk found within this disease process.

There will always be difficulties in treating OSMF patients however this treatment option is a minimally invasive surgical option, does not rely on adjunctive therapies, it is cost and time effective with no reported complications in this study. The role of adipose tissue and its ASCs in the treatment of scars and aberrant wound healing conditions as such in the case of the OSMF, remains to be an exciting and developing field.

This study will hopefully awaken interest into autogenous fat injection as a stand-alone treatment not only for OSMF, but also open the door for further investigations as to where it may fit into Maxillofacial and Oral Surgery and the dental field as a whole.

The role of ASCs as a by-product of autogenous fat injections and their effects on diseased tissues leads to new possibilities for its uses within dentistry. Fat grafting has been in the realm of Plastic surgeons and many other clinicians who have appreciated its uses, it must now be looked at for its potential applications in Maxillofacial and Oral Surgery and Dentistry. It may have a bright future and be a critical tool in the arsenal of dentists and dental specialists.

Chapter 10

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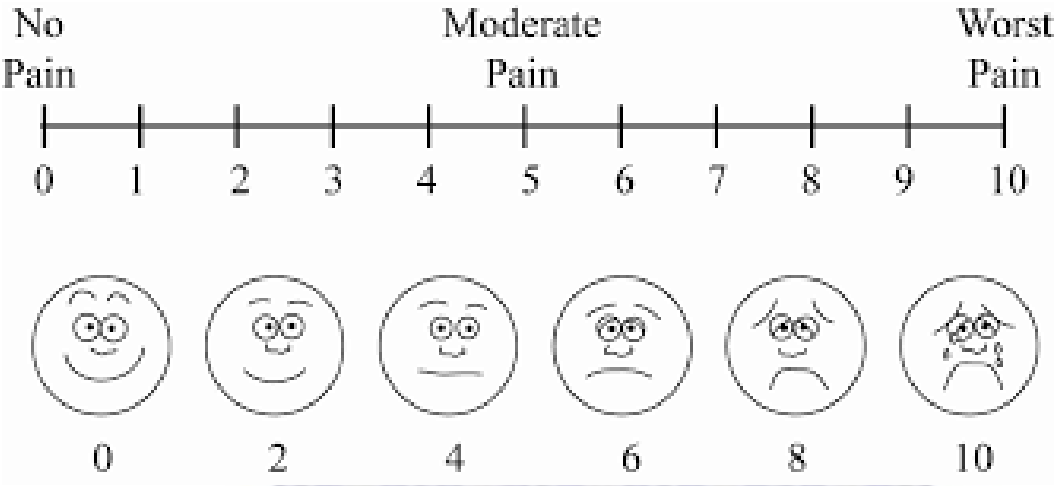
Appendix 1

Visual Analogue scale for Pain (Yale VAS)



Appendix 2

Quality of life questionnaire This questionnaire asks about the effect of your mouth condition on daily life activities (Tadakamadla *et al*., 2017).

Boxes to tick for each question: Not at all /A little /Somewhat /Quite a bit /Very much

1 How difficult was it for you to get your mouth condition diagnosed?

2 How much did the need to visit many doctors for getting your mouth condition diagnosed affect daily life activities?

3 How stressful was it for you to take a variety of treatments before being diagnosed with your mouth condition?

4 How much pain and agony does your mouth condition cause you?

5 How much burning sensation do you experience while having spicy food?

6 How difficult is it for you to open your mouth widely?

7 How much is your oral condition causing you to limit your desired foods?

8 How much is your mouth condition limiting you from enjoying your meals?

9 How much does your mouth condition affect

your taste sensation?

10 How much dryness do you feel in your mouth?

11 How frustrated are you because of your oral condition?

12 How depressed or low do you feel because of your mouth condition?

13 In general, how much is your mouth condition affecting your relationship with family and friends?

14 How much is your mouth condition affecting your satisfaction with life?

15 How scared are you about the possibility of your oral condition turning into cancer?

16 How scared are you about the outcome of this condition affecting your life?

17 How embarrassing is it for you to eat foods at parties, functions, or other social gatherings?

18 How much pain do you experience with treatment of your oral condition?

19 How satisfied are you with the effectiveness of treatment for your mouth condition?

20 How much are your treatment appointments

affecting your daily schedule

Appendix 3

Consent form

Department of Maxillofacial and Oral Surgery

Faculty of Dentistry and WHO Oral Health Collaborating Centre

University of the Western Cape

Cape Town

Title: INTRALESIONAL AUTOGENOUS FAT INJECTION IN ORAL SUBMUCOUS FIBROSIS

The study has been described to me in language that I understand and I freely and voluntarily agree to participate. My questions about the study have been answered. I understand that my identity will not be disclosed and that I may withdraw from the study without giving a reason at any time and this will not negatively affect me in any way.

Participant's name.....

Participant' signature …….

Date.....

Appendix 4

Patient Information

Department of Maxillofacial and Oral Surgery

Faculty of Dentistry and WHO Oral Health Collaborating Centre

University of the Western Cape

Cape Town

Study Title: **INTRALESIONAL AUTOGENOUS FAT INJECTION IN ORAL SUBMUCOUS FIBROSIS**

What is this study about?

This is a research project being conducted by Dr Tashen Gounden from the University of the Western Cape in South Africa. We are inviting you to participate in this research project because you meet the set criterion for the population of interest and your participation will help other people. The purpose of this research project is to evaluate the effect of intra-lesional autogenous fat injections on an individual's oral health-related quality of life.

What will I be asked to do if I agree to participate?

You will be asked to sign a consent form agreeing to take part in the study and will be assigned a study participant number, which will keep you anonymous. The study will involve record keeping of your demographic and medical details. It will also involve recording of your mouth opening/presence or absence of tongue movements/ pain score via visual analogue scale and quality of life (questionnaire) before your operation and 6 months after. The aim is to identify the effectiveness of intralesional autogenous fat injections in patients with oral submucous fibrosis.

Would my participation in this study be kept confidential?

Your personal information will be kept confidential. To help protect your confidentiality you will be assigned a study participant number to identify your data. Only the researchers will have access to your personal data, which will only be used to make the initial group allocation. Your data and any results we obtain will be kept under password protection and in locked cabinets. Your results and opinions will be kept confidential and no personal data will be made public.

What are the risks of this research?

There are minimal risks involved if participating in this research study. These risks are minimal surgical risks such as pain which will be treated with normal pain medication; there could be swelling and bruising in the areas of surgery. There will be no costs involved.

What are the benefits of this research?

The procedure may improve the scarring in your mouth area. You will also have the right to benefit from the researcher knowledge and skills.

Do I have to be in this research and may I stop participating at any time?

Your participation in this research is voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or be discriminated against.

What if I have questions?

Should you have any questions regarding this study and your rights as a research participant or if you wish to report any problems you have experienced related to the study, please contact: Prof Jean Morkel (Supervisor) at jamorkel@uwc.ac.za; tel. 021 937 3087 or Research Ethics Committee at BMREC, UWC, Private Bag x17, Bellville, 7535, Tel: + 27 21 959 4111, Email: research-ethics@uwc.ac.za.

Appendix 5

UNIVERSITY OF THE WESTERN CAPE

FACULTY OF DENTISTRY

PATIENT CONSENT TO CLINICAL PHOTOGRAPHY AND VIDEO RECORDINGS

Surname:	Date of Birth:
Name:	Gender:

I, ... hereby consent to photographs or video recordings being taken of me as requested, I understand that these photographs and recordings will be stored appropriately, treated with the utmost confidentiality and be part of my dental record. I hereby give consent for the images or recordings to be used ONLY for the boxes I have indicated with a tick (√):

☐ **Record purposes and for my/my child's future management**

The photographic images and recordings will form part of the information collected for your care and treatment. This information is handled in accordance with the HPCSA **Booklet 14:** Guidelines on the keeping of patient records.

☐ **Education and training purposes**

The photographic images and recordings may be used for teaching purposes and viewed by health professionals outside of the UWC Faculty of Dentistry. The images may be used for example, in talks, conference presentations, posters or on the Internet to help train other health professionals in the management of dental and oral diseases

☐ **Approved research purposes & publication**

This may involve the photographic images and recordings being used for example in medical or dental publications, journals, textbooks, conference material, e-publications and on the Internet. Images will be seen by health professionals and researchers who use the publications in their professional education. The images may be seen by the general public. Images will not be used with identifying information such as name, however, full confidentiality is not guaranteed.

☐ **Other purposes** (please specify): ……..

- I understand that all efforts will be made to conceal my identity but that full confidentiality cannot be guaranteed.

- I understand that my consent or refusal will in no way affect my dental care.

Patient Signature: ..... Date: .....

Signature: ..... Date: .....

Appendix 6

Record number:

University of the Western Cape

Faculty of Dentistry

INTRALESIONAL AUTOGENOUS FAT INJECTION IN ORAL SUBMUCOUS FIBROSIS

Date of procedure:

*T0- pre-operatively

*T1- 6 months post-operatively

1. Patient Identification

Hospital number:

Birth Date YYYYMMDD/ Age:

Gender (M/F):

Ethnicity:

Disease history:

When did the disease start –

Previous treatment history-

Medical history-

Medications (type and duration)-

Surgical history-

2. General Medical Comorbidities (list)

Co-morbidities	**yes**	**no**	**unknown**	**Treatment (Y/N)**
Asthma				
Hypertension				
Cardiac disease				
Diabetes				
HIV				
Chronic kidney disease				
Chronic liver disease				
Chronic neurological/neuromuscular disease				
COPD/chronic pulmonary disease				
pregnancy				
Immunodeficiency (excluding HIV)				
cancer				
Anaemia				

Other: ……

3. Has there been any complications from the procedure?

Y	**N**

If yes:……...

4. Interincisal mouth opening (calliper)

T0-

T1-

5. presence or absence of restricted tongue movements prior to treatment and six months post-operatively (present vs absent)

T0-

T1-

6. pain score (1-10 visual analogue scale)

T0-

T1-

7. QOL questionnaire

T0

Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q 7	Q 8	Q 9	Q 10	Q 11	Q 12	Q 13	Q 14	Q 15	Q 16	Q 17	Q 18	Q 19	Q 20

T1

Q 1	Q 2	Q 3	Q 4	Q 5	Q 6	Q 7	Q 8	Q 9	Q 10	Q 11	Q 12	Q 13	Q 14	Q 15	Q 16	Q 17	Q 18	Q 19	Q 20

Appendix 7

DEPARTMENT OF MAXILLO-FACIAL AND ORAL SURGERY

Faculty of Dentistry & WHO Oral Health Collaborating Centre

Private Bag X1, Tygerberg 7505

South Africa

Telephone: +27 21 937 3151/50

Fax: +27 21 937 3182

Email: jamorkel@uwc.ac.za (Prof JA Morkel)

jdewet@uwc.ac.za (Ms J de Wet)

For Attention: The CEO

Dear Dr T. T. Khanyile

RE: Application to conduct research study at the Hospital

A Master's student, Dr Tashen Gounden, is conducting research under the supervision of Prof Jean Morkel of the Dept. of Maxillofacial and Oral Surgery. The title of the study is "intralesional autogenous fat injection in oral submucous fibrosis".

We will use our findings to compile our results and complete our research project which will be for an MSc in Maxillofacial and Oral Surgery. Ethical approval will be requested from the UWC Research Ethics Committee, for consideration for registration as an approved research project.

Please do not hesitate to contact me should you require anything further
Yours sincerely

Dr Tashen Gounden
Researcher

Prof Jean Morkel
Supervisor

Appendix 8

UNIVERSITY of the WESTERN CAPE

Department of Institutional Advancement
University of the Western Cape
Robert Sobukwe Road
Bellville 7535
Republic of South Africa

11 May 2021

Dr T Gounden
Maxillo-Facial and Oral Surgery
Faculty of Dentistry

Ethics Reference Number: BM21/03/05

Project Title: Intralesional autogenous fat injection in oral submucous fibrosis

Approval Period: 07 May 2021 – 07 May 2024

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

Permission to conduct the study must be submitted to BMREC for record-keeping.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

KWAZULU-NATAL *PROVINCE* **Appendix 9**

HEALTH

REPUBLIC OF SOUTH AFRICA

DIRECTORATE:

INKOSI ALBERT LUTHULI CENTRAL HOSPITAL
OFFICE OF THE MEDICAL MANAGER
Private Bag X03, Mayville, 4058
800 Vusi Mzimela (Bellair) Road, Mayville,4091
Tel: 031 240 1059 Fax: 031 240 1005 Email: Ursula.john@ialch.co.za

Reference: HREC REF: 17/2019

Enquiries: Medical Management

Dr T Gounden

Maxillo-Facial and Oral Surgery

Faculty of Dentistry

Dear Dr Gounden

RE: PERMISSION TO CONDUCT RESEARCH AT IALCH

I have pleasure in informing you that permission has been granted to you by the Medical Manager to conduct research on: Intralesional autogenous fat injection in oral submucous fibrosis.

Kindly take note of the following information before you continue:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN Department of Health.
3. Kindly ensure that this office is informed before you commence your research.
4. The hospital will not provide any resources for this research.
5. You will be expected to provide feedback once your research is complete to the Medical Manager

.....

Dr A Harrichandparsad Clinical Care Manager

KWAZULU-NATAL PROVINCE **Appendix 10**

Postal Address: Private Bag X9050
Physical Address: 330 Langalibalele Str; PM Burg; 3201
Tel: 0333953189/3123/2805 Fax: 033-3943782
Email address: hrkm@kznhealth.gov.za
www.kznhealth.gov.za
Health Research & Knowledge Management Unit

NHRD Ref: KZ_202106 009

Dear Dr T Gounden

(University of the Western Cape)

The research proposal titled 'INTRALESIONAL AUTOGENOUS FAT INJECTION IN ORAL SUBMUCOUS FIBROSIS' was reviewed by the Kwa-Zulu-Natal Department of Health (KZN-DOH).
The proposal is hereby approved for research to be undertaken at Inkosi Albert Luthuli Central hospital.

All research conducted in KwaZulu-Nata/ must comply with government regulations relating to Covid19. These include but are not limited to: regulations concerning social distancing, the wearing of personal/ protective equipment, and limitations on meetings and social gatherings. Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research. Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires. Provide an interim progress report and final report (electronic and hard copies) when your research is complete to HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200 and e-mail an electronic copy to hrkm@kznhealth.gov.za.

Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study. For any additional information please contact Ms G Khumalo on 033-395 3189,

Yours Sincerely

Dr E Lutge
Chairperson, Health Res
Date: 08/06/2021